

**Modulation of Executive Function in Children with
Attention Deficit/Hyperactivity Disorder by Allelic
Variants of the Catechol-O-Methyltransferase Gene
and Three Varying Doses of Methylphenidate**

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Abstract

An association has been observed between the *catechol-O-methyltransferase* (*COMT*) gene, the predominant means of catecholamine catabolism within the prefrontal cortex (PFC), and neuropsychological task performance in healthy and schizophrenic adults. Since several of the cognitive functions typically deficient in children with Attention Deficit Hyperactivity Disorder (ADHD) are mediated by prefrontal dopamine (DA) mechanisms, we investigated the relationship between a functional polymorphism of the *COMT* gene and neuropsychological task performance in these children. Furthermore, since methylphenidate (MPH), the primary pharmacological drug in ADHD, may exert its effects, at least in part, through PFC dopaminergic pathways, we investigated the relationship between the *COMT* polymorphism and acute neuropsychological and behavioural response to three varying doses of MPH. Children with ADHD showed improvement on measures of both cognitive and motor behaviours under MPH, although improvement within each functional domain appeared to follow distinctive dose-response patterns possibly reflecting different neurobiological pathways. No genotype effects were observed on any measures of cognitive or motor behaviour or on the response of cognitive or motor behaviours to MPH.

Résumé

Une association a été observée entre le gène *catechol-O-methyltransferase* (*COMT*), le moyen prédominant du catecholamine catabolism à l'intérieur du cortex préfrontal (CPF), et la performance des tâches neuropsychologiques chez les schizophrènes adultes en santé. Puisque plusieurs des fonctions cognitives typiquement déficientes chez les enfants avec le Trouble d'Attention avec Hyperactivité (TDAH) sont méditées par le mécanisme de dopamine préfrontal, nous avons examiné la relation entre un polymorphe fonctionnel du gène *COMT* et la performance des tâches neuropsychologiques chez ces enfants. De plus, puisque le méthylphénidate (MPH), l'approche thérapeutique primaire dans le traitement du TDAH, exerce ses effets par le chemin dopaminergique du CPF, nous avons examiné la relation entre le polymorphe *COMT* et la performance de tâches neuropsychologiques et comportementales sur trois doses variantes de MPH dans cette même population. Aucun effet de génotype n'a été observé sur les mesures de cognition et de comportement lorsque les enfants prenaient ou ne prenaient pas de médicament. Les enfants avec le TDAH ont démontré une amélioration sur les mesures cognitives et les fonctions comportementales lors de la prise de MPH, quoique l'amélioration à l'intérieur de chaque domaine fonctionnel parait suivre un modèle distinctif de réponse à la dose, qui reflète peut-être une divergence des chemins pathophysiologiques.

Introduction

1 Rationale

Attention Deficit Hyperactivity Disorder (ADHD) is the most commonly diagnosed psychiatric disorder among school-aged children in North America and research investigating its etiology has identified genetic factors as a significant contributor to the predisposition for the development of this disorder¹⁻³. Environmental factors are also believed to play an important role in the development of ADHD⁴. Although it is not known precisely how these genetic and environmental factors are implicated in increasing the risk for the disorder, it has been proposed that these factors exert their effects through the deregulation of brain dopamine (DA) pathways. Therefore, genes coding for proteins involved in these pathways may be suitable candidate genes for the study of ADHD. One particularly compelling gene is the *catechol-O-methyltransferase* (*COMT*) gene which encodes for the enzyme COMT, an enzyme involved in catalyzing the inactivation of catecholamines such as dopamine⁵ within the prefrontal cortex (PFC)⁶. The *COMT* gene contains a polymorphism with a clear functional effect on the activity of the enzyme⁷. Since deficits in PFC-mediated executive functions are present in children with ADHD⁸, attempts have been made to identify associations between functional polymorphisms of genes implicated within the brain DA pathways, such as the *COMT* gene, and ADHD but have met with modest success^{1,9-12}. Augmenting the strength of these associations may require the identification and investigation of associations between candidate gene polymorphisms and intermediate risk phenotypes, such as deficient executive functioning, which may be closer to the primary sites of disease causation and to gene effects.

Methylphenidate (MPH) is the primary therapeutic intervention in the treatment of ADHD¹³ and appears to exert its effects via the inhibition of DA reuptake resulting in an increase in extracellular DA capable of binding to DA receptors¹⁴. Administration of MPH results in a reduction in the core behavioural¹⁵ and cognitive^{16,17} symptoms of ADHD. Given the impact of DA-related genes such as the *COMT* on cortical dopaminergic pathways and the role of these pathways in mediating neurocognitive functioning^{18,19}, it is reasonable to speculate that these genes may mediate cognitive response to DA agonists such as MPH.

2 Objectives

The aims of our research protocol were to determine:

- I. whether or not a functional polymorphism of the *COMT* gene modulates executive functions in children with ADHD;
- II. the nature of executive function and motor behavioural dose-response curves under methylphenidate in children with ADHD.
- III. whether or not a functional polymorphism of the *COMT* gene modulates executive functions and acute behavioural performance under varying doses of MPH;

3 Hypotheses

1. Given the putative role of *COMT* in DA metabolism within the PFC^{20,21}, we hypothesized that the *Val*^{108/158} *Met* polymorphism of the *COMT* gene will be associated with alterations in performance on tasks of executive function, a behavioural index of PFC integrity and function²². Since dysfunctional DA neurotransmission²³ and deficient neuropsychological task performance²⁴ are both characteristic of children with ADHD, we further hypothesized that this association would be evident within this particular clinical population.

Specifically, ADHD children expressing the high enzymatic activity *Val* allele (H), resulting in reduced PFC DA neurotransmission⁷, will show more pronounced deficits in neuropsychological task performance reflecting executive functions than their low enzymatic activity *Met* allele (L) counterparts.

2. We further hypothesized that an association exists between the *COMT Val*^{108/158} *Met* polymorphism and response of neuropsychological task performance to MPH in children with ADHD, given the dependency of this drug on DA pathways to exert its therapeutic effects¹⁴.

In order to test these hypotheses, we constructed an experimental design consisting of two parts. In Part A of our study, we used three measures of executive function in order to determine whether the *COMT Val*^{108/158} *Met* polymorphism mediates neuropsychological task performance in children with ADHD while they were off medication. Our task battery included: the Wisconsin Card Sorting Test (WCST)²⁵, a measure of set-shifting ability capable of differentiating between ADHD children and controls²⁴ and associated with the *COMT* polymorphism in normal^{26,27} and schizophrenic adults²⁸⁻³⁰; the Tower of London (TOL)³¹, a measure of planning ability, which consistently differentiates ADHD children from controls²⁴, and; the Self-Ordered Pointing Task (SOPT)³², a measure of working memory also capable of differentiating between ADHD children and controls²⁴. In Part B of our study, we used two measures of executive function in order to determine whether the *COMT Val*^{108/158} *Met* polymorphism mediates response to MPH at three varying doses (0.3, 0.5, 1.0 mg/kg) as measured by

neuropsychological task performance in children with ADHD. In addition to the assessment of neuropsychological performance, we included two measures of acute motor behavioural response to methylphenidate in order to study concomitantly cognitive and acute motor behavioural dose-response in children with ADHD under the three doses of MPH. Our cognitive tasks included the SOPT and the WRAML Finger Windows Task, a measure of visual-spatial working memory sensitive to MPH administration in ADHD children³³. Our acute motor behaviour measures included the Restricted Academic Situation Scale (RASS)³⁴, an index of the frequency of specific motor behaviours during the performance of an academic task which differentiates ADHD children from their healthy peers and has shown validity and reliability across medication dosages³⁵, and the Clinical Global Impression (CGI)³⁶ scale, which includes two dimensions: the severity and improvement of hyperactive symptoms during a period of observation.

Literature Review

1 ADHD – An introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a childhood psychiatric disorder characterized by symptoms of inattention, impulsivity and motor hyperactivity afflicting 6-8% of school-aged children in North America^{37,38}. Although ADHD is a disorder with complex and heterogeneous etiology, genetic factors appear to play a significant role in predisposing and perpetuating the development of the disorder as evidenced by twin^{1,39}, family^{2,40,41}, and adoption studies³. A substantial portion of the phenotypic variance in the expression of ADHD appears to be contributed by genetic factors, with heritability estimates exceeding 70 % in some cases⁴². Environmental factors have also been implicated in the determination of ADHD⁴.

Association studies have implicated several susceptibility loci including a 40-base pair (bp) allele of the Variable Number of Tandem Repeats (VNTR) polymorphism of the *SLC6A3* gene⁴³ and a 48-bp repeat polymorphism of the *DRD4* gene⁴⁴. Attempts to replicate these findings have met with modest success possibly owing to the clinical heterogeneity characteristic of the disorder⁴⁵. One method that may act to augment the strength of these associations would be to identify associations between endophenotypic intermediates of ADHD and candidate genes rather than attempting to identify direct links between genetic variations and the entire syndrome of ADHD. Endophenotypes are heritable behavioural traits or neurophysiological characteristics that represent simpler and possibly more homogeneous constructs than a syndrome in its entirety and serve as indices of intermediate changes occurring along the pathophysiological course leading to

the emergence of a disorder. As such, endophenotypes may be closer to the genetic determinants of a disorder thus making the relationship between candidate genes and endophenotypes easier to identify.

2 Dopamine hypothesis of ADHD

Theories of deregulated dopamine (DA) pathways in ADHD have been supported by the efficacy of dopamine agonists in reducing the core symptoms of the disorder⁴⁶. Both mesocortical and mesolimbic DA pathways have been implicated in the pathophysiology of ADHD⁴⁷. The mesocortical DA pathway appears to be integral to prefrontal cortex (PFC)-mediated cognitive functioning through the enhancement of task-related neural activity via D1 receptor activation⁴⁸. Both PET¹⁸ and SPECT¹⁹ imaging studies support a neuromodulatory role for DA in the PFC during tasks of executive function. In addition, administration of DA agonists to the rat PFC acts to enhance working memory in animals⁴⁹. Consistent with this line of thinking, children with ADHD show deficits in performance of tasks of executive function [summarized in a meta-analysis by Sergeant et al. (2002)]²⁴ and significant improvement of performance under methylphenidate (MPH)^{16,17}. These findings have prompted the hypothesis that the overt symptoms of ADHD may be the manifestation of an underlying deficiency in a range of PFC-mediated cognitive domains, including working memory, planning, and set shifting, collectively regarded as executive function^{8,50,51}.

3 Executive dysfunction in ADHD

Executive functions are a set of cognitive control processes that serve to optimize performance during complex tasks and include processes such as attention, response inhibition, planning, and working memory. Of the range of processes categorized as

executive functions, working memory has received considerable attention. Working memory is a function responsible for the active maintenance of temporary, task-relevant information for further processing or recall and is essential for the focusing of selective attention during complex cognitive tasks such as mental calculation and language comprehension⁵². Working memory can be further categorized into a variety of domain-specific models including auditory-verbal and visual-spatial. There is ample evidence to suggest that children with ADHD show considerable deficits in working memory function^{8,53,54}. These findings are consistent with the putative role of mesocortical DA pathways in mediating performance on tasks of executive functioning^{18,19} and the hypothesized contribution of dysfunctional DA neurotransmission to the pathophysiology of ADHD. Studies in animals and humans have shown that working memory, particularly visual-spatial working memory, is mediated, by the PFC^{55,56} and is, at least in part, dopamine dependent^{57,58}. Electrophysiological studies of the P300 event-related potential, which is a sensitive index of the attentional and working memory demands of a task, indicate a specific impairment of visual-spatial working memory in children with ADHD⁵⁰.

4 Endophenotypes – Bridging the gap between genotype and phenotype

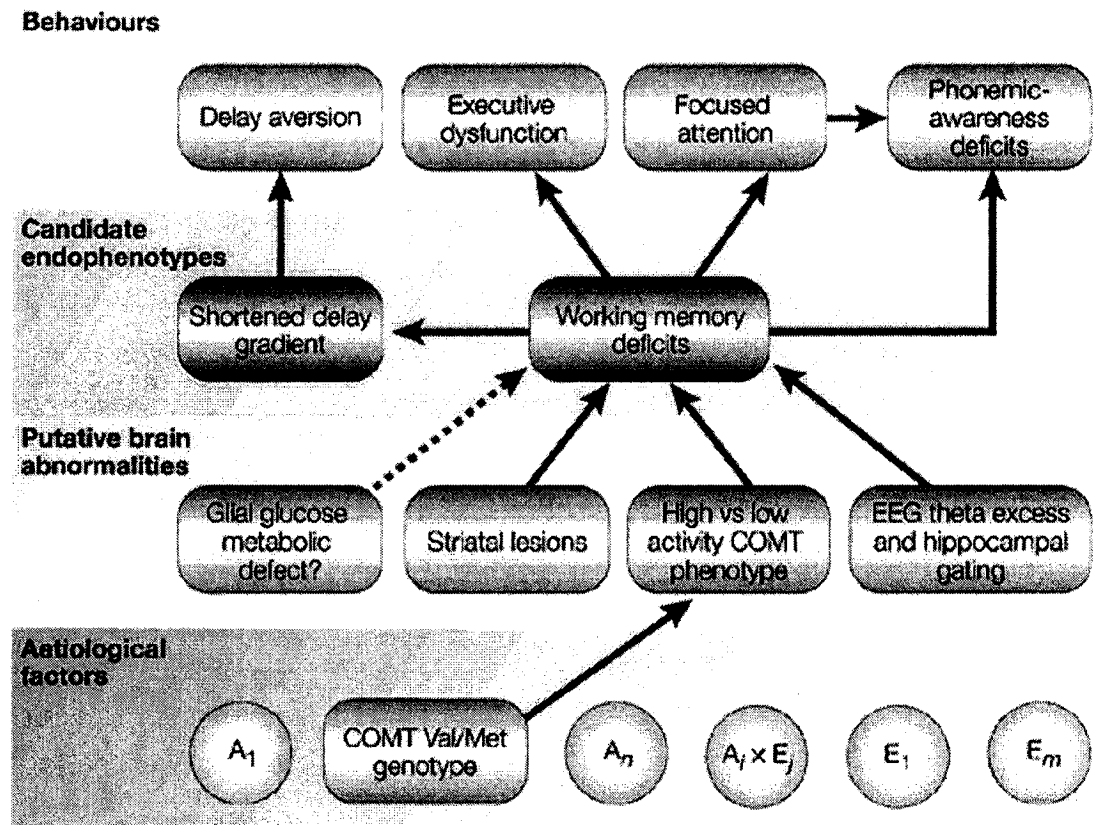
ADHD, like most psychiatric disorders, does not follow classic Mendelian patterns of inheritance. Cornblatt and Maholtra (2001)⁵⁹ warn that the use of symptom-based, diagnostic classification systems may limit one's ability to detect associations or linkages between candidate risk genes and the behavioural manifestations of a complex disorder given the clinical heterogeneity characteristic of psychiatric disorders such as ADHD⁴⁵. This point was elegantly formulated by Gottessman and Gould (2003) who stated: "It

stands to reason that more optimally reduced measures of neuropsychiatric functioning should be more useful than behavioural ‘macros’ in studies pursuing the biological and genetic components of psychiatric disorders”⁶⁰. Endophenotypes are heritable traits that index an individual’s liability for developing or manifesting a given disease and are believed to be more directly related to a disease’s aetiology than dichotomous diagnostic categories⁶¹. In a detailed review of the literature, Castellanos and Tannock (2002)⁵³ concluded that such endophenotypes should be continuously quantifiable, probabilistic in their predictive ability, close to primary sites of causation and grounded in the neurosciences. They further suggested that executive function, and more specifically working memory, represent compelling quantitative traits worthy of consideration in the investigation of the pathophysiology of ADHD (Figure 1).

5 Methylphenidate and the treatment of ADHD

Methylphenidate (MPH), or Ritalin, is the primary medication used in the treatment of ADHD¹³. 70% of children with ADHD receiving MPH show significant reductions in the core symptoms of the disorder⁶² and the short-term behavioural improvements facilitated by MPH have been confirmed by hundreds of studies [summarized in review by Solanto et al. (2001)]¹⁵, with observable improvements occurring as rapidly as thirty minutes following ingestion. The long-term benefits of MPH were investigated in a sample of 579 ADHD children and MPH proved superior to both intensive behavioural therapy and community care interventions over a 14-month period. In addition, MPH treatment proved to be of equal benefit as a combined medication-behavioural therapy intervention⁶³. The primary MPH pathway is believed to involve the inhibition of DA reuptake resulting in an increase in extracellular DA capable of binding to DA

Figure 1 - Working memory deficits as a candidate endophenotype (Castellanos & Tannock, 2002⁵³)



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Castellanos and Tannock (2002)⁵³. Such deficits might arise as a result of brain abnormalities, including striatal lesions and alterations in *catechol-O-methyl transferase* (COMT) activity. Attention-deficit/hyperactivity disorder (ADHD)-associated behaviours that are influenced by working memory might include attentional processes and learning disorders. Broken arrows indicate untested proposed causal links; A₁, dopamine transporter (*DAT1*) polymorphism; A_{2-n}, additive genetic factors; A₃, *catechol-O-methyl transferase* (COMT) Val/Met polymorphism; A_i × E_j, gene-environment interactions; EEG, electroencephalogram; E_{1-m}, environmental factors.

receptors¹⁴. DA acts to decrease background-firing rates and to increase the signal-to-noise in target neurons, thereby enhancing task-specific signalling and improving attention while decreasing distractibility. The PFC, a highly DA-innervated region⁶⁴ intricately involved in executive function (specifically working memory)^{55,56}, is known to be dysfunctional in children with ADHD⁶⁵. Not surprisingly, increases in synaptic DA within this region as facilitated by MPH, have been observed to produce improvements in overall cognitive functioning and executive functions such as working memory^{16,17,33,66}. Evidence exists to suggest that MPH-induced improvement on working memory tasks may be particularly robust within the visual-spatial domain, as has been observed in healthy individuals¹⁶ and children with ADHD^{33,67,68}.

There is also evidence to suggest that children with ADHD show considerable variability in their cognitive response to MPH, with some children even exhibiting adverse drug responses^{69,70}. A number of hypotheses have been proposed in an attempt to explain this variability. Zahrt et al. (1997) suggested that differential cognitive processes require different levels of catecholamines in order for optimal performance to be achieved⁷¹. Observed differences in performance under MPH on tasks measuring working memory processes have also been explained in terms of varying memory load²⁷ since an association has been observed between the memory load demands of a particular task and DA release⁴⁹. One theory proposes that the variability in cognitive drug response to MPH is a result of assessments occurring under varying dose conditions⁷². However, even at a fixed dose this variability appears to persist^{66,73} and therefore attempts have been made to generate a rationale explaining inter-individual differences under fixed medication

conditions. Mattay et al. (2003) attempted to develop such a rationale in terms of a functional polymorphism of a DA gene, specifically the *catechol-O-methyltransferase* (*COMT*) gene, which affects basal dopaminergic tone²⁷. This rationale was based on the observation that the positive effects induced by psychostimulants (such as MPH) on attention and cognition appear to be mediated by DA pathways⁷⁴. In addition, DA-induced improvements in cognitive functioning have been hypothesized to occur within a narrow range of DA concentration in accordance with an inverted “U”-shaped concentration-response curve^{48,58}. This relationship predicts that differential levels of basal DA, as determined by functional polymorphisms of genes involved in DA neurotransmission, will be influential in mediating drug-induced changes in DA concentration and ultimately, cognitive functioning. At the molecular level, it's believed that DA strengthens the effects of strong depolarizing currents and enhances task-related neural activity through the activation of D1 receptors⁴⁸. This, in turn, sharpens the signal and amplifies its effect on a subset of inputs to PFC neurons⁷⁵. However, too much DA disorganizes PFC neural networks by activating inhibitory mechanisms, including the inactivation of N-type Ca^{2+} channels⁷⁵, activation of GABAergic interneurons, and the pre- and post-synaptic reduction of glutamate-mediated synaptic responses⁷⁶. Mattay et al. (2003) provided support for this rationale after observing an association between *Val*^{108/158} *Met* polymorphism of the *COMT* gene and performance on a task assessing working memory under a fixed dose of amphetamine²⁷. Depending on their relative starting positions on the hypothetical, inverted “U”-shaped DA concentration-response curve, as determined by the *COMT Val*^{108/158} *Met* polymorphism, subjects either showed significant improvement or deterioration in performance depending on whether they

expressed allelic variants producing either reduced or enhanced basal DA concentrations, respectively.

6 Cognitive and acute motor response to methylphenidate

The variability in the cognitive response of children with ADHD to MPH has become a particularly attractive and relevant area of research given the clinical importance of prescribing appropriate doses of medication capable of maximizing both behavioural and cognitive improvements. Research investigating the behavioural improvements produced by varying doses of MPH have largely been in agreement that behavioural improvement under MPH follows a more-or-less linear dose-response relationship, with increasing doses producing the most substantial behavioural benefits^{72,77,78}. In a 1977 study, Sprague and Sleator were among the first researchers to suggest that the optimal dose of MPH necessary to maximize behavioural improvement in children with ADHD may not coincide with the optimal dose necessary to maximize cognitive improvement in these children⁷⁹. In fact, using a short-term working memory task, Sprague and Sleator observed that cognitive improvement under MPH followed an inverted “U”-shaped dose-response relationship, with optimal improvement occurring at moderate doses and a steady decline in improvement occurring at increasing doses. This finding was particularly significant since it argued against the typical clinical practice of determining and prescribing dosages of MPH appropriate for ADHD children based upon a single measure, such as parent or teacher reports. In support of these findings, it has been argued that MPH, under certain conditions, might impair cognitive flexibility and produce perseveration of action⁸⁰, as well as interfering with the learning of new material⁸¹. Cognitive flexibility is the ability to shift freely from one concept to another and the

ability to change a course of action according to the demands of a new situation. Support for this theory has been in the form of studies of ADHD children receiving MPH and exhibiting reduced ability to shift mental set⁷⁰, problems with divergent rather than convergent thinking⁸², motor stereotypy⁸³, and behaviour described as “zombie”-like⁸⁴. However, these findings are generally inconsistent and marred with methodological limitations such as limited subject size⁸² and the transient nature of observed effects⁷⁰. Tannock et al. (1995) confirmed the hypothesis of discrepant acute behavioural and cognitive dose-response curves, observing a linear relationship for improvement in motor activity under MPH, in the form of reductions in motoric restlessness, and observing an inverted “U”-shaped relationship for improvement in response inhibition under MPH⁸⁵. However, a variety of other non-linear dose-response relationships were observed for other cognitive functions, such as response execution (“L”-shaped), indicating that dose-response may vary both between and within cognitive domains and that Sprague and Sleator’s polarized view of acute behavioural and cognitive dose-response may be overly simplistic.

Evidence also exists to suggest that, similar to acute behavioural improvement under varying doses of MPH, cognitive improvement may also follow a linear dose-response relationship^{17,72,86}. Douglas et al. (1995) found no adverse effects and increasingly positive effects under increasing doses of MPH on tasks measuring perseveration, divergent thinking and ability to shift mental set⁸⁶. Berman et al. (1999) also observed a linear relationship between performance on a memory task and dose of MPH¹⁷. Interestingly, as MPH dose increased, an inverse correlation between number of errors

produced and time to respond was observed. In other words, as dose increased, children were adapting their strategy in order to favour accuracy over speed. Berman et al. argued that, viewed from the wrong perspective, this result could be misinterpreted as an adverse cognitive reaction to high doses of MPH. Instead, they stressed the value of examining a series of measures within any single cognitive domain in order to generate conclusions regarding drug response.

7 Candidate risk genes and the dopamine pathway

The hypothesized role of a dysfunctional mesocortical dopaminergic pathway in the development of symptoms of ADHD has encouraged the investigation of candidate genes involved in this pathway including *SLC6A3*⁴³, *DRD4*⁴⁴ and, more recently, the *COMT* gene⁹. The *COMT*, encoded by a gene located on chromosome 22q11, catalyzes the inactivation of catecholamines, most importantly DA⁵. A functional polymorphism of this gene, involving a substitution of Valine (*Val*) for Methionine (*Met*) at codon 108/158 (*Val*^{108/158} *Met*), results in a 4-fold variation in enzyme activity, with individuals homozygous for either the *Val* or *Met* allele exhibiting either reduced or preserved levels of DA respectively⁷. Although the dopamine transporter (DAT) is the predominant means of DA termination in most dopaminergic neurons⁸⁷, considerable evidence exists to suggest that the DAT may play a reduced role within the PFC⁸⁸⁻⁹², where other clearance mechanisms may be implicated. Comparison of DA metabolite levels within discrete brain loci in both rats⁶ and monkeys²⁰, as well as the measurement of DA levels in *COMT* knock-out mice²¹, suggest an important functional role for *COMT* in the PFC. If *COMT* is indeed inextricably linked to DA metabolism within the PFC, it is reasonable to assume that variations in enzyme activity, as dictated by the *Val*^{108/158} *Met* polymorphism, may

modulate the performance of tasks of executive functioning in healthy individuals, as well as individuals with reduced PFC basal dopamine levels. In addition, it is also reasonable to assume that, given the impact of psychostimulant drugs such as MPH on the concentration of extracellular DA within the PFC¹⁴, *COMT*-related variations in enzyme activity may also modulate the performance of tasks of executive functioning in both healthy and hypodopaminergic individuals under psychostimulant medication. In support of these assumptions, associations have been reported between the *Val*^{108/158}*Met* polymorphism and performance on the Wisconsin Card Sorting Test (WCST) in healthy adults^{26,27}. Mattay et al. (2003) also identified an association between the *Val*^{108/158}*Met* polymorphism and amphetamine on performance of the WCST in healthy adults²⁷. In adults with Schizophrenia, a disorder characterized by dopaminergic hypofrontality⁹³, associations have also been observed between the *COMT* polymorphism and WCST performance²⁸⁻³⁰. Although one study reported an association between the *COMT* polymorphism and ADHD using a haplotype relative risk design⁹, this study failed to investigate any indices of executive function and several other studies failed to replicate this finding^{1,10-12}. To date, no studies have attempted to investigate potential associations between the *COMT Val*^{108/158}*Met* polymorphism and acute behavioural and cognitive response under MPH in either healthy or clinical populations such as children with ADHD.

Methods

1 Subjects

In Part A of our study, 118 children diagnosed with ADHD were recruited from the Disruptive Behaviour Disorders Program and the children outpatient clinic at the Douglas Hospital. In Part B of our study, 30 ADHD-diagnosed subjects were recruited from these same facilities. They were referred to these specialized care facilities by school principals, community social workers, and paediatricians.

Inclusion criteria required children to be between the ages of 6 and 12 years of age, meeting DSM-IV diagnosis criteria for ADHD⁹⁴. Diagnosis of ADHD was based on a structured clinical interview of parents using the DISC-IV (parental report)⁹⁵, school reports, teacher interviews, and clinical observation of the child. In the majority of cases, mothers were the primary informants for the collection of clinical information. Written reports from the child's school were also available in the majority of cases. Parents completed the Child Behavioural Checklist (CBCL)⁹⁶, a scale that assesses a variety of behavioural domains, and the Conners' Global Index for parents (CGI-P)⁹⁷. Teachers also completed the Conners' Global Index (CGI-T)⁹⁵. Assessments were made while children were free of medication. Exclusion criteria included a history of mental retardation, with an IQ less than or equal to 70 as measured by the WISC-III⁹⁹, and history of Tourette Syndrome, pervasive developmental disorder, psychosis or any medical condition or impairment that may interfere with the child's ability to complete the study.

2 Neurocognitive and behavioural assessment

2.1 Part A

A comprehensive neuropsychological test battery assessing different aspects of the central executive functions was administered to all children by trained research personnel. All children were assessed subsequent to a one-week medication “wash-out” period. Children were permitted to take breaks upon request and, in some cases, testing was carried out over two sessions. On average, the testing procedure lasted 1.5 hours. The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Parents were explained the study and provided written consent. Children were also explained the study and gave their assent to participate as well.

Tests were selected according to their ability to tap into various performance domains of executive function. We restricted the number of tests in each domain in order to balance comprehensiveness with the co-operation of patients. Abstraction and concept formation were evaluated by means of the WCST (perseverative errors)²⁵. In this task, children are required to sort cards according to three different criteria (colour, number, or shape of signs presented on cards). Feedback on whether the child achieved a correct or incorrect match is given after each trial. The matching criterion changes after ten consecutive correct matches and the child has to identify the new matching criterion using the feedback (correct/incorrect) provided to them. Evidence of the reliability and validity of the WCST with various normal and clinical populations has been reported in several studies²⁴. Planning capacity was evaluated using the TOL³¹. This test is used to assess planning and problem solving aspects of executive functioning. The validity and

reliability of the TOL has been reported in numerous studies²⁴. Standardized administration and scoring procedures as well as normative data have been developed for paediatric populations¹⁰⁰. Visual-spatial working memory was evaluated using the representational version of the SOPT³². In this task, series of matrices of 6, 8, 10, and 12 images are presented to the child. The child is asked to select, by pointing, one different image on each page. Errors occur when the child points to images previously selected on the preceding pages. Each set is presented to the child three times. Successful performance on this task involves working memory as well as planning and monitoring skills. Shue & Douglas (1992) have reported significant differences in performance between ADHD children and normal controls on the SOPT¹⁰¹.

2.2 Part B

A double-blind, within-subject (crossover) experimental design was used to assess cognitive and motor behavioural response at MPH doses of 0.3, 0.5 and 1.0 mg/kg (Figure 2). On average, the testing procedure lasted two hours on each dose day and was comprised of an identical pre-treatment and post-treatment cognitive and motor behavioural assessment separated by a 45-minute break. Testing occurred over three consecutive days. In most cases, testing occurred following a two-day wash-out period. For cognitive measures, a baseline assessment was administered two weeks prior to the commencement of the titration protocol. For motor behavioural measures, in addition to the assessment occurring under the three doses of MPH, an assessment was also administered under placebo within the two-week period prior to the commencement of the titration protocol.

**Figure 2 – Double-blind, within-subject (crossover), MPH titration research design
(Part B)**

Day A	Day B	Day C
0.3 mg/kg (or 0.5 mg/kg or 1.0 mg/kg)	0.5 mg/kg (or 1.0 mg/kg or 0.3 mg/kg)	1.0 mg/kg (or 0.3 mg/kg or 0.5 mg/kg)
<u>Assessment</u>	<u>Assessment</u>	<u>Assessment</u>
Finger Windows	Finger Windows	Finger Windows
SOPT	SOPT	SOPT
RASS	RASS	RASS
CGI-I	CGI-I	CGI-I
Pill/45-minute break	Pill/45-minute break	Pill/45-minute break
Finger Windows	Finger Windows	Finger Windows
SOPT	SOPT	SOPT
RASS	RASS	RASS
CGI-I	CGI-I	CGI-I

SOPT = Self-Ordered Pointing Task; RASS = Restricted Academic Situation Scale; CGI-I = Clinical Global Impression Scale-Improvement; MPH = methylphenidate.

Doses were selected in an effort to provide information regarding response to what is generally considered a clinically low dose (0.3 mg/kg), a clinically moderate dose (and the dose most consistently used in clinical and research settings¹⁰²) (0.5 mg/kg), and a clinically high dose (1.0 mg/kg). The order of administration (0.3, 0.5 and 1.0 mg/kg) was counterbalanced and determined by random assignment and researchers were blind to which dose corresponded to which day. Doses of MPH were prepared individually in daily blister packs by a clinical pharmacist not otherwise involved in the study. Response under MPH was assessed 45 minutes subsequent to the ingestion of the pill since this appears to be the period necessary for MPH to attain its maximal cognitive effects¹⁰³. The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Parents were explained the study and provided written consent. Children were also explained the study and gave their assent to participate as well.

Cognitive functioning was assessed using the WRAML Finger Windows Task and the SOPT. Tests were selected according to their ability to tap into the visual-spatial working memory executive function, a performance domain known to be deficient in children with ADHD^{6,53,54} and sensitive to MPH effects in these same children^{33,67,68}. The Finger Windows task is a measure of maintenance and manipulation of visual-spatial working memory¹⁰⁴. In this task, the child is asked to reproduce increasingly difficult series of finger points presented by the examiner. The child is awarded one point for each correctly reproduced series. The task is discontinued after three consecutive errors occur. Performance on this task appears to be sensitive to MPH in children with ADHD and a viable means of differentiating these children from controls³³. The SOPT is also a

measure of visual-spatial working memory, as well as planning and monitoring ability, and is discussed previously.

Acute motor behavioural response to MPH was assessed using the RASS³⁴ and the CGI³⁶. The RASS provides information about the frequency of specific motor behaviours during the performance of an academic task. The child is left alone in a clinic playroom with a set of math problems adapted to his or her academic level and instructed to complete as many problems as possible in a 15-minute time period. Behavioural events are recorded at 30-second intervals according to five behavioural categories: “off task”, “fidgets”, “out of seat”, “vocalizes” and “plays with objects”. Trained research personnel, who monitor the child’s behaviour from another room through a one-way mirror, carry out the assessment. Research personnel are trained until inter-rater reliability reaches a high intraclass correlation coefficient (in our laboratory the intraclass correlation coefficient ranges from 0.97 to 0.99). The RASS has been found to correlate with teachers’ ratings of ADHD symptoms and to discriminate ADHD children from their normal peers³⁵. The RASS has also been found to be reliable and valid for repeated administrations of psychostimulant drugs across medication dosages and placebo³⁵. The CGI is a rating scale that measures the severity of clinical symptoms during the assessment period preceding the administration of MPH and indexes improvement in hyperactivity during the assessment period following the administration of MPH. The child is assigned an acute behavioural improvement score, ranging from 1, “very much improved”, to 7, “very much worse”, based on their level of motor hyperactivity post-medication relative to their level of motor hyperactivity pre-medication.

For clinical purposes, at the completion of the titration, a qualified group of psychiatrists, psychologists and other research personnel assign a cognitive, acute behavioural and overall response score to each day ranging from one to three, one representing optimal performance. The titration code is then broken to reveal which dose corresponded to which day.

3 Molecular genetics

The *Val*^{108/158}*Met* polymorphism of the *COMT* gene was genotyped using a PCR based method as previously described⁷. The PCR was performed in a 25 µl total reaction volume containing 1X PCR buffer, 200uM dNTPs, 200 ng of primers (5'-GCGATGGTGGCACTCCAAGC; 5'-TTGGAGAGGCTGAGGCTGAC), 1 unit of Taq DNA polymerase, and 100 ng of genomic DNA. PCR products were electrophoresed on agarose-TAE gel along with 1kb ad 100bp DNA ladders, visualized under UV-light and coded according to the length of the PCR product. Genotypes were called by two independent and experienced technicians who were blind to all clinical data. No discordance in any of the readings was noted. Children were stratified according to genotype only after all neuropsychological task data was collected.

4 Statistical analyses

The *Val*^{108/158}*Met* polymorphism consists of both the low-activity *Met* (L) and high-activity *Val* (H) alleles. Subjects were stratified into three groups: two homozygous genotype groups (LL, HH) and one heterozygous genotype group (HL).

4.1 Part A

To test for the excess transmission of the risk H allele, we recorded all occurrences of unambiguous transmissions (T) and non-transmissions (NT) of this allele from

heterozygous parents to their affected children. A transmission disequilibrium test $[(T-NT)^2/(T+NT)]$, which follows a χ^2 statistic with one degree of freedom, was then calculated to test departure from the null hypothesis ($T = NT$). An investigation of linkage and within-family association between quantitative phenotypes (standardized WCST perseverative error score, standardized TOL error score, and SOPT error score) was conducted utilizing the Quantitative Trait Disequilibrium Test (QTDT) statistical software package¹⁰⁵.

A one-way analysis of variance (ANOVA) where genotype (LL, HL, HH) was the independent variable and neuropsychological task performance (standardized WCST perseverative error score, standardized TOL total item score) was the dependent variable was performed. For the SOPT, no normalized scores are available and testing procedures involve several levels of difficulty (4). We therefore used a two-way, repeated measure, mixed design analysis of covariance (ANCOVA), where genotype and level of task difficulty were the between and within subjects independent variables, respectively, neuropsychological task performance (SOPT raw error score) was the dependent variable, and age was the covariate. As the TOL also involves multiple levels of task difficulty (12), we repeated the analysis for this test using the same statistical approach as that applied to the SOPT. A one-way ANCOVA, where genotype was the independent variable and age was the covariate, was performed on all other non-standardized measures of neuropsychological task performance (WCST number of categories completed, WCST number of trials to first category, TOL number of problems solved).

4.2 Part B

A two-way, within subject (MPH dose and time) ANCOVA, in which MPH dose (0.3, 0.5, 1.0 mg/kg) and time (pre-treatment, post-treatment) were the independent variables, neuropsychological (SOPT total error score) and acute motor (RASS total item score) task performance were the dependent variables and age was the covariate (since no standardization information exists for these measures), was used to assess response to MPH and dose-response relationships within cognitive and acute motor behavioural functional domains. A two-way, within subject (MPH dose and time) ANOVA, where Finger Windows task standardized score was the dependent variable, and a one-way, within subject (MPH dose) ANCOVA, where CGI-Improvement score was the dependent variable, were also used in the analysis of treatment and dose effects.

Main effects of genotype and treatment and interaction effects were analyzed using a three-way, one between (*COMT* genotype), two within subject (MPH dose and time) ANCOVA, in which genotype, MPH dose and time were the independent variables, SOPT total error score and RASS total item score were the dependent variables and age served as a covariate. An ANOVA of identical design was used in the analysis of the Finger Windows task data (Finger Windows task standardized score). A two-way, one between (*COMT* genotype), one within subject (MPH dose) ANCOVA was used in the analysis of CGI-Improvement scores, where age also served as a covariate. Simple main effects were analyzed by way of planned comparisons. For therapeutic response of motor behaviours and cognitive functions, insufficient sample size ($n = 15$) prevented the analysis of these genetic data using the QTDT. Results were analyzed and reported as mean score \pm standard deviation.

Results

1 Part A

Table 1 shows clinical and demographic information for the children stratified according to genotype [n = 23 for LL (19.5%), n = 66 for HL (56.0%) and n = 29 for HH (24.5%)]. The three groups were similar with regard to age, average household income, severity of behavioural problems as assessed by the CBCL, and mean number of inattention items, mean number of hyperactivity items and distribution of ADHD subtypes according to the DISC-IV. No significant differences existed between the groups in IQ as measured by the WISC-III. Our sample was characterized by a high prevalence of comorbid disorders, particularly oppositional defiant disorder and conduct disorder. The frequency of these disorders was equally distributed between the genotype groups. The proportion of subjects who had never received medication for ADHD within each genotype group was also similar. Although a significant effect of gender was observed between genotype groups ($\chi^2 = 7.39$; $df = 2$, $p = 0.02$), this result was treated as a type I error (false positive) due to the absence of female subjects with the HH genotype and given the relative lack of female representation across all genotype groups. However, given the previously observed association between gender and several polymorphisms at the *COMT* loci¹⁰⁶, increasing the sample size to achieve a more comparable gender representation and distribution would be a valuable revision to the present study.

The genotype distribution conformed to a Hardy-Weinberg equilibrium ($\chi^2 = 0.42$; $df = 2$, $p > 0.05$). 156 parents participated in the study and gave blood samples. Among

Table 1 – Demographic and clinical characteristics of children with ADHD separated according to COMT genotype (Part A)

	LL (23)	HL (66)	HH (29)	p-value
Gender (M/F)	20/3	52/14	29/0	$\chi^2 = 7.39$, df = 2 p = 0.02
Age	9.2 (2.0)	9.0 (1.8)	9.3 (1.7)	$F_{2,115} = 0.21$, p = 0.81
IQ	97.2 (13.7)	97.5 (13.5)	95.6 (13.8)	$F_{2,98} = 0.17$, p = 0.84
CBCL (total score)	68.0 (9.8)	70.9 (10.4)	68.9 (8.9)	$F_{2,112} = 0.87$, p = 0.42
Income (% less than 20K)	32 %	42 %	48 %	$\chi^2 = 1.39$, df = 2 p = 0.50
DISC-IV Inattention Items	7.3 (1.5)	6.9 (2.2)	7.2 (2.3)	$F_{2,113} = 0.46$, p = 0.63
DISC-IV Hyperactivity Items	5.9 (2.4)	6.4 (2.3)	6.4 (2.7)	$F_{2,113} = 0.33$, p = 0.72
DISC-IV ADHD Subtype (I/H/C)	10/3/10	14/13/39	7/3/19	$\chi^2 = 5.68$, df = 2 p = 0.22
Comorbid ODD	13/23	50/66	20/27	$\chi^2 = 3.21$, df = 2 p = 0.20
Comorbid CD	5/23	27/64	8/27	$\chi^2 = 3.57$, df = 2 p = 0.17
Never Medicated	11/22	38/62	18/28	$\chi^2 = 1.17$, df = 2 p = 0.56

CBCL = Child Behavioral Checklist. DISC-IV = Diagnostic Interview Schedule for Children fourth edition. ODD = Opposition Defiant Disorder, CD = Conduct Disorder. ADHD Subtypes: I = Inattentive, H = Hyperactive, C = Combined. Values are mean (SD).

these parents, 76 were heterozygous (M = 43 and F = 33) and transmitted the *Val* allele to their affected children in 28 occurrences, whereas this same allele was not transmitted in 29 occurrences [$\chi^2 = 0.02$; df = 1, $p > 0.05$ (transmission disequilibrium)]. Conversely, parents transmitted the *Met* allele to their affected children in 29 occurrences, whereas this same allele was not transmitted in 28 occurrences [$\chi^2 = 0.02$; df = 1, $p > 0.05$ (transmission disequilibrium)]. In addition, results from the QTDT revealed no evidence of linkage or within-family association between the three quantitative phenotypes and the *COMT* gene.

A one-way ANOVA performed on these data revealed no significant difference between the LL, HL, and HH genotypes according to WCST standardized perseverative error scores [$F_{2,97} = 0.66$, $p > 0.05$](Table 2) and TOL standardized total item scores [$F_{2,99} = 0.97$, $p > 0.05$](Table 2). A repeated-measure, mixed design ANCOVA performed on these data revealed no effect of genotype on SOPT raw error scores [$F_{2,108} = 0.62$, $p > 0.05$] (Table 2), TOL raw item scores [$F_{2,107} = 0.35$, $p > 0.05$], and TOL time to complete each trial [$F_{2,108} = 0.04$, $p > 0.05$]. No genotype by task interaction was observed for SOPT raw error scores [$F_{6,327} = 0.39$, $p > 0.05$], TOL raw item scores [$F_{11,1199} = 1.63$, $p > 0.05$], and TOL time to complete each trial [$F_{11,1210} = 1.65$, $p > 0.05$]. A one-way ANCOVA performed on these data revealed no effect of genotype on WCST number of categories completed [$F_{2,96} = 1.94$, $p > 0.05$], WCST number of trials to first category [$F_{2,96} = 1.04$, $p > 0.05$] and TOL number of problems solved [$F_{2,112} = 1.04$, $p > 0.05$]. No genotype effects were observed when the HL and HH genotype groups were combined into one category and contrasted with the LL genotype (recessive model) on WCST

Table 2 – Neuropsychological task performance in children with ADHD (Part A)

	LL (23)	HL (66)	HH (29)	ES	p-value
WCST	96.3 (15.1)	99.1 (11.8)	100.6 (12.2)	0.31	$F_{2,97} = 0.67, p = 0.52$
TOL	103.3 (16.5)	99.5 (15.1)	103.8 (12.6)	0.03	$F_{2,99} = 0.97, p = 0.38$
SOPT	13.5 (6.9)	15.1 (8.8)	15.8 (8.2)	0.31	$F_{2,108} = 0.62, p = 0.54$

WCST = Wisconsin Card Sorting Test standardized perseverative error score (LL: n = 21, HL: n = 56, HH: n = 23). TOL = Tower of London standardized score (LL: n = 20, HL: n = 55, HH: n = 27). SOPT = Self Ordered Pointing Task error score (LL: n = 23, HL: n = 63, HH: n = 26). ES = Effect size for LL vs. HH. Values are mean (SD).

standardized perseverative error scores [$F_{1,98} = 1.11, p > 0.05$], WCST number of categories completed [$F_{1,97} = 0.01, p > 0.05$], WCST number of trials to first category [$F_{1,97} = 0.36, p > 0.05$], TOL standardized total item scores [$F_{1,100} = 0.42, p > 0.05$], TOL raw item scores [$F_{1,108} = 0.22, p > 0.05$], TOL time to complete each trial [$F_{1,109} = 0.07, p > 0.05$], TOL number of problems solved [$F_{1,113} = 1.33, p > 0.05$] and SOPT raw error scores [$F_{1,109} = 0.85, p > 0.05$].

2 Part B

Table 3 shows clinical and demographic information for the children participating in Part B of the study. Table 4 shows clinical and demographic information for the children for which genetic information was available stratified according to genotype [$n = 6$ for LL (40%), $n = 5$ for HL (33.3%), $n = 4$ for HH (26.7%)]. The three groups were similar with regard to age, gender, average household income, severity of behavioural problems as assessed by the CBCL, and mean number of inattention items, mean number of hyperactivity items and distribution of ADHD subtypes according to the DISC-IV. No significant differences existed between groups in IQ as measured by the WISC-III. Our sample was characterized by a high prevalence of comorbid disorders, particularly oppositional defiant disorder and conduct disorder. The frequency of these disorders was not significantly different in the three genotype groups. The proportion of subjects who had never received medication for ADHD within each genotype group was also similar.

The genotype distribution conformed to a Hardy-Weinberg equilibrium ($\chi^2 = 5.40; df = 2, p > 0.05$). 18 parents participated in the study and gave blood samples. Among

Table 3 – Demographic and clinical characteristics of children with ADHD

(Part B)

Gender (M/F)	25/5
Age	8.6 (1.6)
IQ	96.2 (13.3)
CBCL (total score)	70.7 (6.8)
Income (% less than 20K)	50 %
DISC-IV Inattention Items	7.2 (2.0)
DISC-IV Hyperactivity Items	6.1 (2.4)
DISC-IV ADHD Subtype (I/H/C)	6/5/12
Comorbid ODD	15/25
Comorbid CD	6/25
Never Medicated	8/24

CBCL = Child Behavioral Checklist. DISC-IV = Diagnostic Interview Schedule for Children fourth edition. ODD = Opposition Defiant Disorder, CD = Conduct Disorder. ADHD Subtypes: I = Inattentive, H = Hyperactive, C = Combined. Values are mean (SD).

Table 4 – Demographic and clinical characteristics of children with ADHD separated according to COMT genotype (Part B)

	LL (6)	HL (5)	HH (4)	p-value
Gender (M/F)	6/0	5/0	3/1	$\chi^2 = 2.95$, df = 2 p = 0.23
Age	8.2 (1.9)	9.0 (1.0)	8.8 (1.7)	$F_{2,12} = 0.38$, p = 0.70
IQ	92.3 (12.7)	100.3 (13.3)	100.0 (n=1)	$F_{2,4} = 0.32$, p = 0.74
CBCL (total score)	64.4 (11.0)	74.8 (3.3)	71.0 (4.5)	$F_{2,10} = 2.16$, p = 0.17
Income (% less than 20K)	25 %	40 %	50 %	$\chi^2 = 0.54$, df = 2 p = 0.76
DISC-IV Inattention Items	7.7 (1.0)	8.4 (1.0)	9.0 (0.0)	$F_{2,11} = 2.47$, p = 0.13
DISC-IV Hyperactivity Items	6.0 (2.6)	7.4 (1.5)	7.0 (1.0)	$F_{2,11} = 0.69$, p = 0.52
DISC-IV ADHD Subtype (I/H/C)	3/1/2	1/0/4	0/0/3	$\chi^2 = 4.96$, df = 4 p = 0.30
Comorbid ODD	4/6	2/5	2/3	$\chi^2 = 0.93$, df = 2 p = 0.63
Comorbid CD	0/6	3/5	1/3	$\chi^2 = 4.85$, df = 2 p = 0.09
Never Medicated	2/3	3/5	4/4	$\chi^2 = 2.04$, df = 2 p = 0.36

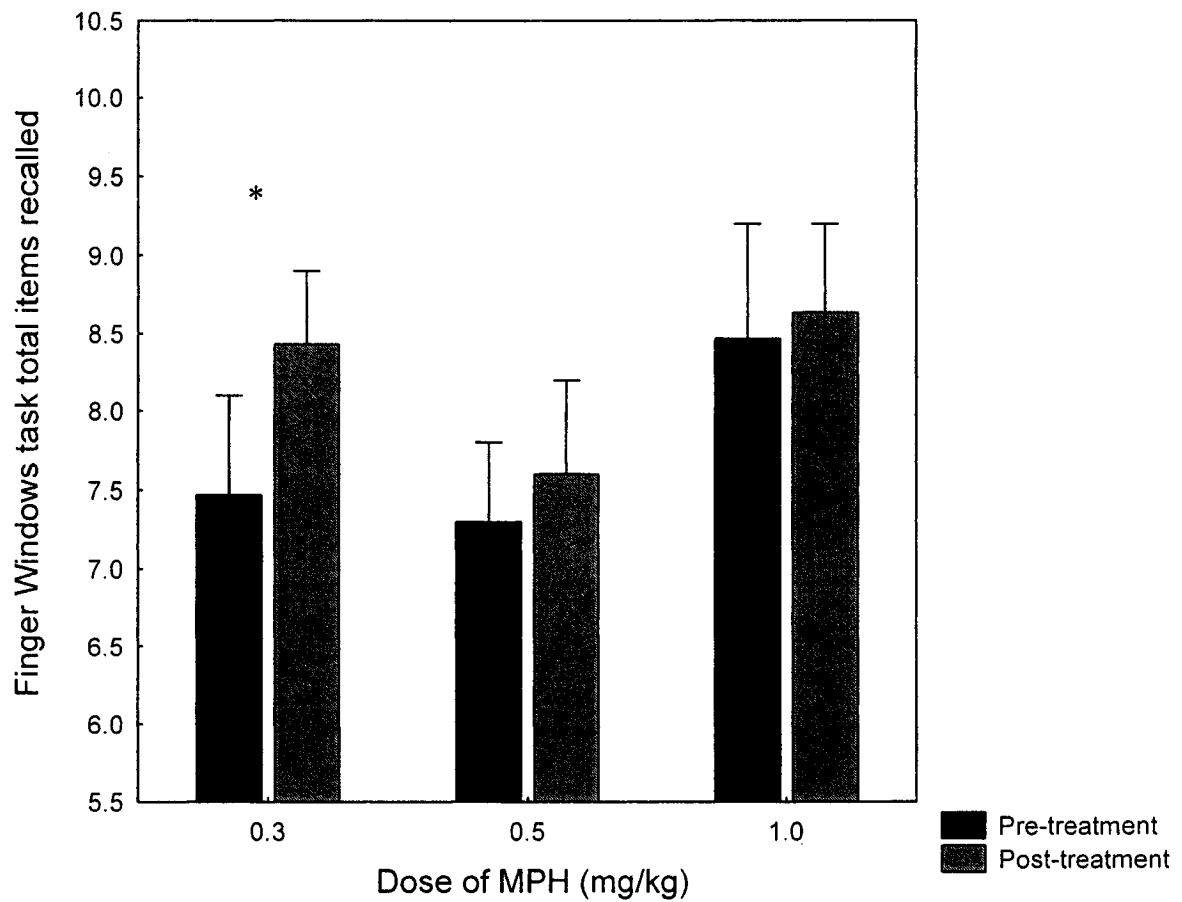
CBCL = Child Behavioral Checklist. DISC-IV = Diagnostic Interview Schedule for Children fourth edition. ODD = Opposition Defiant Disorder, CD = Conduct Disorder. ADHD Subtypes: I = Inattentive, H = Hyperactive, C = Combined. Values are mean (SD).

these parents, 11 were heterozygous (M = 5 and F = 6) and transmitted the *Val* allele to their affected children in 4 occurrences, whereas this same allele was not transmitted in 5 occurrences [$\chi^2 = 0.11$; df = 1, $p > 0.05$ (transmission disequilibrium)]. Conversely, parents transmitted the *Met* allele to their affected children in 5 occurrences, whereas this same allele was not transmitted in 4 occurrences [$\chi^2 = 0.11$; df = 1, $p > 0.05$ (transmission disequilibrium)].

2.1 Neurocognitive assessment

Results from a two-way, within subject (MPH dose and time) ANOVA performed on these data indicate a significant effect of time according to Finger Windows task standardized scores [$F_{1,29} = 4.92$, $p < 0.05$] and are presented in Figure 3. Although our initial analysis revealed no dose by time interaction according to this measure [$F_{2,58} = 0.74$, $p > 0.05$], a marginally significant dose by time interaction was observed when our analysis was limited to subjects with IQ greater than 80 according to the WISC-III [$F_{2,20} = 3.17$, $p = 0.06$]. This revision was made to our analysis since the only other study to date investigating Finger Windows task performance in children with ADHD under MPH excluded children with WISC-III IQ less than 80³³. Planned comparisons of Finger Windows task data revealed a marginally significant improvement in Finger Windows task standardized score under the 0.3 mg/kg dose of MPH only, both when our analysis included all subjects [$F_{1,29} = 4.09$, $p = 0.053$] and when our analysis was limited to subjects with IQ greater than 80 [$F_{2,20} = 5.37$, $p < 0.05$].

Figure 3 – Mean Finger Windows task standardized score (\pm SE) in children with ADHD before and after MPH treatment separated according to dose of MPH



MPH = methylphenidate. P-value corresponds to difference between pre-treatment and post-treatment performance. *represents p-value of 0.053. Asterisks represent significant differences between pre-treatment and post-treatment performance.

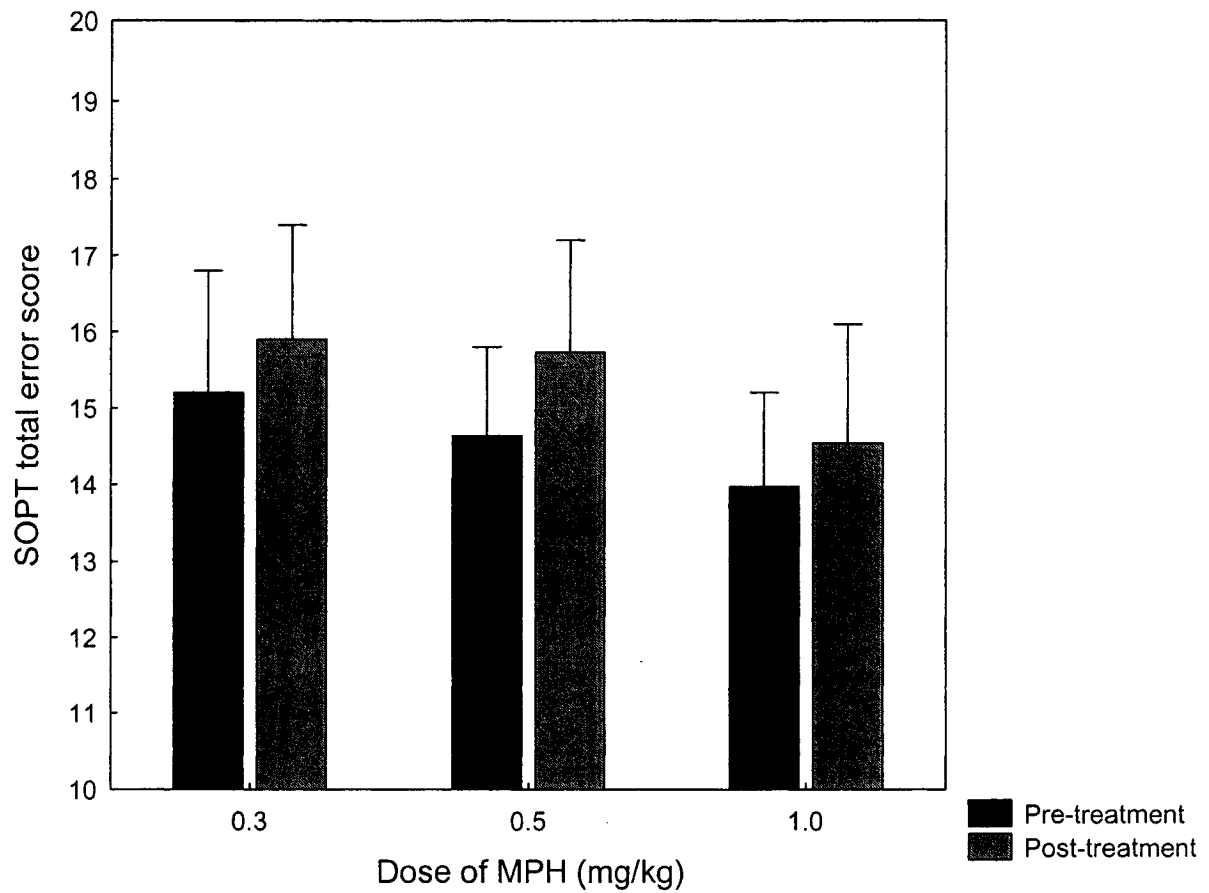
Results from an ANCOVA of identical design performed on the SOPT data indicate no dose by time interaction [$F_{2,56} = 0.12$, $p > 0.05$] and no time effect [$F_{1,28} = 0.72$, $p > 0.05$] according to SOPT total error scores and are presented in Figure 4.

A three-way, one between (*COMT* genotype), two within subject (MPH dose and time) ANOVA performed on these data revealed no genotype by dose by time [$F_{4,24} = 0.98$, $p > 0.05$] or genotype by time [$F_{2,11} = 0.20$, $p > 0.05$] interaction according to Finger Windows task standardized scores. An ANCOVA of identical design performed on these data revealed no genotype by dose by time [$F_{4,22} = 1.52$, $p > 0.05$] or genotype by time interactions according to SOPT total error scores [$F_{2,11} = 2.39$, $p > 0.05$]. Planned comparisons of SOPT data revealed significant improvement in SOPT total error scores under 0.5 [$F_{1,11} = 5.80$, $p < 0.05$] and 1.0 mg/kg [$F_{1,11} = 5.00$, $p < 0.05$] doses of MPH among subjects expressing the LL genotype (Figure 5). When the HL and HH genotype groups were combined and contrasted with the LL genotype, a significant genotype by time interaction was observed on SOPT total error score [$F_{1,12} = 4.88$, $p < 0.05$] (Figure 6). The LL group produced significantly fewer errors during the SOPT than the combined HL/HH genotype group under MPH. No other significant main effects or interactions were observed under the recessive model according to neurocognitive measures.

2.2 Acute motor behavioural assessment

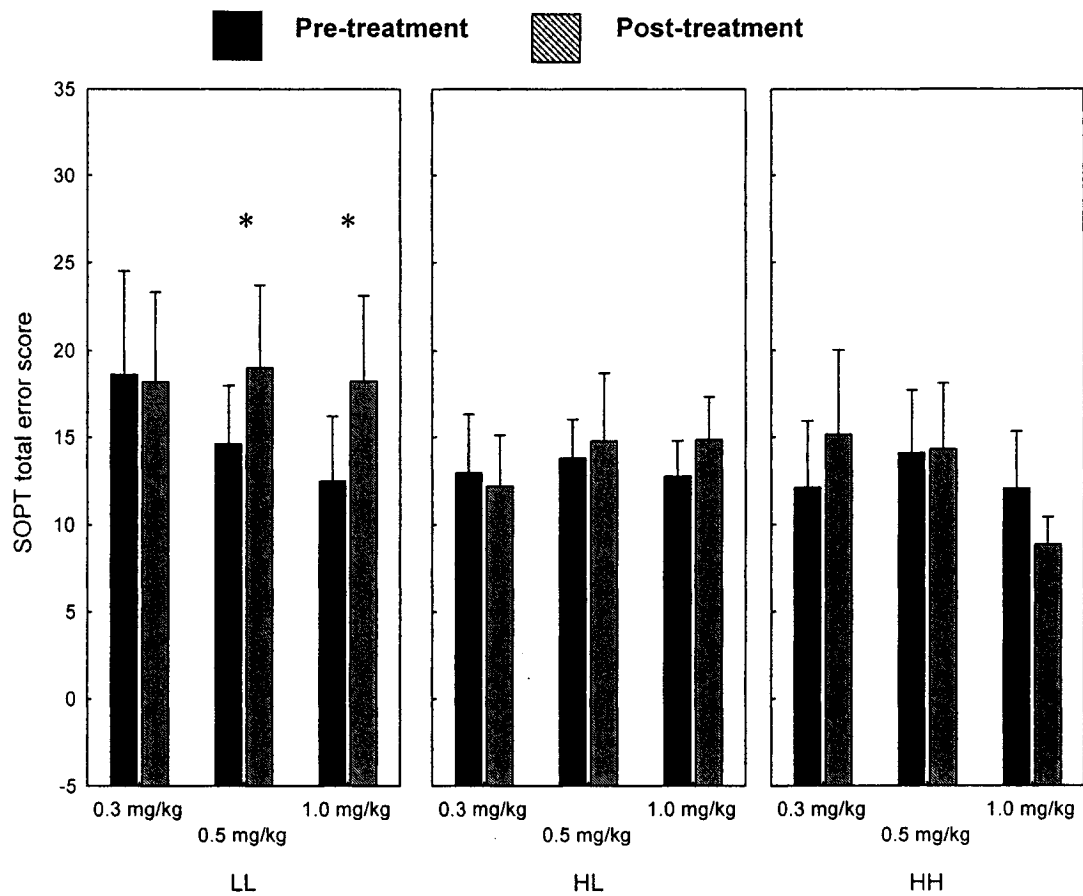
Results from a two-way, within subject ANCOVA performed on these data indicate a significant dose by time interaction [$F_{3,75} = 5.94$, $p < 0.005$] and time effect [$F_{1,25} = 6.16$, $p < 0.05$] according to RASS total item scores and are presented in Figure 7.

Figure 4 – Mean SOPT total error score (\pm SE) in children with ADHD before and after MPH treatment separated according to dose of MPH



SOPT = Self-Ordered Pointing Task; MPH = methylphenidate.

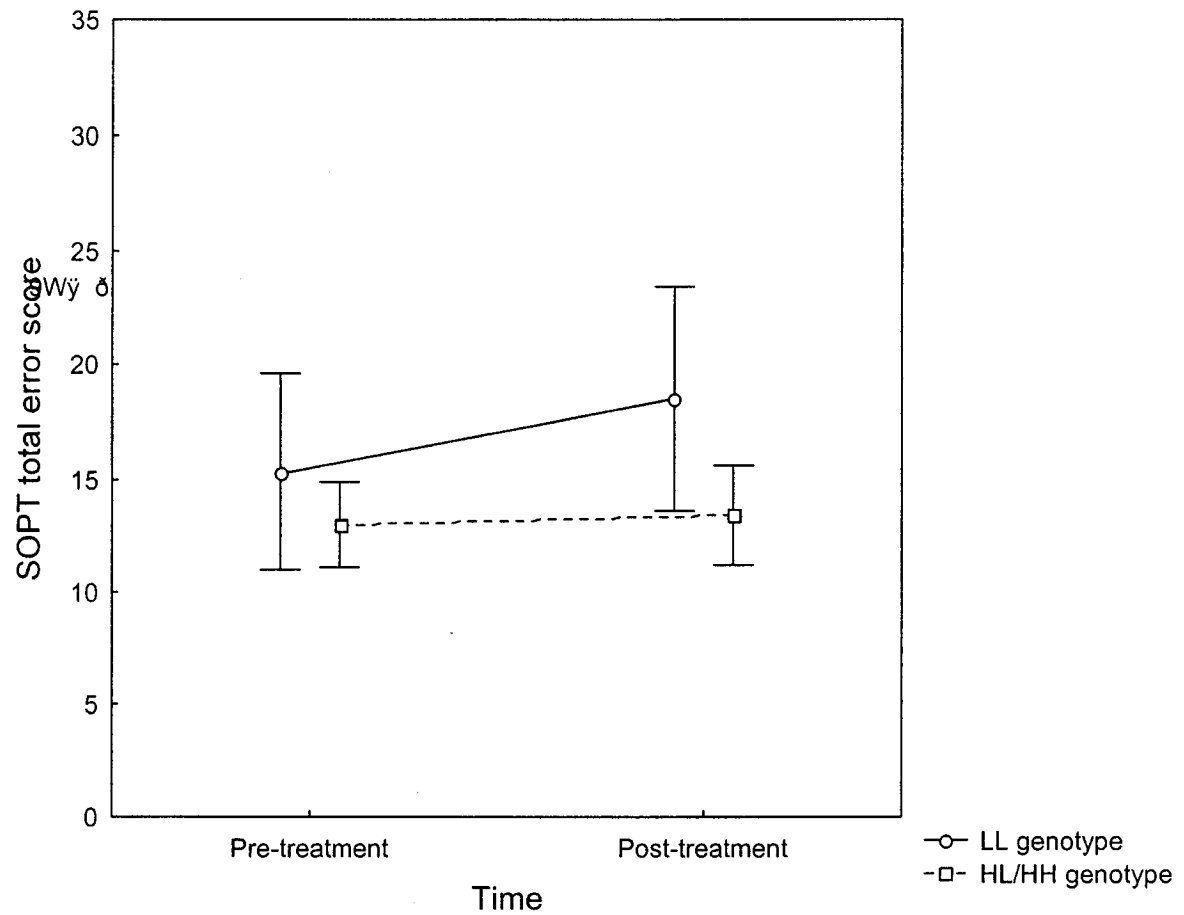
Figure 5 – Mean SOPT total error score (\pm SE) in children with ADHD separated according to COMT genotype and dose of MPH



SOPT = Self-Ordered Pointing Task; MPH = methylphenidate. HL: n = 5; LL: n = 6; HH: n = 4.

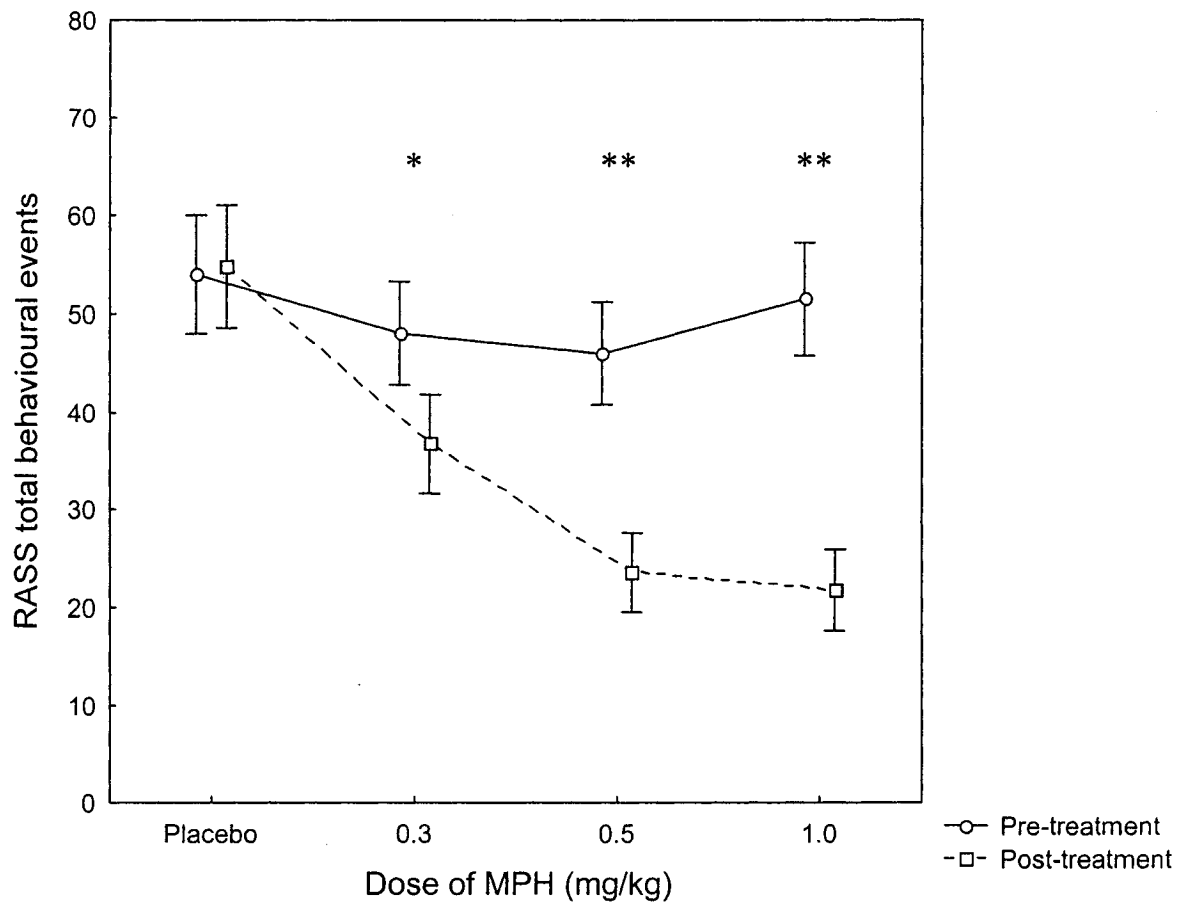
*significant at $p < 0.05$. Asterisks represent significant differences between pre-treatment and post-treatment performance.

Figure 6 – Mean SOPT total error score (\pm SE) in children with ADHD before and after MPH treatment separated according to COMT genotype (recessive model)



SOPT = Self-Ordered Pointing Task; MPH = methylphenidate. LL: n = 6; HL/HH: n = 9. Mean scores represent performance under three MPH dose levels combined.

Figure 7 – Mean RASS total item score (\pm SE) in children with ADHD before and after MPH treatment separated according to dose of MPH



RASS = Restricted Academic Situation Scale; MPH = methylphenidate. *significant at $p < 0.01$.

**significant at $p < 0.001$. Asterisks represent significant differences between pre-treatment and post-treatment performance. Placebo measurements collected independently of MPH titration protocol.

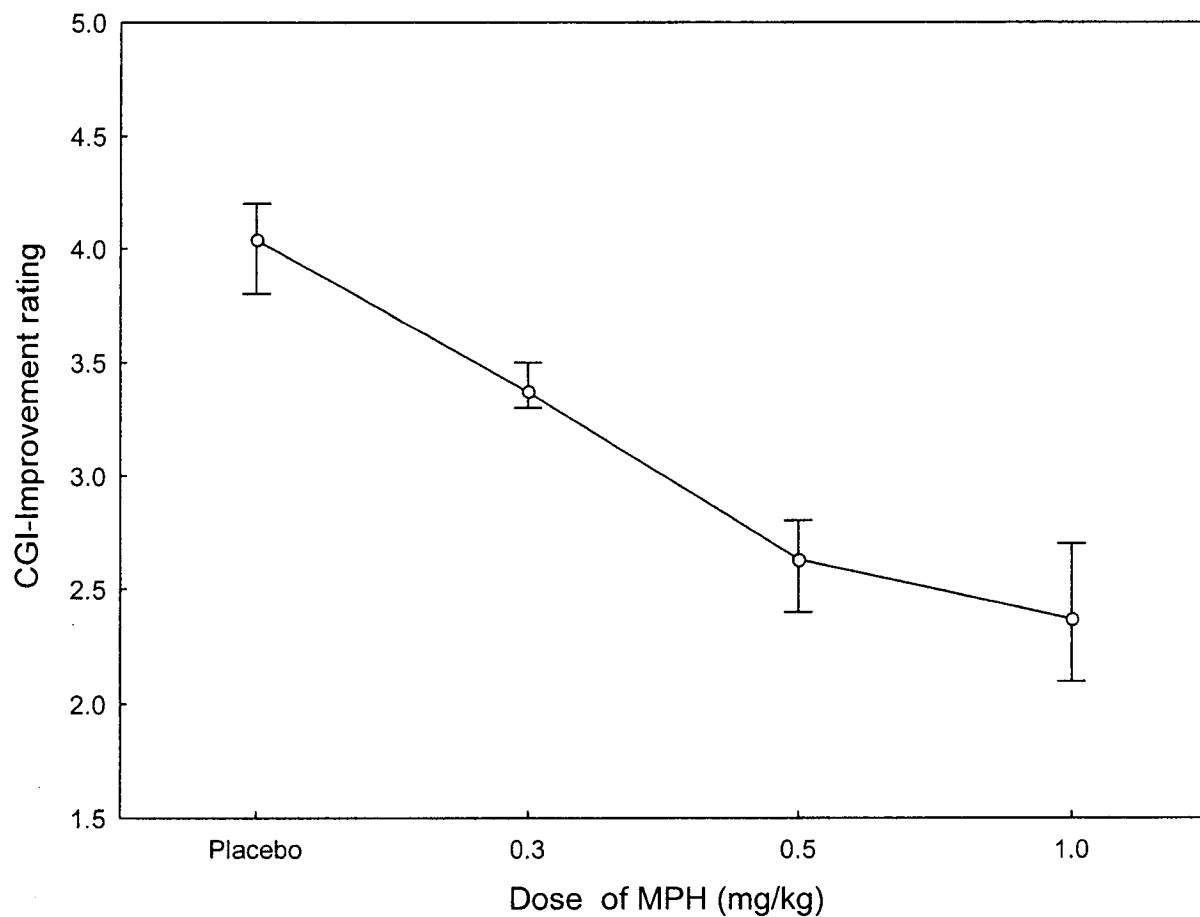
Planned comparisons of RASS data revealed significant improvements in RASS total item scores under 0.3 [$F_{1,25} = 11.58, p < 0.005$], 0.5 [$F_{1,25} = 41.88, p < 0.001$] and 1.0 mg/kg [$F_{1,25} = 30.06, p < 0.001$] doses of MPH, in addition to a significant increase in the degree of improvement on RASS scores between placebo and 0.3 [$F_{1,25} = 4.71, p < 0.05$], 0.5 [$F_{1,25} = 14.59, p < 0.001$] and 1.0 mg/kg [$F_{1,25} = 19.28, p < 0.001$] doses, and between the 0.3 mg/kg dose and 0.5 [$F_{1,25} = 4.89, p < 0.05$] and 1.0 mg/kg [$F_{1,25} = 10.35, p < 0.005$] doses of MPH. To address the possibility that cognitive improvement under the 0.3 mg/kg dose of MPH has some biological relationship to acute motor behavioural improvement under the same dose, we conducted a product-moment correlation analysis on these data and observed no correlation of RASS total item scores and Finger Windows task standardized scores at the 0.3 mg/kg dose of MPH [$r = 0.13, p > 0.05$].

Results from a one-way, within subject (MPH dose) ANCOVA performed on these data indicate a significant dose effect [$F_{3,75} = 5.43, p < 0.005$] according to CGI-Improvement scores and are presented in Figure 8. Planned comparisons of CGI data revealed significant improvements in CGI-Improvement scores between placebo and 0.3 [$F_{1,25} = 8.11, p < 0.01$], 0.5 [$F_{1,25} = 25.26, p < 0.001$] and 1.0 mg/kg [$F_{1,25} = 26.51, p < 0.001$] MPH dose conditions, and between the 0.3 mg/kg and 0.5 [$F_{1,25} = 11.09, p < 0.005$] and 1.0 mg/kg [$F_{1,25} = 15.71, p < 0.001$] MPH dose conditions.

A three-way, one between (*COMT* genotype), two within subject (MPH dose and time) ANCOVA performed on these data revealed no genotype by dose by time [$F_{6,30} = 1.49, p > 0.05$] or genotype by time [$F_{2,10} = 0.55, p > 0.05$] interaction according to RASS total

item scores. A two-way, one between (*COMT* genotype), one within (MPH dose) ANCOVA performed on these data revealed no genotype by time interaction according to CGI-Improvement scores [$F_{6,30} = 0.86$, $p > 0.05$]. In addition, no significant main effects or interactions were observed under the recessive model according to acute behavioural measures.

Figure 8 – Mean CGI-Improvement score (\pm SE) in children with ADHD separated according to dose of MPH



CGI = Clinical Global Impression Scale; MPH = methylphenidate. Placebo measurements collected independently of MPH titration protocol.

Discussion

1 Part A

Previous studies have identified an association between the *COMT* polymorphism and a variety of indices reflecting executive control both in healthy^{26,27} and schizophrenic adults²⁸⁻³⁰. The *COMT* appears to be important to the regulation of dopamine metabolism within the PFC^{6,20,21}. Since the PFC and dopamine pathways have been hypothesized to play an important role in the pathogenesis of ADHD^{43-45,107,108}, we conducted this study in an attempt to test whether the *COMT Val^{108/158} Met* polymorphism, which is known to be associated with a significant change in the catabolic capacity of this enzyme, modulates the risk for ADHD or various indices of executive control. Contrary to our expectations and findings in both healthy^{26,27} and schizophrenic adults²⁸⁻³⁰, an association between the *Val^{108/158} Met* functional polymorphism of the *COMT* gene and neuropsychological task performance reflecting executive control was not observed in children with ADHD. This result is consistent with the findings of a recent case-control study conducted by Mills et al. (2004), which, to our knowledge, is the only other study to investigate the relationship between the *COMT Val^{108/158} Met* polymorphism and neuropsychological task performance in children with ADHD¹⁰⁹. However, this study did not include the WCST, the measure responsible for producing the most consistent results in the previous literature. In addition, we did not identify a biased transmission of either of the two alleles from parents to affected offspring.

The absence of an association between the *COMT Val^{108/158} Met* polymorphism and behavioral indices of executive function in children with ADHD may be explained by the

young age of the population of patients included in the present study. Indeed it is possible that, due to age-related changes in the functional importance of the *COMT* within the prefrontal cortex, this association is observable only in adults. This possibility is supported by data in both rats¹¹⁰⁻¹¹² and humans^{113,114} suggesting that monoamine content and metabolism decrease with age. This age-related decrease may render functions dependent on monoamine content more prone to be dysfunctional at an older age. In addition, evidence from rat studies has indicated a positive correlation between aging and *COMT* activity¹¹⁵⁻¹¹⁷. This observation may suggest that the implication of the *COMT* in the metabolism of dopamine is developmentally regulated, with children relying less on this metabolic pathway than adults. Conversely, it has been reported that DAT density is inversely correlated with age¹¹⁸. Taken together, the presence of an inverse and direct correlation between age and DAT density on the one hand and *COMT* activity on the other hand, may suggest that dopamine metabolism relies more on the DAT than on *COMT* activity in children compared to adults. This hypothesis is compatible with the fact that several studies have identified an association between the DAT^{43,107,119-121}, but not the *COMT*, gene and ADHD.

It is also possible that the negative result observed in the present study is due to a type II error (false negative) secondary to the lack of power of our sample to detect an association. However, using results from the WCST, the variable for which relevant genetic data already exists, we conducted a power analysis and determined that our sample size has sufficient power (80% at $\alpha = .05$) to detect a clinically significant mean

difference of 11.2 on this measure. Furthermore, it is possible that some of the tests used in our assessment are mediated by the PFC but insensitive to PFC DA levels¹²².

An additional limitation of the present study is that some genotype groups included few subjects. Increasing the sample size to achieve larger genotype groups would be necessary to reach firmer conclusions. This is particularly true for female subjects who were significantly underrepresented in the study (as is common to most clinical studies of ADHD). In order to generalize these negative results to females, a more comparable gender representation is required, particularly in view of some previous research indicating that the allelic distribution of the COMT may be gender dependent¹⁰⁶.

2 Part B

In Part B of our study, we investigated the nature of the cognitive and behavioural response of children with ADHD to MPH. Specifically, we were interested in determining which cognitive and behavioural measures are sensitive to MPH and what are the dose-response relationships associated with these measures. Ample evidence exists to suggest that MPH aids in the performance of tasks of both executive³³ and overall behavioural functioning⁶² in children with ADHD but questions regarding the nature of dose-response relationships corresponding to each of these distinct functional domains, i.e. the doses at which optimal performance can be achieved, remain unresolved. Our study attempted to elucidate this rather unclear picture. Consistent with the findings originally presented by Sprague and Sleator in 1977⁷⁹, we observed a significant improvement in cognitive functioning at a low to moderate dose of MPH (0.3 mg/kg), as assessed by the Finger Windows task. Our findings are also consistent with

the study conducted by Bedard et al. (2004)³³, which was the first to observe an improvement in Finger Windows task standardized scores under MPH. In an attempt to replicate the analysis performed in this previous study, we performed an additional analysis including only subjects with IQ greater than 80 as determined by the WISC-III and observed a marginally significant dose by time interaction according to Finger Windows task standardized scores. A significant improvement under MPH on measures of acute behavioural functioning, as assessed by the RASS and CGI, was also observed. Similar to previous studies⁷⁷, improvement on both measures occurred at all dose levels, with the exception of placebo, and followed a more-or-less linear dose-response pattern, with increasing doses producing increasingly more robust improvements. One exception to the latter observation occurred between the 0.5 and 1.0 mg/kg doses, in which no difference in degree of improvement on RASS total item scores and CGI-Improvement scores was observed. This finding suggests a “levelling-off” of the hypothetical MPH dose-response curve and argues in support of the careful titration of MPH doses before prescription of medication as opposed to simply selecting the highest dose under which improvement was observed. It should be noted that the remarkable similarity on RASS and CGI scores under MPH observed in the present study is most likely a reflection of the fact that the CGI is an index of improvement of hyperactive symptoms during the testing period and, given the large portion of the testing period devoted to the RASS, is based largely on behaviours observed during the RASS. We have therefore chosen to confine the remainder of our discussion of behavioural improvements under MPH to the RASS.

The present study improves upon the study conducted by Bedard et al. (2004)³³ in that our analysis included both pre- and post-treatment measures of cognitive functioning for each testing day in an effort to control for any within subject variability resulting from factors such as level of motivation or fatigue that could potentially influence the child's performance during the testing procedure. Bedard et al. identified an effect of MPH treatment on children with ADHD according to Finger Windows task standardized scores at dose levels of approximately 0.28, 0.43 and 0.59 mg/kg relative to placebo, with the number of correctly recalled items increasing linearly with dose. Their analysis of MPH treatment effect involved the comparison of Finger Windows task scores collected subsequent to the administration of three doses of MPH with scores collected subsequent to the administration of a placebo dose on an independent testing day. This protocol prevents the exclusive attribution of cognitive improvement to the drug treatment since it is impossible to determine whether the child's performance would have improved relative to placebo irrespective of administration of MPH. It is therefore more reasonable to conclude that, given our improvement upon this previous design, the most robust cognitive improvements, as assessed by the Finger Windows task, occur under low to moderate doses of MPH exclusively, as opposed to occurring in a linear dose-response pattern, whereby higher doses of MPH produce the most significant improvements in task performance.

Our observation of optimal cognitive improvements occurring at low to moderate doses of MPH in children with ADHD does run contrary to a previous study of the affects of MPH on working memory which identified a linear improvement in performance with

increasing doses of MPH up to 0.9 mg/kg in these children¹⁷. This study provided a neurocognitive assessment under both placebo and MPH on each testing day in an effort to control for within subject variability over the testing period. This discrepancy in findings may be explained in terms of the differential nature of the specific cognitive processes required to perform each task. The memory task used in the study by Berman et al. (1999) was a visual-memory search task that placed heavy demands not only on working memory but also on attentional shifting and self-regulation. The Finger Windows task used in our study is strictly a measure of visual-spatial working memory. It is possible that the differential cognitive processes tapped by these two tasks require different levels of catecholamines, such as DA, in order for optimal performance to be achieved⁷¹. Therefore, it is reasonable to speculate that task-specific dose-response curves may exist, both between and within cognitive and behavioural functional domains.

The fact that improvement under MPH was observed for the Finger Windows task but not the SOPT may also be explained in terms of the differential nature of the specific cognitive processes required to perform different tasks. Despite the fact that the Finger Windows task and the SOPT are both considered measures of visual-spatial working memory, the SOPT appears to tap into other complex cognitive processes as well. The SOPT requires subjects to continuously select different exemplars from a set on successive occasions. Collins et al. (1998) suggest that performance of this task requires: a) active working memory in order to maintain and update a list of exemplars during each trial; b) inhibitory control in order to avoid reselection of prior exemplars, and; c) planning ability in order to formulate a sequence of responses¹²². These additional

processes may be modulated by other neurotransmission pathways and thus a simple increase in extracellular DA (or norepinephrine), as facilitated by MPH, may not suffice to produce improved performance on this task. Results from our genetic analysis suggest that under certain neurobiological conditions, such as in the case of individuals expressing the LL genotype, higher doses of MPH should be able to facilitate improvements on the SOPT however, in most cases, it appears as if other neurobiological conditions must be satisfied as well. It is also possible that, since no normative data exists for the SOPT, our inability to observe improvements in performance under MPH may be a reflection of the fact that our sample is performing similarly to their age-matched healthy counterparts and thus further improvement with medication is limited.

It has been suggested that cognitive improvements in children with ADHD under MPH occur secondary to the ability of this drug to reduce the putative core behavioural symptoms of the disorder. By reducing inattention, impulsivity and motor hyperactivity, MPH in turn improves goal-oriented behaviour such as that required to successfully perform tasks measuring cognitive functioning. This rationale would explain why, similar to studies investigating acute behavioural dose-response relationships⁷⁷, several studies have observed linear cognitive dose-response relationships⁷² in children with ADHD. The present study argues against the existence of synonymous dose-response relationships for cognitive and acute behavioural improvements under MPH in children with ADHD. Behavioural improvements clearly appear to be following a linear pattern while cognitive improvements do not. If cognitive improvements were simply a result of an improvement in goal-oriented behaviour, we would have expected to see improvement on both the

Finger Windows task and the SOPT. The fact that no improvement in SOPT scores under MPH was observed in the present study, in addition to the fact that no correlation exists between improvement in RASS total item scores and Finger Windows standardized scores under the 0.3 mg/kg dose of MPH, suggest the presence of distinctive pathophysiological loci of control for both acute behavioural and cognitive pathways.

Part B of our study also attempted to build upon results collected in the first part of our study. After failing to observe an association between the *COMT Val^{108/158} Met* polymorphism and neuropsychological task performance in children with ADHD in Part A of our study, we were interested in investigating whether or not this polymorphism, which is known to play a putative role in the modulation of DA neurotransmission within the PFC⁶, modulates neuropsychological response in these same children to MPH, a known DA agonist and the most commonly prescribed medication in the treatment of ADHD¹³. Contrary to findings in healthy adults under amphetamine²⁷, an association between the *COMT Val^{108/158} Met* polymorphism and neuropsychological task performance in children with ADHD under three varying doses of MPH was not observed. Furthermore, no association was observed between the *COMT* polymorphism and measures of overall acute behavioural functioning in these same children under similar drug conditions. To our knowledge, this is the first study to investigate this polymorphism and cognitive and acute behavioural response to MPH in children with ADHD. It should be noted however that the findings presented in this study are strictly preliminary in nature given our relatively small sample size.

The lack of association observed between the *COMT* polymorphism and neuropsychological task performance in children with ADHD under MPH may be understood in terms of the rationale presented in the Discussion section of Part A of our study. Indeed, the young age of our population and the relatively reduced dependence of children on the COMT for DA metabolism may explain our inability to identify an association between the *Val*^{108/158} *Met* polymorphism and neuropsychological task performance, both on and off medication. However, our small genetic sample size, in which one genotype group contains only four subjects, prevents the generation of any definitive conclusions concerning cognitive drug response in children with ADHD.

Despite the lack of observed genetic interactions and overall main effects, a number of interesting and unexpected results were observed over the course of our statistical analyses. Subjects expressing the LL genotype, which results in an increase in PFC DA neurotransmission, showed significant improvement in SOPT total error score under both 0.5 and 1.0 mg/kg doses of MPH. In addition, a significant genotype by time interaction was observed according to SOPT total error scores when subjects expressing the LL genotype were contrasted with subjects from a combined HL-HH genotype group. The LL genotype group showed significant improvement in SOPT scores under MPH while the combined HL-HH group did not. Taken together, these findings may suggest that successful performance on the SOPT may be facilitated by comparatively higher levels of DA neurotransmission such as those afforded by the combination of the high basal dopaminergic tone characteristic of individuals expressing the LL genotype and higher doses of MPH, i.e. between 0.5 and 1.0 mg/kg. Collins et al. (1998) argued that self-

ordered sequencing tasks might be prefrontally mediated but independent of mesocortical DA systems¹²². Our findings argue that that the mesocortical DA system may not be a necessary contributor to the successful performance of self-ordered sequencing tasks but, under certain physiological conditions, may play an important role in modulating performance on neurocognitive tasks such as the SOPT.

Conclusions

This study does not support the involvement of the *Val*^{108/158} *Met* polymorphism of the *COMT* gene in increasing the risk for ADHD or in modulating several indices of executive functions in children with ADHD, both on and off MPH. This result is contrary to previous findings in both healthy and schizophrenic adults and may be related to developmental specificities. Increasing the sample size of children genotyped for the *COMT Val*^{108/158} *Met* polymorphism will be an important revision to the present study in order to address whether or not this gene is implicated in the cognitive response to MPH. In addition, this study suggests that MPH produces improvements in both acute behavioural and cognitive functioning. However, these improvements appear to occur under different pharmacological conditions and thus careful titration of MPH dose is required in order to maximize therapeutic benefits within each of these functional domains.

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Appendix

Douglas Hospital Research Ethics Board approval certificate (see attached).

October 29, 2003

Dr. Natalie Grizenko
Douglas Hospital Research Centre
Bond Pavilion

Subject : **Protocol 99/22 : Clinical and Pharmacogenetic Study of Attention Deficit with Hyperactivity Disorder (ADHD)**
Annual Renewal & Amendment

Dear Dr. Grizenko;


We acknowledge receipt of the revised consent forms you submitted for the above protocol as requested by the REB.

As Chairperson, I have examined these consent forms and find them satisfactory. I therefore give final approval to your request for annual renewal and amendment to this study.

This research project is therefore approved for another year.

Sincerely yours,



 for:
Serge Gauthier, M.D., F.R.C.P(c)
Chairperson
Douglas Hospital
Research Ethics Board
/lb

October 17, 2003

Dr. Natalie Grizenko
Douglas Hospital Research Centre
Bond Pavilion

Subject : **Protocol 99/22 : Clinical and Pharmaco-genetic Study of Attention Deficit with Hyperactivity Disorder (ADHD)**
Annual Renewal & Amendment

Dear Dr. Grizenko;

At its meeting of October 14, 2003 the Douglas Hospital Research Ethics Board examined the annual report and request for amendment submitted for the above protocol and found them satisfactory. However, it is mentioned that the consent forms have been revised but copies were not annexed. Therefore, copies of the new consent forms will need to be submitted to the REB before final approval can be granted.

Sincerely yours,



for:
Serge Gauthier, M.D., F.R.C.P(c)
Chairperson
Douglas Hospital
Research Ethics Board
/lb

**Study Protocol # 99/22: Clinical and pharmaco-genetic study of
Attention Deficit Hyperactivity Disorder (ADHD)**

Ridha Joobar, Natalie Grizenko, et al.

In light of the examination of preliminary data from our ongoing research as well as some practical issues, we hereby submit an amendment to the above research protocol titled "Clinical and pharmaco-genetic study of Attention Deficit Hyperactivity Disorder (ADHD)". This amendment is comprised of three points:

1. Determination of the optimal dose of methylphenidate for each child:

Our current research protocol consists of a double-blind placebo-controlled within subject crossover trial in children between 6 and 12 yrs of age. Each treatment (0.5 mg/kg of MPH and placebo) is administered for a period of 1 week. At the end of the two week trial, we break the treatment code, examine the child's results and present feedback to parents on whether methylphenidate is recommended as treatment for their child.

This protocol has proven to be clinically very useful and parents have generally expressed their satisfaction at having participated. However, we believe that in order to determine the optimal dose needed to treat patients, modification to the study protocol is necessary. This revision would involve the inclusion of three additional therapeutic response evaluation days with the purpose of examining the titration effects of varying doses of methylphenidate (MPH) in ADHD children, as well as contributing to the further development of a comprehensive and valid symptom profile of executive dysfunction in these individuals.

On each of these three evaluation days, we intend to repeat two components of our original behavioural evaluation of treatment response: the Restricted Academic Situation Scale (RASS) and the Clinical Global Impression Score, in order to comparatively assess therapeutic response at doses of 0.3, 0.5, and 1.0 mg/kg of MPH. Preliminary findings in our lab have supported the sensitivity of these two measures to behavioural changes under MPH.

In addition, two neuropsychological tasks saturated in demand for executive processes typically deficient in ADHD children, the Self-Ordered Pointing Task (SOPT) and the WRAML Finger Windows: Forward and Backward task, will be included in order to evaluate cognitive treatment response to MPH, as well as to further investigate pharmacological titration effects. The SOPT has been observed to clearly distinguish between normal and ADHD children (Shue & Douglas, 1992) and has shown modest

sensitivity to the effects of MPH (Douglas *et al.*, 1988). ADHD children also have been observed to demonstrate increased performance under MPH on the WRAML Finger Windows task (Tannock *et al.*, in preparation).

Each medication trial will occur on a different successive day in accordance with a triple-blind, randomized, within-subjects design. These three days of assessments will take place in the week immediately following the original two week crossover trial.

The advantage afforded by the potential observation of a functional dose-response relationship will be to provide optimal and individualized therapeutic regimen for those individuals who ultimately prove responsive to MPH. Findings would also help to discern between those individuals truly non-responsive to MPH and those individuals requiring higher doses in order to exhibit significant behavioural and neurocognitive benefits. The inclusion of both behavioural and neuropsychological measures of responsivity, in addition to aiding in the explication of the phenotypic variance characteristic of this disorder, will help to resolve the issue of the potentially differing dose-response relationships of these two symptomatic dimensions of ADHD.

As this procedure adds three days of testing, we thought that it is preferable to test the usefulness of this additional testing in patients who are attending the day hospital of the Disruptive Behaviour Disorder Program (DBDP) at the Douglas Hospital. Those subjects included in the amended protocol will receive a revised version of the revised consent form (see attached). Each of the three additional evaluation days will be organized identically, apart from the dosage of MPH administered. This will consist of behavioural and neuropsychological pre-medication evaluation (roughly 30 minutes in duration), administration of one of the three dosage levels of MPH or placebo, and a post-medication evaluation 45 minutes after the pill is ingested. If we found that these three days of testing will permit a better titration of the treatment with MPH, we will extend this part of the study to all patients, including those recruited in the out patient clinics (provided that an ethic approval will be granted for this extension).

2. Elimination of part of the study exploring the effects of methylphenidate on sleep

Our preliminary results regarding the effects of methylphenidate on sleep indicate that Methylphenidate has minor effects on the quality of sleep. It was also found that children who respond to methylphenidate and those who do not are not differentially affected with regard to their sleep by methylphenidate. We estimate that we have answered this question and there is no need to continue this part of the study. A manuscript on this part of the study is currently submitted for publication. We therefore propose to eliminate this part of our study from this point on.

3. Revisions of the information and consent forms

Revisions of the information and consent forms have been made to accommodate the changes in our protocol discussed in the points 1 and 2. In addition, although we have

mentioned in our previous information forms that, if the parents agree, information from the study will be provided to the child's treating team, we wanted to make it more explicit in the consent form. In this revised version of the consent form we mention explicitly which results of the clinical and neuropsychological evaluations would be provided to the treating team.

We also take advantage of this revision to correct some minor errors in the previous forms, particularly paying close attention to the conformity between the English and French versions.

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