CHAPTER 6

Acute effects of methylphenidate in children with ADHD on a neurocognitive test battery: a randomized, placebo controlled trial

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Abstract

RATIONALE The use of neurocognitive tasks as biomarker of drug effects increases the efficiency of pharmacological research. The non-invasive nature of such tasks makes them particularly attractive for application in the pediatric population, as risks and burden of scientific research in this group should be minimal.

OBJECTIVES To establish whether acute effects of methylphenidate can be measured with a neurocognitive test battery (Neurocart) in children with attention deficit hyperactivity disorder (ADHD), to establish how performing these tasks is tolerated by these children, to measure acute cardiovascular effects of methylphenidate, and to measure methylphenidate concentrations in saliva.

METHODS We performed a randomized, placebo-controlled, 2-way cross-over study with 20 children aged 8-12 years with a confirmed diagnosis of ADHD. Data were collected on two separate study days after administration of either methylphenidate or placebo.

RESULTS Acute effects of methylphenidate could be demonstrated in five of the tasks from the Neurocart test battery (body sway, saccadic reaction time, smooth pursuit, finger tapping task and adaptive tracking task). The study was well-tolerated by participating children. A significant rise in heart rate (4/min) and blood pressure (4.5 mmHg systolic, 3.1 mmHg diastolic) was observed 60-120 minutes after methylphenidate administration. Saliva analyses showed maximum methylphenidate concentrations of 25.23 (±22.67) ng/mL at 120 minutes post-dose, with an apparent elimination half-life of approximately 120 minutes.

CONCLUSIONS Acute neurocognitive and cardiovascular effects of methylphenidate can be measured with the Neurocart test battery. This study also demonstrates the feasibility of performing data-intensive studies in this age group with little burden or risk to participants.

Introduction

In the research field of clinical pharmacology, biomarkers are frequently utilized to study drug effects. The use of biomarkers is of increasing importance in the field of drug evaluation and drug development, as the efficiency of research can be increased, and data generated by studies employing biomarkers can be used in PK/PD modeling as well as bridging studies. One form of such biomarkers is the result of neurocognitive tasks in subjects before and after administration of drugs with known or suspected central nervous system (CNS) effects. The Neurocart is a test battery consisting of a set of well-known neurocognitive tasks, with extensive validation in virtually all classes of psychotropic drugs, including those acting on the dopaminergic and serotonergic system (1, 2).

Utilizing the Neurocart in the pediatric age group would be particularly attractive, as it provides a non-invasive method of gathering data on the size and the time course of an effect in the setting of a drug trial. Minimal risk and burden are a requirement for drug trials in minors as stated by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (3). With a legal framework now in place forcing industry to evaluate new drugs across all pediatric age groups (4), the need for validated and non-invasive biomarkers in the field of pediatric drug research is substantial.

In order to evaluate the suitability of the Neurocart test-battery in the pediatric age group, we performed a randomized placebo-controlled trial in children with Attention-Deficit Hyperactivity Disorder (ADHD) currently receiving treatment with methylphenidate (MPH). Methylphenidate was considered a good candidate for this study, as measurable effects on several tasks incorporated in the Neurocart were anticipated based on

previous experience with compounds with an effect on the dopaminergic system, and importantly, methylphenidate treatment can be temporarily withdrawn without any apparent risks to the patients involved. In fact, the British National Institute for Health and Clinical Excellence (NICE) guideline suggests to periodically suspend treatment in order to assess the child's condition while off medication (5). ADHD is a behavioral disorder characterized by symptoms of inattention, hyperactivity and impulsivity. It is one of the more common psychiatric disorders in childhood, with an estimated prevalence of 5% in school-age children (6). A leading theory postulates a deficit in behavioral inhibition, resulting in disruption of working memory, motor control, and sustained attention (7). Imaging studies in ADHD have shown hypoactivity in the prefrontal cortex and the anterior cingulate (8, 9). These brain structures are modulated mainly by the catecholaminergic neurotransmitters. MPH is thought to exert its effect mainly through an increase of dopamine levels in the synaptic clefts in the prefrontal cortex and basal ganglia by blocking dopamine reuptake (10, 11). On this theoretical basis, one would expect measurable effects of methylphenidate to be distinguished from placebo in neurocognitive testing. Indeed, a recent review identified improvement of performance on a wide range of neurocognitive tasks varying from 50-83.3% of published studies. The authors noted considerable variability of results between studies, caused among others by methodological limitations and problems associated with repeated neurocognitive testing (12).

The current study provided an opportunity to gather useful data on a number of additional parameters. Previous studies have demonstrated EEG changes after prolonged treatment of ADHD with stimulant medication in children (13, 14). This study provided the opportunity to study acute pharmaco-EEG after administration of methylphenidate.

The stimulant action of methylphenidate is known to elicit a small but statistically significant rise in blood pressure (15, 16). Cardiovascular effects of stimulant medication have come under increased interest since concerns have been raised that these effects may expose treated children to an increased risk of cardiovascular mortality (17). Therefore, we monitored cardiovascular parameters, providing data on acute effects of methylphenidate on heart rate and blood pressure.

In order to establish a relationship between methylphenidate levels and observed effects, pharmacokinetic data are required. Blood sampling in the paediatric age group in the setting of a non-therapeutic study is undesirable. Previously published literature shows that methylphenidate can be measured in saliva of children (18).

With this study, we intended to demonstrate whether tasks incorporated in the Neurocart test battery are suitable for measuring acute methylphenidate effects in children with ADHD. By simultaneously obtaining saliva methylphenidate concentrations, we might be able to build a model establishing a pharmacokinetic-pharmacodynamic (PK-PD) relationship in a future analysis.

Subjects and Methods

This was a randomized, placebo-controlled, 2-way cross-over study. Twenty children aged 8-12 years, with a confirmed DSM-IV diagnosis of ADHD, currently receiving treatment with methylphenidate were eligible for participation. Children treated with other drugs with known psychotropic effects were excluded. The Dutch Central Committee on Research involving Human subjects approved the study protocol. In compliance with the Declaration of Helsinki, written informed consent was obtained from the parents or legal guardians of the participating patients.

STUDY DRUGS

Capsules containing 5 mg of methylphenidate hydrochloride and matching placebos were prepared by the hospital pharmacy of the Leiden

University Medical Center. Participating children continued on their usual dose of methylphenidate; an equivalent regimen was calculated in case of children treated with sustained release formulations. Participants were randomized to either 7 days placebo followed by 7 days methylphenidate or vice versa. Randomization was performed using a 2 by 2 Williams square randomization procedure.

NEUROCOGNITIVE TASKS

Two single-trial computerized versions of the classic *colour-word Stroop tasks* were presented to the test subjects (19). In the first trial, 20 coloured items are presented at random. The subjects were asked to respond as quickly and accurately as possible by pressing the keys 1, 2 or 3 on the numerical pad with the index finger, middle finger and ring finger of the dominant hand, corresponding with the correct answer. In the second trial, which appeared directly after the first trial, 20 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were again asked to respond as fast as possible by pressing the keys 1, 2 or 3 on the numerical pad, corresponding with the correct answer. In both trials, reaction times and the number of correct responses were recorded.

The *left/right distraction task* is a parametric version of the Stroop colour-word response conflict task. The words Left and Right were displayed either at the left or the right side of a computer screen. Response instructions are to respond quickly (by pressing a corresponding button) to the meaning of the word irrespective of its location. The output parameters are the response time and the number of correct responses.

The *adaptive tracking test* is a pursuit-tracking task. A circle of known dimensions moves randomly about a screen. The test subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. In contrast to non-adaptive tracking methods, this leads to a constant and individually adapted challenge throughout the procedure. Performance is scored after a fixed period. The adaptive tracking test was performed as originally described by Borland and Nicholson (20, 21), using customised equipment and software (Hobbs, 2004, Hertfordshire, UK). The average performance and the standard deviation of scores over a 3.5-minute period is used for analysis. This 3.5-minute period includes a run-in time of 0.5 minute, in this run-in time the data is not recorded.

The measurement of *saccadic eye movements* was originally described by Baloh et al (22). In this study we used the nystagmo stimulator from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan) for stimulus display, the program for signal collection and the AD-converter from Cambridge Electronic Design (CED Ltd., Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, USA) and the sampling and analysis scripts were developed at the CHDR (Leiden, the Netherlands). Disposable electrodes were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Head movements were minimised with the aid of a head support placed opposite the target. The target consists of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity of all *correct* saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

The same system as used for saccadic eye movements was also used for measurement of *smooth pursuit*. For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5

degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The time in which the eyes are in smooth pursuit of the target were calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as parameter.

Finger tapping has been adapted from the Halstead Reitan Test Battery (23), and evaluates motor activation and fluency. In this test speed of finger tapping is measured for the index finger of the dominant hand; a session contains five 10 second trials. Feedback on performance is given by a counter in the centre of the screen, while the amount of taps of each 10 second trial is shown on the screen in between the trials. The space bar is used as tapping device. The volunteer is instructed to tap as quickly as possible with the index finger and to rest the wrist on the table. The mean tapping rate and the standard deviations for the dominant hand are used for statistical analysis.

The *body sway* meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway is measured with an apparatus similar to the Wright ataxiameter (24). With a string attached to the waist, all body movements in the sagittal (forward/backward) plane over a period of 2 minutes are integrated and expressed as mm sway on a digital display. Subjects were instructed to keep the eyes closed to eliminate the contribution of vision to postural control. Before starting a measurement, subjects were asked to stand still and comfortably, with their feet approximately 10 cm part and their hands in a relaxed position alongside the body.

EEG

EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artifacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artifacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha- (7.5-11.5 Hz) and beta- (11.5-30 Hz) frequency ranges.

VITAL SIGNS

Blood pressure and heart rate were recorded using automated oscillometry.

QUESTIONNAIRES

At the end of each treatment week, parents and teachers completed a validated ADHD questionnaire. The questionnaire consists of 18 items, rating core symptoms of ADHD on a scale of 0 (behavior does not occur) to 4 (behavior occurs very often) (25).

PHARMACOKINETICS

saliva samples were obtained using the Polyester Salivette swab system (Sarstedt AG, Nümbrecht, Germany). This is a commercially available product designed specifically for collection of saliva specimens. It contains a roll of polyester which is held in the oral cavity for several minutes. After

collection, the swabs were centrifuged and the saliva was stored at -80 degrees Celsius until analysis. Analysis was performed on a validated GC/MS assay with a lower limit of quantitation (LLOQ) of 3 ng/mL. Samples measured below the LLOQ were set at 50% of the LLOQ (ie 1.5 ng/mL) for analysis purposes.

ASSESSMENT SCHEDULE

On day 7 of each of the study weeks, one or two assessments were performed before administration of the morning dose of methylphenidate, after which assessments were performed according to the schedule as represented in figure 1.

STATISTICAL ANALYSIS

a mixed model analysis of variance was performed to establish whether significant treatment effects could be detected on pharmacodynamic endpoints. The analysis was performed with fixed factors: treatment, time, occasion and treatment by time; random factors: subject, subject by treatment and subject by time. The averaged pre-dose measurements were used as covariate in the analysis. The pre-values were also analysed seperately, with the same model, to investigate a possible long-term effect of the MPH of the previous week. Least Square means estimates were calculated, as were contrasts between the two treatment conditions with 95% confidence intervals and p-values of the contrasts. Body sway results were log-transformed prior to analysis to correct for non-normal distribution of the data. A post-hoc analysis was performed on cardiovascular measurements, including only measurements 60-120 minutes post-dose, as the largest effect size was anticipated around maximum MPH concentrations. A second post-hoc analysis was performed to establish the effect of age on the results of different tasks. This analysis was performed with a mixed model regression analysis of age on a task (fixed factor: treatment; random factor: treatment by age, subject; covariate: age). The slope of the regression line of age on the respective variables was calculated, a slope significantly different from 0 would indicate an age effect. Finally, data from the current study were compared with data from 15 healthy children aged 8-12 years obtained in a previous study (Klein et al, submitted). For practical purposes the first three post-dose measurements from this study were compared with three consecutive measurements obtained in healthy children. The data were analyzed with a repeated measures model analysis of variance, with group, time and group by time as fixed factors, and time as repeated factor within subjects with a compound symmetry covariance structure and age as covariate. Contrasts between the three groups (ADHD on methylphenidate, ADHD on placebo, and healthy children) were calculated. Analyses were performed with SAS for windows version 9.1.2 (SAS Institute Inc., Cary, NC, USA). Results of pharmacokinetic analyses were calculated with Microsoft Excel 2007 (Microsoft co., Redmond, WA, USA) and are presented as mean ± SD.

Results

Twenty patients (18 male, 2 female; aged 10.5 ± 1.2 years) were recruited from several outpatient clinics. Of the 20 recruited patients, 10 were on a slow-release form of methylphenidate and 10 were on immediate release methylphenidate prior to study participation. The mean total daily dose prior to study enrolment was 26.6 ± 11.9 mg (range 10-54 mg). All patients completed the study.

Pharmacokinetic analyses showed methylphenidate concentration in saliva with a mean Cmax of 25.23 (±22.67) ng/mL at t=120 minutes post-dose, with an apparent elimination half-life of approximately 120 minutes (figure 2).

The analysis of the pre value showed no significant difference on any of the neurocognitive tasks. Results of the total analysis of neurocognitive

tasks are shown in table 1 and graphical representations of change from baseline are shown in figures 3a-e. Body sway was significantly reduced in the MPH condition. The eye movement tasks also showed a MPH effect: saccadic reaction time was reduced in the MPH condition and smooth pursuit performance increased. No differences were observed on saccadic inaccuracy and saccadic peak velocity. Adaptive tracking performance increased after MPH compared with placebo. Reaction times for colour naming in the Stroop task tended to increase during MPH; no difference was observed on reaction times in the conflict situation. The number of correct responses tended to increase on MPH in both the Stroop basic condition and the conflict condition. No differences were observed in the number of correct responses during the left/right distraction tasks, a trend toward shorter reaction times was observed during MPH. A slight but statistically significant increase in finger tapping performance was observed in the MPH condition. Variability of tapping frequency (standard deviation of mean tapping rate), was not influenced by MPH.

Analysis of EEG data revealed no significant differences between the ADHD and placebo study days (data not shown).

Comparison of the data with the data from a study in healthy children showed statistically significant contrasts in the body sway and adaptive tracking task, with task results of children with ADHD treated with MPH moving toward results of healthy children (table 3 and figures 4a-e).

Cardiovascular measurements showed increased blood pressure and heart rate after MPH. An analysis using only measurements obtained 60-120 minutes post-dose showed larger contrasts (table 2 and figures 5a-c).

The ADHD questionnaires completed by parents and teachers revealed a clear MPH effect as expected. Mean symptom score as rated by parents (n=19) was 25.4 during MPH and 42.2 during placebo ($p<0.0001$). Mean symptom score as rated by teachers (n=14) was 17.9 during MPH and 37.6 during placebo ($p<0.0001$).

The post hoc analysis investigating whether task results were influenced by age showed a significant age effect on the adaptive tracking task (slope of the regression line: 1.90 ±0.89; $p<0.05$, figure 6). An age effect was not observed in the other tasks (data not shown).

Discussion

This study investigated acute effects of methylphenidate in children with ADHD as compared with placebo. The effects covered a range of neurocognitive functions, as well as EEG and cardiovascular parameters. Several neurocognitive tasks showed clear effects of methylphenidate. Effects on blood pressure and heart rate could also be demonstrated. Rather than performing a single measurement in a treatment- or placebo condition, multiple measurements were performed in the hours following drug administration, thereby providing effect profiles over time.

Postural stability increased during MPH treatment. A previous study in children with ADHD only demonstrated improvement of postural stability during a dual task condition (the second task being a cognitive task), not during a single task condition. This observation seemed to support the theory that MPH effect on postural stability might be mediated through effects on dopamine networks in the frontal and prefrontal cortex, which are implicated in working memory and dual tasking (26, 27). In this sense, the MPH-induced postural improvement in our study would be related to beneficial effects on mental concentration. Alternatively, MPH effect might be mediated through direct dopaminergic influence on brain areas involved in motor and balance control. The movement effects of dopaminergic medication are well known, for instance of increasing dopamine levels in the striatum in patients with Parkinson's disease. Effects on postural stability in patients treated with ADHD may be mediated through a similar pathway (26). Findings from our current study seem to support the latter theory, as MPH effects were observed without increased working

memory workload. In this case, improved postural stability could be a manifestation of reduced motor restlessness.

MPH effects on oculomotor control demonstrated in this study (both smooth pursuit eye movements and visually guided saccades) included faster saccade initiation, and the ability to maintain smooth pursuit at higher stimulus velocities. Saccadic inaccuracy and saccadic peak velocity were not affected by MPH. Impairments of oculomotor control, as well as MPH effects on oculomotor tasks in children with ADHD have been demonstrated in several studies (28-32). Oculomotor control is a complex process, controlled by a number of brain areas including the posterior parietal and frontal cortex, basal ganglia, cerebellum, superior colliculus and brain stem reticular formation (31). The frontal eye fields form a component of this network located in the precentral gyrus in the frontal lobe, an area with a high density of dopaminergic receptors (33). The frontal eye fields are involved in both visual fixation and saccade initiation, thus altered performance on smooth pursuit and saccadic reaction time might be anticipated when administering dopaminergic compounds such as MPH.

We also demonstrated an increase in adaptive tracking performance after MPH administration was demonstrated. Adaptive tracking requires several skills including sustained attention, eye-hand motor coordination, and fine motor skills. Several studies using a pursuit task similar to our adaptive tracking task were performed in ADHD children and normal controls, demonstrating impaired performance in ADHD children (34, 35). Abilities such as fine motor coordination, nonverbal working memory and planning have been attributed to the broader concept of executive functioning, which is seemingly mediated by the prefrontal lobes of the frontal cortex (7, 36). Again, increased performance after MPH treatment as demonstrated in our study may be attributed to increased dopaminergic tone in the frontal cortex.

The left/right distraction task and the Stroop Color-Word task are two examples of neurocognitive tests targeting the phenomenon of response interference. When a stimulus with a single dimension is presented (e.g. a coloured rectangle), response time is faster than with a stimulus containing two conflicting or discongruent dimensions. (e.g. the word blue printed in red ink). Lower scores on the two-dimensional discongruent condition compared with the unidimensional stimulus reflect the interference effect, which is presumably caused by response conflict (37). When comparing children with ADHD to normal controls, a meta-analysis showed higher naming speed in the unidimensional condition in controls than in children with ADHD. Differences in the interference effect were inconsistent (37). Studies looking at MPH effects on Stroop performance in children with ADHD generally observed faster colour and word naming (single dimension stimulus), and inconsistent results on Stroop interference (38-41). No significant MPH effects were observed in the parameters studied in our study. Of note, the response times in the Stroop basic colour-naming condition tended to increase rather than decrease, contrary to previous findings. With essentially unchanged reaction times in the conflict condition, the smaller difference between the two conditions under MPH is caused by slower colour-naming rather than response interference. This could be related to reduced impulsivity. However, these observations need to be interpreted with caution, as they contradict previously published studies and differences were not statistically significant.

A small but statistically significant increase in tapping performance was observed during MPH. Motor timing in tapping tasks has been shown to be impaired in ADHD as compared to controls, and MPH has been shown to improve motor timing (42-44). The increased tapping performance observed in our study may be attributed to enhanced timing, or to better motor control. However, this is hard to judge, as we assessed maximum tapping frequency rather than paced or constant tapping.

This study failed to demonstrate any statistically significant EEG changes. EEG studies performed in children with ADHD have demonstrated differences as compared to controls, including increased theta in the frontal regions, and decreased alpha and beta activity in posterior regions.

Some studies have shown EEG normalization after MPH (13, 14). The small sample size number and the limited number of EEG-electrodes used may have reduced the ability to demonstrate EEG changes in this study.

Pharmacokinetic analyses demonstrated a profile consistent with previous work by other groups (18, 45). The variability is large, but this is to be expected with the heterogeneity of our study population (different doses used, and age range implicating different volumes of distribution between subjects). Before concentration-effect modelling can be undertaken, a pharmacokinetic model will be required including prediction of plasma levels. Ethical and practical issues withheld plasma sampling in our study. However, we expect to resolve this issue by studying plasma-saliva relations in a group of healthy volunteer adults, permitting future concentration-effect modelling.

Blood pressure (both diastolic and systolic) and heart rate were all shown to increase following MPH administration, with a maximum effect at 60-120 minutes post-dose. These findings were not unexpected, as methylphenidate is a stimulant drug with known adrenergic properties. Indeed, previous studies have repeatedly demonstrated a cardiovascular response to methylphenidate administration (15, 46). The design of our study with repeated measures following MPH administration demonstrates a clear concentration-effect relationship, with maximum blood pressure response occurring at the anticipated Tmax of MPH.

This study has shown, as expected, measurable effects of methylphenidate in children across a range of neurocognitive tasks. This study also demonstrates the feasibility of performing data-intensive studies in this age group with little burden or risk to participants. Based on these results, one could consider performing follow-up studies comparing for example the effect profiles of immediate-release methylphenidate and controlled-release methylphenidate. Assuming a valid PK model can be constructed with the obtained saliva PK samples, these studies could again include saliva PK monitoring, thereby enabling PK-PD modelling on the obtained dataset.

TABLE 1 Least square mean estimates of neurocognitive tasks during MPH or placebo, contrasts with 95% confidence interval, and change from baseline for placebo and MPH.

	LS Means Estimates		Contrasts	Change from baseline	
Variable	**Placebo**	**MPH**	**Placebo vs MPH (95% CI)**	**Placebo**	**MPH**
Body sway (mm)	751	475	-36.8% (-49.3%, -21.1%) p=0.0004	13.87%	-27.99%
Saccadic Inaccuracy (%)	6.7	6.5	-0.2 (-0.9, 0.5) p=0.5763	-0.3	-0.5
Saccadic Peak Velocity (deg/sec)	531.8	526.1	-5.8 (-18.2, 6.7) p=0.3364	-18.0	-23.8
Saccadic Reaction Time (sec)	0.237	0.223	-.014 (-.026, -.001) p=0.0305	0.011	-0.002
Smooth pursuit (%)	37.8	43.9	6.1 (2.3, 9.9) p=0.0034	-0.9	5.2
Adaptive tracking (%)	7.47	9.71	2.24 (0.85, 3.63) p=0.0032	-0.89	1.35
SD adaptive tracking (%)	2.57	2.65	0.08 (-0.21, 0.36) p=0.5560	0.09	0.17
L/R distraction correct	27.1	27.2	0.1 (-1.4, 1.6) p=0.9149	0.1	0.2
L/R distraction incorrect	3.3	3.5	0.2 (-1.1, 1.4) p=0.7870	-0.2	-0.0
L/R distraction rt correct (msec)	943.6	898.5	-45.0 (-119, 29.2) p=0.2176	7.5	-37.5
L/R distraction rt incorrect (msec)	758.9	730.9	-28.1 (-125, 68.8) p=0.5436	-20.7	-48.7
Stroop Basic correct	17.4	18.0	0.6 (-0.4, 1.6) p=0.2046	-0.8	-0.1
Stroop Basic correct rt (msec)	674.1	724.5	50.4 (-8.1, 108.9) p=0.0868	-123.4	-73.0
Stroop Conflict correct	16.9	17.6	0.7 (-0.4, 1.9) p=0.2048	-1.2	-0.5
Stroop Conflict correct rt (msec)	909.9	904.1	-5.8 (-95.7, 84.0) p=0.8927	-88.4	-94.3
Tap dominant hand	53.02	54.75	1.73 (0.55, 2.91) p=0.0081	-0.04	1.70
SD tap dominant hand	4.35	4.31	-0.04 (-0.99, 0.92) p=0.9339	0.46	0.42

TABLE 2 Results (least square means estimates) of cardiovascular measurements MPH or placebo, contrasts with 95% confidence interval, and change from baseline for placebo and MPH.

	LS Means Estimates		Contrasts	Change from baseline	
Variable	Placebo	MPH	Placebo vs MPH	Placebo	MPH
Heart rate-overall (bpm)	79.4	80.7	1.3 (-1.6, 4.3) p=0.3505	3.5	4.8
Heart rate-Tmax (bpm)	76.2	80.3	4.0 (0.5, 7.5) p=0.0255	0.3	4.4
Systolic blood pressure-overall (mmHg)	108.8	110.7	1.9 (-0.8, 4.7) p=0.1556	3.1	5.1
Systolic blood pressure-Tmax (mmHg)	106.5	111.1	4.5 (1.3, 7.8) p=0.0077	0.8	5.4
Diastolic blood pressure-overall (mmHg)	62.6	63.8	1.2 (-0.4, 2.8) p=0.1195	2.1	3.3
Diastolic blood pressure-Tmax (mmHg)	63.0	66.1	3.1 (1.0, 5.2) p=0.0043	2.5	5.6

TABLE 3 Calculated contrasts between children with ADHD on 2 treatment conditions and healthy children. Estimates of difference with 95% confidence intervals and the respective p-values are presented. (NS: not significant).

	Contrast	Estimate of difference (95% CI)	p-value
Body Sway	ADHD placebo – healthy	100.9 (49.8 to 169.5)%	< 0.0001
	ADHD MPH – healthy	36.4 (1.6 to 83.0)%	0.0394
	ADHD MPH – ADHD placebo	-32.1 (-41.1 to -21.8)%	< 0.0001
Saccadic reaction time	ADHD placebo – healthy	0.01 (-0.01 to 0.03) sec	NS
	ADHD MPH – healthy	-0.004 (-0.024 to 0.015) sec	NS
	ADHD MPH – ADHD placebo	-0.014 (-0.023 to -0.005) sec	0.0022
Smooth Pursuit	ADHD placebo – healthy	4.1 (-4.8 to 13.0)%	NS
	ADHD MPH – healthy	8.9 (0.1 to 17.8)%	0.0488
	ADHD MPH – ADHD placebo	4.8 (2.5 to 7.1)%	<0.0001
Adaptive Tracking	ADHD placebo – healthy	-8.07 (-10.85 to -5.29)%	<0.0001
	ADHD MPH – healthy	-5.98 (-8.76 to -3.20)%	<0.0001
	ADHD MPH – ADHD placebo	2.09 (0.90 to 3.28)%	0.0007
Tap dominant hand	ADHD placebo – healthy	-1.5 (-5.34 to 2.35)/10sec	NS
	ADHD MPH – healthy	0.02 (-3.82 to 3.86)/10 sec	NS
	ADHD MPH – ADHD placebo	1.52 (0.71 to 2.32)/10 sec	0.0003

FIGURE 1 Study assessment schedule. (time in minutes; the timepoint 0' indicates methylphenidate administration).

	-60'	-30'	0'	30'	60'	90'	120'	150'	180'	210'	240'	300'	360'
Saliva sample		⁝⁝		⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝
CNS block*	⁝⁝ **	⁝⁝			⁝⁝		⁝⁝		⁝⁝		⁝⁝		⁝⁝
cardiovascular		⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝
EEG	⁝⁝	⁝⁝			⁝⁝		⁝⁝		⁝⁝		⁝⁝		⁝⁝

* The CNS block consists of consecutive administration of the neurocognitive tasks described in the methods section
** This block is intended as a training session. Some tasks (Saccadic Eye Movements and Stroop) were performed twice during this block to eliminate learning effects.

FIGURE 2 Methylphenidate levels in saliva (ng/ml) over time.

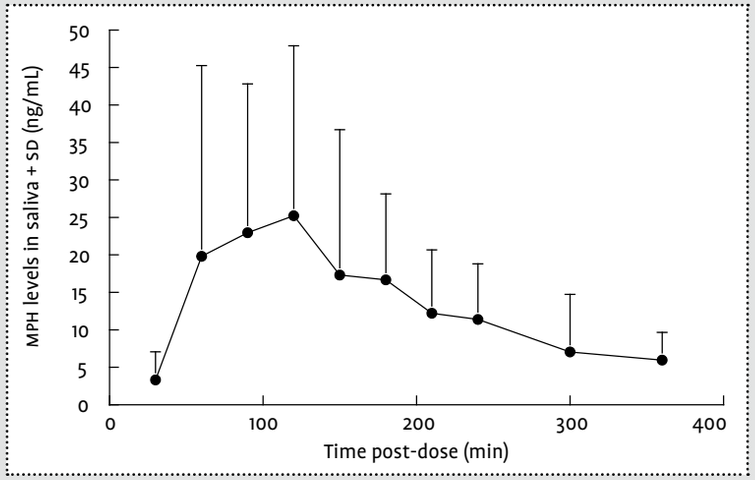
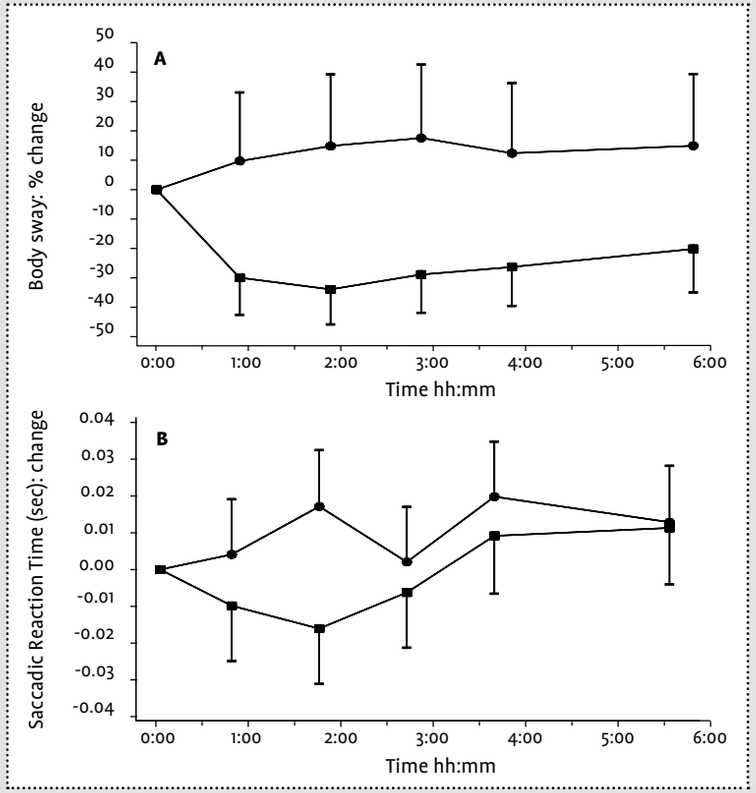


FIGURE 3A-E Change from baseline profile of neurocognitive tasks. Shown data are least square mean estimates, corrected for pre-dose measurements. Square: methylphenidate. Circle: placebo.



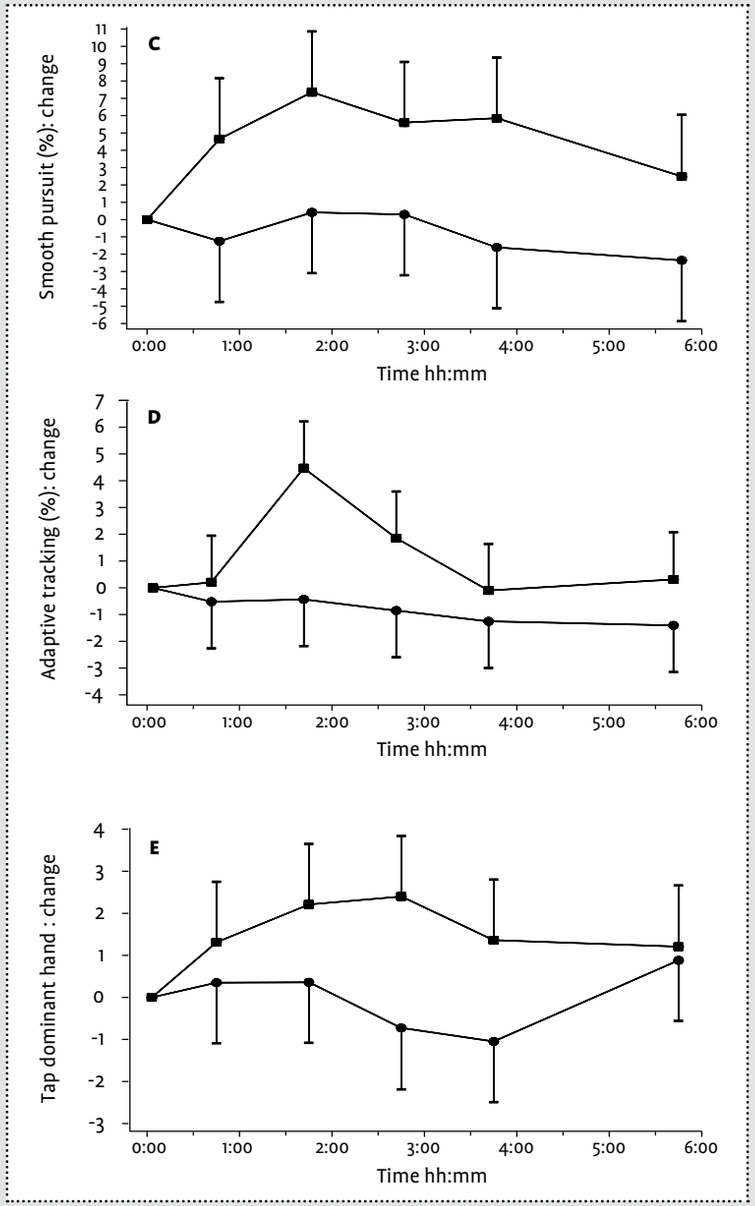
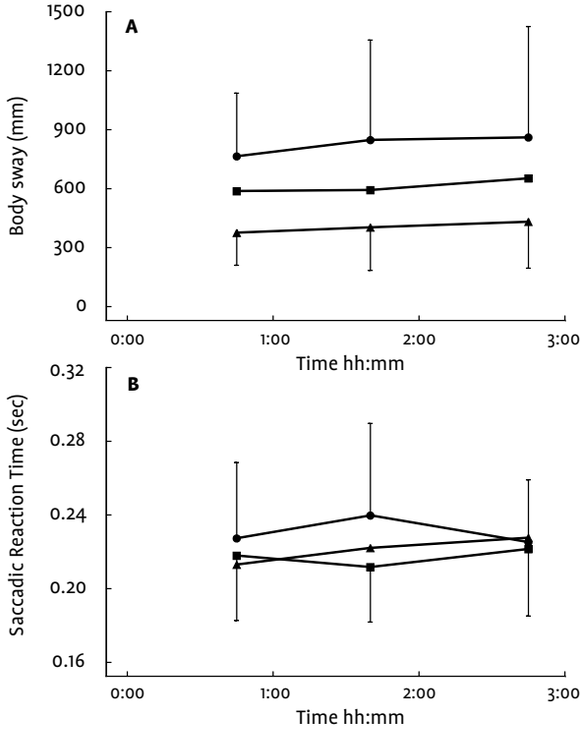


FIGURE 4A-E Comparison of the first 3 test runs after placebo (circle) and methylphenidate (square) to 3 consecutive test runs in healthy children (triangle) with SD error bars.



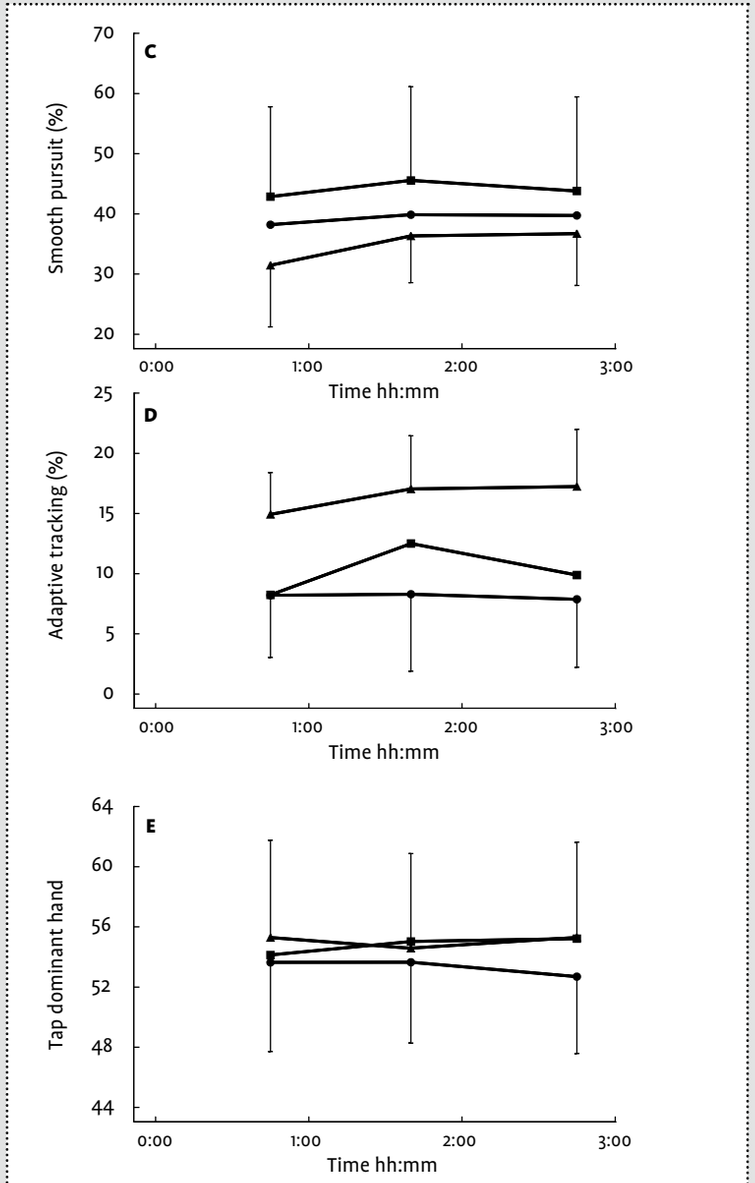


FIGURE 5A-C Change from baseline profile of vital signs. Shown data are least square means estimates, corrected for pre-dose measurements with SD error bars. Square: methylphenidate. Circle: placebo.

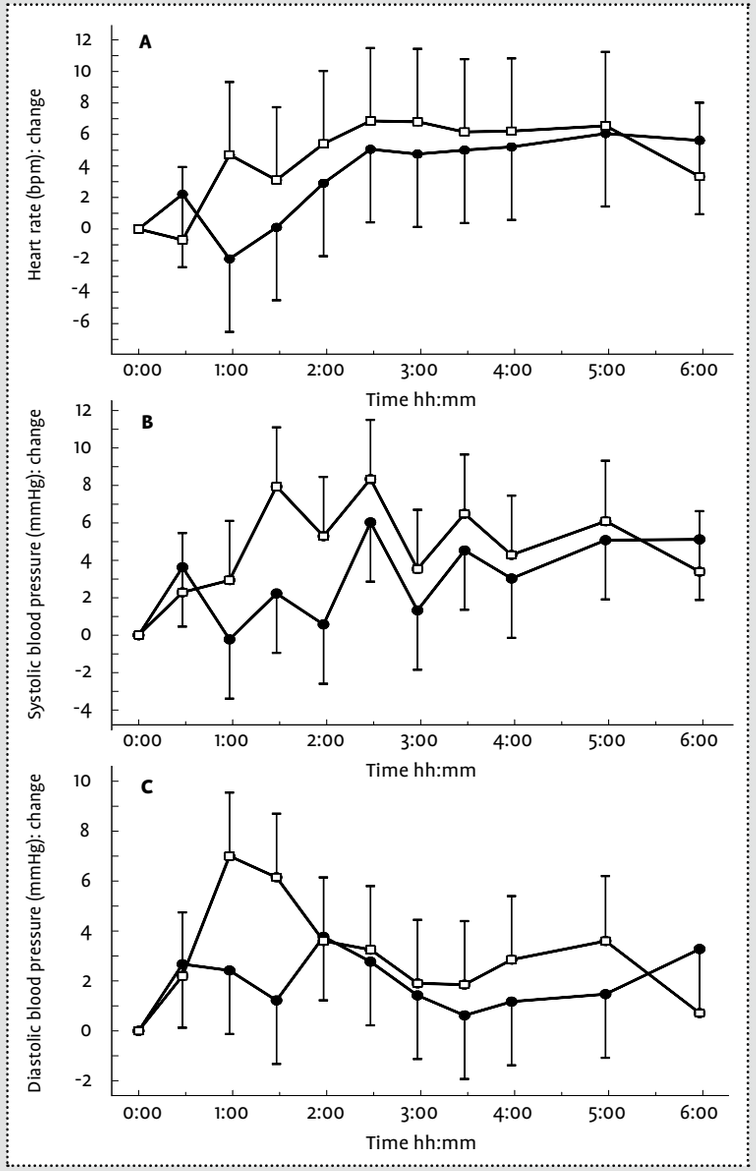
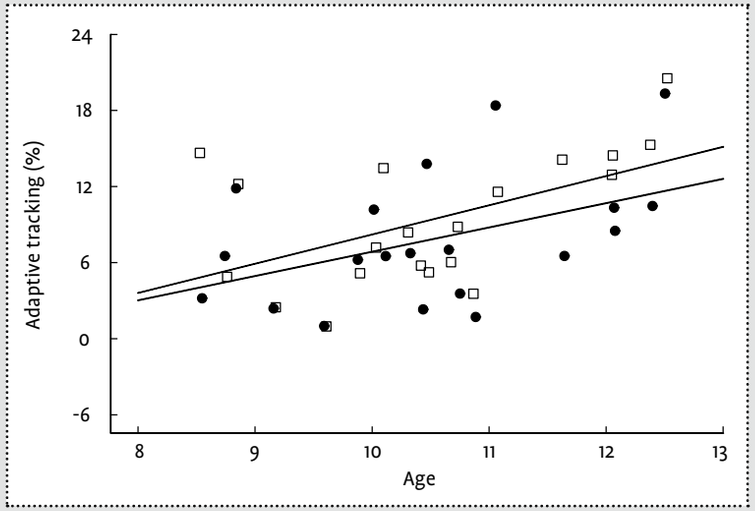


FIGURE 6 Regression line of age on adaptive tracking performance for both placebo (dots) and methylphenidate (squares).



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