ATTENTION - DEFICIT/HYPERACTIVITY DISORDER – ALTERATIONS OF MOTOR BEHAVIOUR AND DOPAMINERGIC TRANSMISSION

by

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Cover illustration: The PET image showing distribution of [11C]PE2I binding in the human brain.

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Felix, qui potuit rerum cognoscere causas

Vergilius, Georgicae, II, 490
Abstract

Altered catecholaminergic neurotransmission in the brain has long been thought to be of importance in the regulation of motor behavior and cognitive performance in children who had symptoms of distractibility, impulsivity and clumsiness. The dopamine theory of AD/HD has mainly been substantiated by: i) the effects of psychostimulants, which target the dopamine transporter (DAT), and thereby reduce impulsiveness and inattentiveness, increases the striatal cerebral blood flow and functional activity, and ii) the evidence from linkage studies associating the AD/HD syndrome with allelic variations of genes encoding the dopamine transporter and, possibly, the dopamine D4 receptors. However, the central regulatory mechanisms of dopamine neurotransmission in AD/HD have not been established yet.

The main aim of this thesis was to examine the dopaminergic system in vivo in children with AD/HD by using positron emission tomography (PET). Twelve adolescents with AD/HD and ten young adults were investigated applying the double-tracer paradigm. Presynaptic and postsynaptic dopamine markers, DAT and dopamine D2 receptors, were mapped using the radioligands $[^{11}C]$PE2I and $[^{11}C]$raclopride. In the group of adolescents with ADHD, we also investigated relationship between the central dopamine markers and behavioral/cognitive performance. A new radioligand $[^{11}C]$PE2I that has a high affinity and selectivity to the central DAT was used. Its favourable signal-to-background ratio enabled us to quantify the DAT density in the human striatum and midbrain; cross-validation of quantification methods of $[^{11}C]$PE2I binding permitted its application in the clinical study.

The PET measurements showed that the DAT and DRD2 density in the striatum did not differ between adolescents and young adults once a correction had been made for age. Thus, the initial findings of increased DAT in the striatum in AD/HD reported in the literature were not confirmed. The decreased regional density of DAT found in the substantia nigra/ventral tegmentum rather suggests a shift in the focus of the pathophysiology of AD/HD to the midbrain structures. In addition, positive correlations between hyperactivity levels and the density of dopamine markers in the striatum support similar reports by other authors and provide evidence for the involvement of the dopaminergic system in the pathophysiology of AD/HD.

The AD/HD syndrome is a heterogenous diagnostic entity, and it is still a matter of debate whether perception and movement coordination problems are a constituent part of it. We investigated movement coordination problems from the perspective of motor control theories with a load lifting task, providing measurements of manipulative movements and associated postural adjustments. Fifty two children were investigated, including an additional control group of younger children, with the intention of addressing the developmental aspects of motor behavior. The results showed that children with AD/HD and developmental coordination disorder, or both, have a deficit in the programming of their motor behavior that is related to the severity/complexity of the syndrome, but which does not correspond to the motor performance of younger children. The deficient parametric control of the motor output, and lack of temporal coordination between the lifting movement and the postural responses limited the adaptation of the motor behavior to the environment.
Publications

This thesis is based on the following publications. They will be referred to in the text by their respective Roman numerals.


IV. Jučaitė A., Fernell E., Haldin C., Forssberg H., Farde L. Reduced dopamine transporter binding in the midbrain of adolescents with ADHD; association between dopamine transmission markers in the striatum and motor hyperactivity. (manuscript)
### List of abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AD/HD</td>
<td>Attention-Deficit/Hyperactivity Disorder</td>
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<tr>
<td>DCD</td>
<td>Developmental Coordination Disorder</td>
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<td>AD/HD+</td>
<td>combination of AD/HD and DCD</td>
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<td>MND</td>
<td>Minor Neurological Disorder</td>
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<td>APA</td>
<td>Anticipatory postural adjustments</td>
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<td>CBCL</td>
<td>Child Behavioral Checklist</td>
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<td>DSM</td>
<td>Diagnostic and Statistic Manual of Mental Disorders</td>
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<tr>
<td>ICD</td>
<td>International Statistical Classification of Diseases and Related Health problems</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>SPET</td>
<td>Single photon emission tomography</td>
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<td>BP</td>
<td>Binding potential</td>
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<td>IV</td>
<td>intravenous</td>
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<td>ROI</td>
<td>region of interest</td>
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<td>TAC</td>
<td>time activity curve</td>
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<tr>
<td>CM</td>
<td>compartment model</td>
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<td>SRTM</td>
<td>Simplified Reference Tissue Model</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
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<td>EEG</td>
<td>electroencephalography</td>
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<td>EMG</td>
<td>electromyography</td>
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<tr>
<td>GF</td>
<td>grip force</td>
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<td>LF</td>
<td>load force</td>
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<td>COP</td>
<td>center of pressure</td>
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<td>COG</td>
<td>center of gravity</td>
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<td>SR</td>
<td>specific radioactivity</td>
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<td>FWHM</td>
<td>Full Width Half Maximum</td>
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<tr>
<td>ABSS</td>
<td>automated blood sampling system</td>
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<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
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<tr>
<td>DRD1, DRD2,</td>
<td>dopamine receptor D1, D2, D3, D4, D5</td>
</tr>
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<td>DRD3, DRD4, DRD5</td>
<td></td>
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<tr>
<td>HVA</td>
<td>homovalinic acid</td>
</tr>
<tr>
<td>SN</td>
<td>substantia nigra</td>
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<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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<tr>
<td>PCBs</td>
<td>polychlorinated biphenyls</td>
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<tr>
<td>DAT</td>
<td>dopamine transporter</td>
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<td>CPT</td>
<td>continuous performance task</td>
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<td>RT</td>
<td>reaction time</td>
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<td>VSWM</td>
<td>visuo-spatial working memory</td>
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### Units:

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<tr>
<td>Bq</td>
<td>becquerel, activity (referred to a radionuclide, equal to one reciprocal second, s⁻¹)</td>
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<td>Ci</td>
<td>curie</td>
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<tr>
<td>N</td>
<td>newton, unit of force, m·kg·s⁻²</td>
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INTRODUCTION

I. GENERAL INTRODUCTION

“Passionate, deviant, spiteful and lacking inhibitory volition”

Still, 1902, Lancet (on children with abnormal behaviours)

Attention-deficit/hyperactivity disorder (AD/HD) is a diagnostic category comprising a particular constellation of cognitive deficits and atypical behaviours. The cardinal symptoms expressed are: impaired attention, excessive motor activity, impulsivity and distractability.

AD/HD as a diagnostic entity

The concept of the diagnosis “AD/HD” may serve as an example of the history of the medical thought over the past hundred years. The initial observations of deviant overactivity and conduct disorders were once judged to be a “defect of moral self-control” (Still, 1902). The subsequent decades were framed by belief in brain damage that was not extensive enough to cause severely handicapping neurological conditions, such as cerebral palsy and seizures, but which nevertheless resulted in signs of neurological dysfunction. The terms “minimal brain damage”, “minimal brain dysfunction”, “minor neurological dysfunction” and “minor neurological disorder” have long been in use and the latter, indeed, is still used (Hadders-Algra 2002, Fily et al. 2003). Here, clinical neurological investigations and symptoms are linked predominantly to a single group of aetiologies, related to the perinatal brain damage. Over time, however, the extent of the problems recognised in the group of children with atypical behaviour has broadened, revealing a great complexity of neurodevelopmental disorders. There was increasing need to classify the apparent cognitive and behavioural symptoms. With the introduction of DSM-III in 1980, the significance of cognitive deficits was emphasized in this group of children. The focus shifted to the inattention as a central and primary symptom, and the category was assigned the name ADD — Attention Deficit Disorder, with the description “with” or “without hyperactivity”. In the subsequent revision of the diagnostic criteria, DSM-III-R (1987), cognitive problems and deviant behaviour were linked into a single whole, Attention-Deficit Hyperactivity Disorder. Seven years later this coexisting set of symptoms was liberated by the introduction of subtypes of Attention-Deficit/Hyperactive Disorder “predominantly inattentive” and “predominantly hyperactive-impulsive” and “combined” (DSM-IV, APA, 1994). The ICD-10 (WHO, 1993) diagnostic system introduces the term “Hyperkinetic disorder”. It is diagnosed using the same symptoms, however, in a different cluster. In addition, the presence of mood and anxiety disorders excludes AD/HD diagnosis. Altogether, the ICD-10 criteria are more restrictive and, in contrast to the DSM-IV system, they have high specificity, low sensitivity and, ultimately, lead to underestimation of the prevalence of AD/HD.

AD/HD: categorical vs dimensional approach. As a diagnostic category formulated in the DSM-IV classification scheme (1994, APA) “AD/HD” firstly serves the purpose for convenience of communication, and helps to cope with the complex information. Diagnosis of AD/HD is formed as a cluster of different syndromes and is, by and large, a heterogenous disorder, the validity of which is still questioned. There is ongoing debate that concerns: i) the separability of attention-deficit and hyperactivity-impulsivity profiles, ii) the delimitations of the categorical approach and iii) the coexistence of other neurological, cognitive and behavioural problems (developmental coordination disorder, conduct disorder, etc.). Meanwhile, research into the causes and pathophysiology of AD/HD requires a representative and reasonably homogenous group of subjects for precision and the reproducibility of the results. In such a case, dimensional approach can be used, which perceives a symptom, like hyperactivity,
Diagnostic criteria for Attention-Deficit/Hyperactivity Disorder

A. Either (1) or (2):
   (1) six (or more) of the following symptoms of inattention have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:

   Inattention
   (a) often fails to give close attention to details, makes careless mistakes in schoolwork or other activities
   (b) often has difficulty sustaining attention in tasks or play activities
   (c) often does not seem to listen when spoken to directly
   (d) often does not follow through on instructions and fails to finish schoolwork (not due to oppositional behavior or failure to understand instructions)
   (e) often has difficulties in organizing tasks and activities
   (f) often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework)
   (g) often loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books, or tools)
   (h) is often easily distracted by extraneous stimuli
   (i) is often forgetful in daily activities

   (2) six (or more) of the following symptoms of hyperactivity-impulsivity have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:

   Hyperactivity
   (a) often fidgets with hands or feet or squirms in seat
   (b) often leaves seat in classroom or in other situations in which remaining seated is expected
   (c) often runs about and climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)
   (d) often has difficulty playing or engaging in leisure activities quietly
   (e) is often “on the go” or often acts as if “driven by a motor”
   (f) often talks excessively

   Impulsivity
   (g) often blurs out answers before the question has been completed
   (h) often has difficulty awaiting turn
   (i) often interrupts or intrudes on others (e.g., butts into conversations or games)

B. Some hyperactive-impulsive or inattentive symptoms that caused impairment were present before age 7 years.
C. Some impairment from symptoms is present in two or more settings (e.g., at school [or work] and at home).
D. There must be clear evidence of clinically significant impairment in social, academic, or occupational functioning.
E. The symptoms do not occur exclusively during the course of a Pervasive Developmental Disorder, Schizophrenia, or other Psychotic Disorder and are not better accounted for by another mental disorder (e.g., Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder).

DSM-IV, 1994 (314.01 AD/HD, Combined Type, 314.00 AD/HD, Predominantly Inattentive Type, 314.01 AD/HD, Predominantly Hyperactive-Impulsive Type)

Diagnostic criteria for 315.4 Developmental Coordination Disorder

A. Performance in daily activities that require motor coordination is substantially below chronologic age and measured intelligence. This may be manifested by marked delays in achieving motor milestones (e.g., walking, crawling, sitting), dropping things, “clumsiness”, poor performance in sports, or poor handwriting.
B. The disturbance in Criterion A significantly interferes with academic achievement or activities of daily living.
C. The disturbance is not due to a general medical condition (e.g., cerebral palsy, hemiplegia, or muscular distrophy) and does not meet criteria for Pervasive Developmental Disorder.
D. If Mental Retardation is present, the motor difficulties are in excess of those usually associated with it.
to be a feature with a continuous distribution in the population. There is, however, no distinct boundary between the two approaches. In this thesis, we have viewed AD/HD as a category with a set of dimensional symptoms.

**Comorbidity issues.** The real picture of AD/HD is even more complex. This is because of the coexistence of a broad spectrum of neurodevelopmental disorders such as conduct (CD) and oppositional-deviant disorders, language and reading deficits, motor disorders, autistic spectrum disorders, anxiety, etc. (Kadesjö and Gillberg, 2001). The key issue is whether the numerous disorders represent varied manifestations of the same disorder (e.g., AD/HD, DCD, dyslexia) or simultaneous presence of two unrelated conditions, for example owing to genetic co-segregation (ex. Tourette syndrome and AD/HD (Comings et al, 1996)). Alternatively, one disorder may increases the risk of having another, e.g., difficulty coping with AD/HD symptoms could lead to anxiety, depression and slower learning (Rutter et al, 1997, Angold et al, 1999, Gillberg 1998). Yet another outlook is that different clinical syndromes are linked and just expressed during different periods of development. For example, combined type AD/HD could develop with age into obsessive-compulsive, conduct disorders and, in combination with CD may develop into drug or alcohol abuse (Flory and Lynam, 2003).

The syndrome addressed specifically in this thesis is the Developmental Coordination Disorder (DCD). This occurs in every second child with AD/HD (Barkley 1997, Gillberg 1998). A broader overview is provided in the upcoming section on the coordination of movements (see p. 7).

**Theories of AD/HD**

The development of the classification systems parallels changes in the concepts of AD/HD. Different and contradictory models have been suggested. Neuropsychological schools view AD/HD as a disorder arising from a cognitive deficit, e.g., interruption of frontal “executive” functions (Barkley 1997). Four main aspects of executive dysfunction have been highlighted as impaired in AD/HD: the working memory, self-directed speech, inhibition and self-control. The inability to inhibit behaviour was suggested as a core deficit and a precursor of later development of executive dysfunctions (Barkley 1997). However, no follow-up studies were performed to prove this hypothesis. Another model of AD/HD within the framework of executive dysfunction was developed by Sergeant and Van der Meere (Sergeant, 2000, review). These authors suggested that AD/HD is a disorder of cognitive-energetic state regulations. Failure to modulate arousal and activation, and to maintain effort during the performance of a task is impaired in AD/HD. Yet another independent approach has developed from investigations of the reward systems (Sagvolden et al, 1998, Johansen et al, 2002). According to this motivation-based model of impulsivity/hyperactivity, alterations in behaviour develop from the heightened sensitivity to delayed reward and development of delayed aversion\(^3\). As a consequence, afflicted children attempt to avoid delay, and direct their attention to different stimuli because they are unable to count for future rewards (Kuntsi et al, 2001, Sonuga-Barke et al, 1998, Solanto et al, 2001).

In contrast to the search for a “core deficit” or a single impaired brain region several integrative models of AD/HD have been suggested. Sonuga-Barke (2003) argued that the two previously mentioned models of executive dysfunction and altered motivation should be treated as complementary in understanding of AD/HD, despite the fact that they are dissociable. Another, “two-stage” model of AD/HD has been postulated by McCracken (1991) and subsequently developed by Pliszka et al. (1996) in an attempt to integrate neurochemical alterations in AD/HD and relate them to impaired cognitive processes. The principle underlying this model suggests a fundamental chemical dysregulation in the brainstem nuclei, leading to excessive tonic activity in the locus ceruleus (the norepinephrine system) and, as a consequence, disinhibition of neurons in the raphe nucleus (the serotonin system) and facilitation of the firing of dopamine neurons in the ventral tegmental area. As a result, deficits develop in the posterior attention system (the attention shifts), the anterior

\(^3\)Executive function (EF) — the “ability to maintain a problem-solving set for attainment of a future goal” (Luria, 1966). EFs include set-shifting, set maintenance, interference control, inhibition, integration across space and time, planning, and working memory. Cortex-specific action selection is control to EF.

\(^4\)Aversion to delay — negative emotional response to delay.
attention system (focused and sustained attention) and reward system. Different approaches and areas of knowledge have recently been combined into a single stratified system linking genetic-environmental etiological factors to the AD/HD symptoms via endophenotypes\(^7\) (Castellanos et al, 2002). It has been suggested that the endophenotypes such as working memory, temporal processing and shortened delay gradient are central to AD/HD research.

**Aetiopathogenesis of AD/HD**

The aetiology and the pathophysiological mechanisms of AD/HD still are largely unknown and difficult to investigate owing to the aforementioned heterogeneity of phenotype. At present, AD/HD is viewed as the result of an interaction between genetic endowment and environmental influences. Findings from adoption and twin studies have indicated that genetics plays a major role in the aetiology of AD/HD, with a heritability index of \( \lambda = 0.75-0.91 \), one of the highest found among neurodevelopmental disorders (Levy et al, 1997, Thapar et al, 1999, Todd et al, 2001). The molecular aetiology of AD/HD has been extensively explored and a few genome-wide scans for gene loci involved in AD/HD have been performed (Fisher et al, 2002, Ogdie et al, 2003, Bakker et al, 2003) yielding candidate regions on the 16p13, 5p13 and 17p11.1-17q11 chromosomes. Meta-analysis studies (Faraone et al, 2001) have confirmed that the most commonly replicated findings were associations of the dopamine D4 receptor, DRD4, and the dopamine transporter, DAT1, genes and AD/HD traits. AD/HD patients having 7-repeat allele\(^6\) DRD4 were prone to an obsessive-compulsive profile, impulsiveness, higher hyperactivity levels as measured by an actigraph and shorter reaction time (Langley et al, 2004) and high novelty seeking behaviour (LaHoste et al, 1996). Genes coding for noradrenaline and nicotine receptors have also been studied. However, there are no conclusive results as yet. The gene encoding 5HT 1B receptors has been related to hyperlocomotor activity in animal studies (Adams et al, 2004). A recent magnetic resonance spectroscopy (MRS) study evaluating the effects of treatment of AD/HD evidenced involvement of the glutamate system (Carrey et al, 2002). Furthermore, a family-based association analysis has provided evidence of a relationship between the glutamate receptor gene, GRIN2A, polymorphism and an increased risk of having AD/HD (Turic et al, 2004).

Despite substantial efforts, no single gene has thus been conclusively associated with AD/HD. Rather, the phenotype of AD/HD resembles patterns consistent with a complex trait where a polygenic inheritance and environmental factors exert a shared influence. Furthermore, the AD/HD phenotype may represent a final common pathway with multiple aetiologies. Of interest in this respect is the study of Kahn et al, from 2003, which showed that homozygosity for the 10-repeat allele of the DAT1 gene was associated with hyperactivity/impulsivity only in the presence of maternal perinatal smoking. In addition, unfavourable perinatal factors (such as hypoxia, infection and trauma, low birth weight) may lead to a non-optimally wired brain. It has been suggested that these factors might increase the risk of developing AD/HD (Sooarani-Lusung et al, 1993, Lou et al, 1996, Mick et al, 2002), especially if the insults involve the basal ganglia (Lou et al, 1996). Neurotoxic factors, like prenatal exposure to polychlorinated biphenyls (PCBs) have also been associated with working memory deficits, impulsivity, and poor concentration (Jacobson and Jacobson, 2003).

Currently there is a belief that disturbances of the fronto-striatal circuits form the basis of AD/HD. The most robust findings supporting the fronto-striatal theory come from MRI-based morphometric studies. The possible anatomical landmarks of the AD/HD patient group are: i) a reduced global brain volume (reduced by 4%) and ii) regional volumetric differences, and in particular, a decreased volume of the caudate nucleus, the rostral area of the corpus callosum and the cerebellar vermis (Hynd et al, 1993, Castellanos et al, 1996b). Longitudinal developmental studies of brain morphometry have also pointed to the caudate nucleus and the frontal cortex as potential brain regions associated with AD/HD (Castellanos

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\(^7\)Endophenotype — a trait that is associated with the expression of an illness in the population. It is heritable, manifests itself in illness and is believed to represent the genetic liability of the disorder among non-affected subjects. Endophenotypes can be biochemical, neurophysiological, neuroanatomical, cognitive or neuropsychological (Gottesman et al, 2003).

\(^6\)Alleles — versions of the gene at corresponding loci on a pair of homologous chromosomes.
et al., 2002). Studies into the function of the brain have reported regional striatal hypoperfusion and hyperperfusion in primary sensory and sensorimotor cortical regions (Lou et al., 1989, Kim et al., 2001), decreased perfusion in the prefrontal cortex during the performance of attention-demanding tasks (Amen and Carmichael, 1997), and altered patterns in the fronto-striatal activation in response to inhibition task (Durston et al., 2003).

Taken together, there is considerable evidence for preferential involvement of the prefrontal-dorsolateral and medio-orbital cortical areas and for striatal structures in the pathophysiology of AD/HD. A model of imbalance in multiple parallel frontal-subcortical circuits (Alexander et al., 1990) has been suggested to understand the coexistence of cognitive, behavioural and motor symptoms of AD/HD.

✦✦✦

The focus of this thesis has been on different aspects of motor behaviour of children with AD/HD. Two different perspectives were adopted. In the first part, the movement coordination problems that children with AD/HD often experience were addressed. The neurophysiological basis of motor problems was studied from the perspective of cognitive neuroscience, e.g., anticipation and internal neural representations. In the second part of the thesis, we investigated the neurobiological basis of motor hyperactivity and investigated its relationship with the alterations in the central dopamine system.

II. MOVEMENT COORDINATION AND AD/HD: ASPECTS OF NEURAL CONTROL

Movement coordination from a motor control perspective

Most of us have an intuitive “gestalt” sense of what constitutes coordinated movements. We can easily detect when a clumsy motor action is performed clumsily, and we can identify abnormal motor behaviours. An unusual gait is obvious to the eye, although it is sometimes difficult to describe what is so atypical about it. Views on what truly constitutes movement coordination are diverse. Movement coordination may be understood as the organisation of movement components in a harmonious action, e.g., reaching and grasping.

A particular kind of coordination exists between movement and posture. Usually we are aware of making movements that serve a particular intention (e.g., throwing something or reaching for an object). However, we are not aware of the consequences of these intentional movements on our posture. Voluntary movements disturb the equilibrium of the whole body by introducing inertial forces which change position of the body’s centre of gravity (COG).

One of the aims of the postural control system is to keep the projection of the COG on the ground steady. In other words, it needs to stabilise the body parts against any internal or external perturbations. The central nervous system has two modes to control postural stability. After external perturbations, it uses the feedback mode of control, e.g., loss of balance induces corrective postural reactions via the sensory system. In voluntary movement, another, predictive, feed-forward strategy is used. The central nervous system has the ability to foresee loss of postural stability and plan anticipatory postural adjustments (APAs) (Massion 1992). The functional aim of these adjustments is to prevent or decrease the loss of balance by counteracting perturbing forces.

Posture – is defined by the position of the different body segments at a given instant due to a tonic muscle activity. Postural control – active maintenance of a given posture on the basis of sensory reafference (Paillard, 1971, Gurflinkel and Shli, 1973)
A feed-forward organisation of movement is using memory representations (Fig. 1) that carry information about previous experiences, e.g., the shape, weight, size and friction of an object that has been previously manipulated (Johansson and Cole, 1992). These memory representations resemble working memory, i.e., they are continuously updated and can be retrieved and used in planning and correction of ongoing movement. This concept has been broadly investigated in studies on precision grip, both in adults and during development (Johansson, 1996, Forsberg et al, 1992, 1999). When organising the motor commands and setting the parameters for the upcoming movement, the central nervous system is using representations of the relevant object in extra-personal space, as well as internal models of the subject’s own motor apparatus (the position of the body, arm, etc.). In summary, a continuous interaction between the central motor programs, sensory feedback and motor memory is a prerequisite for coordinated movement.

The investigations of postural control in our study were limited to the anticipatory postural adjustments (APAs). They are defined as muscle activation patterns occurring shortly before or concomitantly with the onset of self-initiated movement (Belenky et al, 1967). APAs are integral part of the motor activity and are characterised by direction-specificity and adaptation to the requirements of the task (Diener et al, 1992). The pattern of APAs varies in accordance with other movement parameters, such as the velocity of movement and the body segments involved (Bouisset and Zattara, 1987, Horak and Nashner, 1986, Nashner and Forsberg, 1986, van der Fits et al, 1999). APAs are not always present, they may be reduced or eliminated when a movement slows down, since then sensory feedback correction mechanisms can take over. Body support may also preclude the necessity of APAs. They are not found during externally induced postural perturbations, unless they are cyclic and predictable (Massion, 1992, Kaluzy and Wiesendanger, 1992, Aurin and Latash, 1995, Eliasson et al, 1995, Dietz et al, 1993).

Anticipatory postural adjustments have most often been investigated with the focus on the movement of the extremities: for locomotion, arm or leg movements or for the performance of bimanual tasks.

According to the framework of control system theory there are two classes of control systems: feedback and feed-forward. The feedback system measures the controlled variable (i.e., the output) and compares it with the desired value. If there is a discrepancy between the two, the output is corrected after the error has occurred. Feed-forward control systems anticipate the effect of environmental disturbance and apply predictive adjustments. Motor systems have characteristics of both feed-forward and feedback control systems.

Postural reactions and adjustments are the products of motor programs. Motor programs are comprised of a set of muscle commands that are structured and sent to the muscles with the appropriate timing and in the appropriate sequence. For practically all movements, the forces of agonist and antagonist muscles are planned and organised into so-called muscle synergies. The motor programs operate in a feedforward mode.
(Hugon et al, 1982, Bouisset and Zattara, 1997, Aruin and Latash, 1995, Schmitz et al, 2002). As yet, no investigations have been made of the relationship between fine voluntary movements, such as precision grip and postural responses. Neural organisation and the control of manipulative finger movements and posture are distinct and investigated separately. Little is known about the coordination of those two relatively independent motor systems.

**AD/HD and developmental coordination disorder**

In a clinical setting, soft neurological signs and motor problems (such as poor handwriting, difficulties performing tasks requiring precise fine movements and balance, and poor visuo-motor coordination) are seen in many children with AD/HD (Barkley, 1997). They are of clinical importance since the addition of motor syndrome to AD/HD diagnosis leads to a considerably poorer prognosis (Gillberg and Kadesjö, 2003, Gillberg 2003).

Moderate and minor movement problems in children have been noted in the two diagnostic systems, “Specific developmental disorder of motor function” (ICD-10, 1993), “Developmental Coordination Disorder” (DSM-III-R, 1987) and previously popular term “clumsy child syndrome” (Henderson and Hall, 1982, Taft and Barowsky, 1989) all denote movement coordination problems below the appropriate age and intelligence levels. Developmental dyspraxia is yet another term used. It is understood as the impairment of the ability to plan and carry out motor actions (Dewey, 1995).

A longstanding discussion is whether there is a link between motor clumsiness and the behavioural manifestations of AD/HD since they overlap more often than by chance alone. Clumsiness in children with AD/HD has been considered by some authors to be secondary to impulsivity and inattention. A series of studies has been performed in which motor activity was investigated and explained within an information processing framework. The most replicated finding was prolonged reaction time and increased variability of the reaction time. These results were interpreted as slower motor decision and motor preparation problems in children with AD/HD (van Der Meere et al, 1992, Rubia et al, 1999).

Another line of studies has been conducted on the aspects of sensorimotor integration in AD/HD, with a focus on the kinaesthetic abilities. Whitmont and Clark, 1996 showed that children with AD/HD have poorer kinaesthetic acuity, which correlated with fine motor skills, but not with the severity of the AD/HD. In contrast, Piek et al, 1999 found correlations between inattentiveness, impulsivity and the level of movement function (using Movement ABC scores). However, they could not replicate the findings of decreased kinaesthetic sensitivity in children with AD/HD.

**DCD** is also an umbrella term and covers a heterogeneous phenotype. DCD is intertwined with attention and language deficits. Commonly encountered problems in children with DCD include: 1) visuospatial deficits, 2) attention and memory deficits, 3) fine motor and language deficits and 4) movement sequencing deficits (see review, Visser 2003).

Developmental coordination disorder has also been considered to be the result of sensory processing problems, a deficit in sensorimotor integration (Ayers 1972). Bairstow and Laszlo, 1981, have suggested deficient kinaesthetic sensation, whilst Lord and Hulme 1987a, could not confirm these findings and have suggested that the visual system and visuo-motor integration are one of the sources of clumsiness (Lord and Hulme 1987b, Sigmundsson, 2003, review).

In the group of children with DCD, cerebellar and basal ganglia pathology was inferred, based on temporal deficits in a finger tapping task (Lundy-Ekman et al 1991). No functional neuroimaging studies have been performed to examine the motor problems of children with DCD or AD/HD and no brain morphometric studies exist on children with DCD. At present, the specificity of the movement component in AD/HD, and, indeed, in developmental disorders in general, remains unclear and an understanding of its neurophysiological basis awaits further studies.

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8Kinaesthesia — system of sensory feedback related to the perception of movement.
III. THE Dopamine SYSTEM AND AD/HD: FROM NEUROCHEMICAL INDIVIDUALITY TO BEHAVIOUR

Dopamine is a phylogenetically old neurotransmitter intrinsic to brain function and behavior. It is of central importance in normal behaviours, such as movement, reward associated behaviour and emotions. Abnormal patterns of dopamine neurotransmission have been suggested to underly several neurological or psychiatric disorders, e.g. Parkinson’s and Huntington’s diseases, schizophrenia, drug abuse and AD/HD.

The dopaminergic system
Macroanatomy. Dopamine neurons are aggregated in nine distinct clusters: the ventral midbrain (A8-9-10), diencephalon (A11-15) and telencephalon (A16-olfactory bulb, A17 and the retina). There are three major nuclei in the brain which contain dopaminergic cell bodies: 1) the substantia nigra, pars compacta (SN, A9), located in the ventral midbrain; 2) the ventral tegmental area, VTA or A10, lying medial to SN; and 3) the arcuate nucleus of the hypothalamus, or A11-15 (in the diencephalon). Smaller groups of dopaminergic neurons are located in the retina and the olfactory bulb.

The dopaminergic projections from these neurons are distributed throughout the anatomically segregated neuronal systems that control motor, limbic and cognitive aspects of behaviour. The dopaminergic projections form three major pathways: 1) the nigrostriatal pathway, containing over 80% of all dopaminergic innervation, involved in the control of movement; 2) the mesolimbic pathway, with neurons from VTA synapsing in the nucleus accumbens and amygdala, engaged in emotions; and 3) the mesocortical pathway, originating in the VTA and terminating in the prefrontal cortex, anterior cingulate, entorhinal cortices, largely involved in cognitive functions. In addition, several shorter pathways have been identified: 4) the tuberoinfundibular pathway from the hypothalamic nucleus to the anterior pituitary, contributing to neurohumoral regulation on lactation; 5) the mesohippocampal tract that originates in the SN-VTA and terminates at the hippocampus,

![Diagram of dopaminergic axons](image)

involved in memory formation, and 6) the mesofrontal tract, from the SN to the prefrontal cortex, involved in reward systems.

Neurotransmission including the synthesis-storage-release-receptor binding and neurotransmitter uptake or degradation, is a highly controlled process. The complex balance of this cascade determines the intensity of monoaminergic signalling. Two units of the neurotransmission system have been investigated in the present work, i.e., the dopamine transporter, located at the presynaptic site of the dopaminergic neuron, and the dopamine D2 receptor density at the postsynaptic site.

Dopamine transporter; distribution and function. The topology of the dopamine transporter shows that it is a plasma membrane protein, with 12 transmembrane domains. It is localized only on dopaminergic neurons and is considered to be their phenotypic marker. Dopamine transporter is encoded by a single gene (Giros et al, 1991).
The DAT distribution in the human brain in vitro has been mapped using immunohistochemistry and autoradiography techniques. The highest levels of DAT expression were found in the striatum and midbrain, and significantly less in the frontal cortex and hypothalamus and with low levels in the olfactory bulb and the pituitary (Nirenberg et al., 1996, Ciliax et al., 1995, Hall et al., 1999). DAT loss with age has been reported in vitro (Bannon and Whitty, 1997). DAT distribution in human brain also has been investigated in vivo using PET and age related decline in DAT density by 6.6% has been reported in a group of 126 subjects from 18 years to 88 years of age (van Dyck et al., 2002a). However, little is known about the developmental changes of DAT in human brain. The post-mortem study indicates considerable increase of dopamine transporter expression during first two years and regression during adolescence (Haycock et al., 2003). Of interest here are experimental animal studies, suggesting not only changes in the level of expression of DAT, but also rather late structural and functional maturation of peptide, so there may be specific developmentally determined modes of dopamine transmission at a younger age (Patel et al., 1994, Jones et al., 1996).

Different expression of DAT in various populations of dopamine neurons suggests different region-specific types of dopamine transmission regulation. There is thus classical type in the striatum in contrast to the paracrine or volume transmission type of signalling in the midbrain and the neocortex (Vizi 2000). Recently it has been suggested that the function of DAT may parallel the transmission type, i.e., it may have a reuptake function in the striatum and be involved in release in the midbrain (Falkenburger et al., 2001).

The principal mechanism for terminating neurotransmission is removal of dopamine from the synaptic cleft by backward transport into presynaptic neurons, a function performed by the dopamine transporter. The overall aim of the reuptake system is to maintain a constant level of neurotransmitter at the synapse (or in the narrow range). Animal models have shown that genetic elimination of DAT leads to a considerably prolonged clearance time, elevated extracellular levels of dopamine and altered neuronal firing properties (Jaber et al., 1997, review). Thus, the constant level of DAT is of physiological significance and they are achieved by self-regulation of its own expression in response to neurotransmitter levels. In addition, neural activity, growth factors, hormones, environmental factors and pharmacological agents can modulate DAT activity. It can also be regulated at the genetic level.

**Dopamine D2 receptor; distribution and function.** In 1905, the British physiologist Langley was first to postulate that most drugs, hormones and transmitters produce their effects by interacting with specific sites on the cell membrane, that we now know as receptors.

Two families of dopamine receptors have been characterized in the brain, DRD1 and DRD2. They differ in the link to enzyme adenylyl cyclase — DRD1 is positively linked and DRD2 family is negatively linked to it, which indicates that they will have a different effect on the second messenger systems in the cell (i.e., one has a stimulatory effect and the other an inhibitory one). DRD2 family is further subdivided into DRD2, DRD3, DRD4 forms. The DRD1 family includes DRD1 and DRD5 receptors.

The precise anatomical location of the dopamine receptors in the human brain has been mostly fully established for the dopamine D2 receptors. There is a density gradient of receptors in the decreasing order - the striatal structures, the thalamus, the midbrain, the neocortex. The dopamine DRD2 receptor distribution in the neocortex is uneven—varying between high values in the temporal lobes to minute receptor densities in the occipital lobes (Suhara et al., 1999, Cselenyi et al., 2002). DRD3 have a different anatomical distribution, being absent in the dorsal striatum, but abundant in the ventral limbic regions. However, so far there are no agonists available for dopamine D3 receptors and they are indistinguishable from dopamine D2 receptors in in vivo measurements. The other member of dopamine D2 receptor family, largely implicated in the pathogenesis of ADHD is dopamine D4 receptor. It has eight polymorphic variants in humans (Barta et al., 2001). It is found at a high density in the limbic cortex and the hippocampus and is absent from the motor regions of the brain (Matsumoto et al., 1996). It’s visualization in vivo meanwhile is not available.

The major functions of dopamine receptors are: recognition of specific transmitter-dopamine and activation of effector, leading to an altered cell membrane potential and changes in the biochemical state of the postsynaptic cell. It is worth mentioning that neurotransmission via dopamine receptors, being metabolotropic receptors, is not sufficient to cause action potential, but modulates neurotransmitter release.
electrical excitability and the neural firing properties. In addition, dopamine receptors can function as autoreceptors. Structurally autoreceptors belong to the dopamine D2 receptor family and can be found on axon terminals, dendrites and cell bodies. Stimulation of the autoreceptors in the somatodendritic cell region slows down the firing rate of the dopaminergic neuron (Bunney et al, 1991), stimulation of the autoreceptors on the nerve terminal inhibits the synthesis and release of dopamine while upregulates its uptake (Wu et al, 2002).

AD/HD and dopaminergic hypothesis

The neurochemical basis of then called minimal brain dysfunction was first postulated about thirty years ago (Wender, 1972). At this time, both noradrenergic and dopaminergic systems were thought to play a role and the “catecholaminergic” hypothesis was proposed. Successful treatment of patients with impulsivity/hyperactivity using psychostimulants, and the paradoxical calming effect shifted the focus of research to the dopaminergic system, firstly to the search for the genes involved.

The most replicated results today are the association between the dopamine transporter, and the dopamine D4 receptor gene polymorphisms and AD/HD (Faraone et al, 2001, Kent 2004). Linkage genome scans have pointed to other susceptibility genes, for example, DRD5, SNAP-25 (Fisher et al, 2002, Bakker et al, 2003). Association studies have singled out DRD2, tyrosine hydroxylase and DOPA-decarboxylase (Kirley et al, 2002). Even if the contribution of these genes to AD/HD syndrome in general seems to be small, the positive associations suggest dopamine signalling to be involved in the brain dysfunctions of AD/HD. For instance, homozygosity of the 10-repeat allele of the DAT1 gene has been associated with: i) poor response to methylphenidate and a significantly higher regional cerebral blood flow in the medial frontal and left basal ganglia when compared to children without this genotype (Rohde et al, 2003), and ii) poor performance in sustained attention tasks and an unusual pattern of cortical EEG response to methylphenidate (Loo et al, 2003).

The positive effect stimulants have on vigilance was reported in a pivotal study by Rapoport et al, 1974. Since then, many studies have shown the robust effects of stimulants in short-term treatment of the behavioral symptoms of AD/HD (Porrino and Rapoport 1983, Vaidya et al, 1998). The mechanisms by which the stimulants act appeared to be complex and are not completely understood. It is known that methylphenidate and amphetamine inhibit the dopamine transporters (Amara and Kuhar, 1993), block the reuptake of dopamine and noradrenaline and increase the release of these monoamines (Elia et al, 1990). PET-imaging studies have further elucidated the mechanisms underlying the action of stimulants, confirming that the dopamine transporter is blocked, thereby significantly enhancing the extracellular dopamine level, an effect that is dependent on the initial levels of dopamine in the system and the context (for example, the amount of stress the person is under) (Yoikow et al, 2002a, b).

A whole range of animal models simulating hyperactive behavior has been produced. Models based on the genetic and neurotoxin-effect both induced changes in the dopamine system. Overall, the results appear to be paradoxical and do not explain the mechanism of hyperactivity since both the hypodopaminergic state (SHR or neonatally 6-OHDA lesioned rat) and hyperdopaminergic state (DAT-knockout-mouse) could lead to hyperlocomotor behaviour. However, these results do serve as evidence that changes in the dopamine signalling play a role in motor behavior (see Davids et al, 2003, Russell, 2002 for extensive reviews).

The dopaminergic system in humans can be studied in two ways: indirectly, through measurement of dopamine metabolites in plasma, urine, cerebrospinal fluid or skin fibroblast culture, or directly, using molecular imaging techniques in vivo, e.g., positron emission tomography (PET) and single photon emission tomography (SPET). Studies investigating the peripheral metabolism of dopamine in AD/HD patients have shown increased levels of homovanillic acid (HVA) in the cerebrospinal fluid, which were positively related to the severity of hyperactivity and the response to stimulants (Castellanos et al, 1994, 1996a). Lower HVA levels excreted over 24 h by hyperactive children and an increase in HVA excretion in response to d-amphetamine have also served as arguments for evidence of the involvement of the dopamine system in (Shekim et al, 1983). Recently, a few elegant studies have shown a relationship between the cognitive and physical load on children with AD/HD and the response of the catecholamine
system (by the level of dopamine-noradrenaline metabolite excretion) (Wigal et al, 2003, Konrad et al, 2003). Thus, they also support the view that functioning of the dopamine system determines the maladaptive stress-related responses in children with AD/HD.

Molecular neuroimaging studies have provided preliminary direct information about the central dopamine system in patients with AD/HD. At present, results have been obtained for a few initial studies on the dopamine transporter, dopamine D2 receptor densities and dopamine synthesis function both in adults and children. The first reports from SPET-studies have shown increased dopamine transporter density in the striatum in adults (Dougherty et al, 1999, Krause et al, 2000). These results were not replicated, however, and unaltered and even decreased DAT density in the striatum was reported later (van Dyck et al, 2002, Volkow et al, 2002). Age-specific, and possibly compensatory effects in the dopamine system in adults with AD/HD may be inferred from the studies of Ernst et al, 1998, 1999. The authors have reported that, whilst adolescents with AD/HD have an increased Fluoro-DOPA ratio in the right midbrain, by adulthood a decreased Fluor-DOPA ratio in the prefrontal cortex is recorded. Later studies on children with AD/HD have investigated the density of dopamine D2 receptors using treatment challenges. It has been shown that the dopamine D2 receptor density in the striatum predicts the response to treatment with methylphenidate and that the drug-induced release of dopamine correlated with the severity of AD/HD (Rosa Neto et al, 2002, Ilgen et al, 2001). An increased DAT density in the striatum of also in children with ADHD has been reported (Cheon et al, 2003). Thus, initial molecular neuroimaging studies suggest that AD/HD symptoms are related to the changes in the signalling of the dopaminergic system. There is discrepancy between the findings, however, and it may be related to differences in phenotype, effects brought about by drugs, or to the different radioligands and neuroimaging techniques used (Table 1).

Hence, each of the research results mentioned provides combined evidence that hyperactive behavior and cognitive symptoms may be related to a deficient modulatory function of the dopamine system. Studying the principles of the physiology of dopamine signalling in the midbrain dopaminergic neurons, Grace, 1991, introduced the concept of balance between the tonic and phasic responses in the regulation of dopamine levels. In this respect, Grace (2001) has proposed a model for the dopaminergic dysfunction in AD/HD. He suggests that because of reduced stimulation from the prefrontal cortex, children with AD/HD have low tonic dopaminergic activity in the limbic regions. As a consequence, the low tonic stimulation of inhibitory autoreceptors causes high phasic activity in the nucleus accumbens and perhaps also in other subcortical areas, which, in turn, leads to dysregulation of the motor and impulse control. If this supposition is correct, the effect of psychostimulants could be due to increase of tonic dopamine levels and the decrease in phasic activation.
Table 1. Description of changes of central dopaminergic markers in patients with ADHD.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects (age)</th>
<th>Method: PET/SPECT, ligand</th>
<th>Results: dopamine markers</th>
<th>Results: clinical correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ernst et al., 1996</td>
<td>17 AD/HD, 23 yrs, 33-46 yrs</td>
<td>PET, $[^{18}F]DOPA$</td>
<td>Lower $[^{18}F]DOPA$ ratio in the prefrontal areas, gender-specific asymmetry</td>
<td>Negative correlation to ADHD severity in childhood</td>
</tr>
<tr>
<td>Dougherty et al., 1999</td>
<td>6 AD/HD, 24-51 yrs, treated with stimulants, 30 control - database</td>
<td>SPECT, $[^{123}I]Altropane$</td>
<td>70% higher $[^{123}I]Altropane$ binding in the striatum</td>
<td>-</td>
</tr>
<tr>
<td>A. Krause et al., 2000</td>
<td>10 AD/HD 22-63 yrs, pre-post treatment, MPH 4 weeks, 15mg/day, control-database</td>
<td>SPECT, $[^{99mTc}]TRODAT-1$</td>
<td>1. higher DAT density in the striatum 2. MPH decreases DAT</td>
<td>-</td>
</tr>
<tr>
<td>B. Dressel et al., 2000</td>
<td>17 AD/HD, continuum of study A, 21-64 yrs control-database</td>
<td>SPECT, $[^{99mTc}]TRODAT-1$</td>
<td>1. higher DAT density in the striatum 2. MPH decreases DAT</td>
<td>Correlation between decrease in DAT binding after treatment and improvement of clinical symptoms</td>
</tr>
<tr>
<td>Van Dyck et al, 2002b</td>
<td>9 AD/HD, 25-56 yrs, 8 treated mean 37 days, control-database</td>
<td>$[^{123}I]b$-CTI</td>
<td>No difference to control</td>
<td>No significant correlation with clinical symptoms. No correlation to treatment effects</td>
</tr>
</tbody>
</table>

| Children                         |                                                                               |                           |                                                                 |                                                                  |
| Ernst et al., 1999               | 10 AD/HD 10 control 12-17 yrs                                                | PET, $[^{18}F]DOPA$       | Higher $[^{18}F]DOPA$ ratio in the right midbrain              | Positive correlates with hyperactivity score (Conners score)       |
| Ilgin et al., 2001               | 9 AD/HD 9.8±2.3 yrs,                                                        | SPECT, $[^{123}I]IBSOM$, 3 mg of MPH, 0.5-1.5mg/kg | DRD2 density in the striatum significantly higher than hypothetical mean for the control group; appr. 30% decrease after MPH, effects depending on baseline | The higher the baseline BP, the higher the reduction of hyperactivity after treatment (Conners teacher rating scale) |
| Rosa Neto et al, 2002            | 8 AD/HD 14.2±2.4 yrs                                                        | PET, $[^{1}C]raclopride$, 6 subjects, MPH, 0.3mg/kg p.os, after 30 min 2nd PET scan | 7.5% decrease in $[^{1}C]raclopride$ binding in the striatum after treatment | Baseline BP - no correlates. Ratio of decrease in BP after treatment correlated with commission errors on Go/No Go task |
| Cheon et al., 2003               | 9 AD/HD 9±2.1 yrs, 6 control, 10.3±2.9 yrs                                  | SPECT, $[^{123}I]IPT$     | Higher DAT binding ratio                                       | No significant correlates                                        |
| Lou et al., 2004                 | 6 AD/HD 12-14, born preterm                                                 | PET, $[^{1}C]raclopride$, CBF at birth | Negative correlation between CBF at birth and DRD2 density in the striatum | Negative correlation to RT and RT variability (TOVA)              |

BP- binding potential. CBF- cerebral blood flow. MPH- methylphenidate. RT- reaction time. DRD2- dopamine D2 receptors.
AIMS

The overall aim of this thesis was to examine motor behaviour in children with AD/HD and to investigate central dopaminergic neurotransmission as the putative neurobiological basis for the clinical symptoms of AD/HD.

Studies on motor behaviour

* The synergy between fingertip forces and anticipatory postural adjustments in a load lifting task was studied in young adults to whether shared memory representations are present

* This synergy between the precision grip and the associated postural adjustments was investigated in children with neurodevelopmental disorders (AD/HD, AD/HD+, DCD) was investigated. The parametric control of the forces exerted, the temporal coordination between the precision grip and postural adjustments, and the capacity to adapt to altered weight of the object were examined.

Studies on dopaminergic neurotransmission

The role of dopaminergic neurotransmission in the pathophysiology of AD/HD was tested by:

* investigating the receptor kinetics of the new radioligand binding selectively to the dopamine transporter ([123]PE2I) and cross-validating the methods of quantification

* evaluating the integral function of the nigrostriatal system of adolescents with AD/HD, measuring dopamine transporter and dopamine D2 receptor binding with a PET-system (using the radioligands [123]PE2I and [123]raclopride)

* searching for a relationship between regional changes in the DAT and dopamine D2 receptor density and the neurocognitive-behavioral performance of adolescents with AD/HD.
SUBJECTS AND METHODS

I. SUBJECTS

The work conducted for this thesis is presented in four papers. Subjects investigated consisted of 25 young adults and 74 children (Table 2). PET-studies have been conducted on 22 participants (12 adolescents with AD/HD and 10 adults), with a total of forty four PET-measurements. The studies were performed at the Motor Control Laboratory at the Institute of Woman and Child Health (studies I and II) and the Department of Clinical Neuroscience at Karolinska Institutet (studies III and IV) subsequent to being approved by the Ethics and Radiation Safety Committees at the Karolinska Hospital. The subjects participated after giving oral and written informed consent in accordance with the Helsinki Declaration.10

<p>| Table 2. Characteristics of subjects |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>No. of subjects</th>
<th>Age</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>23 to 37 years</td>
<td>Healthy; 6 female, 9 male</td>
</tr>
<tr>
<td>II</td>
<td>62</td>
<td>5.5 to 11 years</td>
<td>Typically developing children, children with AD/HD, DCD, AD/HD+; 17 female and 45 male</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>19 to 38 years</td>
<td>Healthy, all male</td>
</tr>
<tr>
<td>IV</td>
<td>22 (8 from study III)</td>
<td>12 to 15 years, 19 to 38 years</td>
<td>adolescents with AD/HD, young adults; all male</td>
</tr>
</tbody>
</table>

The clinical investigations on children with AD/HD, DCD, AD/HD+ included: i) verification of the diagnosis, based on DSM-IV criteria (studies II and IV); ii) screening for other neurodevelopmental comorbidities by collecting medical-genetic anamnesis, using the Brown questionnaire (Brown, 1996) (study IV) and Child Behaviour Checklist (CBCL, Achenbach, 1991) (study IV); iii) performance of the Touwen examination for minor neurological symptoms (Touwen, 1979) and the Movement ABC test (Movement Assessment Battery for Children; Henderson and Sugden, 1992) (study II); and iv) investigation of IQ using WISC-III-R (Wechsler, 1999) (study IV). The typically developing children completed a questionnaire regarding their medical history. They underwent a clinical neurological examination and were tested with the, Movement ABC test (study II). Adults participating in the study on movement coordination (study I) had no history of neurological or psychiatric disorders. In the PET-studies, none of the adult subjects had a medical history of somatic or psychiatric disorders, or history of smoking or drug abuse. They also underwent a routine blood analysis, with unremarkable results, and exhibited no brain abnormalities on magnetic resonance imaging (MRI). Adolescents with AD/HD participating in the PET-study were screened using routine clinical blood tests and brain MRI.

II. METHODS

A number of different techniques and methods have been used. Here, they are divided in two groups based on the different areas of research.

Studies on neural control of motor behaviour

Kinetic and electromyographic recordings. Studies I and II are based on the acquisition of kinetic data, i.e., on measurements of the force output (Figure 1). An instrumented object for gripping, the grip-instrument, was used to record the fingertip forces produced by the thumb and the index finger when

10 World Medical Association Declaration of Helsinki, WMA General assembly, Helsinki, Finland, 1964, revision 2000
handling an object. Transducers in the grip-instrument measure two forces: the grip force, GF (the force normal to the surface), and the load force, LF (the vertical lifting force) (sensitivity 0.05 N, sandpaper contact surfaces) (Johansson and Westling, 1984). The grip-instrument had a slot in its base into which blocks of different mass, but identical appearance could be inserted. The subjects were standing on the force plate (platform with built-in sensors) that was used to record ground reaction forces in the anterior-posterior, lateral and vertical directions. In addition, a pilot study aimed to determine the local motor organisation of the movement. For that purpose, surface electromyography signals (EMG) were recorded from the ten hand, trunk and leg muscles. All signals were sampled at 400 Hz, digitised at 12-bit resolution, and stored and analysed using the SC/Zoom laboratory computer system (Department of Physiology, Umeå University, Sweden). The EMG signals were amplified and rectified using a root mean square processor.

**Experimental procedure.** The subjects stood unsupported and as still as possible on a force platform (Kistler, No.9281C, edition No.96) with their feet close together and their right arm held out in front, flexed at 90° in the shoulder. The instrumented object was between the thumb and index finger (Johansson and Westling, 1984), but without touching it (Fig. 3A). Each subject performed four series of 13 fast, self-induced lifts of the object. The series contained different weights of the object. The weights and the order of presentation were: 500, 1000, 1500 and 2000 g in Study I. Weights were adjusted for children, 300, 700, 500 and 1000 g, in Study II. The subjects performed some practice trials before the recordings started. The first trial of each series was not included in the analysis due to adjustments to the current weight.

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**Figure 3.** Schematic diagram of the experiment (A). The typical trial shows the muscle responses and the resultant shifts of the centre of pressure in conjunction with a load lift (B). EMG signals are from the following muscles: mm. deltoideus (DEL), abductor pollicis brevis (APB), dorsalis interosseus (FDI), extensoris thoracis (TE), extensoris lumbarum (LES), hamstrings (HAM), gastrocnemius (GAS). There were no consistent responses from the m. tibialis anterior and m. rectus abdominis. T₀ denotes the time of the lift-off, i.e., the moment at which the load force overcomes gravity. The vertical dashed line demarcates the “anticipatory window”, i.e., the time during which pre-movement events in the central nervous system occur and are represented in the output parameters, namely muscle responses and forces are generated. The fingertip forces were preceded by brisk phasic activation of the dorsal trunk and leg muscles. Muscle forces generated by the early EMG activity resulted in the development of an angular impulse during the preloading and loading phase of the lift, consisting of forward-backward excursions of the center of foot pressure.
Data analyses
The grasping and lifting movements were characterised by the load and grip force profiles and the first time-derivatives of the of the grip and load forces (dGF, the grip force rate, and dLF, the load force rate, respectively). Programmed movements are recognized by their bell shaped velocity profile with only one peak, i.e. no corrective movements, as seen in Figs. 4.

![Diagram](image)

**Figure 4.** Single trial of an adult subject presenting fingertip forces and center of pressure displacements and their first time derivatives. Parameters used for the analysis are marked with boxes. Anticipatory time window is demarcated by shaded area. T₀ – time of lift-off.

Anticipatory postural adjustments were described by the displacements of the center of foot pressure (COP) and the rate of displacement (dCOP). The COP was computed from the ground reaction forces. In other words, the COP is the neuromuscular response induced by the central nervous system to correct for the imbalances of the body’s center of gravity (COG).

The analysis of the registered signals focused on the biomechanical events that occur prior to the lift. The parameters taken into account were: 1) the amplitudes of the fingertip forces, of the first forward peak of the COP displacement and of the positive peaks of their first time-derivatives (dGF, dLF and dCOP) and 2) the timing of the onset of the COP displacement and the relative timing of the peaks of the derivative signals compared to lift-off (T₀) (Fig.2).

At T₀ the load force applied on the grip instrument equals the weight of the instrument and from the moment of lift-off. It is the moment when we obtain the full somatosensory feedback information about the weight of the object. This moment was chosen as a reference point in the analysis. All events occurring before this instant were considered to be of a programmed nature. In the group of children, this time was adjusted to T₀ + 50 ms.

Statistics
The effect of weight on the various parameters within each group was evaluated using the analysis of variance, where the subject was entered as the covariant. A 2-way ANCOVA was applied for the evaluation of weight and group interactions. Post hoc analyses were performed with the Newman-Keuls Test. A within-subject relationship between manual forces and COP displacements was estimated using the Pearson Product-Moment correlation. In addition, differences in the rate of occurrence of neurological symptoms or the presence of trials with anticipatory postural behaviour, between the four groups of children were tested with the Chi-square or Mann-Whitney U Test. Differences in the onset of COP-
displacement between children with a different neurological condition were tested with the t-test. Throughout the analysis, differences and correlations with a p < 0.05 were considered to be statistically significant.

**Molecular imaging studies**

**Positron emission tomography**

Positron emission tomography (PET) is an imaging technique that uses radiolabeled molecules to image biological processes in the living brain. It can be used to visualize and quantify different biochemical processes, e.g., like the metabolism of endogenous substances, enzyme activity, receptor distribution and occupancy, and neuronal and vesicular uptake systems (Hallidin et al, 2001). Owing to the selectivity of the radioligands used and the sensitivity of pico-nanomolar range, PET makes it possible to measure protein molecules. PET is applied in the investigations of normal behaviors and the pathophysiology of neuropsychiatric disorders, monitoring of pathological conditions and development of new psychopharmacological agents. Recent advances include increase in the spatial resolution of the PET-systems and the development of new radiotracers which should make it possible to visualize signal transduction mechanisms and gene expression in the human brain (Hallidin et al, 2001, Lammerstma, 2002, Eberling et al, 2003, Ernst et al, 2003).

The principles of PET imaging are based on the use of so-called radiopharmaceuticals (radionuclides) that emit positrons (i.e., positively charged electrons). As a first step, an extra proton is accelerated into the nucleus (in the present studies, into carbon atoms) using a cyclotron. The substance to be used as the radiotracer is then synthesized by incorporating the unstable isotope into the precursor. The radiotracer obtained is then injected intravenously. In tissue, the unstable isotope is broken down, and emits positrons. In collision with an electron, each positron emits two γ-rays, in almost opposite directions. These γ-rays are recorded by coincidence detection, i.e., simultaneous detection by two opposite detectors in the PET system are required for a signal to be valid. The radioactive decay is counted over time and the distribution of radioactivity within the brain slice is reconstructed to produce images by using mathematical algorithms (Malison et al, 1995).

In summary, PET provides measurements of regional tissue radioactivity. Specialized mathematical data treatment is used in the next step to translate these measurements into quantitative parameters, like receptor binding potentials and receptor density (Bmax).

1. **Data acquisition.**

The PET system used was a Siemens ECAT Exact HR, which operates in 3D mode. It provides 47 sections with a centre to centre distance of 3.125 mm. The intrinsic in-plane spatial resolution was 3.8 mm with 4.0 mm Full Width at Half Maximum (FWHM) axially (Wienhard et al, 1994). A Hanning filter of 2.0 mm FWHM was used in image reconstruction. The image matrix size was 128x128x47, and the voxel size was 2.02x2.02x3.125 mm. An attenuation correction was done using the transmission scan data obtained for each subject before the PET measurements were performed.

The MRI system used was the GE Sigma, of 1.5 Tesla, and the standard pulse sequence was the fast spin-echo method (TR= 400 msec, TE =9 msec, T1-weighted images). The field of view was 26 cm, and the image matrix size was 512x256.

A head fixation system with an individualised plaster helmet was prepared and used for both MRI and PET measurements to ensure that the same head position was used for the two imaging modalities and to avoid movement artifacts (Bergström et al, 1981).

Two radioligands were used in the PET-studies. [11C]raclopride and [11C]PE21 were synthesized by methylation of precursors using [11C]methyl triphlate (Hallidin et al, 1991, 2003). In each PET experiment the subject lied recumbent on the bed with his head in the PET-system. A cannula was inserted into the right cubital vein (study III and IV) and another cannula into the left brachial artery (study III). A sterile physiological phosphate buffer (pH=7.4) solution containing [11C]PE21 was injected as a bolus over a 2 s period into the cubital vein. The cannula was then immediately flushed with 10 ml saline solution.
The radioactivity in the brain was measured for 63 minutes. Acquisition started immediately after the intravenous injection and consisted of a preprogrammed sequence of 15 frames. The frame sequence was comprised of three 1 minute frames followed by four 3 minute frames and eight 6 minute frames.

To obtain the arterial input function (study III), an automated blood sampling system was used for the first 5 minutes of PET measurement (Eriksson et al, 1988). Subsequent arterial blood samples (each of 2 ml) were drawn manually at the midpoint of each frame until the end of the PET data acquisition and at 4, 10, 20, 30, 40 and 50 min after iv injection of $[^{11}C]PE2I$. These samples were used to determine the fractions of plasma radioactivity corresponding to unchanged $[^{11}C]PE2I$ and labelled metabolites (Halldin et al, 2003).

2. Image processing.

The radioligand $[^{11}C]PE2I$ has excellent image contrast characteristics (Fig. 5), that facilitate delineation of anatomical structures. The regions of interest (ROI) for the caudate nucleus, the putamen and the cerebellar cortex, were drawn on MRI images and manually transferred to the reconstructed PET images. The ROI for the substantia nigra/ventral tegmentum were drawn at the level of the upper midbrain.

![Figure 5. The sequence of procedures involved in the analysis after iv injection of $[^{11}C]PE2I$: regions of interest drawn on MRI (A), superimposed on the PET image (B), and the time-activity curves calculated (C).](image)

The ROIs were drawn in three adjacent transaxial sections and the data were pooled to calculate the average radioactivity concentration for the whole volume of interest. To obtain regional time activity curves (TACs), the regional radioactivity was calculated for each frame, and corrected for decay and plotted over time.

3. Quantitative analysis

The signal recorded by the PET-system provides a single measure — the total amount of radioactivity in the tissue (for the selected brain region), $C_t$. However, it is a composite measure; the signal contains information about the specifically bound ligand ($C_s$), non-specifically bound ligand (adhering to protein fragments, lipids; $C_{non}$), and the free (unbound) ligand in the tissue ($C_f$) (Frost et al, 1989). In addition, intravascular cerebral blood forms another physiologically separate component. Furthermore, there is a continuous exchange of the injected substance between the physiological states (bound-unbound) and also tissues. In addition, the brain signal depends on the delivery of the radioligand, i.e., on the time course of the radioactivity in the arterial plasma. Hence, the second variable measured during the PET-experiments was the radioactivity in the arterial plasma, $C_p$ (Maguire and Leenders, 2002).

Both the total radioactivity in the brain tissue and the radioactivity in the arterial plasma, are used to describe the observed data and to derive parameters indexing the receptor density, such as the binding potential (BP) and the volume distribution. For this purpose, the concept of “compartments”, i.e., physiologically distinguishable pools of tracer, is used. The transfer of the radioligand between the compartments (plasma-tissue-receptors) is described by first order rate constants ($K_1$, $k_2$, $k_3$, $k_4$) (Fig. 6).
The rate constants $K_i$ and $K_t$ correspond to the influx and outflux rates for radioligand diffusion through the blood-brain barrier, respectively. $^{1^1C}$-PE21 is assumed to be free to diffuse from the blood to the brain tissue. The rate constants $k_i$ and $k_t$ describe receptor-ligand interactions and correspond to the rates of radioligand transfer between the compartments for free ($C_i$) and specifically bound ($C_t$) radioligands, respectively. The rate constants $k_s$ and $k_b$ correspond to the rates of radioligand transfer between the compartments for nonspecifically bound radioligands.

PET-measurements can be interpreted using several biomathematical modeling procedures. In brief, the so-called input function (the arterial plasma curve or the reference brain region time activity curve, TAC) is used to mathematically model the expected tissue TAC or the tissue response function. These predicted results obtained are then compared with the measured data using fitting procedures. The values that best explain the measured data are used to derive binding parameters.

\[ \begin{align*}
C_i & \xrightarrow{k_i} C_t \\
\text{2CM} & \\
C_s & \xrightarrow{k_s} \text{sum} \\
C_b & \xrightarrow{k_b} C_t & \text{3CM} \\
C_s & \xrightarrow{k_s} \text{sum} \\
C_b & \xrightarrow{k_b} C_t & \text{4CM}
\end{align*} \]

**Figure 6.** Configuration of two (2CM), three (3CM) and four compartment (4CM) kinetic models

In study III, the kinetic model (direct method, using arterial input function) and three derived approaches (indirect quantitative methods, using reference region as an input function) were used and compared in an attempt to select the most appropriate method for applied quantitative studies. The binding potential (BP) was the parameter compared.

**Kinetic analysis**

The classical way to evaluate radioligand binding to receptors in the brain is to use the four-compartment model shown in Fig. 6 (Frost et al, 1989). It is a common assumption that there is a rapid exchange of radioligand between $C_i$ and $C_s$ compartments, and, because of this, they can be treated as one compartment representing nondisplicable radioligands in the brain (Wong et al, 1986). The simplified three-compartment model (3-CM) (Lammertsma et al, 1996) was used to describe the time-course of regional $^{1^1C}$-PE21 binding in the striatum and the midbrain. The time curve representing the radioactivity of unchanged $^{1^1C}$-PE21 in arterial plasma, i.e., the metabolite-corrected curve, was used as the input function.

In addition, if the association and dissociation from the status of specific binding are rapid when compared to the transport parameters $K_i$ and $K_t$, the model may collapse into two compartments: a single tissue compartment containing free, nonspecifically bound and specifically bound ligands and a second plasma compartment (Koeppe et al, 1991). In this model, there are just two rate constants, $K_i$ and $K_t$.

With the aim of examining whether the 3-CM or 2-CM is preferable to describe $^{1^1C}$-PE21 kinetics in the putamen, the midbrain and the cerebellum, we compared the statistical parameters using three methods: the Akaike information criterion, the Schwarz criterion and F statistics (Farde et al, 1989, 1998).

**Distribution volume**

Theoretically the distribution volume provides the ratio of the concentrations of radioactivity, i.e., the ratio of the total tissue volume to the volume occupied by tracer at the equilibrium. Equilibrium is the state of the system at the time when the number of molecules being exchanged between two compartments is the same. The $^{1^1C}$-PE21 binding was analysed using the concept of “total distribution volume”, $V_t$ (Koeppe et al, 1991, Lammertsma et al, 1996, Farde et al, 1998) and computed using rate constants, obtained from kinetic analysis.

**Linear graphical analysis**

The kinetic analysis approach views radioactive decay as a non-linear function and uses a sum of exponential functions to describe the tissue response. Alternative, less laborious ways of treating the
experimental data have been developed. One of them is linear graphic analysis for reversible ligands, or
the Logan plot (Logan et al., 1990). The underlying principle is the transformation of nonlinear functions
into linear ones by means of integrals of time activity curves from exchangeable pools.
11C-PE2I binding was estimated using a linear graphical analysis (Logan et al., 1990) with both direct
(arterial plasma curve) and indirect (cerebellum time activity curve) input functions. The slope was
determined by using the data from the time interval 24 to 60 min after the iv injection.

The reference tissue models
Yet another approach has been developed with the aim of omitting invasive arterial blood sampling
procedures. In the reference tissue model (Lammertsma et al., 1996), receptor binding in the target region
(the compartment with both specific and non-specific radioligand binding) is estimated indirectly using
the time activity curves for the cerebellum (the reference region) as an input function. The validity of this
model relies on two assumptions: i) that influx and outflux rates over the blood-brain barrier (K1/k2) are
the same in both regions and ii) that the cerebellum has a negligible density of receptors of interest
(Lammertsma et al., 1996). The equation includes the BP and is solved in a convolutional manner by
fitting to experimental data in a least squares sense. However, the estimation of parameters using this
method gives high standard deviations, and therefore, it was not used in study III. Instead, a simplified
reference tissue model (SRTM, Lammertsma and Hume, 1996) was applied in which the tissue ROI is
approximated by a single compartment and only three parameters are required for a fit: R1 (delivery
relative to the reference tissue), k2 (the rate constant from tissue to plasma), and BP.

The transient equilibrium approach
The condition of transient equilibrium is defined as occurring when the derivative of Cb(t) is zero (Farde
et al., 1989). Theoretically, at that instant, the number of molecules associating to the receptors is equal to
the number that dissociate from them. The concentration of radioactivity in the cerebellum was used as an
estimate for Cn(t), Cb(t) was defined as C(t) minus Cn(t). This method was applied to the estimation of
BP in the midbrain. Given the flat shape of Cb(t) and the difficulty of defining a single peak of
radioactivity in the striatum, the last frame of the equilibrium analysis was used to estimate BP (Olsson
et al., 2001).

The cerebellum as a reference region
The cerebellar cortex is a region with no established density of dopamine transporter in the human brain in
vitro or in vivo (Hall et al., 1999, Madras et al., 1998, Haldin et al., 2003). In a region devoid of specific
binding sites, a two-compartment model should be sufficient to describe the time-activity curves. The 2-
CM and 3-CM were both applied to the cerebellum to evaluate if the binding in the cerebellum can be
regarded as a single-tissue compartment.

Methodological aspects

Error sources
PET resolution. The PET system used was the Siemens Ecatt EXACT HR with an in-plane resolution of
3.8 mm and 4 mm Full Width Half Maximum (FWHM) axially (Wienhard et al., 1994). The PET
resolution provides several limitations. Firstly, the quantification of the radioligand binding in the small
anatomical structure may be underestimated since artifact-free image reconstruction is only possible for
objects greater than 0.5×FWHM (Eriksson et al., 1990). Secondly, near the edges of the objects two other
phenomena occur, the spillover effect (whereby a false positive signal can be obtained) and the partial
volume effect (where a false negative signal is measured), which also have to be taken into account
(Eriksson et al., 1990). The latter may also lead to underestimation of radioligand binding. Special
programs to correct for the partial volume effects are now being introduced, but they were not available at
the time of the present study. Limitations of resolution, however, cause systematic errors and should not
influence group comparisons. In addition, the volumes of interest, in the striatum and midbrain did not
differ for the groups in study IV.
Quantitative analysis is a long stepwise procedure, during which errors can be introduced at each step. In the estimation of the arterial plasma input function, the weakest link is the metabolite correction. Determination of labeled metabolites is prone to high errors when radioactivity counts are low and, in the case of \(^{[1]}\text{C}\)PE2I, this was the case after approximately 20 minutes. This has a strong impact on the values of the rate constant \(k_3\). Other significant factors are: the estimation of background counts, calibration of the blood count systems and the PET system, correction for delay and the cerebral volume component. All these issues are of general character and were dealt with in accordance with the established procedures. In addition, study III dealt with the choice of model to be used. In that respect, we must balance the precision and the bias, i.e., the more complex the model is, the lower the precision (owing to statistical uncertainty), but a more simplified method introduces bias. Hence, methods that require the fitting of many parameters, as does the four compartment kinetic model and the reference tissue model (Lannerstina et al, 1996), were not included in the analysis. The method of choice for the estimation of \(^{[1]}\text{C}\)PE2I binding in the applied study (study IV) was the simplified reference tissue model (SRTM), since the standard errors for BP in the regions with a high and low density of dopamine transporter were the smallest.

Sample size and test-retest reliability. Another limitation in PET studies is the small sample size, which may lead to low statistical power and ultimately preclude the drawing of clear conclusions. This limitation is caused by fundamental issues, such as expensive and labour-intensive experiments, data analysis, and the invasive procedures for the patients. Thus, in practice, increasing the sample size would only be possible by conducting multi-center studies.

However, several approaches can be taken to evaluate the reliability of findings. In group comparisons, reproducibility can be predicted by a statistical parameter - effect size. In study IV, the significantly lower DAT density in the midbrain of adolescents with AD/HD had a very large effect size, ES=0.89\(^1\). The reproducibility of the findings is also characterized by test-retest reliability. For the \(^{[1]}\text{C}\)raclopride, the test-retest reliability in the striatum has been reported to be 96-98% (Nordström et al, 1992, Nyberg et al, 1996). At present, the radioligand \(^{[1]}\text{C}\)PE2I does not have any test-retest data.

Ethical considerations. Ethical questions are of particular importance when working with children, a group with weakened autonomy. Written information was provided to the children and their parents. In addition, to prepare them for the PET-study, the children and parents were met and carefully instructed about the equipment, and the whole procedure was demonstrated. The family was allowed a considerable time to make a decision about participation in the study. Care was taken to avoid establishing a direct doctor-patient relationship. Written and oral consent was asked of one of the parents and a child. Ethical considerations in study IV determined the choice of the subjects in the control group — young adults. The comparisons were interpreted in the context of established age effects on the density of dopamine markers.

\(^1\)The formula used for calculation of the effect size (d) was the difference in the means for the two groups divided by their average SD: 
\[ d = \frac{X_1 - X_2}{\sqrt{SD_1^2 + SD_2^2}/2} \]
Neurocognitive investigations

In addition to clinical investigations, the group of adolescents with AD/HD (study IV) were administered a set of specialized neurocognitive tests. The aim was to evaluate the core symptoms (inattention, impulsivity and hyperactivity) in an objective and standardized way.

Inattention is a problem in which the person “misses” information or does not register it in his or her working memory for further processing. Attention was examined using the Continuous Performance Test (CPT), and simple and choice reaction time tests (SRT and CRT). The Continuous Performance Test measures the processing speed, sustained attention and impulse control. A wide variety of CPT tasks are used with different presentation methods (auditory, visual and verbal) and with measures of simple reaction time measures or choice reaction time to two or more stimuli that require different responses. The latter type of stimulus presentation (response to eight-pointed star and inhibition to five-pointed star) was used in the computer-administered CPT of the present study (Fig. 7B) The test provided parameters: omission errors (that is, false negative responses to the presentation of the target), commission errors (making false positive responses), response latency and variability (Teicher et al, 1996).

During the performance of the CPT task the hyperactive behavior was quantified with an infrared motion analysis system (Qualys, Glastonbury CT, Connecticut, USA) (Fig.7A). The analysis included the movement area, the frequency of movement, the displacement (taken as the total distance moved by the marker) and a spatial scaling exponent (a measure of the complexity of the movement path). In addition, a simplified version of the reaction time and choice reaction time tests was administered (using visual stimulus), since high variability in the visual RT task is a consistent finding for the AD/HD group (Stuss and Benson, 1984, Westerberg et al, in press).

The dopamine system is known to modulate performance of the working memory, evidenced both in experimental animal studies and in humans (Castner et al, 2000, Abi-Dargham et al, 2002). Thus we included working memory task in the cognitive battery administered. The visuo-spatial working memory (VSWM) was evaluated using the paradigm developed and tested in behavioral and neuroimaging studies in our group (Klingberg et al, 2002). It was found to be a sensitive discriminator of cognitive performance in the AD/HD group (Westerberg et al, in press). In brief: visual stimuli (circles) are presented on a four by four grid on a computer screen with increasing load (i.e., an increasing number of circles). The working memory capacity was determined by the highest level passed (that is, the sum of the number of correctly indicated circles). Both the RT and VSWM tasks used E-prime software for stimulus presentation (Psychology Software Tools Inc, Pittsburgh, USA).

Statistics

A comparison between the regional binding potentials (BP) for [11C]PE2I and [11C]raclopride for the groups was made using the Student’s t test. However, as stated above, the density of dopamine markers in the brain is known to decrease with age (van Dyck et al, 2002a). Thus, in a second step, the age effect on the radioligand binding in the control group was evaluated using linear regression analysis. Potential associations between cognitive and motor performance and [11C]PE2I and [11C]raclopride BP were assessed with the Spearman rank correlation analysis (R) and the Mann-Whitney U test. In all analyses, the statistical significance was set at p < 0.05.
RESULTS AND COMMENTS

Programming of the precision grip and associated postural adjustments in adults and children with neurodevelopmental disorders

Studies I and II focus on the mechanisms of postural control in association with the precision grip in 'normal' and pathological conditions, including developmental coordination disorder and AD/HD. From research on the sensorimotor control of the precision grip, we know that correct adjustments of fingertip forces are achieved through a build-up and continuous updating of internal neural representations of the object’s physical properties (i.e., its weight, size and friction) (Johansson, 1996, Forssberg et al., 1992). In the studies presented here, firstly, we asked whether adaptation of whole body postural adjustments to environmental stimuli is governed by the same principles of sensorimotor control as the precision grip, i.e., whether the postural adjustments are linked to neural representations of the lifted object. And, if so, the formation and shared use of motor memory may be one of the models for understanding movement coordination control systems. Secondly, we questioned whether the clumsiness observed in children with neurodevelopmental disorders may be related to the deficient motor memory systems, and in particular to deficient anticipatory control mechanisms and poor movement adaptation.

The load-lifting task was used as an experimental paradigm to investigate the integrity of the precision grip and associated internally triggered postural responses. The subjects lifted a “grip instrument” whose weight was varied. Such load-lifting movement requires coordination between multiple joints (the shoulder, hips, knees and ankle), and involves many degrees of freedom. The pilot study showed that arm movement induced postural perturbations, the net effect of which, as seen on kinematic recordings, was movement around the ankle joint, so-called ankle strategy (Horak and Nashner, 1986). A few small hip and/or knee flexions could be observed in individual trials, but with no evident pattern to them. Thus, further description of motor behaviour was reduced to kinetic variables, that is, to force trajectories, representing the final outcome of movement and which are sufficient to describe the basic principles of movement control.

The main characteristics of programmed movement, namely, unimodal force rate profiles with force rate peaks occurring before the onset of the lift, were present both for the fingertip forces and postural adjustments. The similar parametric control of the fingertip forces and APAs and their synchronised output were the main findings of the study. This may suggest that two different motor programs, for control of dextrous manipulation and posture are coupled, into a “functional synergy”. Both of these components of movement are adapted by using the information from the neural representations of the objects in our environment.

In the second study, when motor coordination problems in children were investigated, we predicted that the children would fail to induce the appropriate anticipatory postural adjustments, and that they would not couple their fingertip forces and APAs. Children with AD/HD, DCD and the combination, AD/HD+, were investigated using the load-lifting paradigm. Their performance was compared to age-matched and younger control groups (in total, five groups comprised of 52 children were involved). Three basic interrelated mechanisms of movement control were explored: i) anticipation, ii) coordination, and iii) adaptation (gain control).

The results of the study showed that children with AD/HD, DCD and AD/HD+ had developed anticipatory control of fingertip forces and that voluntary load lifting could induce anticipatory postural adjustments. However, clinically observable motor coordination problems were associated with underlying neurophysiological deficits. The pattern of deficits was most obvious in children who had severe and complex neurological symptoms, i.e., the children of AD/HD+ group (Fig. 8).
On a group level, the children with AD/HD+ differed from the age-matched control group in their: i) excessive grip force, ii) the later onset and smaller amplitude of the postural adjustments, iii) the absent coordination between the rate of change of the fingertip forces and the postural adjustments, and iv) the reduced ability to adapt the motor output. In other words, they had deficient parametric control of their fingertip forces and the COP adjustments and they were less successful in adapting to the stimulus modifications (the weight of the grip instrument).

**Developmental aspects of motor coordination**

The observations of anticipatory postural adjustments in response to fast pointing movements have been shown as early as by 15 months of age (Van der Fits et al, 1999). These anticipatory postural adjustments become functionally significant at 6-7 years as indicated by studies on the development of APAs gait initiation and bimanual tasks (Hirschfeld and Forssberg, 1992, Grasso et al, 1998, Ledebt et al 1998, Assante et al, 2000, Schmitz et al, 2002). The efficient pattern typical of APAs in adulthood is achieved quite late, into adolescence (Forssberg et al, 1992).

In the present study, at the age of 5-7 and 8-11 years children were able to elicit anticipatory postural adjustments in the experimental load-lifting task. At both 5-7 and 8-11 years, the children adapted their hand force output to correspond to the increasing weight, the same was true for the adjustments in the amplitude of the COP (Fig. 9). The timing characteristics, however, only showed a tendency towards adaptation, possibly indicating later maturation of the temporal control systems. Interestingly, the coordination of manual forces and postural adjustments seems to occur later than their isolated development, since, at 8-11 years of age, only half of the children coordinated their fingertip forces and COPs.

The pattern of the motor output in children of the younger age did not differ from the group of older children and did not resemble the altered motor parameters of children with AD/HD+. Thus, there was no evidence to support the idea of developmental delay in children with AD/HD and DCD. Rather, the findings indicate altered neural mechanisms of motor control that may explain the results of the follow-up
studies of children with DCD, showing this to be a disorder that continues into adulthood (Cousins and Smyth, 2003, Cantell et al, 2003).

**Cognitive aspects of motor coordination in AD/HD**

*Neural representations and movement control.* There is a longstanding discussion about whether the poor motor behaviour of some children with AD/HD depends on cognitive deficits (such as distractability and lack of inhibition) or whether they have a specific disturbance in their sensorimotor control system. The most robust, replicated finding was the increased grip force (Pereira et al, 2000, 2001). This deficit could be related to narrowed cortical networks (Ehrsson et al, 2001, Kuhtz-Buschbeck et al, 2001) typically used for precision grip and inefficient use of additional cognitive operations for planning and implementing particular movement. Atypical parametric control of both the grip force output and COP displacements, a tendency for decreased frequency of APAs, and a decreased number of coupled manual-posture responses indicate that the feedforward control of movements in this group of children is deficient. One of the deficits may be inefficient build-up and/or use of shared neural representations of the objects in our environment. Further support for this view comes from the decreased visuo-spatial and phonological working memory capacity in children with AD/HD (Westerberg et al, in press, Norrelgren et al, 1999). These findings are in line with clinical observations of the co-occurrence of cognitive and motor deficits, indicative of a tight relationship between the ‘cognitive’ and ‘motor’ circuitries. Thus, the results of this study support the view that children with AD/HD have executive dysfunctions including working memory deficits involving the motor systems.

Internal representation models also include the build up and maintenance of internal representations of the body scheme and visuospatial representations of the environment that specify the coordinates for planned action. Problems in the elaboration of an internal representation of a sensory-motor system may, thus, influence motor adaptation, and thereby motor skill acquisition (Berthoz and Viaud-Delmon, 1999). Wilson et al, 2002 have proposed that the slow and variable motor performance characteristic of developmental coordination disorder is a result of deficits in the internal representation of movement and have argued for imagery training in children with DCD that may improve this predictive process. Of interest in this respect are the correspondence between the model of internal representations serving for coordination of eye-hand tracking, proposed by Sarchilli et al, 1999 and the findings of impaired eye-hand coordination in children with AD/HD (Eliasson et al, 2004).

**Attention and posture.** Given the widely accepted integrative approach to cognitive processes and motor action, there are surprisingly few studies that have tested the effect of cognitive parameters on the coordination of movements and, specifically, on postural control. This may be partly related to the notion of postural control as a highly automated perception-action system which requires minimal resources in terms of attention. However, recent studies on healthy young adults have challenged this idea and shown that cognitive tasks may interfere significantly with postural stability. For instance, the performance of arithmetic subtraction task lowers the amplitude of the measured postural adjustments made in response to platform perturbations while standing (Rankin et al, 2000) and tasks increasing the cognitive load lead to decreased postural stability (Pellechia 2003, Maylor et al, 2001, Riley et al, 2003). In the group of children with DCD, Pick et al, 1999 have found correlations between attention and Movement ABC scores, and have, therefore, suggested that inattentiveness might be a predictor of movement coordination problems. It would be of clinical importance to further test the motor behavior of children with AD/HD and DCD using cognitive load, dual-task paradigms.

**Neuropysiological aspects of motor coordination**

The load-lifting paradigm was advantageous for simultaneous investigation of the precision grip and postural behaviour. We observed different alterations of the precision grip and of the whole body postural adjustments in children with neurodevelopmental disorders. It could be related to different basic neural control mechanisms sub-serving the precision grip and posture in standing.

**Precision grip.** Anticipatory control of the grip force is described by the timing of the peak grip force rate. In children with AD/HD+, the grip force peaked before the onset of movement. The peak grip force was higher in children with ADHD+. Thus, the grip force was targeted to a higher default grip force, as if operating on a different threshold level. This could reflect shift in the threshold arising from abnormal
gating and modulation of sensory afferent input which are functions that may be involving the basal ganglia (Schwarz et al. 2001, Serrien et al. 2001). A highly increased grip force has also been reported in patients with cerebellar lesions (Babin-Ratte et al, 1999). This is not surprising since experimental animal studies have shown that the frequency of discharge of cerebellar Purkinje cells encodes the weight and friction of an object (Espinoza and Smith, 1990).

Alternatively, the high grip force may be related to the perception that the load is heavier than it really is. It may also be a compensation for disturbed sensory information as seen in the case of diminished cutaneous afferent information associated with anesthesia (Johansson and Westling, 1984) or deafferentation (demyelinating neuropathy; Nowak et al, 2003). An explanation of the increased grip may be that it is a compensatory strategy, emerging to avoid slips. Fatigue may also affect the sensation of heaviness, but we controlled for fatigue during the experiment by introducing rest periods. Furthermore, there was no effect of the trial number on the force output.

**Posture.** The organisation of postural adjustments in children with neurodevelopmental disorders was affected in both the amplitude and temporal domains. To our knowledge, no analogous studies in children with neurodevelopmental disorders have been performed. For that reason, we looked into the literature on the investigations of postural disturbances in adult subjects with defined primary regional pathology of the brain. Patients with Parkinson’s disease had decreased amplitude of the initial COP during anticipatory postural adjustments in a leg-lifting paradigm (Lee et al, 1995). The following neurophysiological mechanism has been suggested by the authors: impaired gating of the sensory input through the basal ganglia has the consequence of lowering the amplitude of the muscle contractions resulting in smaller postural excursions. Delayed latencies of COP adjustments, the lack of a phasic component in EMG responses when performing a task involving raise up on tip-toes were reported in subjects with cerebellar disease (Diener et al, 1990). Interestingly, among children with DCD there is a subgroup showing clinical cerebellar symptoms which correlate to the increased variability of performance in the finger tapping task (Lundy-Ekman et al, 1991). Disturbed central timing mechanisms have been suggested by the authors. As a consequence, disturbed synchronization and sequencing of muscle activations were thought to be responsible both for the variability of motor responses and changes in amplitude. Too late activation of agonist muscles may lead to an increase in the grip force (Williams et al, 1992), thus indicating that altered cerebellar mechanisms can not only underly postural disturbances, but also fine manipulative activity. As known from experimental studies, the developmental loss of climbing fiber innervation from cerebellar Purkinje cells occurs at a particular postnatal critical period and may result is a mild but persistent loss of motor coordination (Kakizawa et al, 2000).

Similarities between disturbances of motor output in children with AD/HD+/DCD and with different neurological disorders of known localisation of primary pathology, support the idea that AD/HD+/DCD problems have a clear neural basis. Clinical analogies may suggest a deficit of motor control arises from the pathology of the striatum and/or cerebellum. There are no neuroimaging studies on children with DCD so far, but decreased basal ganglia and cerebellar volumes in children with AD/HD have been reported (Bergen et al, 1998), suggesting a possible contribution from these regions to the disturbed mechanisms of fine manipulative behaviour and postural control.

**Specificity and individual variability**

How specific are the documented disturbances of motor control to AD/HD and DCD and other neurodevelopmental disorders? The pattern of altered motor behaviour was not distinguishable between the AD/HD, DCD or ADHD+ groups, thus pointing to a rather non-specific syndrome of motor disturbances that can be present in individual cases in any of the groups. With respect to other neurodevelopmental disorders, only children with autism have been investigated using a bimanual task, and deficient anticipatory control of postural adjustments has been found (Schmitz et al, 2003). But it has to be kept in mind that information from the behavioural studies is limited to the fact that only the variables associated with the final outcome of a movement were measured. Once a deeper look using neuroimaging methods is possible, the question of specificity may be examined in greater depth. An example of this is the imaging of the cerebellum. Cerebellar hypoplasia is found in autism, schizophrenia and AD/HD. However, different lobules are affected, for example, I and V in schizophrenia, VI and VII
in autism, VIII and X in AD/HD and could have different implications for function, depending on their different anatomical connectivity (Ivry, 2003, review).

One of the important issues in AD/HD research is its remarkable phenotypic heterogeneity, as discussed in the introduction. The analysis and interpretation of the results may thus be approached from two perspectives. One way to evaluate the biological reality is to concentrate on the individual variability and explore its patterns. Such an approach was taken in previous studies conducted in our group by Pereira et al, 2000, 2001. In the present study, the individual performance was variable and was presented with the emphasis on clinical symptoms (Fig.10). An increase in the amplitude of the grip force and the variability, a decrease in the amplitude of the center of pressure and delayed onset of the COP displacement were observed more often in those children who had more complex neurological symptoms. Another way is to focus on the analysis of the group data and establish the principle alterations in motor behaviour. This approach was used in the present study and presented in the paper II.

**Figure 10.** The relationship between the clinical neurological symptoms and behavioral measurements. (Number 1 denotes the group of children with movement coordination problems (dysdiadochokinesis, poor finger-nose and knee-heel tests), number 0 - the group of children with no coordination problems).

In summary, deficits in the motor control of children with developmental disorders were aspecific. At present, we cannot specify the break-point in the neural systems contributing to the impairment, since only the final motor output was investigated, i.e., the forces. The pathophysiological dysfunction may occur at several levels of the nervous system: erroneous acquisition of sensory information, disturbed gating of sensory information (i.e., a “shift in the “threshold” from the appropriate value) or an inability to use relevant information. Whether there is a common denominator for all motor dysfunctions observed presently is still an open question. Combining the knowledge of the distributed and variable pattern of individual neuroanatomical changes, the broad neural circuitry involved in the pathophysiology of AD/HD and the multi-level organization of neural control of motor behavior would rather suggest that the motor problems in the group of children with AD/HD and DCD are distributed and multimodal. Differences in the motor behavior between and within groups of children could reflect differences in the severity of the disorder or in the dominant clinical pattern, i.e., whether it corresponds to fine and/or gross motor problems. Nevertheless, the results of the group analysis show clear differences in the motor performance of children in the AD/HD+ group, indicating that there is a neural substrate for more severe motor disturbances. This diminishes the possibilities for motor adaptation and learning, and could explain the persistence of motor problems over the lifespan.
Mapping dopaminergic markers *in vivo* in adults and children with AD/HD

The PET studies reported here had a four-fold aim: i) to map the regional distribution of dopamine transporter in the human brain; ii) to quantify the binding kinetics of the new radiopharmaceutical \([^{11}C]P E 2 I\), which is selective for the dopamine transporter, and to evaluate its suitability for measurements of the dopamine transporter in the human brain; iii) to examine the dopamine transporter and dopamine D2 receptor binding in adolescents with AD/HD; and iv) to search for the relationship between the density of the central dopamine markers and cognitive functions and clinical symptoms of AD/HD.

The present study showed a favourable signal-to-background ratio for \([^{11}C]P E 2 I\) binding in the human striatum and the midbrain. The concentration of radioactivity was highest in the striatum, lower in the midbrain and very low in the thalamus, neocortex. The time activity curves for the caudate, putamen and midbrain regions could be described by kinetic compartment model. For the cerebellum, the 3-CM was statistically preferred, suggesting the presence of kinetically distinguishable nonspecific binding. We suggest that the nonspecific binding, in part, could contribute to the slightly higher BP-values obtained using kinetic compartment methods than more indirect methods. Among the indirect methods of quantification of \([^{11}C]P E 2 I\) binding, the simplified reference tissue method (SRTM) showed the lowest coefficient of variance and was thus chosen for the quantifications of the binding potential (BP) in the clinical study.

There were three main findings in the study of adolescents with AD/HD: i) there was no significant difference between the DAT and DRD2 density in the striatum of adolescents with AD/HD and young adults (Fig. 11A). The density of the DAT and dopamine D2 receptors in the striatum was numerically higher in the group of adolescents with AD/HD, but only by 5-6%, a value that might be attributed to the age effect; ii) DAT binding in the midbrain of adolescents with AD/HD was significantly lower compared to young adults (revealed by a conservative effect size of 0.79, after Bonferroni corrections for multiple comparisons, p=0.04, Fig. 11B); iii) the DAT and DRD2 density in the striatum correlated positively with the level of motor hyperactivity and negatively with the cognitive performance of adolescents with AD/HD. In particular, significant positive correlations were found between the DRD2 density in the right caudate nucleus and motor hyperactivity.

In summary, the lower BP-values for the dopamine transporter in the midbrain indicate neurochemical alterations at the site of the dopaminergic nuclei, which are the origin of the numerous pathways of the widespread dopaminergic innervation to the striatum, limbic system and the neocortical areas. The deficit in the maintenance and regulation of the homeostasis of dopamine in these neurons could be of significance in understanding the mechanisms of heterogenous phenotype of AD/HD.

**Asymmetry, Region-specificity**

Different structures of the brain have specific developmental patterns. Neuroaatomical asymmetries are commonplace in a typically developing brain, and loss of asymmetry or reversal in different areas of the brain may indicate specific brain structures important in pathophysiology of AD/HD. A particular issue for children with AD/HD has been difference in the size and asymmetry of the caudate nucleus. A normal developmental pattern of right to left asymmetry and a substantial age-related volume decrease (of up to 13%) in the group of 6 to 19 year old children has been reported by Castellanos et al. (1994, 1996). With respect to this control group, children with AD/HD differed in terms of the loss of asymmetry and the absence of age-related effects (Castellanos et al, 1994, 1996b). This was substantiated by a lack of typical age-related asymmetry such as that expected for auditory event-related potentials (Oades et al, 1996). Reversed asymmetry, i.e., a smaller volume of total left caudate and caudate head in the group of children with AD/HD has been later reported by Filipek et al, 1997 in a smaller group sample and using
Figure 11. Comparison of $[^{11}]$C]PE2I and raclopride binding in the striatum (A) and the midbrain (B) of adolescents with ADHD and young adults (comprising the comparison group).

different methodological approaches. The authors also reported a less favorable response to psychostimulants in the children with reversed caudate asymmetry. In addition, the significance of interhemispheric asymmetry has been supported by: i) functional brain imaging studies linking increased right frontal rCBF to impaired response inhibition (Langleben et al, 2001), ii) lateralized frontal activation patterns from EEGs (Baving et al, 1999). A series of such results advocated that there is a dysfunction of the right sided prefrontal-striatal systems in children with AD/HD (Schrimsher et al, 2002, Stefanatos and Wasserstein, 2001, review).

But are these structural differences associated with the laterality of the neurochemical changes? Deteriorated protein cascades have been found in localized cortical areas in, for example, schizophrenia (Shirikawa et al, 2001). Hints of neurochemical asymmetry and loss of this asymmetry in AD/HD has been addressed in all PET studies, however, at present there is too little information to draw any conclusions.
Table 3. Dopamine D2 receptor and dopamine transporter binding characteristics.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Comparison group (n=10)</th>
<th>AD:HD group (n=12)</th>
<th>Two-tailed t-test (df=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Dopamine transporter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right caudate</td>
<td>8.07</td>
<td>1.46</td>
<td>8.75</td>
</tr>
<tr>
<td>Left caudate</td>
<td>8.08</td>
<td>2.73</td>
<td>8.41</td>
</tr>
<tr>
<td>Right putamen</td>
<td>9.93</td>
<td>3.30</td>
<td>12.31</td>
</tr>
<tr>
<td>Left putamen</td>
<td>9.63</td>
<td>2.52</td>
<td>9.47</td>
</tr>
<tr>
<td>Right midbrain</td>
<td>1.58</td>
<td>0.34</td>
<td>1.26</td>
</tr>
<tr>
<td>Left midbrain</td>
<td>1.58</td>
<td>0.35</td>
<td>1.33</td>
</tr>
<tr>
<td><strong>Dopamine D2 receptors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right caudate</td>
<td>2.81</td>
<td>0.62</td>
<td>3.08</td>
</tr>
<tr>
<td>Left caudate</td>
<td>3.09</td>
<td>0.54</td>
<td>3.28</td>
</tr>
<tr>
<td>Right putamen</td>
<td>3.73</td>
<td>0.64</td>
<td>3.94</td>
</tr>
<tr>
<td>Left putamen</td>
<td>3.66</td>
<td>0.51</td>
<td>3.92</td>
</tr>
</tbody>
</table>

There were no significant hemispheric differences in the density of dopamine markers between the groups (Table 3). The asymmetry index (AI, (right-left)/(right+left)) was higher in the group of adolescents with AD:HD compared to adults, and significantly higher for the asymmetric DAT density in the putamen, however the latter results were driven by the high DAT-values in the putamen in one subject. There was no clinical explanation for this finding, other than the fact that the child had severe symptoms of AD:HD.

The distribution of dopaminergic markers throughout the brain and even across the same region is uneven, as has been shown in the detailed map of dopamine D2 receptor distribution in humans in vivo (Suhara et al, 1999, Cselényi et al, 2002) and the DAT distribution in the present PET-study, as well as the receptor density gradients evident within the distinct anatomical structures, for example in the striatum (Garnett et al, 1987). These findings show that there may be different kinds of functional organization of the dopamine system and that there are different regulatory mechanisms in the different regions of the brain. Moreover, electrophysiological studies have shown that, for example, DAT can change its function, depending on the environmental context, i.e., it may serve for dopamine release instead of typical reuptake function (Falkenburger et al, 2001). Psychostimulants may, thus, have a different effect on the dopaminergic transmission in different brain regions, or subregions of the striatum (Martinez et al, 2003). Not all dopaminergic areas have been investigated in the present study, e.g., the ventral striatum. In addition, no search was conducted to identify a subregional receptor distribution in the striatum. However, it can be seen that the complexity of neurochemical systems and innervation circuitry probably precludes from very generalized concepts of ‘hypo-or hyperdopaminergic status’ of the brain being drawn for patients with AD:HD.

**Pre-postsynaptic considerations**

One novelty of the present PET-study was the measurement of two central dopamine markers in the same individual, i.e., at the pre- and postsynaptic site. Correlation analysis showed region-specific results: i) there was no significant correlation between the DAT and dopamine D2 receptor availability in the...
striatum in either of the groups (AD/HD, r=0.38, p=0.2; adults, r=0.12, p=0.8); ii) the DAT availability in the midbrain and the striatum was positively correlated and statistically significant for the midbrain-caudate nucleus in both groups (adult group, r=0.65, p=0.04; AD/HD, r=0.63, p=0.03). The correlation pattern did not differ between the AD/HD and control groups.

Not much is known about the balance between DAT and dopamine D2 receptors in the human brain. It has been shown that in different disorders the relationship between these pre- and postsynaptic proteins may change differently and may thus even be helpful for the differential diagnosis. For instance, DAT binding is decreased in Parkinson’s disease (PD), multiple systemic atrophy (MSA) and progressive supranuclear palsy (PSP). The DRD2 density in the striatum and the caudate to putamen ratio increase in PD, but decreases in MSA (Kim et al, 2002).

It may be speculated that the lack of a relationship between pre- and postsynaptic dopamine markers in the striatum represents high interindividual variability in the dopamine “balance”, so relationships may not be seen in our small sample. Alternatively, this pre-postsynaptic complex may be differentially regulated by in fact different cells and thus relationship between pre-and postsynaptic units is not linear. This result may be in agreement with the studies in vitro showing that DAT expression can vary between the DA cell groups (Shimada et al, 1992; Niremberg et al, 1996). In contrast, DAT binding between the midbrain and the striatum correlated significantly and may then represent similar expression of DAT in the dendritic tree and terminals of the same neuron.

**Neurocognitive correlates**
The critical role of central dopamine in the cognitive and emotional functions has been investigated in normal adults. A positive association between various cognitive abilities, including attention, response inhibition, and age-dependent dopamine D2 receptor density in both the caudate nucleus and the putamen has been reported (Buckman et al, 2000; Volkow et al, 1998). Studies on the changes of dopamine markers and their relation to the cognitive deficits in neuropsychiatric disorders have just started. A negative association between the DRD1 receptor density and working memory has been reported in association with schizophrenia (Abi-Dargham et al, 2002). In our study, adolescents with AD/HD had negative correlations between DAT, DRD2 binding in the caudate nucleus, the putamen, and IQ subscores, the working memory capacity (Table 4). However, it is still difficult to specify the actual dopamine contribution to cognitive functions in general and specifically in children with AD/HD. The mechanisms behind these relationships have yet to be explored and may be related to the atypical frontostriatal function found in children with AD/HD, including the decreased capacity to engage basal ganglia (Vaidya et al, 1998). Importantly, altered dopaminergic neurotransmission in the striatum may not only influence behaviour, but also contribute to the cognitive dysfunctions of children with AD/HD.

**Final remarks and future perspectives**

Children with AD/HD display a wide variety of symptoms that may be attributed to changes in the different functional systems of the brain: deficits in frontal executive processes, altered reinforcement mechanisms, and changes in the movement control. Individual profiles comprised of the altered cognitive abilities and behavior of children may be related to the individual combinations of genetic and environmental factors affecting brain development.

Many areas of clinical and experimental research have provided evidence that alterations in the dopamine system are important in the genesis of AD/HD symptoms. The main effects reported are related to the decrease in the children’s capacity to adapt their behavior to environmental stimuli, as has been also supported in the present thesis in regarding the motor systems. Children with AD/HD and DCD preserved the ability to perform the motor task, however they lack capacity to adapt. In addition, our results of the PET-study may suggest a paradigm shift in understanding of AD/HD, i.e., a change from the main focus on the cortico-striatal activity to the closer examination of the midbrain, the intersection of all monoaminergic systems.
Multiple receptor mapping, zooming into subregional changes and a combination of morphological and functional neuroimaging approaches are probably not too far into the future. In the search for specific central neurochemical changes in AD/HD, the nearest studies would be to investigate the extrastriatal dopamine D2 receptor, the dopamine D1 receptor density and noradrenergic system using the presently available radioligands and pharmacological challenges. Dopaminergic dysfunction is implied in a number of other neurodevelopmental disorders. Thus cross-disorder neurochemical studies (e.g., AD/HD and Tourette syndrome) may give additional clues to the specificity of the AD/HD symptoms. The important limitation faced in PET-studies is the number of subjects, therefore a multicenter study would be of considerable value, especially with respect to children.

Behavioural studies on AD/HD have proved to be as important. There are many brain functions to be explored, in particular the relationship between different processes, such as cognitive, perception and motor functions. The influence of neurochemical systems on their regulation could be tested using psychostimulants, and new pharmaceuticals, such as inhibitors of noradrenaline transporter.

Understanding complex human behaviour in the health and illness is a challenge that needs unified effort from professionals in multiple disciplines. The search for new links between genetics, pharmacogenetics, neuroimaging and endophenotypes suggests promising research strategies for the near future.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AD/HD (n=12)</th>
<th>Correlation with DAT binding:</th>
<th>Correlation with D2 receptor binding:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>right caudate</td>
<td>left caudate</td>
</tr>
<tr>
<td>WISC-III- R test:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IQ</td>
<td>83.4 (22.2)</td>
<td>-0.13</td>
<td>-0.63*</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>85.5 (18.7)</td>
<td>-0.14</td>
<td>-0.68*</td>
</tr>
<tr>
<td>Performance</td>
<td>85.2 (23.7)</td>
<td>-0.10</td>
<td>-0.51(t)</td>
</tr>
<tr>
<td>Verbal understanding</td>
<td>89.2 (20.2)</td>
<td>-0.11</td>
<td>-0.67*</td>
</tr>
<tr>
<td>Perception organisation</td>
<td>89.9 (23.0)</td>
<td>-0.02</td>
<td>-0.41</td>
</tr>
<tr>
<td>Attention</td>
<td>80.3 (11.5)</td>
<td>-0.21</td>
<td>-0.34</td>
</tr>
<tr>
<td>Speed</td>
<td>77.9 (18.6)</td>
<td>-0.25</td>
<td>-0.74**</td>
</tr>
<tr>
<td>DSM-IV scale (score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention</td>
<td>12.1 (2.8)</td>
<td>-0.11</td>
<td>0.53(t)</td>
</tr>
<tr>
<td>Hyeractivity</td>
<td>16.7 (3.5)</td>
<td>0.05</td>
<td>0.37</td>
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<tr>
<td>Impulsivity</td>
<td>5.8 (2.2)</td>
<td>-0.39</td>
<td>-0.18</td>
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<tr>
<td>Total</td>
<td>34.7 (6.9)</td>
<td>-0.15</td>
<td>0.34</td>
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<tr>
<td>Brown scale</td>
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<tr>
<td>Attention score</td>
<td>16.1 (5.1)</td>
<td>0.15</td>
<td>0.30</td>
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<tr>
<td>CBCL</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Internalizing criteria</td>
<td>3.2 (2.3)</td>
<td>-0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>Externalizing criteria</td>
<td>20.0 (8.1)</td>
<td>-0.34</td>
<td>-0.22</td>
</tr>
<tr>
<td>Visuo-spatial working memory (level)</td>
<td>5.5 (1.4)</td>
<td>0.21</td>
<td>-0.15</td>
</tr>
<tr>
<td>Reaction time (RT) task</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commissions</td>
<td>1.3 (1.1)</td>
<td>0.76**</td>
<td>0.08</td>
</tr>
<tr>
<td>RT</td>
<td>22.5 (3.8)</td>
<td>-0.37</td>
<td>0.15</td>
</tr>
<tr>
<td>RT delta</td>
<td>76.1 (27.1)</td>
<td>0.26</td>
<td>0.10</td>
</tr>
<tr>
<td>Continuous performance task (CPT)</td>
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<td></td>
</tr>
<tr>
<td>Commission errors</td>
<td>19.9 (11.8)</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Omission errors</td>
<td>12.7 (20.4)</td>
<td>0.42</td>
<td>0.33</td>
</tr>
</tbody>
</table>

r-values presented. *p<0.05, ** p<0.01
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---

*Des mots que tu as tant cherchés, les plus beaux, les plus justes,*  
*Des mots qui changeraient le monde,*  
*Qui le réinventeraient...*  
*Tu les trouves par hasard, au tournant d’une rue.*  
*...Et à nouveau tu les oubliées.*

*Patrice de La Tour du Pin*
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