

Research Report

THE DOSE-DEPENDENT EFFECT OF METHYLPHENIDATE ON PERFORMANCE, COGNITION AND PSYCHOPHYSIOLOGY

NICHOLAS J. COOPER*

*The Brain Resource Company and the Brain Resource International Database
Ultimo, NSW 2007, Australia
nick.cooper@brainresource.com*

HANNAH KEAGE

Flinders University, Bedford Park, SA 5001, Australia

DANIEL HERMENS

*The Brain Dynamics Center, University of Sydney and
Westmead Hospital, NSW 2145, Australia
Discipline of Psychological Medicine, University of Sydney, NSW 2006, Australia*

LEANNE M. WILLIAMS

*The Brain Dynamics Center, University of Sydney and
Westmead Hospital, Westmead, NSW 2145, Australia
Discipline of Psychological Medicine, University of Sydney, NSW 2006, Australia*

DAVID DEBROTA

*Lilly Research Laboratories, Eli Lilly & Company
Indianapolis, IN 46285, USA*

C. RICHARD CLARK

*Cognitive Neuroscience Laboratory, Flinders University
Bedford Park, SA 5001, Australia*

*Corresponding author.

EVIAN GORDON

*The Brain Resource Company and the Brain Resource International Database
Ultimo, NSW 2007, Australia*

*Convenor, Integrative Neuroscience, Brain Dynamics Center
University of Sydney and Westmead Hospital, NSW 2145, Australia*

*Discipline of Psychological Medicine, University of Sydney
NSW 2006, Australia*

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The effects of methylphenidate (MPH) on 32 healthy human male volunteers (aged 18 to 25 years, mean age = 22.26) were examined using a within-subject design. Each participant attended six testing periods, held once per week. Within each testing period, three repeat testing sessions were undertaken: pre-medication, on-medication and two hours post-medication. In these sessions, dose was manipulated (placebo, 5 mg, 15 mg or 45 mg) according a double-blind placebo design. In this report, we focus on behavioral, autonomic arousal (heart rate, skin conductance) and psychophysiological (ERP) data acquired during the working memory task. We found increased autonomic arousal (heart rate, skin conductance and blood pressure) with MPH. A linear reduction in reaction time, omission errors and target P3 latency, and a corresponding increase in background P3 amplitude was observed with increased MPH dose. The relationship between these measures supported an increase in performance and underlying brain function with MPH. To our knowledge, this is the first paper to use behavioral, arousal and electrophysiological measures in an integrative approach to study the effects of MPH on healthy adults.

Keywords: Methylphenidate; P3; arousal; CPT; ERP; Dose.

1. Introduction

The majority of research into the cognitive neuropsychological and behavioral effects of methylphenidate (MPH) has focused on children, especially those diagnosed with Attention Deficit/Hyperactivity Disorder (ADHD) [for example, see 7, 12, 19]. The few studies that have investigated the effects of MPH in healthy adults report that MPH is associated with behavioral improvements in cognition, emotion and performance during *simple* tasks, including reaction time, vigilance, response selection and subjective affect — consistent with the results from childhood ADHD research [4, 6, 20, 28, 41]. However, while childhood ADHD studies have typically reported similar MPH-related improvements in performance for *complex* cognitive tasks, comparatively inconsistent findings have been found for healthy adults for these tasks [1, 5, 8, 9, 10, 13, 32]. Thus, the extent to which MPH improves complex cognitive processing and performance in healthy adults remains unresolved.

MPH has also been examined with regard to its effects on psychophysiological measures of brain function and autonomic arousal. Previous psychophysiological studies of adolescent and childhood ADHD have reported improvements in such measures following MPH, which correspond to those observed for cognition and

performance [7, 11, 16, 18]. For instance, MPH has been found to “normalize” excess electroencephalograph (EEG) theta activity, and to amplify event-related potentials (ERPs) to both easy and hard tasks. MPH has also been found to increase skin conductance (as an index of autonomic arousal) in these children [45], consistent with its stimulant properties.

Studies of psychophysiology and MPH in healthy subjects have revealed a somewhat inconsistent pattern of results, akin to the mixed findings for cognition and performance. For instance, whilst some studies have found that MPH affects the P3 ERP component in adult samples, other studies have failed to find an effect [1, 5, 32]. Several studies have used the Continuous Performance Task (CPT), which involves selectively attending to infrequent stimuli (referred to as “targets”) — the background component P3 is reported to assess aspects of working memory [11]. In other lines of research, behavioral studies suggest that the CPT is sensitive to medication effects [22, 29]. Evidence from double-blind, placebo-controlled studies with healthy adults suggest that the enhancing effects of MPH are apparent only during difficult versions of the CPT. MPH has not been found to affect the amplitude of the P3 during “easy” versions of the CPT, yet produced a significant increase in LPC during a “difficult” version of the CPT [13], and reduced errors in MPH-treated compared to placebo subjects [1]. Moreover, MPH-treated subjects displayed larger P3’s following targets and backgrounds during the difficult version of the CPT. This is suggestive of an enhancement of working memory updating processing. As this enlargement of peaks followed both targets and backgrounds, this finding suggests that the additional attentional resources provided by MPH are not specific to selective attention, but instead produce a more generalized improvement in working memory [13].

In the paired-associate learning (PAL) task, a similar increase in the P3 component following task response has been found, even though performance on this task was unexpectedly not found to improve [6]. By contrast, another study using the PAL did not reveal an MPH effect on P3 amplitude [31], which was interpreted as possibly reflecting practice effects. Despite some inconsistency in these findings, evidence that the P3 component may be influenced by MPH is consistent with observations that this component is modulated by noradrenergic function, activated by MPH [26].

One consistent finding in these studies has been an increase in task-related heart rate (HR) with MPH [5, 13], reflecting an enhancement of autonomic arousal. It has been suggested that changes in brain function with MPH may be due to the effect of arousal on enhancing attention [27]. Consistent with this proposal, other studies have observed a robust relationship between MPH administration and increases in HR, indicating increased levels of arousal [21, 39]. MPH has also been found to affect motivational states, which in turn impact on performance [39]. These findings accord with the longstanding relationship between states of physiological arousal and performance described in cognitive psychology [39, 44]. To date, evidence concerning

the relationship between MPH and arousal suggests that MPH serves to modulate arousal, consistent with evidence that autonomic arousal is also modulated by noradrenergic mechanisms [2, 43].

Volkow *et al.* [39] used Positron Emission Topography (PET) to estimate Dopamine Transporter (DAT) occupancies and found occupancies of 12% for 5 mg, 40% for 10 mg, 54% for 20 mg, 72% for 40 mg and 74% for 60 mg. They reported that 50% occupancy was required for MPH to have therapeutic effects for ADHD. This study utilized doses of 5 mg, 15 mg, 45 mg and placebo. By interpolation from the Volkow study results, 15 mg should correspond with about 50% occupancy and 45 mg with around 73%, which seems to be close to saturation point. Thus, the Volkow claim that 50% occupancy is required for therapeutic effects can be tested in the current study by looking at the dose threshold for significant changes in performance.

The studies outlined above typically employed a single measure of brain function or autonomic arousal, and were usually focused on a particular MPH dose, thereby restricting the opportunity to explore the inter-relationships between brain function, autonomic arousal, cognition and performance in response to various doses of MPH. The current Brain Resource International Database (BRID) study focused on the integration of behavioral and psychophysiological measures of both central and autonomic function, acquired during the CPT, to investigate more fully the dose-related effects of MPH. Healthy adults were examined, since previous results for this group have tended to be contradictory. Psychophysiological measures included task-related indices of both central and autonomic function: the Late Positive ERP Component and the phasic skin conductance response (SCR), a measure of autonomic responsiveness [2, 30]. To investigate dose-dependent relationships, these measures were recorded during the CPT under four MPH dose conditions (5 mg, 15 mg, 45 mg and placebo).

2. Method

2.1. *Subjects*

Thirty two healthy human male volunteers (aged 18 to 25 years, mean age = 22.26) were recruited from two sites (Sydney and Adelaide, Australia).^a

2.2. *Design*

A within-subject, double-blind, placebo controlled, design was employed (summarized in Fig. 1). Each participant attended six testing periods, held once per week (each testing day was seven days apart). In each testing period, there were three repeat testing sessions undertaken: pre-medication, on-medication and two hours post-medication. In these sessions, dose was manipulated (placebo, 5 mg, 15 mg or 45 mg), according to the double-blind placebo design. Dose was counterbalanced

^aThe potential effect of site was addressed in the analyses.

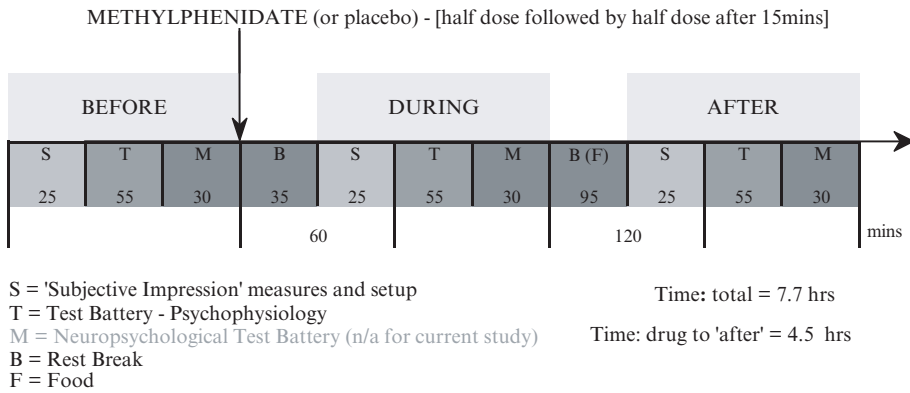


Fig. 1. Schematic of testing procedure.

within subjects. Only four weeks were required to counterbalance dose. The additional two weeks were used to repeat test each dose (counterbalanced within subjects), to determine reproducibility of dose findings. Total number of testing sessions was therefore 18 (6 periods \times 3 sessions each period).

At each visit, subjects were assessed with a battery of electrophysiological and neuropsychological measurements, as outlined in Sec. 2.3.

2.3. Data acquisition

2.3.1. Working memory task

The focus of this report is on performance, psychophysiology and autonomic measures recorded during the Continuous Performance Test (CPT), in which working memory ERPs were examined. A series of letters (B, C, D or G) were presented to the subject, each for 200 ms and separated by an interval of 2.5 seconds. If the same letter appeared twice in succession, the subject was asked to press buttons with the index finger of each hand. Speed and accuracy of response were equally stressed in the task instructions. A total of 125 stimuli were presented; 85 being non-target letters and 20 being target letters (i.e., repetitions of the previous letter).

The task is an adaptation of an earlier one-back design [11] that requires the participant to repeatedly update target identity during the processing of background stimuli. Given that targets are defined as consecutive repeats of any letter, each stimulus becomes a potential target. Earlier work [11] has shown a distinctive late positive component to background stimuli in this task that reflects the updating of working memory with target identity. This component has been termed the P450 [11], and occurs at a latency similar to the more conventional P3 that is also obtained in this task to target stimuli. In the present study, these two late positive components are referred to as the background P3 and target P3, respectively.

Test measures recorded during the CPT working memory task included performance (reaction time and accuracy, including errors of commission/omission), psychophysiology (event-related potentials, ERPs), and autonomic measures (skin conductance level, SCL; skin conductance response, SCR; heart rate, HR; respiratory rate, RR) — all recorded concurrently.

Behavioral (performance) measures

The interval between a letter stimulus presentation and the subsequent button press (reaction time) was recorded. The total number of times subjects failed to button-press during the working memory task became the score for errors of omission. Similarly, errors of commission was defined as the total number of times that subjects button-pressed to a background letter.

Autonomic measures

Skin conductance was recorded via a pair of silver-silver chloride electrodes with 0.05 M sodium chloride gel placed on the distal phalanges of digits II and III of the left hand. The electrode pairs were supplied by a constant voltage (Grass SCA1) and the current representing conductance was transformed to a voltage by the transducer and recorded using a DC amplifier. The ECG recording electrode was positioned on the inside of the left wrist; positioned at the radial pulse. ECG was referenced by two non-cephalic sites and used to estimate HR. A respiration belt was positioned just below the armpit line (above clothing) to sense the contraction and expansion of the chest wall. The respiration, ECG and skin conductance channels were sampled at 500 Hz, with 22-bit resolution digitization (Neuroscan Nuamps). A low pass filter was applied prior to digitization with a cut-off at 100 Hz.

Skin conductance was decomposed into SCL as a function of time, and SCR components. The “tonic arousal” slope of SCL (microSiemens/second or $\mu\text{S}/\text{sec}$) was estimated during both the eyes open (EO) and auditory oddball tasks. This measure was calculated by fitting an exponential curve to the EDA time series and then taking the initial slope of the fit as characterizing the rate of change of the exponential baseline.

SCRs (“phasic arousal”/“orienting”) were quantified using a method that enables overlapping SCRs to be separated. This method assumes the driving nerve signal has a shorter time constant than the skin conductance responses, enabling peaks in the driving signal to be separated and isolated. SCRs were defined as responses greater than $0.05 \mu\text{S}$ and time locked to each target stimulus, with the onset of the SCR occurring within 1 to 3 seconds of a target stimulus.

Heart rate was scored automatically using the Pan/Tompkins real-time ORS detection algorithm [25].

Psychophysiological acquisition and scoring

Subjects were seated in a sound and light attenuated room with an air-conditioned ambient temperature of $24 \pm 1^\circ\text{C}$. An electrode cap was used to acquire data from the Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T3, T4, T5, T6, Pz, P3, P4, O1, O2, and Oz scalp sites. Averaged earlobes serve as reference. Horizontal eye movement potentials were recorded using two electrodes, placed 1cm lateral to the outer canthus of each eye. Vertical eye movement potentials were recorded using two electrodes placed on the middle of the supraorbital and infraorbital regions of the left eye. Skin resistance at each site was $< 5\text{ k}\Omega$. A continuous acquisition system was employed. The Gratton procedure for EOG artifact correction was applied followed by thresholding to reject contaminated epochs (those exceeding $\pm 100\ \mu\text{V}$). The sampling rate was 500 Hz. A 100 Hz low-pass filter was applied to the signals prior to digitization [15].

Conventional ERP averages were formed at each recording site in relation to target and background stimuli. All target stimuli with a correct button response (only) were included in the target average. Similarly, only background stimuli without a button press were included in the background average. Before averaging, each single-trial waveform was filtered at 25 Hz with a Tukey or cosine taper to 35 Hz, above which frequency no signal was passed. For each stimuli waveform, the peaks (amplitude and latency) of the N1, P2, N2 and P3 ERP components were identified (relative to a pre-stimulus baseline average of -300 to 0 ms) at each site. ERPs were scored using an automated algorithm, which was validated by experienced scorers (for P3 only, as data for the other components was not used), and had over 98% accuracy. The algorithm used the following latency windows as a guide to determining characteristic component peaks for both background and target ERPs: N1 (70–120 ms), P2 (120–220 ms), N2 (120–300 ms), P3 (220–550 ms).

2.4. Statistical analyses*2.4.1. Data dimensions*

A total of 22 variables were obtained from the measures recorded for this study, for a total of 18 testing sessions. A summary of the experimental dimensions is provided in Table 1.

Table 1. Data dimensions.

Total subjects = 32	(16 subjects by two sites)
Number of sessions per subjects = 18	(6 days by 3 sessions per day)
Total number of sessions = 564	(32 subjects by 18 sessions)
Number of behavioral measures = 3	
Number of arousal measures (ECG, SC, BP) = 7	(midline sites [3] \times target/background [2])
Number of EEG (ERP) measures = 12	\times amplitude/latency [2])
Total Measures = 22	

2.4.2. *Analysis methods*

Linear mixed models

The main analysis method employed was linear mixed modeling. Mixed models account for fixed effects, random effects and repeated effects within the same framework and allow multiple measurements for each subject. It is necessary to use these models, in context of the current experimental design, because there are overlapping levels of within subjects factors (e.g., *dose* and *period*) as well as between subjects factors. This combination is beyond the scope of regular ANCOVA.

The basis (the fixed effects) of these models is ANCOVA. Statistical tests for the main effects of interest are taken from this fixed effects portion of the model. In addition to the fixed effects, the structure of the experimental design is modeled by random and repeated effects (variance components). There are two repeating factors (*time*, and either *period* or *dose*) but most statistical packages only allow for one, so the second repeating factor is modeled by a random factor (which is statistically equivalent). The random factor in this model specifies a variance component for each subject. The repeated factor (*time*) fits a variance component which accounts for the correlation between session two and session three of each testing day. In order to determine which variable would become the random factor, *period* was tested against *dose* to determine the best model (if *dose* was used, the *return* effect was also tested for possible inclusion).

The overall mixed effect model encompasses fixed, random and repeated effects, and is not associated with an overall “p value” or statistic. Models either converge (successive iterations produce a set of parameter values which conform to all the conditions specified for the model), or do not, and apart from this gross criteria, models can be evaluated using Information Criteria (IC). IC are relative, rather than absolute, measures of model fit and can only be used to compare different models for the same variable. Accordingly, the strategy for analysis was to find the best fitting mixed effect model for each variable, and to focus on the fixed effects results which were associated with a p value.

Tukey step down trend test

The Tukey TCH Trend Test [35] involves regressing a dependent measure onto three alternative carriers for trend shapes (Ordinal, Logarithmic and Arithmetic) with baseline as a covariate. The error term is the within groups mean square and the trend shape is chosen by the carrier with the highest F value. Initially, the process is performed for all four doses, then if the four dose trend is statistically significant, then a three dose trend from 15 mg down to 0 mg is tested, then 5 mg versus placebo. The highest dose which is not significantly different from placebo is labeled the NOSTASOT dose (No Statistical Significance Of Trend). For further detail on this procedure, see [35].

Relational analyses

For variables which showed a significant dose effect in the linear mixed models generated in the univariate analyses, interrelationships were explored. Only data from the baseline session conducted in the morning was used to generate correlations. Examination of these correlations in the treatment condition would be counterproductive as simultaneous dose effects on the measures being correlated would produce a confounding influence on the correlations.

3. Results**3.1. Univariate analyses**

Eleven of twenty two mixed effect models tested had a significant fixed effect of *dose* ($p < .05$) indicating that these measures were sensitive to MPH when all model parameters were taken into account; baseline performance (*baseline*), practice effects (*period*), time of day effects (*time*), and so forth. These results for fixed effects analyses are summarized in Table 2.

All of the variables with significant *dose* effect in the mixed effects model (except SCL) also showed a significant step-down dose response trend [35]. The trend results from the Tukey Tests and the corresponding dose response effects from the linear mixed models, for behavioral, arousal and psychophysiological domains, are discussed below.

3.1.1. Behavioral (performance) measures

Omission Errors showed a decreasing logarithmic trend according to the Tukey Step Down procedure ($F = 43.99$, $p < .001$): increasing dose was associated with less errors of “omission”. Furthermore, we also found that there was a significant *dose* effect for reaction time: the Tukey procedure revealed a significant ordinal trend where increasing dose was associated with faster reaction time ($F = 22.71$, $p < .001$). A graphical representation of model LS Means for behavioral variables with significant Tukey dose trends are presented in Figs. 2(a) and 2(b). Such LS Means graphs show the means across doses corrected for other significant model factors affecting each measure (e.g., practice [*period*] effects). Of the behavioral measures, only Commission Errors showed no consistent dose response to MPH (see Table 2). This is probably due to a ceiling effect which has previously been found using the CPT with adult samples [29]. There is strong evidence for such a ceiling effect in the current study given that 74% of second sessions were completed with one commission error or less.

3.1.2. Autonomic measures

All arousal measures employed showed significant *dose* effects in the mixed effects models, except for diastolic blood pressure (BP) and average peak latency of SCRs

Table 2. Summary of *dose*, *baseline* and *time* effects for all models: Results for some key fixed effects in the Linear Mixed Models. “—” indicates that the variable was discarded because it was both not significant and not involved in a higher order interaction which was significant.

Measure Name	Dose	Baseline	Time	df
<i>Behavioral Measures</i>				
Reaction Time	<.001	<.001	0.02	181
Omission Errors	<.001	<.001	0.002	181
Commission Errors	—	0.223	<.001	181
<i>Arousal Measures</i>				
Blood Pressure Systolic I	<.001	<.001	<.001	192
Blood Pressure Diastolic I	—	<.001	0.011	190
Heart Rate	<.001	<.001	<.001	184
Number of SCRs	<.001	<.001	—	158
Average Amplitude of SCRs	0.004	<.001	<.001	158
Average Peak Latency of SCRs	—	0.003	—	157
SCL	0.02	—	0.019	135
<i>ERP Measures</i>				
ERP Targets P3 Latency (Fz)	0.019	0.001	—	172
ERP Targets P3 Latency (Cz)	0.002	<.001	0.294	171
ERP Targets P3 Latency (Pz)	0.047	<.001	0.333	171
ERP Targets P3 Amp. (Fz)	—	<.001	0.018	172
ERP Targets P3 Amp. (Cz)	—	<.001	0.009	171
ERP Targets P3 Amp. (Pz)	—	<.001	0.001	171
ERP Backgrounds P3 Latency (Fz)	0.067	<.001	0.002	181
ERP Backgrounds P3 Latency (Cz)	—	<.001	<.001	178
ERP Backgrounds P3 Latency (Pz)	0.354	0.005	<.001	183
ERP Backgrounds P3 Amp. (Fz)	—	<.001	—	181
ERP Backgrounds P3 Amp. (Cz)	0.05	<.001	0.312	178
ERP Backgrounds P3 Amp. (Pz)	0.349	<.001	0.031	183

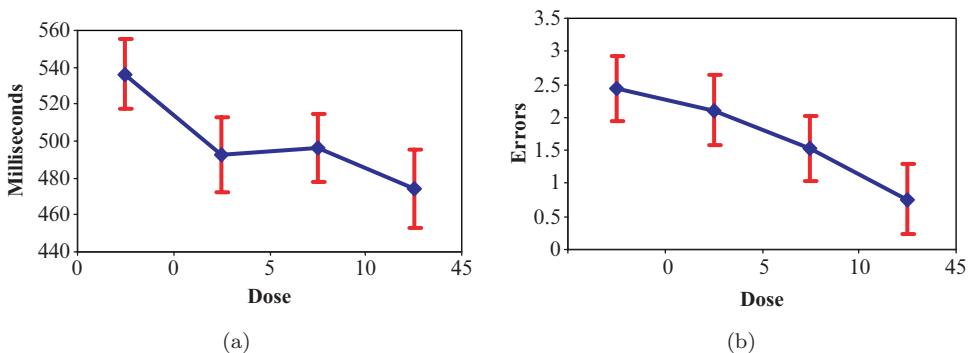


Fig. 2. (a) Reaction time; (b) omission errors.

(see Table 2 for summary of model fixed effects). The Tukey Test for HR revealed a significant logarithmic trend ($F = 66.83, p < .001$), where increasing dose was associated with faster HR during the working memory task. Systolic BP showed an ordinal trend: increasing dose was associated with increasing pressure ($F = 10.71,$

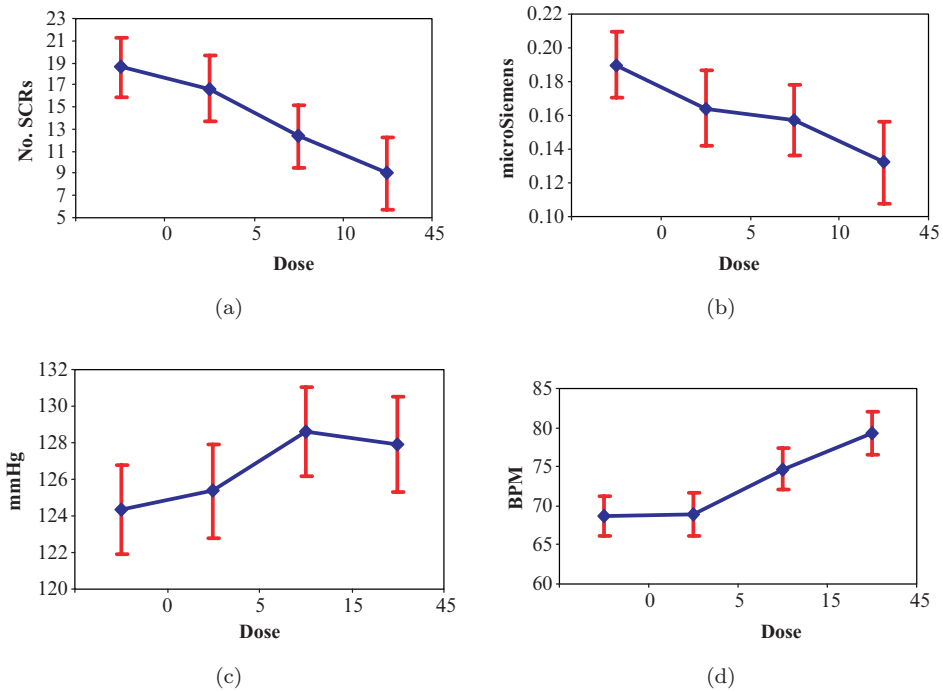


Fig. 3. (a) Number of SCR's; (b) Average amplitude of SCR's; (c) Systolic blood pressure; (d) Heart rate.

$p < .001$). The number of SCR's and the amplitude of these responses both decreased with increasing dose. The Tukey Trend Tests for these two measures revealed logarithmic and ordinal trends ($F = 24.41$, $p < .001$; $F = 14.64$, $p < .001$), respectively. Whilst there was a significant *dose* effect for SCL in the mixed effects model (with increasing LS Means for SCL as dose increased), SCL did not show a significant Tukey trend for dose ($F = 0.78$, $p > .05$). A graphical representation of model LS Means for arousal variables with significant Tukey dose trends can be seen in Figs. 3(a)–3(d).

3.1.3. Electrophysiological measures

Four from twelve ERP variables showed significant *dose* effects in the mixed effects models. No measures of target amplitude and no measures of background latency were statistically significant. For P3 target latency, all three midline sites had a significant *dose* effect in the mixed effects models and furthermore they showed significant Tukey trends: Fz, logarithmic, $F = 8.20$, $p < .01$; Cz, ordinal, $F = 9.78$, $p < .01$; Pz, ordinal, $F = 8.97$, $p < .01$. For P3 background amplitude only the site Cz showed a significant *dose* effect. The Tukey trend test for the Cz site revealed a logarithmic trend ($F = 10.17$, $p < .01$); the Pz site was only just non-significant for a ordinal trend ($F = 3.83$, $p = .051$). While *dose* was non-significant in the mixed effects model for background amplitude at Pz, the higher order interaction

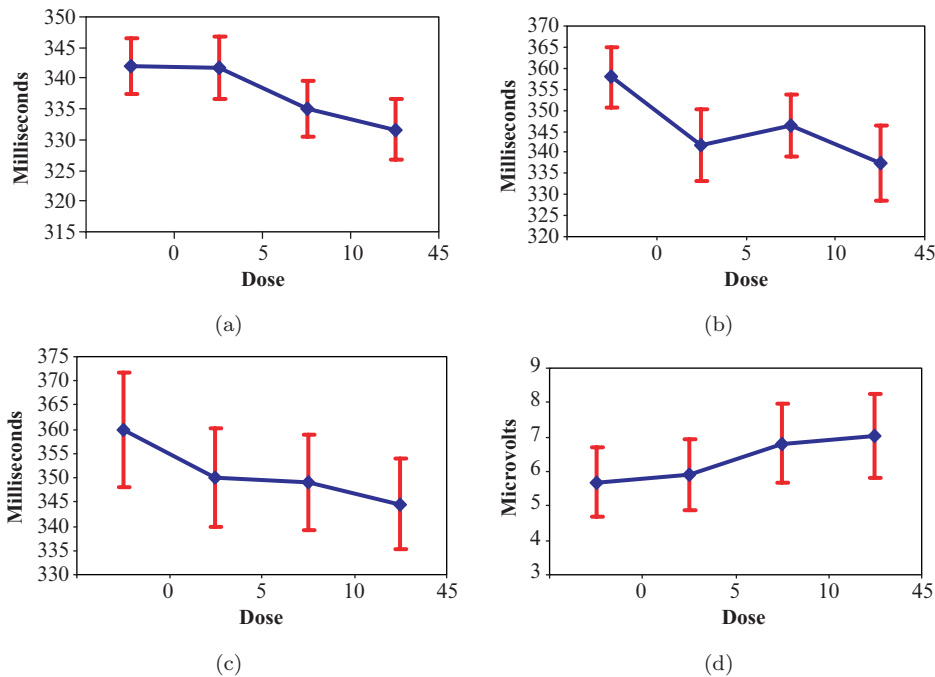


Fig. 4. (a) P3 target latency (Fz); (b) P3 target latency (Cz); (c) P3 target latency (Pz); (d) P3 background amplitude (Cz).

*dose*time*, was significant. See Figs. 4(a)–4(d) for graphical representation of model LS Means for electrophysiological variables with significant Tukey dose trends. The results for the Tukey tests showing significant dose trends are summarized in Table 3.

3.2. Relationships between working memory measures

Given the concurrent univariate changes in measures across increasing doses of MPH, it would greatly aid interpretation of such a profile of dose responses to know the relationships between these measures in a baseline condition. Presented in Table 4 are the Pearson correlations between all the measures showing significant Tukey step down trends. Systolic BP was omitted because this measurement was taken at rest prior to commencement of the working memory task, whereas all other measures were taken dynamically and simultaneously during the task.

No correction for multiple testing was made for the correlation matrix. If a full Bonferroni $k = 36$ adjustment had been applied, all correlations exceeding .3 would have remained significant. Given the sample size of over 150, combined with strict control of confounds in this study, it was thought that even small (italicized) correlations might have functional significance. Caution should be exercised in the interpretation of small correlations ($r < .3$).

The highest correlations (as would be expected) were between measures from the same domain (i.e., arousal, behavioral, psychophysiological). Faster reaction time

Table 3. Results of Tukey step down tests for variables with significant dose effects.

Variable Name	4 Level Trend (p)	Shape (0-45)	3 Level Trend (p)	Shape (0-15)	45 mg(vs) Placebo	15 mg(vs) Placebo	5 mg(vs) Placebo	NoStaSoT Dose
<i>Behavioral Variables</i>								
Working Memory RT	0.0000	Ordinal	0.0007	Ordinal	0.08	0.36	0.34	5 mg
Working Memory Omission Errors	0.0000	Logarithmic	0.0000	Arithmetic	0.00	0.12	0.74	5 mg
<i>Psychophysiological Variables</i>								
WM ERP Targets P3 Latency (Fz)	0.0045	Logarithmic	0.0288	Arithmetic	0.02	0.04	0.52	5 mg
WM ERP Targets P3 Latency (Cz)	0.0019	Ordinal	N/S		0.01	0.14	0.04	15 mg
WM ERP Targets P3 Latency (Pz)	0.0030	Ordinal	N/S		0.03	0.13	0.06	15 mg
WM ERP Backgrounds P3 Amp.(Cz)	0.0016	Logarithmic	0.0298	Arithmetic	0.12	0.68	0.44	5 mg
<i>Arousal Variables</i>								
Blood Pressure Systolic I	0.0012	Ordinal	0.0009	Arithmetic	0.01	0.01	0.33	5 mg
WM SCR Average Amplitude	0.0002	Ordinal	0.0119	Ordinal	0.001	0.077	0.275	5 mg
WM Number of SCRs	0.0000	Logarithmic	0.0004	Logarithmic	0.000	0.001	0.715	5 mg
WM Heart Rate	0.0000	Logarithmic	0.0000	Arithmetic	0.000	0.000	1.000	5 mg
WM SCL	N/S		N/S		0.655	0.85	1.000	45 mg

Table 4. Correlations between dose affected variables in the baseline condition. This table shows the Pearson correlation coefficients between the working memory variables with significant dose trends. The sample size for each correlation is greater than 150 and up to 192 depending on levels of pair-wise missing data. With $N > 150$: correlations with $r > .14$ have $p < .05$ (italicized); when $r > .2$, $p < .01$ (bold), when $r > .3$, $p < .000001$.

Measure	Omissions	R. Time	SCR Amp	Num SCRs	Heart Rate	Target Fz	Target Cz	Target Pz
Reaction Time	0.525							
SCR Amplitude	0.005	0.025						
Number SCRs	0	0.054	0.649					
Heart Rate	0.018	<i>-0.146</i>	-0.051	-0.031				
P3 Target Latency Fz	0.027	0.204	-0.001	-0.059	<i>-0.155</i>			
P3 Target Latency Cz	-0.037	0.053	-0.008	-0.057	-0.104	0.585		
P3 Target Latency Pz	-0.11	-0.103	-0.002	<i>-0.149</i>	0.022	-0.445	-0.663	
P3 Background Amp. Cz	-0.326	-0.369	-0.119	-0.066	0.03	0.122	0.008	-0.023

was associated with fewer errors of omission ($r = .525$, $p < .001$). Faster reaction time and fewer errors of omission were also associated with raised P3 background amplitude at the central midline site Cz (RT: $r = -.37$, $p < .001$; Err: $r = -.326$, $p < .001$). Faster reaction time was also weakly associated with increased HR ($r = -.15$, $p < .05$) and earlier P3 latency to targets at site Fz ($r = .20$, $p < .01$). As well as the relationship with reaction time, increased HR was also weakly associated ($r = -.16$, $p < .05$) with earlier P3 latency to targets at the frontal midline site (Fz).

More SCRs were associated with increased amplitude of SCRs ($r = .65$, $p < .001$), and also weakly associated ($r = -.15$, $p < .05$) with earlier P3 latency to targets at the posterior midline site (Pz). As would be expected, there were strong interrelationships between the three midline sites for P3 target latency, with highly significant correlations between .4 and .7. There were only weak relationships between these target P3 measures and the other measures in the table. Most notably, there was no relationship with the other ERP measures (background amplitude at Cz). The interrelationships between measures at baseline are summarized in Table 4.

4. Discussion

There was a consistent dose effect of MPH across behavioral, central and autonomic measures, providing the first convergent evidence that these measures may tap complementary interactions between arousal and performance. Task-related behavioral performance improved, physiologic arousal (indexed by HR) increased and brain function (ERPs) were reduced in latency and increased in amplitude, consistently with MPH dose, suggesting that focal attention was enhanced with increasing MPH-dependent arousal.

4.1. Effects of MPH on behavioral performance

Reaction time decreased ordinally with increasing MPH dose, whilst the number of omission errors decreased logarithmically over dose. Such MPH-related decreases have been reported in both samples of ADHD children [7, 12, 19] and adults [1, 3, 6]. Brumaghim and Klorman [5] and Coons *et al.* [13] however, did not show any improvement in performance during a complex cognitive task following MPH administration.

The minimum dose which showed therapeutic effects for most measures was 15 mg (see NOSTASOT column in Table 3). This result supports the findings of Volkow *et al.* [37] who reported, based on DAT occupancies, that 50% [~ 15 mg] was the threshold for therapeutic effects (in ADHD).

4.2. Effects of MPH on arousal

The increase in HR (logarithmic) and systolic BP (ordinal) in response to MPH provided support for our predictions. This finding is also consistent with previous

observations that HR and/or BP (both systolic and diastolic) increase in response to MPH in adults [21, 38, 40] and children [3, 31, 42] via noradrenergic and dopaminergic effects [38]. The null result concerning diastolic BP was in direct contrast to the results of a study by Brown *et al.* [3], where it was reported that an increase diastolic, but not a systolic, BP occurred following MPH administration. This discrepancy may well be due to age differences in the study subjects (the Brown study sample consisted of boys with ADHD, whereas the current sample was comprised of healthy adult males, aged 18 to 25).

With regard to skin conductance measures, SCL did not show a statistically significant dose response trend, although a significant dose effect was observed in the ordinal mixed models. Increasing dose was associated with increased SCL. The SCRs did show significant dose trends. An ordinal effect was obtained for the average amplitude of SCRs and a logarithmic effect for the number of SCRs. These effects were in the opposite direction than predicted — both amplitude and number of SCRs decreasing as MPH dose increased. A similar result was also obtained by Zahn *et al.* [45], who reported that stimulant drugs decreased SCRs in both normal children and children with minimal brain dysfunction (early DMS terminology for ADHD), whilst increasing HR. One possible explanation is that because the baseline (SCL) increases with administration of MPH, a ceiling effect reduces the size and number of detectable SCRs.

4.3. Effects of MPH on the background and target P3s

Previous research has reported that MPH reduces latency and/or increases amplitude of the P3 component in ADHD samples [16, 33, 34, 36]. In the current study, following targets, P3 latency at the midline sites Fz, Cz and Pz decreased in logarithmic, ordinal and ordinal fashions, respectively, with increasing MPH dose. However, this MPH-related latency decrease was not sustained following backgrounds. This suggests that MPH selectively accelerates updating in working memory to task-relevant stimuli (i.e., targets) which require a fast motor response but no short term memory retention, but does not accelerate updating to stimuli that require no motor action but do require short term memory retention (i.e., backgrounds).

Results from background P3 amplitude analyses provide further support for the proposal that MPH selectively focuses attentional resources. Following backgrounds, P3 amplitude increased at Cz logarithmically, however there were no significant effects on the corresponding P3 amplitude following targets. Given the task required participants to hold on-line background stimuli information for comparison with the following stimuli, but did not require target stimuli information to be held on-line, this result again suggests that working memory updating with newly relevant information was selectively enhanced by MPH. Unlike the acceleration of stimulus categorization which occurred following targets, there was a task-relevant enhancement of the short term memorization process for backgrounds [11, 14, 17].

In terms of comparison with results from adult samples during CPT working memory task, the results are partially consistent with those of Coons *et al.* [13]. Coons reported that MPH (20 mg) significantly increased the LPC at Cz and Pz during a “difficult” version of the CPT following both targets and backgrounds. The currently study only found an amplitude increase for backgrounds, but this discrepancy might be explainable by a difference in methodology. Coons utilized a classic CPT-BX task whereby participants viewed letters on a screen, pressing a button to the letter X, thereby not necessitating the holding on-line of information for comparison (as the current study’s task involved). Thus the CPT-BX task may not have required targets and backgrounds to be processed differently within the brain at such an early stage of processing that the LPC represents.

Coons also found no significant amplitude change, for targets or backgrounds, for two easier versions of the CPT. Coons suggested that only in longer or more difficult tasks would subjects benefit from the extra “attentional resources” produced by MPH [13]. This finding is consistent with the amplitude increase found in the present study despite the current study’s implementation of an “easy” version of the CPT. This is because the current task was part of a longer battery of tests and subjects would presumably be experiencing some degree of fatigue (by the time subjects were completing their first medicated session of the day they would already have undergone three hours of testing) and thus this easy test may have been equivalent to a more difficult or longer version of the CPT.

4.4. Relationships between arousal, performance and MPH

This paper is the first to our knowledge to simultaneously test the effect of increasing doses of MPH on arousal, ERPs and behavioral measures, in healthy adults. Utilizing such a profile of measures provides an opportunity for an integrative insight into the effects of MPH on healthy subjects.

A holistic interpretation of all working memory measures employed in this study can be aided by taking into account the baseline correlations between these measures. Given that these measures are all recorded simultaneously within the same task, the question could be raised as to whether the observed changes across domains occurred in isolation or whether these measures are linked so that change in one domain might imply change in another. In order to determine the degree to which these measures are linked pre- and post-MPH, the differing relationships between the dose affected variables will be discussed in context of their baseline correlations.

Firstly, in summary of the univariate analyses, when MPH dose increased, P3 latencies to targets decreased, P3 amplitude to backgrounds increased, reaction time quickened and omission errors decreased, HR and SCL increased, and skin responsivity decreased in the form of lowered SCR amplitude and frequency.

In the baseline condition, faster reaction time was associated with fewer errors of omission. This finding contrasts with the archetypal idea of a speed/accuracy

trade-off, because speed and accuracy on this task improve concurrently. Due to simplicity of the task, one factor that might contribute to both faster reaction time and improved accuracy could be motivation (concentration). Poorer performances on the task may reflect boredom, given that healthy adults (such as the present cohort) should have no difficulties in completing the task [29]. With increasing dose of MPH, performance on both speed and accuracy improved, which suggests that MPH enhanced subjects' motivation for the task. This idea is supported by Volkow *et al.* [39], who found that MPH increased the saliency of a mathematical task, and that performance improved as a consequence of increased motivation. Similar results were found by Kollins *et al.* [21], who reported that subjects' self-report data on the Drug Effects Questionnaire (DEQ) indicated increases in motivation (also alertness, vigor, "good effects", jitteriness and a drug "high"), following 20 mg and 40 mg doses of immediate release MPH.

The next strongest correlate of reaction time in the baseline condition was P3 amplitude to backgrounds (at site Cz). Greater amplitude was associated with faster reaction time at baseline. As expected (since MPH was shown to both increase the amplitude of the P3 and to quicken reaction time), with increasing dose of MPH, this relationship strengthened ($r = -.55$, $p < .01$, for post-hoc correlation at the 45 mg dose). This might suggest a link between the P3 component and processing speed, except that increasing P3 amplitude was also associated (with similar r value) with fewer errors of omission. Thus the P3 amplitude has shown an association with task performance rather than speed or accuracy in isolation. One possible explanation is that P3 amplitude is a biomarker for motivation/attention. It might be argued that the P3 is causally related to reaction time only, and that the correlation between the P3 and errors emerges because of the stronger correlation between the two behavioral measures. Such an argument does not necessary carry much weight, since a .5 order correlation is (probably) not large enough to have this moderating effect. This is demonstrated by the latency data which did show specificity between errors and RT. P3 latency to targets correlated positively with RT, but did not correlate with omission errors. P3 amplitude to backgrounds as a marker of "motivation" might also be consistent with the findings of Kotchoubey [23], who argued that amplitude was increased for rare stimuli even in a passive condition — perhaps such stimuli are more motivating to attend to.

Other, although much weaker, associations with reaction time in the baseline condition were found with both HR and P45 latency to targets (Fz). Interestingly, there was also a similarly sized correlation between HR and P3 target latency (Fz). Increased HR (arousal) was associated with both faster reaction time and earlier P3 latency. These correlations suggest the possibility of a three way link between heart rate (arousal), ERP latency and reaction time.

SCR amplitude increased as the total number of SCRs (frequency) during the baseline condition increased. This finding suggests that subjects with greater tonic responsivity would tend to show both increased amplitude and frequency

of SCRs (not simply one or the other). The implication is that amplitude and frequency of SCRs are tied together, and that we would not expect much variation in one without a concurrent change in the other. A weak relationship was also observed between number of SCRs and P3 latency to targets. More SCRs were associated with earlier latency, but given the small size of the correlation, this finding is feasibly due to an indirect relationship with an unknown mediating variable.

Given the substantial correlations between the three P3 latency sites (targets), the similar dose responses amongst the three are unsurprising. Potentials measured at different locations on the scalp would be expected to be highly correlated, given that every source affects all scalp sites to some extent through volume conduction. No relationship was observed between P3 amplitude for backgrounds and the P3 latency measures for targets. This suggests that the observed MPH effects on background amplitude and target latency of P3 ERPs are independent despite the fact that these are similar measures taken from the same task.

From the results of this study, it can be seen that the effects of MPH are characterized by a general increase in performance; instantiated as both an increase in task speed and accuracy (high level behavioral performance), as well as earlier target P3 latency and increased background P3 amplitude (low level cognitive performance). This performance increase was accompanied by an increase in arousal (HR, SCL), alongside a decline in responsivity (SCRs). Importantly, this is the first study to our knowledge, which demonstrates an unambiguous profile of cognitive enhancement in normal subjects using MPH. The increase in performance might be attributed to the increase in arousal, were it not for the fact that there were no substantial relationships found between arousal and performance in the baseline condition (there was a weak negative association between HR and reaction time). This also compliments the proposal that MPH may improve performance in healthy subjects by increasing levels of motivation and sustained attention required for the continuous performance (working memory) task.

By assessing the profile of findings for arousal, performance and electrophysiological variables, it has been possible to elucidate the effects of methylphenidate on the brain that may not have been possible to glean from separate studies using isolated measures. For instance, the three way relationship observed between reaction time, P3 latency for targets and HR was only uncovered because arousal, ERPs and reaction time were measured simultaneously. Further research is suggested to help understand the functional implications of this finding. The association between P3 amplitude to backgrounds with both errors of omission and reaction time implicated this ERP component as a biomarker for motivation. Further research will be required to determine the significance of this finding. It is clear, however, given the issues emerging from these relationships between different measures, that an integrative neuroscientific approach is invaluable to uncovering complex brain-body interrelationships.

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References

- [1] Aman MG, Vamos M, Werry JS, Effects of methylphenidate in normal adults with reference to drug action in hyperactivity, *Aust NZ J Psychiat* **18**(1):86–88, 1984.
- [2] Boucsein W, *Electrodermal Activity*, Plenum Press, New York, 1992.
- [3] Brown RT, Wynne ME, Slimmer LW, Attention deficit disorder and the effect of methylphenidate on attention, behavioral, and cardiovascular functioning, *J Clin Psychiat* **45**(11):473–476, 1984.
- [4] Brown WA, Psychologic and neuroendocrine response to methylphenidate, *Arch Gen Psychiat* **34**(9):1103–1108, 1977.
- [5] Brumaghim JT, Klorman R, Methylphenidate's effects on paired-associate learning and event-related potentials of young adults, *Psychophysiology* **35**(1):73–85, 1998.
- [6] Camp-Bruno JA, Herting RL, Cognitive effects of milacemide and methylphenidate in healthy young adults, *Psychopharmacology* **115**(1–2):46–52, 1994.
- [7] Chabot RJ, Orgill AA, Crawford G, Harris MJ, Serfontein G, Behavioral and electrophysiologic predictors of treatment response to stimulants in children with attention disorders, *J Child Neurol* **14**(6):343–351, 1999.
- [8] Clark CR, Geffen GM, Geffen LB, Role of monoamine pathways in the control of attention: Effects of droperidol and methylphenidate in normal adult humans, *Psychopharmacology* **90**:28–34, 1986.
- [9] Clark CR, Geffen GM, Geffen LB, Catecholamines and attention in humans: studies in normal humans, *Neurosci Biobehav R* **11**(4):353–364, 1987.
- [10] Clark CR, Geffen LB, Geffen GM, Monoamines in the control of state-dependent cortical functions: evidence from studies in animals and humans, *N Neurobiology* **12**:487–502, 1984.
- [11] Clark CR, Orr RS, Wright E, Weber D, Working memory updating to visual verbal stimuli: a high resolution ERP study, in Koga Y, Nagata K, Hirata K (eds.), *Brain Topogr Today* (pp. 173–178), Elsevier Science, Amsterdam, 1998.
- [12] Clarke AR, Barry RJ, McCarthy R, Selikowitz M, Croft RJ, EEG differences between good and poor responders to methylphenidate in boys with the inattentive type of attention-deficit/hyperactivity disorder, *Clin Neurophysiol* **113**(8):1191–1198, 2002.
- [13] Coons HW, Peloquin LJ, Klorman R, Bauer LO, Ryan RM, Perlmutter RA, Salzman LF, Effect of methylphenidate on young adult's vigilance and event-related potentials, *Electroen Clin Neuro* **51**(4):373–387, 1981.
- [14] Fabiani M, Karis D, Donchin E, P3 and recall in an incidental memory paradigm, *Psychophysiology* **23**(3):298–308, 1986.
- [15] Gratton G, Coles MG, Donchin E, A new method for off-line removal of ocular artifact, *Electroen Clin Neuro* **55**:468–484, 1983.

- [16] Holcomb PJ, Ackerman PT, Dykman RA, Cognitive event-related brain potentials in children with attention and reading deficits, *Psychophysiology* **22**(6): 656–667, 1985.
- [17] Hruby T, Marsalek P, Event-related potentials — the P3 wave, *Acta Neurobiol Exp* **63**(1):55–63, 2003.
- [18] Jonkman LM, Kemner C, Verbaten MN, Van Engeland H, Camfferman G, Buitelaar JK, Koelega HS, Attentional capacity, a probe ERP study: differences between children with attention-deficit hyperactivity disorder and normal control children and effects of methylphenidate, *Psychophysiology* **37**(3):334–46, 2000.
- [19] Klorman R, Brumaghim JT, Methylphenidate reduces abnormalities of stimulus classification in adolescents with attention deficit disorder, *J Abnorm Psychol* **101**(1): 130–138, 1992.
- [20] Koelega HS, Stimulant drugs and vigilance performance: a review, *Psychopharmacology* **111**(1):1–16, 1993.
- [21] Kollins SH, Rush CR, Pazzaglia PJ, Ali JA, Comparison of acute behavioral effects of sustained-release and immediate-release methylphenidate, *Exp Clin Psychopharm* **6**(4):367–374, 1998.
- [22] Kornetsky C, The use of a simple test of attention as a measure of drug effects in schizophrenic patients, *Psychopharmacologia* **24**(1):99–106, 1972.
- [23] Kotchoubey B, Do event-related brain potentials reflect mental (cognitive) operations? *J Psychophysiol* **16**:129–149, 2002.
- [24] Lim CL, Gordon E, Rennie C, Wright JJ, Bahramali H, Li WM, Clouston P, Morris JG, Dynamics of SCR, EEG, and ERP activity in an oddball paradigm with short interstimulus intervals, *Psychophysiology* **36**(5):543–51, 1999.
- [25] Pan J, Tompkins WJ, A real-time QRS detection algorithm, *IEEE Trans. Biomed Eng BME-32* (**3**):230–236, 1985.
- [26] Pineda JA, Foote SL, Neville HJ, Holmes TC, Endogenous event-related potentials in monkey: the role of task relevance, stimulus probability, and behavioral response, *Electroen Clin Neuro* **70**(2):155–171, 1988.
- [27] Pliszka SR, McCracken JT, Maas JW, Catecholamines in attention deficit hyperactivity disorder: Current perspectives, *J Am Acad Child Adolesc Psychiatry* **35**:264–272, 1996.
- [28] Rapport M, Kelly K, Psychostimulant effects on learning and cognitive function: Findings and implications for children with attention deficit hyperactivity disorder, *Clin Psychol Rev* **11**(1):61–92, 1991.
- [29] Riccio CA, Waldrop JJM, Reynolds CR, Lowe P, Effects of stimulants on the continuous performance test (CPT): Implications for CPT use and interpretation, *J Neuropsych Clin N* **13**(3):326–335, 2001.
- [30] Sergeant J, The cognitive-energetic model: an empirical approach to attention-deficit hyperactivity disorder, *Neurosci Biobehav R* **24**(1):7–12, 2000.
- [31] Solanto MV, Conners CK, A dose-response and time-action analysis of autonomic and behavioral effects of methylphenidate in attention deficit disorder with hyperactivity, *Psychophysiology* **19**(6):658–667, 1982.
- [32] Strauss J, Lewis JL, Klorman R, Peloquin LJ, Perlmutter RA, Salzman LF, Effects of methylphenidate on young adults' performance and event-related potentials in a vigilance and a paired-associates learning test, *Psychophysiology* **21**(6):609–621, 1984.

- [33] Sunohara GA, Malone MA, Rovet J, Humphries T, Roberts W, Taylor MJ, Effect of methylphenidate on attention in children with attention deficit hyperactivity disorder (ADHD): ERP evidence, *Neuropsychopharmacol* **21**(2):218–228, 1999.
- [34] Taylor MJ, Voros JG, Logan WJ, Malone MA, Changes in event-related potentials with stimulant medication in children with attention deficit hyperactivity disorder, *Biol Psychiat* **36**:139–156, 1993.
- [35] Tukey JW, Ciminera JL, Heyse JF, Testing the statistical certainty of a response to increasing doses of a drug, *Biometrics* **41-1**:295–301, 1985.
- [36] Verbaten MN, Overtom CCE, Koelega HS, Swaab-Barneveld H, van der Gaag RJ, Buitelaar J, Van Engeland J, Meth H, Methylphenidate influences on both early and late ERP waves of ADHD children in a continuous performance test, *J Abnorm Child Psychol* **22**(5):561–578, 1994.
- [37] Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS, Hitzemann R, Pappas N, Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate, *Am J Psychiat* **155**(10):1325–1331, 1998.
- [38] Volkow ND, Wang GJ, Fowler JS, Molina PE, Logan J, Gatley SJ, Gifford A, Ding YS, Wong C, Pappas NR, Zhu W, Swanson JM, Cardiovascular effects of methylphenidate in humans are associated with increases of dopamine in brain and of epinephrine in plasma, *Psychopharmacology* **166**(3):264–270, 2003.
- [39] Volkow ND, Wang GJ, Fowler JS, Telang F, Maynard L, Logan J, Gatley SJ, Pappas N, Wong C, Vaska P, Zhu W, Swanson JM, Evidence that methylphenidate enhances the saliency of a mathematical task by increasing dopamine in the human brain, *Am J Psychiat* **161**(7):1173–1180, 2004.
- [40] Volkow ND, Wang GJ, Gatley SJ, Fowler JS, Ding YS, Logan J, Hitzemann R, Angrist B, Lieberman J, Temporal relationships between the pharmacokinetics of methylphenidate in the human brain and its behavioral and cardiovascular effects, *Psychopharmacology* **123**:26–33, 1996.
- [41] Weiss B, Laties VG, Enhancement of human performance by caffeine and the amphetamines, *Pharmacol Rev* **14**:1–36, 1962.
- [42] Wilens TE, Biederman J, Lerner M, Group CS, Effects of once-daily osmotic-release methylphenidate on blood pressure and heart rate in children with attention-deficit/hyperactivity disorder: results from a one-year follow-up study, *J Clin Psychopharm* **24**(1):36–41, 2004.
- [43] Yamamoto K, Ozawa N, Shinba T, Hoshino T, Functional influence of the central noradrenergic system on the skin conductance activity in rats, *Schizophr Res* **13**(2): 145–50, 1994.
- [44] Yerkes R, Dodson J, Relation of strength of stimulus to rapidity of habit-formation, *J Comp Neurol Psychol* **18**:459–482, 1908.
- [45] Zahn TP, Abate F, Little BC, Wender PH, Minimal brain dysfunction, stimulant drugs, and autonomic nervous system activity, *Arch Gen Psychiat* **32**(3):381–7, 1975.