Behavioral response to methylphenidate challenge: Influence of early life parental care and personality

Doctoral Thesis

by

Veronika Engert

Faculty of Psychobiology

Department of Clinical and Theoretical Psychobiology

University of Trier, Germany

Supervisors:

Prof. Dr. Dirk H. Hellhammer, University of Trier, Germany

Prof. Dr. Jens C. Pruessner, McGill University, Quebec, Canada

October 2006

Acknowledgements

In the past years, I have been indebted to many a colleague who have provided me with support and friendship.

I am very grateful to my supervisors, Dr. Jens Pruessner and Dr. Dirk Hellhammer, for their invaluable intellectual guidance and patience, which have guided me throughout my work. Thank you for all your trust and encouragement.

Special thanks to Michael Meaney whose ideas have always been a great source of inspiration.

This work would not have been possible without the enormous help of Claudia Buss, Katarina Dedovic, Cathrine Lord, Najma Khalili Mahani and Mehereen Wadiwalla who have always been there when I needed them.

Wolff Schlotz has guided me through times of statistical confusion. Thank you very much.

Last but not least, I gratefully acknowledge the financial support received from the German Konrad Adenauer Foundation, without which this work could not have been realized.

Contents

List of figures

List of tables

List of abbreviations

ADHD	Attention deficit/hyperactivity disorder
ADS	Alcohol Dependence Scale
ANOVA	Analysis of variance
BDI	Beck Depression Inventory
DAST	Drug Abuse Screening Test
ED50	Median effective dose
fMRI	Functional Magnetic Resonance Imaging
GSES	General Self Efficacy Scale
h	hour
Km	Substrate concentration resulting in the half-maximal rate for an enzyme
mg	Milligram
mm	Millimeter
μM	Micromole
MPH	Methylphenidate
n	number of observations
nM	Nanomole
NMDA	N-methyl-d-aspartate
PBI	Parental Bonding Inventory
PET	Positron Emission Tomography
RSES	Rosenberg Self Esteem Scale
SD	Standard deviation
STAI	State-Trait Anxiety Inventory
TPQ	Tridimensional Personality Questionnaire
WCST	Wisconsin Card Sorting Test

1 THEORY

1.1 Introduction

Psychological neuroscience research aims at identifying the neurochemical correlates of psychological disorders. Given the high monetary and technical costs of imaging procedures, which allow the direct observation of brain processes, it is sensible to test a-priori hypotheses by means of indirect measurement methods. The first step to establishing an indirect measurement method is to find a behavioral paradigm that is sensitive for the neurochemical system of interest. Although the implementation of any such behavioral paradigm can only provide indirect insight into the underlying neurochemical mechanisms, it may reveal important information about the investigated relationships and allow the revision of initial hypotheses. Once a behavioral paradigm has been elaborated, it can be applied and validated in the context of imaging techniques such as Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI). Eventually, validated paradigms help to identify deviances in the regulation of neurochemical systems on the basis of predetermined behavioral characteristics and may therefore be utilized in a cost-efficient manner in a multitude of participants.

The major objective of the present work was to design an indirect measurement method, which would allow the investigation of relations between observable behavior and dopamine regulation. As our core behavioral measure we examined performance in a card-sorting task known to require prefrontal executive functions such as strategic planning, organized searching, cognitive set shifting, goal directed behavior and the modulation of impulsive responding. In order to exert an influence on the dopamine system, basal task performance was systematically manipulated using two dopamine-dependent variables: monetary incentive and the indirect dopamine agonist methylphenidate.

The upcoming theoretical chapters will provide an overview of the neurotransmitter dopamine, its central pathways and binding sites, outline the relationship between dopamine and both reward and methylphenidate, and eventually illustrate a functional model of subcortical dopamine release, which will be repeatedly referred to throughout this work.

1.2 The dopamine system

1.2.1 The neurotransmitter dopamine

Dopamine is a catecholamine, synthesized from tyrosine through the actions of two enzymes. First, tyrosine hydroxylase, the rate-limiting enzyme in the process, converts L-tyrosine to dihydroxy-L-phenylalanine, or L-dopa. Then, aromatic amino acid decarboxylase converts L-dopa to dopamine (Stanwood and Zigmond 2000). Synthesization takes place in the neuron, where dopamine is concentrated in vesicles for its later Ca2+-dependent release into the synaptic cleft and extrasynaptic space (Tupala and Tiihonen 2004).

1.2.2 Overview of dopamine pathways

There are three major dopamine pathways originating from the substantia nigra and ventral tegmental area of the midbrain (Figure 1). Axons of dopamine cells in the substantia nigra form the nigrostriatal projection, and provide dopaminergic innervation of the caudate nucleus and putamen (comprising the striatum). Mesolimbic and mesocortical dopamine pathways arise from the ventral tegmental area. The mesolimbic dopamine neurons innervate subcortical limbic regions such as the nucleus accumbens of the ventral striatum, olfactory tubercle and amygdala. The mesocortical neurons provide dopaminergic afferents to prefrontal, cingulate and entorhinal cortex (Stanwood and Zigmond 2000). Many lines of evidence suggest that midbrain dopamine

neurons play a pivotal role in mediating motivated behavior and the reinforcing effects of rewarding stimuli (including most drugs of abuse) (for a review see Ikemoto and Panksepp 1999; Schultz 2002).

Eventually, the tuberoinfundibular dopamine system, which connects hypothalamus and pituitary gland, is involved in the regulation of the hormone prolactin from the anterior pituitary (Stanwood and Zigmond 2000).

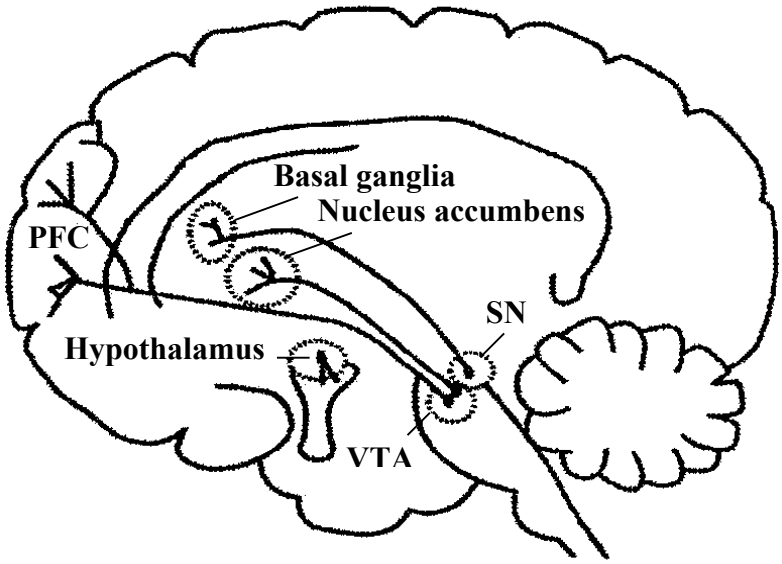

Figure 1. Schematic view of sub/cortical regions, with dopamine pathways highlighted. SN: Substantia nigra; VTA: Ventral tegmental area; PFC: Prefrontal cortex. Adapted and reproduced from Nigg (2005).

1.2.3 Dopamine binding sites

1.2.3.1 Dopamine D1-like receptors

There are two D1-like receptors subtypes: D1 and D5 receptors. D1 receptors are found at high levels in the dopamine-rich regions of the brain: the substantia nigra, striatum, nucleus accumbens and olfactory tubercle. D5 receptors are found at lower levels and with a relatively

restricted distribution in the hippocampus, thalamus and cerebral cortex (Hall et al 1994; Joyce and Murray 1994). On a functional level, D1 receptors are believed to mediate dopamine actions in movement control and cognition. The function of D5 receptors is currently not understood (Joyce and Murray 1994; Tupala and Tiihonen 2004).

1.2.3.2 Dopamine D2-like receptors

Within the D2-like receptor subfamily, two splice variants (D2L and D2S) have been identified. It has been proposed that D2L acts mainly at postsynaptic sites, whereas D2S serves presynaptic autoreceptor functions (Usiello et al 2000).

The D2-like receptors are comprised of D2, D3 and D4-receptor subtypes. The D2 receptor is the predominant D2-like subtype in the brain and, alike the D1 receptor, found at high levels in the dopamine-rich brain regions, especially the striatum and nucleus accumbens (Hall et al 1996; Hall et al 1994). D3 receptors are substantially less abundant than D2 receptors and found particularly in the nucleus accumbens, olfactory tubercle, and cerebral cortex (Sokoloff and Schwartz 1995). D4 receptors are distributed in the amygdala, hippocampus, hypothalamus and frontal cortex (Tupala and Tiihonen 2004).

The D2 receptor is involved in mediating the effects of dopamine in movement control and prolactin as well as growth hormone secretion from the anterior pituitary gland (Balldin et al 1993; Joyce and Murray 1994). The D3 receptor is able to play an autoreceptor role in transfected cells. Animal studies suggest D3 receptor stimulation to be involved in the inhibition of locomotion as well as striatal dopamine synthesis and release. However, the functions of D3 and D4 receptors are currently not well understood (Tupala and Tiihonen 2004).

1.2.3.3 The dopamine transporter

The dopamine transporter, or re-uptake carrier, is a presynaptically located protein. It is responsible for the elimination of dopamine from the synaptic cleft and perisynaptic areas, whereby it transports the neurotransmitter back into the cell. This re-uptake process constitutes the most effective way to terminate dopamine interactions with both pre and postsynaptic receptors (Hoffman 1994). Autoradiographic studies of dopamine uptake in animals and humans have indicated that the distribution of the dopamine transporter is mainly confined to striatal areas, with the highest densities observed in caudate nucleus, putamen and nucleus accumbens (Hall et al 1999b; Tupala et al 2001a; Tupala et al 2001b). Lower densities have been located in the substantia nigra, septal nuclei, retina, olfactory tubercle, median eminence, hypothalamus, and posterior pituitary gland (Hoffman 1994).

1.3 Involvement of dopamine in reward processing

Discovery of the electrical brain self-stimulation phenomenon by Olds and Milner in 1954 (Olds and Milner 1954) played a major role in initiating the idea of central reward mechanisms, although the neural identity of these mechanisms was still obscure. Since then, animal and human studies exploring the relationship between the dopamine system and reward have disclosed a very specific pattern of neural activation. Unit recordings in fully awake monkeys identified that initial contact with primary appetitive stimuli activated midbrain dopamine neurons. With repeated exposure, neural responses to food reward habituated, revealing unpredictability to be an important feature of midbrain dopamine responses. Dopamine neurons are thus activated during the learning phase but stop responding after full acquisition of various reward-delivering tasks. However, gradually, the mere presentation of predictive reward cues was shown to trigger

dopamine neuron firing (for a review see Schultz 2002). In line with the animal data, brain imaging studies in humans, which aimed at midbrain dopamine neuron terminals, showed that striatal regions (especially the medial caudate and the nucleus accumbens) were selectively recruited by the anticipation of monetary reward in well trained goal directed motor tasks (Knutson et al 2001a; Koepp et al 1998). Activation subsided during the delivery of rewarding outcomes (Knutson et al 2001b).

Dopamine is not only involved in the processing of natural rewards. In effect, most drugs of abuse share the potential to increase brain dopamine levels (Di Chiara and Imperato 1988). Phillips et al. (Phillips et al 2003) investigated the pattern of nucleus accumbens dopamine activity during cocaine self-administration in rats. Using fast-scan cyclic voltammetry he could further unveil the relationship between dopamine, (drug-)reward, and motivated behavior on a subsecond timescale. In rats trained to lever-press for cocaine injections, a brief dopamine pulse was detected a few seconds before the animals became interested in approaching and pressing the lever. Dopamine levels continued to rise after this initial pulse and eventually peaked a few seconds after cocaine delivery. The drug-associated test chamber had thus become a predictive cue of cocaine reward and triggered dopamine neuron firing. This naturally evoked dopamine pulse, in turn, triggered reward-seeking behavior (lever pressing). Eventually, the lever pressing was followed by another neurotransmitter pulse. A dual role of dopamine in reward processing can be derived from these findings: dopamine acts as a reward for behavior that precedes its release and subsequently triggers pursuit of the same reward in reaction to its release (Self 2003).

Another important conclusion that can be drawn from Phillips et al's study is that the neural activation patterns following dopamine-increasing drugs differ from those following natural rewards. In the lever-pressing rats, neural responses to cocaine were not subjected to habituation.

Contrarily, time-locked to every operant response for the drug, a dopamine signal occurred. This typical habituation-resistance to drug reward has been hypothesized to abnormally strengthen stimulus-drug associations, resulting in the attribution of excessive motivational value to stimuli associated with drug availability (Di Chiara et al 1999).

1.4 The indirect dopamine agonist methylphenidate

Methylphenidate is a central nervous system stimulant used clinically in the treatment of attention deficit/hyperactivity disorder (ADHD), a childhood psychiatric condition characterized by severe overactivity, impulsiveness and inattention (Swanson et al 1998). In patients with ADHD, methylphenidate reduces hyperactivity and improves executive function (Conners 2002; Faraone et al 2004; Mehta et al 2004; O'Driscoll et al 2005). Typical positive performance effects in healthy adults following single oral methylphenidate doses include the improvement of vigilance, reaction time and working memory (Camp-Bruno and Herting 1994; Cooper et al 2005; Elliott et al 1997; Mehta et al 2000).

Despite the widespread use of methylphenidate, its precise neurochemical mechanisms of action are still under debate. For the most part, methylphenidate's influence on dopamine neurotransmission is thought to play a crucial role in its behavioral and cognitive actions. The indirect dopamine agonist binds to the dopamine transporter and consequently increases extracellular transmitter levels. Given an estimated ED50 (median effective dose) of 0.25mg/kg for oral methylphenidate, therapeutic drug doses (0.3-0.6mg/kg) can be expected to occupy more than 50% of the dopamine transporter (Volkow et al 1998). Highest specific methylphenidate binding was found in terminal regions of the mesolimbic pathway (caudate-putamen, nucleus accumbens, olfactory tubercle and bed nucleus of the stria terminalis) (Unis et al 1985).

Accordingly, these dopamine-rich subcortical regions have often been hypothesized to mediate the drug's therapeutic actions (Seeman and Madras 2002; Seeman and Madras 1998; Volkow et al 2005). However, next to its dopamine-specific influence, methylphenidate increases extracellular levels of the neurotransmitter noradrenalin by blocking its reuptake (Gatley et al 1996; Kuczenski and Segal 1997). Recent animal studies could show that increased dopamine and noradrenalin efflux is linked to improved cognitive function following therapeutic drug doses (Arnsten and Dudley 2005; Berridge et al 2006). By demonstrating the involvement of noradrenergic neurotransmission and cortical regions in methylphenidate's therapeutic actions, these findings pose a dual challenge to the above hypothesis.

Increases in heart rate and blood pressure are characteristic side effects of methylphenidate after single oral and intravenous drug doses as well as after long-time treatment (Rapport and Moffitt 2002; Turner et al 2003; Volkow et al 2003). Although these cardiovascular drug effects have been linked mainly to the noradrenergic system, changes in striatal dopamine seem to be crucially involved (Volkow et al 2003). Moreover, methylphenidate affects mood and arousal. Generally, the subjective drug effects are believed to be more reliably provoked by large and fast dopamine increases (as after insufflation or intravenous drug administration) (Volkow and Swanson 2003), but nevertheless have been shown to occur after administration of oral drug doses (Chait 1994).

1.5 Grace's tonic/phasic model of dopamine system regulation

After delineating the relationship between the neurotransmitter dopamine and both reward and the indirect agonist methylphenidate, a theoretical concept regarding the functionality of subcortical dopamine release can be presented. This concept is based upon the work of Grace (1991, 1995), who differentiates two independently regulated dopamine releasing processes.

1.5.1 Phasic dopamine stimulation

Phasic release refers to the transient release of dopamine, produced by action potentials of dopamine neurons in response to behaviorally relevant (e.g. rewarding) external stimuli (Miller et al 1981; Schultz 1986; Schultz and Romo 1990). Phasic release is competent to set free dopamine levels in the μM range (May et al 1988). This large amplitude, but brief pulse of dopamine into the synaptic cleft is suggested to activate postsynaptic dopamine receptors and evoke dopamine-dependent behavioral responses (Fibiger et al 1987; Gratton et al 1988). Before phasic dopamine diffuses into the extrasynaptic space, it is rapidly (within seconds) removed from the synaptic cleft by high capacity re-uptake systems (for a review see Grace 1991; Grace 1995).

1.5.2 Tonic dopamine stimulation

Unlike spike-dependent neurotransmitter increases reached within the synaptic cleft, dopamine levels in extrasynaptic fluid appear to range between only 10-50nM (Church et al 1987; Sharp et al 1986). This extrasynaptic neurotransmitter concentration is under strong homeostatic control, as it is maintained even after the 6-hydroxydopamine-induced depletion of up to 80% of striatal dopamine (Abercrombie et al 1990; Robinson and Whishaw 1988). Despite its presence in low concentrations, extrasynaptic dopamine seems to cause a steady-state partial activation of D2-like

dopamine autoreceptors, which are located on dopamine neuron terminals. Any changes in extrasynaptic neurotransmitter levels are detected and counterregulated by these very sensitive autoreceptors. Because of its tight control and slow time course of change, this phenomenon has been labeled tonic dopamine regulation (for a review see Grace 1991; Grace 1995).

Tonic dopamine release is regulated via presynaptic N-methyl-d-aspartate (NMDA) receptors in a spike-independent manner by glutamatergic prefrontal cortical afferents. Although studies have shown a near absence of axo-axonic synapses in the striatum, dopamine terminals are frequently directly apposed to glutamate containing synapses (Bouyer et al 1984; Freund et al 1984). Extrasynaptic concentrations of glutamate are within the ED50 of the NMDA-receptors located at presynaptic dopamine sites (Korf and Venema 1985; Sands and Barish 1989). The dopamine terminal is therefore "bathed" in a cloud of glutamate at low concentrations, which acts on the sensitive presynaptic NMDA autoreceptors without initiating postsynaptic effects.

A quandary arises from the suggested mechanism of tonic dopamine release. If the synaptic terminal is capable of removing micromolar concentrations of dopamine from the synaptic cleft, how would the very low concentrations of glutamate-induced neurotransmitter release escape the reuptake process? One potential explanation is related to the kinetics of the uptake enzyme. In order for an uptake process to rapidly clear a space of large neurotransmitter concentrations, the rate constant of this process (i.e. the Km, defined as the substrate concentration resulting in the half-maximal rate for an enzyme) would have to be quite large. It has, in fact, been estimated to be approximately 100nM for the dopamine transporter. Since the uptake rate is maximally efficient with a substrate concentration above the Km, low tonic dopamine concentrations should be capable of diffusing from the synaptic space in a relatively unhindered fashion (for a review see Grace 1991; Grace 1995).

1.5.3 Tonic dopamine stimulation modulates phasic neurotransmitter release

As illustrated above, the presence of tonic dopamine in the extrasynaptic space provides a background stimulation of the sensitive presynaptic dopamine autoreceptors. Abnormal activation of these autoreceptors will trigger inhibition of neurotransmitter synthesis (Kehr et al 1972) and attenuation of phasic release (Farnebo and Hamberger 1971). In the case of dopamine dysregulation, tonic autoreceptor stimulation can thus be used to induce homeostatic changes. Importantly, by selectively activating corticostriatal glutamatergic afferents, the cortex may be capable of dynamically regulating the relative phasic dopamine responsivity via its actions on tonic dopamine levels. This configuration would be consistent with experimental evidence suggesting a suppressive influence of the frontal cortex on subcortical dopamine systems (for a review see Grace 1991; Grace 1995).

1.6 Study rationale

The basic objective of this work was the design of an indirect measurement method, which would allow assessing behavioral correlates of dopamine regulation. As a basal measure we registered the participants' performance in a card sorting task known to require prefrontal executive functions. The task included a monetary reward component, which was hypothesized to directly stimulate midbrain dopamine activity. In order to provoke an increase in tonic neurotransmitter levels, the indirect dopamine agonist methylphenidate was administered. By this means, both mechanistic processes of subcortical dopamine release suggested in Grace's tonic/phasic model of dopamine system regulation were manipulated independently of each other. We could thus investigate how performance was influenced by monetary reward, methylphenidate and the combination of both stimulators. Moreover, we analyzed differences in responsivity patterns as a

function of two selected, dopamine-associated variables: parental care experiences in early life and the personality dimension of Novelty Seeking.

In the upcoming empirical chapters, we will initially report on the investigation of different behavioral responsivity patterns to reward and methylphenidate as a function of parental care experiences in early life. Subsequently, we will report on how reward and methylphenidate interact on a behavioral level. The influence of personality on behavioral responsivity to reward and methylphenidate will be subject of the third empirical chapter. Since all the collected data stem from the same participant sample, we will eventually describe and discuss how parental care and personality interact to influence behavior.

2 EMPIRICAL STUDY

2.1 Behavioral response to methylphenidate challenge: Influence of early life parental care

2.1.1 Summary

Poor family environment in early life is a risk factor for multiple forms of mental health disorders. In the animal model, manipulations of pup-dam interactions alter the regulation of the pup's mesocorticolimbic dopamine system. Findings regarding behavioral responsivity to dopamine stimulation after early maternal deprivation are inconsistent. In human research, adults reporting low maternal care experiences in early life have been shown to exhibit heightened stress-induced mesoaccumbens dopamine release. The present investigation explored the relationship between quality of parental care and the behavioral response to reward and a low therapeutic (20mg) dose of the indirect dopamine agonist methylphenidate. 43 male university students accomplished a card-sorting task involving a monetary reward component in a double-blind, placebo-controlled crossover design. After methylphenidate challenge, participants with high parental care experiences featured impaired performance accuracy in the reward condition of the utilized task. The reward-induced performance accuracy of low parental care participants was contrarily improved. Other than expected, activity after either reward, methylphenidate or the combination of both stimulators was not influenced by parental care. We conclude the distinct drug responsivity patterns across parental care groups to be mediated by prefrontal (cognitive) rather than striatal (activity-related) drug effects. Altogether, this is the first human study to show that the behavioral response to methylphenidate challenge interacts with parental care experiences during critical developmental periods.

2.1.2 Introduction

Early family adversity may profoundly influence mental health throughout lifetime (Bifulco et al 1991; Brown and Anderson 1991; Deminiere et al 1989). Experiences of abuse or emotional neglect represent exceptionally severe forms of early family adversity. However, already subtle variations in the perception of parental love and care during critical development periods may have substantial effects on physical and mental wellbeing across adulthood. In the 35-year follow-up of the Harvard Mastery of Stress Study, Russek and Schwartz (1997) found that individuals who had rated their parents as less caring while in college were more likely to suffer from chronic illness, including alcoholism and drug abuse, in midlife. Low parental care has also been identified as a risk factor for an early onset of substance use among adolescents (Gerra et al 2004) and has been associated with an increased incidence of mood and anxiety disorders (Carter et al 2001; Overbeek et al 2004; Parker et al 1995; Silove et al 1991).

Modification of central dopamine regulation represents one potential mechanism by which exposure to adverse developmental conditions might promote psychopathological vulnerability. The neurotransmitter dopamine plays an essential role in the processing of rewarding stimuli. Dopamine is released in response to natural rewards and most drugs of abuse, and midbrain dopamine activity both reinforces and elicits approach behavior towards saliency (Ikemoto and Panksepp 1999; Schultz 2002). Dysregulations within nigrostriatal and mesocorticolimbic dopamine transmission and associated alterations of reward sensitivity are involved in the psychopathology of addiction and ADHD (Blum et al 2000; Sonuga-Barke 2005).

In the animal model, manipulations of pup-dam interactions exert long-lasting effects on central dopaminergic functioning. Naturally occurring variations in maternal care (licking and grooming) have been shown to determine a right-hemispheric blunting of the medial prefrontal cortex

dopamine stress response (Zhang et al 2005). Adult rats reared under the constraint of repeated maternal separation or social isolation exhibited decreased basal dopamine turnover in the medial prefrontal cortex (Heidbreder et al 2000; Matthews et al 2001), increased basal dopamine levels in the nucleus accumbens (Hall et al 1998), as well as increased nucleus accumbens dopamine responsivity to both pharmacological challenge (Hall et al 1998; Hall et al 1999a) and acute stress (Brake et al 2004; Fulford and Marsden 1998). Behavioral findings, which mainly focus on locomotor activity, are contradictory since different study protocols determine opposite effects. Following maternal separation, adult female rats showed delayed acquisition of conditioned locomotor response to food presentation (Matthews et al 1996b) and attenuated locomotor response to a low dose of d-amphetamine (Matthews et al 1996a). In studies administering relatively higher drug doses, maternal separation was also found to induce hyperactivity to cocaine (Brake et al 2004; Kikusui et al 2005). Following social isolation, rodents developed enhanced locomotor response to conditioned reward (Jones et al 1990) and d-amphetamine (Hall et al 1998; Kehoe et al 1998).

There is only one human study available, which explored the link between early life parental care and dopamine regulation. In line with preclinical data, this PET study revealed that adults with self-reported low maternal care experiences exhibited elevated nucleus accumbens dopamine release in response to a psychosocial stress task when compared to a "high care" group (Pruessner et al 2004). The present investigation explored the relationship between quality of self-reported early life parental care and behavioral responsivity to reward and pharmacological dopamine stimulation in healthy male young adults. We examined performance in a card-sorting task involving a monetary reward component following a low therapeutic (20mg) oral dose of the indirect dopamine agonist methylphenidate. Both reward (Knutson et al 2001a; Koepp et al 1998;

Schultz 2002) and methylphenidate (Arnsten and Dudley 2005; Berridge et al 2006; Volkow et al 1998) have been shown to trigger striatal and prefrontal dopamine release. Our objective of applying two different dopamine stimulators was to maximize dopamine neurotransmission without provoking drug-induced ceiling effects in performance. Moreover, we were interested in the examination of how reward and treatment would interact on a behavioral level (see next investigation). Participants were screened for parental care measured by the Parental Bonding Inventory (PBI; Parker et al 1979) and assigned to either a high or low care group.

It is difficult to generate a directional hypothesis regarding the activity-related effects of reward and methylphenidate. Generally, the stimulation of striatal dopamine produces changes in activity. The effect of methylphenidate on activity is however biphasic. At higher doses the drug induces hyperactivity (Hughes and Greig 1976; Scheel-Kruger 1971). At low therapeutic doses it reduces activity in hyperactive animals (Luthman et al 1989; Steiner et al 1986) and ADHD patients (Solanto 1984; Solanto 1986). The situation is further complicated by the discrepancy of previous animal research as to whether maternal deprivation caused decreased or increased locomotor responsivity to dopamine stimulation. Thus, assuming a link between parental care and striatal dopamine levels, we expected differential activity responses to reward and methylphenidate depending on early life parental care. However, no directional hypothesis was specified.

Since methylphenidate not only influences activity, but also improves executive function in patients with ADHD (Faraone et al 2004; Mehta et al 2004; O'Driscoll et al 2005) and healthy adults (Camp-Bruno and Herting 1994; Cooper et al 2005; Mehta et al 2000), we further expected an altogether positive drug effect on cognitive performance in the utilized task. An inverted-u shaped function has been shown to describe the relationship between stimulation of prefrontal D1

dopamine receptors and cognitive effects (Arnsten and Dudley 2005; Granon et al 2000). In accordance, a recent animal study directly linked prefrontal dopamine efflux after low methylphenidate doses to improved cognitive performance (Berridge et al 2006). Thus, assuming decreased prefrontal dopamine levels after maternal deprivation, we additionally expected the low care group to have a greater cognitive benefit from methylphenidate challenge.

2.1.3 Materials and Methods

2.1.3.1 Participants

We recruited male university students by posting ads on the electronic billboard of McGill University. Upon initial contact, 258 subjects were asked to complete the PBI (Parker et al 1979), 43 of those met our inclusion criteria (which were based upon Parker's cut-off scores for high and low parental care, see below) and were enrolled in the study. Scores of parental overprotection, which are also assessed by the PBI, were controlled for across the treatment by parental care groups (see below). Participants had a mean age of 22.2 years (SD 2.07). The study was approved by the local Research Ethics committee. All procedures were carried out with the adequate understanding and written consent of the participants.

2.1.3.2 Study design and procedure

The study consisted of a total of three visits. During their first visit, participants completed a set of psychological questionnaires (see below) and performed a short psychiatric interview (First et al 1990) as well as a detailed physical examination including blood and urine tests to verify the absence of psychiatric and medical conditions. A double-blind, randomized placebo-controlled crossover design was applied such that in each parental care group one half of the participants

received methylphenidate on the first and placebo on the second testing day, the other half received the reciprocal order of treatments (Table 1). The testing sessions were conducted between 0900h and 1400h with the two appointments separated by three to fourteen days (mean interval between the first and second session was six days). Participants were asked to abstain from recreational drug intake for at least three days prior to testing, from alcohol consumption the evening before, from eating and drinking coffee within three hours and from smoking within the hour of testing (smoking habits, alcohol and recreational drug use were controlled across the treatment by parental care groups, see below). An oral methylphenidate dose of 20mg (2x10mg) was administered. While being sufficiently low to prevent behavioral ceiling effects, a 20mg dose is clinically relevant and has been shown to induce significant cognitive effects in healthy volunteers (Camp-Bruno and Herting 1994; Elliott et al 1997). As both peak brain uptake and maximal behavioral effects can be expected approximately 60 to 120 minutes following drug ingestion (Shaywitz et al 1982; Volkow et al 1998), testing proceeded 80 minutes after treatment. Blood pressure and heart rate were monitored at 5 minutes pre-, as well as 70 and 120 minutes post-treatment to control for methylphenidate's cardiovascular influence. Succeeding each cardiovascular measurement, participants were asked to complete a set of Visual Analogue Rating Scales to examine drug effects on mood and arousal.

Table 1. Study design: distribution of participants to treatment by parental care groups. MPH 1: methylphenidate challenge in session 1; MPH 2: methylphenidate challenge in session 2.

	High care		**Low care**	
	MPH 1	**MPH 2**	**MPH 1**	**MPH 2**
Number of participants	10	13	11	9

2.1.3.3 Measurements

2.1.3.3.1 The Parental Bonding Inventory

The PBI (Parker et al 1979) is a standard instrument to retrospectively measure parental educational style. The 48-items questionnaire assesses care and overprotection received independently from mother and father during the first 16 years of life, resulting in four subscales (mother care, mother overprotection, father care, father overprotection). The subscales can be combined to generate four types of parental bonding: high care – low overprotection (conceptualized as optimal bonding), low care – low overprotection (conceptualized as absent or weak bonding), high care – high overprotection (conceptualized as affectionate constraint) and low care – high overprotection (conceptualized as affectionless control). Parker suggests the assignment to "high" or "low" categories based on cut-off scores of 27 and 24 for mother and father care and 13.5 and 12.5 for mother and father overprotection, respectively. Long-term stability of the PBI was demonstrated in a 20-year follow-up study on the perception of parenting in a non-clinical sample (Wilhelm et al 2005). Further evidence for test-retest reliability emerges from clinical studies in depressed patients, whose PBI scores proved to be stable despite significant changes in levels of depressed mood (Lizardi and Klein 2005).

2.1.3.3.2 Control variables

We performed the Mini Structural Clinical Interview (First et al 1990), a semi-structured diagnostic interview based on the Diagnostic and Statistic Manual of Mental Disorders, to verify the absence of psychiatric conditions in our participant sample. A survey assessing stress events during childhood and adolescence was applied to avoid confounding effects of parental care and childhood trauma. The overprotection scale of the PBI (Parker et al 1979) assessed self-reported

overprotection participants received independently from their mothers and fathers during their first 16 years of life. The Alcohol Dependence Scale (ADS; Horn et al 1984) and the Drug Abuse Screening Test (DAST; Skinner 1982) provided information on alcohol dependence and drug abuse. All participants were asked to specify the last time they had used recreational drugs within the past three months, and smokers were asked to specify their daily average number of cigarettes.

2.1.3.3.3 The monetary reward task

A computerized monetary reward task was developed based upon the Wisconsin Card Sorting Test (WCST; Grant and Berg 1948; Heaton et al 1993), using an Apple Macintosh™ computer and the program SuperCard (Solutions EtCetera, Pollock Pines, CA, USA) (Pruessner 2004) As in the WCST, stimulus- and response cards depicted figures of varying forms (triangles, stars, crosses, circles), colors (red, green, yellow, blue) and numbers of figures (one, two, three, four). Four stimulus cards, representing the following characteristics, were permanently displayed on the top of the screen: one red triangle, two green stars, three yellow crosses and four blue circles (Figure 2). One at a time, the 64 response cards were produced in randomized succession and had to be matched with one of the aforementioned stimulus cards. Participants received immediate feedback whether a match was correct, but were not told the selected matching rule (which was always either color, figure or number of figures). After a randomized number of correct responses (ranging between a minimum of three and a maximum of eight) the rule changed, requiring the participant to develop a new matching strategy. Matching rules were selected in randomized sequence, except that the same rule was never presented consecutively. Pace of card presentation was determined by the participant's reaction speed. Overall playtime amounted to 15 minutes,

composed of a 5-minute practice phase followed by 5 minutes of non-rewarded and 5 minutes of rewarded playing. During the rewarded phase of the game participants gained 0.10$ for every correct response; the total reward (which could amount up to 12$) was displayed in a feedback window.

The WCST is considered a measure of executive function, requiring strategic planning, organized searching, cognitive set shifting, goal directed behavior and the modulation of impulsive responding (Heaton et al 1993). Because of its sensitivity to the effects of frontal lobe lesions, the WCST is often referred to as a measure of prefrontal functioning. With regard to the development of the current paradigm, we were specifically interested in assessing how the activation of dopamine neurotransmission (by means of monetary reward and methylphenidate challenge) would influence performance in a WCST-like task. The program registers the numbers of total, correct and incorrect responses and the ratio of correct to total responses (the success rate). The number of total responses is determined by overall activity in response to the task. The number of correct responses is determined by the numbers of total and incorrect responses: once a matching rule has been discovered, a correct response only requires the application of the discovered rule. Thus, the higher total and the lower incorrect responding, the more correct responses will be achieved throughout the game. The number of incorrect responses is reflective of cognitive performance: whenever a matching rule has changed, the information about the last valid matching rule must be combined with the information of at least one to three subsequent responses to detect the new matching rule. Eventually, the success rate represents an additional indicator of performance accuracy, which is independent of overall response-activity. Our objective was to construct a task that was sufficiently challenging for a population of university

students to prevent ceiling effects in performance, yet allowed for an output increase in the reward condition.

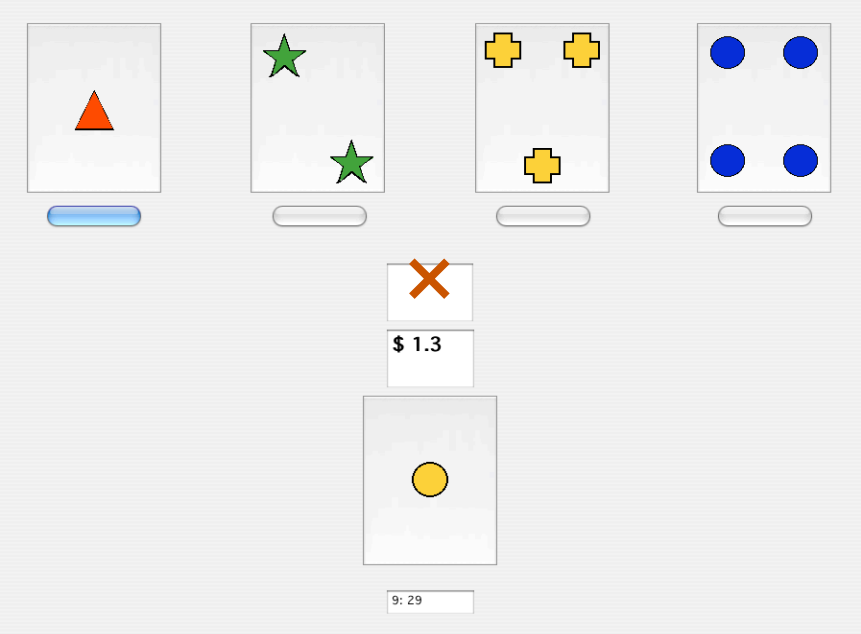

Figure 2. The monetary reward task.

2.1.3.3.4 Visual Analogue Rating Scales

Methylphenidate-induced changes in ratings of mood and arousal were assessed using Visual Analogue Rating Scales (Norris 1971). Participants indicated the point that best represented the perception of their current state on 100mm horizontal lines anchored by word descriptors at each end. The following 16 descriptor pairs were listed: alert/drowsy, excited/calm, strong/feeble, clear headed/fuzzy, well coordinated/clumsy, energetic/lethargic, contented/discontented, tranquil/troubled, quick witted/mentally slow, relaxed/tense, attentive/dreamy,

proficient/incompetent, happy/sad, amicable/antagonistic, interested/bored and gregarious/-withdrawn.

2.1.3.3.5 Psychological variables

Given the effect of parental care on several mental disorders, participants were asked to complete a selection of psychological questionnaires. The Beck Depression Inventory (BDI; Beck 1987) was used to assess characteristic attitudes and symptoms of depression. The Rosenberg Self-Esteem Scale (RSES; Rosenberg 1989) and the General Self-Efficacy Scale (GSES; Jerusalem and Schwarzer 1992) were administered to evaluate self esteem (the sense of one's value) and self efficacy (the sense of personal competence in stressful situations), respectively. The state and trait scales of the State-Trait Anxiety Inventory (STAI; Spielberger 1983) aimed at the participants' recent and general tendency to respond with anxiety to perceived stress.

2.1.3.4 Data analysis

2.1.3.4.1 Data manipulation

The succession of non-reward and reward conditions in the utilized card-sorting task was not randomized (non-reward always preceded reward). It was therefore necessary to adjust the reward effect for a practice effect. To calculate the practice effect, the 5-minutes non-reward and reward conditions were each divided into two phases of 2 minutes and 30 seconds and the numbers of total, correct and incorrect responses and the success rate were assessed in every phase. Practice-induced changes were then calculated by subtracting performance in phase 1 from performance in phase 2 for non-reward and reward conditions separately. As a last step, the practice-induced mean performance changes achieved across phases 1 and 2 of the non-reward

condition were added to the original scores of the non-reward condition and the practice-induced mean performance changes achieved across phases 1 and 2 of the reward condition were subtracted from the original scores of the reward condition. Importantly, this manipulation of the data influenced only the main effect of reward. All interactions between reward and treatment or between care, reward and treatment were not affected.

In order to illustrate this procedure, we created an artificial data set for a 2x4 mixed factorial design using an Apple Macintosh™ computer and the program MATLAB 7.0 (The MathWorks Inc., Natick, MA, USA). Normal distributions of random numbers varying around a fixed value for each cell of the design matrix were generated (see Pruessner et al. 2003 for a description of the general procedure). The dataset was designed such that two groups (each with n=100) were assigned four conditions representing two subsequent phases of a non-reward and a reward condition, respectively. The dependent variable was labeled “number of total responses”.

2.1.3.4.2 Main analysis

This study followed a placebo-controlled, counterbalanced crossover design. Crossover designs bear the potential problem that practice may confound the interpretation of drug effects (Elliott et al 1997). In an initial analysis we therefore examined the practice effect across testing sessions using two-tailed paired sample t-tests for behavioral data (the numbers of total, correct and incorrect responses and the success rate averaged over non-reward and reward conditions). We additionally examined the influence of the time-point of drug administration on the behavioral drug effect using two-way mixed ANOVAs with the between-subjects factor “time-point of drug administration” (methylphenidate in session 1 vs. session 2) and the within-subjects factor “treatment” (placebo vs. methylphenidate).

Eventually, independent analyses were performed for the two testing sessions using separate three-way mixed ANOVAs with the between-subjects factors “care” (high vs. low) and “treatment” (placebo vs. methylphenidate) and the within-subject factors “reward” (non-reward vs. reward) for behavioral data, respectively “time” (-5 vs. +70 and +120 minutes) for subjective and cardiovascular data. Psychological variables (mood, self-esteem, self-efficacy and anxiety) across parental care groups were analyzed using one-way independent ANOVA. Significant interactions were further investigated using simple effects analyses or two-tailed Bonferroni-corrected paired sample t-tests. The relationship between parental care and psychological measures was additionally examined using Pearson’s correlation method. Post-hoc effect size analyses were performed according to the formula provided by Cohen (1988).

2.1.4 Results

2.1.4.1 Artificial data set

Figure 3 shows the group means and standard errors for non-reward and reward conditions and for each phase within non-reward and reward conditions. According to the above delineated procedure, practice effects of 9.36 and 8.34 total responses across phases 1 and 2 were calculated for non-reward and reward conditions, respectively. The score 9.36 was added to the original score of the non-reward condition and the score 8.34 was subtracted from the original score of the reward condition. The such corrected means are also presented in figure 3.

Before correcting for the practice effect, we found main effects of reward ($F_{(1,198)}$=65.50, p<.000) and group ($F_{(1,198)}$=13.33, p<.000) and an interaction of reward and group ($F_{(1,198)}$=7.98, p=.005). After correcting for the practice effect, the main effect of reward was no longer existent

($F_{(1,198)}$=1.38, p=.242), whereas the main effect of group ($F_{(1,198)}$=13.33, p<.000) and the reward x group interaction ($F_{(1,198)}$= 7.98, p=.005) remained unchanged.

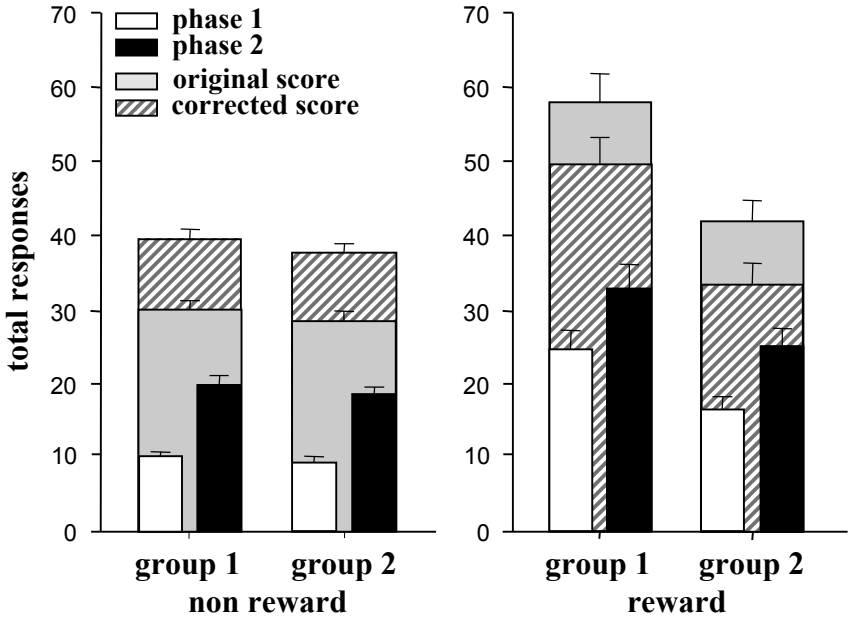

Figure 3. Artificial data set: means and standard errors for the original scores, the original scores divided into two practice phases each and the practice-corrected scores across groups and reward conditions.

2.1.4.2 Control variables

None of the participants had a current psychiatric disorder, had ever been diagnosed with ADHD or had taken methylphenidate for therapeutic purposes. Moreover, none of the participants had experienced severe childhood maltreatment (sexual, physical, emotional abuse or emotional neglect). Treatment by parental care groups were matched in terms of self-reported overprotection, weight and sociodemographic variables (age, level of education). Smoking habits (number of smokers per group and number of cigarettes per smoker), habitual alcohol

consumption (ADS scores) as well as recent and habitual recreational drug use (DAST scores) did not differ across treatment by parental care groups (Table 2).

Table 2. Control variables: means and standard deviations for control variables in the treatment by parental care groups. MPH 1: methylphenidate challenge in session 1; MPH 2: methylphenidate challenge in session 2.

	High care		**Low care**	
	MPH 1	**MPH 2**	**MPH 1**	**MPH 2**
Number of participants	10	13	11	9
Mean parental overprotection score	12.35	10.27	13.68	14.28
	(9.62)	(6.39)	(9.16)	(6.59)
Weight in pounds	174.00	160.08	165.64	161.67
	(24.24)	(16.21)	(25.92)	(24.20)
Age	22.40	21.85	22.36	22.22
	(1,71)	(2,12)	(2.94)	(1.20)
Number of smokers	2	2	3	2
Cigarettes/day	3.70	2.08	1,27	2.44
	(8.38)	(5.40)	(2,37)	(5.25)
Alcohol Dependence Scale score	5.80	6.45	4,18	3.71
	(3.99)	(5.52)	(3.16)	(2.43)
Drug Abuse Screening Test score	1.20	1.18	1.27	1.43
	(1.23)	(1.33)	(0.90)	(1.81)
Number of participants using recreational drugs (past 3 months)	5	5	5	4

2.1.4.3 Behavioral variables

Justifying the calculation of two independent analyses for the two testing sessions, initial data analysis revealed a strong positive main effect of practice for the numbers of total responses ($t_{(42)}$=-8.73, p<.000), correct responses ($t_{(42)}$=-6.88, p<.000) and the success rate ($t_{(42)}$=-3.16, p=.003) across testing sessions 1 and 2. Moreover, the time-point of drug administration significantly influenced methylphenidate's behavioral effect (interaction of treatment and time-point of drug administration for the number of total responses: $F_{(1,41)}$=78.05, p<.000, correct responses: $F_{(1,41)}$=46.56, p<.000 and the success rate: $F_{(1,41)}$=9.74, p=.003). With methylphenidate administration in the first testing session, the number of total and correct responses and the

success rate were decreased in the drug as compared to the placebo condition. With methylphenidate administration in the second testing session, the drug's performance-decreasing effect was superposed by the practice effect, such that the number of total and correct responses and the success rate were increased in the drug as compared to the placebo condition.

2.1.4.3.1 Behavioral variables in session 1

The data describing the behavioral responses of the total participant sample to reward, methylphenidate and their interactions will be reported in chapter 2.2.4.2 of the next investigation. Briefly, reward consistently improved performance in the first testing session. During rewarded as compared to non-rewarded playing, the number of correct responses was increased, whereas the number of incorrect responses was decreased. Consequently, reward increased the success rate. There was a reward by treatment interaction, such that methylphenidate lowered the reward-induced rise in performance to the non-reward level by significantly decreasing the number of correct responses and the success rate, and marginally decreasing the number of total responses, respectively increasing the number of incorrect responses achieved with reward. Methylphenidate thus equalized non-reward and reward-related performance.

When investigating the influence of parental care in the first testing session, we found a three-way interaction of reward, treatment and care, which was significant for the number of incorrect responses ($F_{(1,39)}$=9.28, p=.004, f^2=.10, ω^2=.09; Figure 4) and marginal for the success rate ($F_{(1,39)}$=3.82, p=.058, f^2=.03, ω^2=.03; Figure 5). Simple effects analyses calculating the treatment effect across both parental care groups for reward and non-reward conditions separately revealed that in the reward condition, participants from the high care group increased their number of

errors ($F_{(1,39)}$=6.24, p=.017) and consequently decreased their success rate ($F_{(1,39)}$=6.75, p=.013) after methylphenidate as compared to placebo administration. Reward-induced performance accuracy (incorrect responding and success rate) in the low care group was not significantly affected by methylphenidate challenge. In the non-reward condition, methylphenidate as compared to placebo administration had no significant performance effect on either the high or the low care group. Considering that the overall drug effect manifested itself by inhibiting the reward-induced performance improvement, it is of interest to additionally observe how methylphenidate's influence on reward responsivity differed across parental care groups. We therefore calculated paired samples t-tests (Bonferroni-corrected _-level = .0125) for the reward effect across all four possible combinations of treatment and care. Participants from the high care group significantly decreased their number of incorrect responses ($t_{(12)}$=4.00, p=.002) and increased their success rate ($t_{(12)}$=-3.98, p=.002) in the reward as compared to the non-reward condition after placebo administration. After methylphenidate administration, errors ($t_{(9)}$=-1.43, p>.180) and success rate ($t_{(9)}$=0.74, p>.400) remained unchanged with reward. Participants from the low care group exhibited the almost opposite drug responsivity pattern. The number of incorrect responses decreased ($t_{(10)}$=3.84, p=.003) and the success rate increased ($t_{(10)}$=-2.42, p=.036) in the reward as compared to the non-reward condition after methylphenidate administration. After placebo administration the number of errors remained unchanged ($t_{(8)}$=1.55, p>.150), whereas the success rate nevertheless increased with reward ($t_{(8)}$=-2.42, p=.042). It must be noted that the rises in success rate achieved in the low care group were not statistically significant considering the Bonferroni-corrected _-level of .0125. Altogether, reward-induced performance in the high care group was explicitly deteriorated (correct responses were replaced by incorrect responses) with methylphenidate. The low care group contrarily exhibited a trade-off between correct and incorrect responding with the drug.

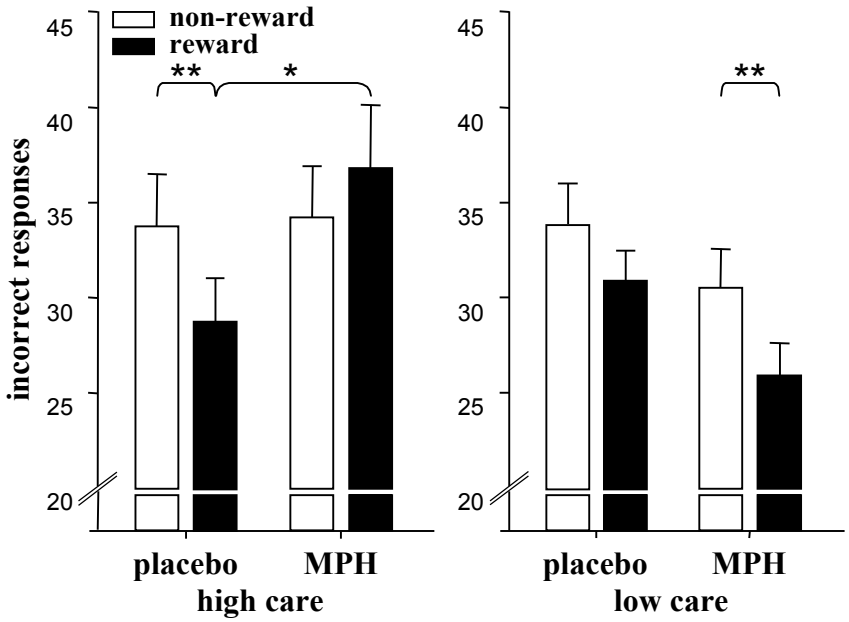

Figure 4. Means and standard errors for the interaction of reward, treatment and parental care for the number of incorrect responses in session 1. MPH: methylphenidate. *p<.05; **p<.0125.

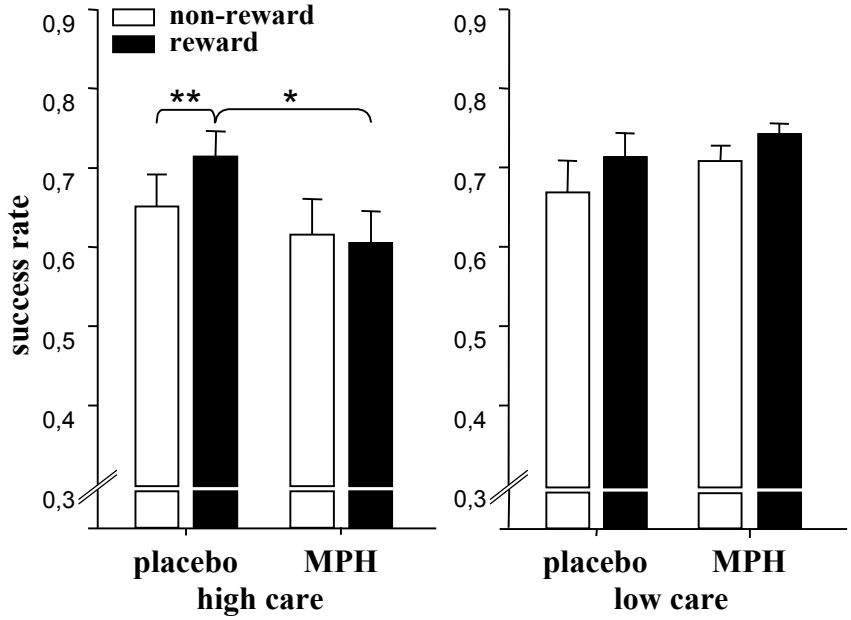

Figure 5. Means and standard errors for the interaction effect of reward, treatment and parental care for the success rate in session 1. MPH: methylphenidate. *p<.05; **p<.0125.

2.1.4.3.2 Behavioral variables in session 2

In the second testing session reward no longer improved overall performance. Contrarily, the numbers of total and incorrect responses were increased and the success rate decreased in the reward as compared to the non-reward condition. Methylphenidate had no influence on overall performance. Since no interaction of treatment and parental care emerged, data will be reported in chapter 2.2.4.2 of the next investigation.

2.1.4.4 Subjective and cardiovascular variables

Measures of mood and arousal were increased after methylphenidate as compared to placebo administration in both testing sessions. Heart rate was increased with methylphenidate in the second testing session. Since no interaction of treatment and parental care emerged, data will again be reported as part of the next investigation (see chapters 2.2.4.3 and 2.2.4.4).

2.1.4.5 Psychological variables

In the calculated one-way independent ANOVA, parental care had no effect on the assessed psychological measures. We took a closer look at the relationship between care and psychological measures in an additional correlation analysis. Both mother and father care turned out to be positively associated with RSES self esteem (mother care: r=.35, p=.028; father care: r=.32, p=.044) and negatively associated with STAI trait anxiety (mother care: r=-.47, p=.002; father care: r=-.44, p=.005) scores. Furthermore a positive correlation between mother care and GSES scores (r=.38, p=.018) and marginal negative correlations between mother care and BDI (r=-.32, p=.051) as well as state anxiety (r=-.31, p=.055) scores emerged.

2.1.5 Discussion

Goal of the present investigation was to examine behavioral responsivity to monetary reward and pharmacological dopamine stimulation depending on the quality of early parent-child relationships. Results showed that in the first testing session monetary reward improved performance in the utilized card-sorting task. Parental care groups did not differ in their overall reward response. Likewise independent of parental care, a low therapeutic (20mg) methylphenidate dose equalized non-reward and reward-related performance by decreasing the number of total and correct responses achieved in the reward condition. Diverging drug responsivity was found in high and low parental care groups regarding reward-induced performance accuracy (the number of incorrect responses and the success rate). Comparing the treatment effect across parental care groups in the reward condition showed that methylphenidate impaired performance accuracy in the high care participants, but failed to exert a significant influence in the low care participants. Additional comparisons of the reward effect across all four combinations of treatment and care showed that methylphenidate inhibited the reward-induced rise in performance accuracy in the high care participants, but improved it in the low care participants.

What do these results imply with regard to our hypotheses? Based on previous animal research, we had expected differential activity in response to reward and methylphenidate depending on early life parental care. This hypothesis was not confirmed: the number of total and correct responses after reward, methylphenidate and their combination did not differ across high and low care groups. We had further expected the low parental care group to have a greater cognitive benefit from methylphenidate challenge. This hypothesis was confirmed: the inhibition of reward-induced correct responding emerged in a different context in the two care groups. In the

high care group, correct responses were replaced by incorrect responses. In the low care group, a trade-off between correct and incorrect responses took place.

By means of the present study, we can only speculate on the underlying neurochemical mechanisms of the delineated results. Regarding stimulation-induced activity, the lack of differential patterns of total responding following either reward, methylphenidate or their combination suggests that there was no behaviorally relevant difference in striatal dopamine levels and/or receptor stimulation across parental care groups. Regarding the cognitive effects of methylphenidate challenge, impairment as found in the high parental care group contradicted our expectation with regard to the drug's general effect. Interestingly, previous studies have not consistently determined a positive methylphenidate impact on performance either. Both the lack of an effect (Bray et al 2004; Turner et al 2003) and performance decrement (Elliott et al 1997) have been observed in healthy adults after 20mg drug doses. Elliott et al. suggested that methylphenidate's deteriorating effect might be related to the time-point of its administration, such that in novel situations the drug facilitates cognitive performance, whereas an impulsivity-increasing effect dominates in familiar situations. This explanation cannot account for our results, which were obtained in a novel situation. Considering the inverted-u shaped relationship between stimulation of prefrontal dopamine receptors and cognitive performance (Arnsten and Dudley 2005; Berridge et al 2006; Granon et al 2000), it is however possible that high care participants functioned on an optimal prefrontal dopamine level in the absence of methylphenidate. Given the combined impact of reward and the drug, the peak of optimal stimulation might have been exceeded, thus determining impaired performance accuracy. In contrast, the methylphenidate response as found in the low parental care group was concordant with our expectation of the drug's general effect on cognitive performance. Again considering the inverted-u shaped

relationship, the low care participants might have functioned on a relatively decreased prefrontal dopamine level in the absence of methylphenidate. Drug challenge might have consequently raised prefrontal dopamine closer to the peak of the inverted u-function, thus determining improved performance accuracy.

In the second testing session, behavioral responses to monetary incentive and methylphenidate challenge changed considerably. For one thing, reward no longer improved performance. Contrarily, total and incorrect responses were increased and the success rate was decreased with reward. We suggest this lack of reward-induced performance improvement to be due to a ceiling effect for the number of correct responses. In order to increase correct responding after the non-reward condition, participants might have had to increase total responding, which, in turn, might have caused an increase in impulsivity (see chapter 2.2.5 of the next investigation for a more detailed discussion). Secondly, methylphenidate no longer affected reward-related performance when administered in the second testing session. The three-way interaction of reward, treatment and parental care was consequently not replicated in the cross over trial. This lack of behavioral drug effect might have originated from changes in reward perception. Participants were familiar with the testing situation, they had discovered the basic principle of the task and knew the approximate amount they would be able to win. The reward had therefore lost its unpredictability, which is thought to be an essential condition for phasic dopamine cell firing (Schultz 2002). Accordingly, it has been repeatedly demonstrated that dopamine neurons are activated by rewards only during the learning phase but that they stop responding after full acquisition of various reward-delivering tasks (Ikemoto and Panksepp 1999; Schultz 2002). Altogether, the fact that learning drastically influenced methylphenidate's performance effect indicates a limited suitability of the utilized task for repeated measures designs.

Methylphenidate consistently increased measures of mood and arousal across both testing sessions. The question arises, why these subjective drug effects were not differentially influenced by parental care given that the dopamine-containing brain regions involved in their mediation (Udo de Haes et al 2005; Volkow et al 1999) correspond to the brain regions affected by early rearing experiences. One explanation could be that the stimulated changes in mood and arousal were not sufficiently strong to reveal potential differences between care groups. Since, generally, the reinforcing effects of methylphenidate are believed to be more reliably provoked by large and fast dopamine increases mimicking phasic dopamine firing (Volkow and Swanson 2003), the detection of group differences may require intravenous injection, smoking or insufflation of the drug.

Surprisingly, in the calculated ANOVA, parental care had no effect on any of the assessed psychological symptoms. In the study conducted by Pruessner et al. (2004) a main effect of maternal care on self-esteem and trait anxiety had contrarily been detected. However, we found significant bivariate correlations between both maternal and paternal care scores and psychological measures, whereby maternal care exerted the stronger influence. Since experimental group selection in the Pruessner study considered only the maternal care score of the PBI, a generally stronger influence of the mother as an attachment figure could explain the incongruity between the studies. In accordance with this hypothesis, several investigations have pointed out that parenting experiences with one's mother were more consistently associated with adult mental disorders (Enns et al 2002; Enns et al 2000). We can conclude from the non-significant ANOVA results that the behavioral differences determined across parental care groups were independent of the assessed psychological symptoms.

It needs to be critically addressed that, next to increasing brain dopamine levels, methylphenidate increases levels of the neurotransmitter noradrenaline by blocking its re-uptake (Kuczenski and Segal 1997), an action which is especially prominent in the dopamine transporter-poor prefrontal cortex and was shown to be involved in the drug's cognitive effects (Arnsten and Dudley 2005; Berridge et al 2006). Yet, as we found methylphenidate to selectively influence reward responsivity and specifically dopamine neurotransmission has been implicated in the processing of reward (Ikemoto and Panksepp 1999; Schultz 2002), we conclude methylphenidate's performance effects to be primarily dopamine-dependent. Since this study employs only indirect, behavioral measures of dopamine activity, the influence of alternative neurotransmitter systems can, however, not be ruled out. Another point of criticism relates to the utilized card-sorting task. Due to the applied modifications, our task – as opposed to the WCST – did not allow the distinction between different types of errors (failure to maintain set, perseverative and non-perseverative errors). With this additional information it would have been possible to determine the cognitive influence of methylphenidate more precisely. Eventually, our practice effect adjustment did not account for the possibility of a non-linear practice effect. In future applications of the task, the succession of non-reward and reward conditions should be changed systematically.

In summary, we could show, for the first time in humans, that the behavioral response to methylphenidate challenge interacts with parental care experiences during critical developmental periods. We could thus extend the observation of an elevated nucleus accumbens dopamine stress response in relation to low early life maternal care (Pruessner et al 2004). Other than expected, behavioral differences in the drug response seemed to be mediated by prefrontal (cognitive) rather than striatal (activity-related) drug effects. The presented findings imply some intriguing

clinical hypotheses. Considering the relationship between low parental care experiences and substance ab/use (Gerra et al 2004; Russek and Schwartz 1997), it may be speculated whether the quality of behavioral drug responses constitutes a predictor for future vulnerability to abuse, as has been shown for the magnitude of the initial positive subjective response to cocaine (Davidson et al 1993). Moreover, considering that the methylphenidate response as found in the low care group is reminiscent of what would be anticipated in ADHD patients following a 20mg drug dose (reduced activity and improved performance accuracy in response to saliency), the question arises whether similar neurochemical dysregulations may exist in both. Given the inability to replicate the critical three-way interaction of reward, treatment and parental care in the cross over trial of this study, more research is however needed to ascertain the clinical significance of our findings. Future studies should examine whether the association of parental care and performance can be found in an independent cohort (preferably using a task, which is less sensitive for learning effects) and whether this association generalizes to other psychostimulant drugs.

2.2 Methylphenidate modulates the behavioral response to monetary reinforcement

2.2.1 Summary

Methylphenidate is a central nervous system stimulant used in the treatment of ADHD. Regarding methylphenidate's therapeutic action, research emphasizes its capacity to block dopamine transporters, thereby increasing extracellular dopamine levels. However, discordance exists regarding the exact mechanism of drug action. Seeman and Madras (2002, 1998) propose that consequent to elevating tonic dopamine levels in striatal brain regions, therapeutic doses of methylphenidate inhibit stimulus-triggered phasic transmitter release, leading to reduced postsynaptic receptor stimulation and psychomotor activation. Volkow et al. (2005) hypothesize methylphenidate to amplify stimuli-induced striatal dopamine increases in magnitude and duration, thus enhancing the stimuli's salience value and driving attention. To validate these hypotheses, we investigated the effect of a low therapeutic (20mg) oral methylphenidate dose on behavioral responses to incentive stimulation. 43 male university students accomplished a card-sorting task involving a monetary reward component in a double-blind, placebo-controlled crossover design. Data analysis revealed a rise in success rate during the rewarded as compared to the non-rewarded task condition: the number of correct responses was increased, whereas the number of incorrect responses was decreased. Methylphenidate inhibited this reward response by lowering the number of correct responses and the success rate to the non-reward level. However, in a subgroup of participants it improved performance accuracy in response to reward. Our findings suggest that in those individuals, who experience a behavioral drug benefit, methylphenidate's therapeutic action comprises a combination of the activity-reducing and attention-improving properties suggested by Seeman and Madras and Volkow et al.

2.2.2 Introduction

The central nervous system stimulant methylphenidate is one of the most frequently used medications in the treatment of ADHD, a childhood psychiatric condition characterized by severe overactivity, impulsiveness and inattention (Swanson et al 1998). Methylphenidate increases tonic dopamine concentrations by blocking dopamine transporters – an effect, which seems to be crucial for its therapeutic benefit (Kuczenski and Segal 1997; Volkow et al 1998). However, discordance exists regarding the precise neurochemical mechanisms underlying the drug's therapeutic action. Especially two theories, established by Seeman and Madras (2002, 1998) and Volkow et al. (2005, 2002, 2004), both of which hypothesize the therapeutic action of methylphenidate to be primarily induced by dopamine-enhancing effects in striatal brain regions, seem to be inconsistent with one another.

The hypothesis of biphasic methylphenidate action by Seeman and Madras is based on the tonic/phasic model of dopamine system regulation (Grace 1991; Grace 1995). According to Grace, dopamine release in subcortical brain regions is regulated via two independent mechanisms: spike-dependent phasic release (which highlights the saliency of stimuli) and tonic release (which, under the homeostatic control of presynaptic dopamine autoreceptors, sets the overall responsiveness of the dopamine system). Seeman and Madras hypothesize that by elevating baseline dopamine levels, therapeutic methylphenidate doses (0.3-0.6 mg/kg) primarily act on presynaptic dopamine autoreceptors, which, in turn, lower impulse-dependent phasic dopamine release. Salient stimuli would consequently trigger reduced phasic neurotransmitter discharge, which would result in reduced activation of postsynaptic receptors and attenuated psychomotor activity in response to the stimulus.

Using PET, Volkow et al. (2004) showed that neither the administration of methylphenidate, nor a salient stimulus alone elicited a detectable striatal dopamine increase. Only the combination of both stimulators caused a significant neurotransmitter response. Concluding from this and related findings, the authors postulate methylphenidate to amplify weak stimuli-induced dopamine increases in magnitude and duration, thus enhancing the stimuli's motivational salience value and driving attention and cognitive performance.

Goal of the present investigation was to validate the outlined hypotheses of methylphenidate action on a behavioral level in a group of healthy male young adults. For this purpose, we examined performance in a card-sorting task and how this performance was influenced (a) by monetary reward, which we expected to initiate phasic dopamine release in striatal structures (Knutson et al 2001a; Koepp et al 1998), (b) by a low therapeutic (20mg) dose of oral methylphenidate, which we expected to enhance baseline dopamine availability (Volkow et al 1998) and (c) by the combination of the two stimulators.

With reward, we expected an increase in the number of correct responses and a decrease in the number of incorrect responses achieved in the utilized card-sorting task. According to Seeman and Madras' activity-focused hypothesis of methylphenidate action, we expected a less pronounced reward-induced increase in activity after methylphenidate as compared to placebo administration. The reward response should therefore trigger a less pronounced increase in the number of correct responses after methylphenidate challenge. The number of incorrect responses with reward should decrease as after placebo administration. According to Volkow et al.'s attention-focused hypothesis of methylphenidate action, we expected a more pronounced reward-induced improvement of cognitive performance after methylphenidate as compared to placebo administration. The reward response should therefore trigger a more pronounced decrease in the

number of incorrect responses after methylphenidate challenge. The number of correct responses with reward should increase as after placebo administration.

2.2.3 Materials and Methods

2.2.3.1 Participants

43 male university students were recruited by posting ads on the electronic billboard of McGill University. These were the same participants who attended the previously described investigation on the association between parental care experiences in early life and behavioral responsivity to methylphenidate challenge (see previous investigation) and thus scored either high or low in parental care measured by the PBI (Parker et al 1979). Participants had a mean age of 22.2 years (SD 2.07). The study was approved by the local Research Ethics committee and all procedures were carried out with the adequate understanding and written consent of the participants.

2.2.3.2 Study design and procedure

The study design and procedure have been described in detail in chapter 2.1.3.2 of the previous investigation. In summary, a double-blind, randomized placebo-controlled crossover study design was applied such that one half of the group (21 participants) received methylphenidate on the first and placebo on the second testing day, the other half (22 participants) received the reciprocal order of treatments. An oral dose of 20mg (2x10mg) methylphenidate was administered. Blood pressure and heart rate were monitored at 5 minutes pre-, as well as 70 and 120 minutes post-treatment to control for methylphenidate's cardiovascular influence. Succeeding each cardiovascular measurement, participants were asked to complete a set of Visual Analogue Rating Scales to examine drug effects on mood and arousal.

2.2.3.3 Measurements

2.2.3.3.1 Control variables

Next to verifying the absence of psychiatric conditions in our participant sample, general and recent alcohol and recreational drug ab/use as well as smoking behavior were assessed (see chapter 2.1.3.3.2 of the previous investigation for a list of the utilized semi-structured diagnostic interview and questionnaires).

2.2.3.3.2 The monetary reward task

A computerized monetary reward task was developed based upon the WCST (Grant and Berg 1948; Heaton et al 1993), using an Apple Macintosh™ computer and the program SuperCard (Solutions EtCetera, Pollock Pines, CA, USA) (Pruessner 2004). A detailed description of the task can be found in chapter 2.1.3.3.3 of the previous investigation. Briefly, response cards depicting figures of varying forms, colors and numbers of figures had to be matched with four different stimulus cards. Participants received immediate feedback whether a match was correct, but were not told the selected matching rule. After a randomized number of (three to eight) correct responses, the rule changed, requiring the participant to develop a new matching strategy. Overall playtime amounted to 15 minutes (5 minutes of practice, non-rewarded and rewarded playing). The program determines the numbers of total, correct and incorrect responses and the ratio of correct to total responses (the success rate).

2.2.3.3.3 Visual Analogue Rating Scales

Methylphenidate-induced changes in ratings of mood and arousal were assessed using Visual Analogue Rating Scales (Norris 1971). Participants indicated the point that best represented the perception of their current state on 100mm horizontal lines anchored by word descriptors at each end (see chapter 2.1.3.3.4 of the previous investigation for a list of the utilized 16 descriptor pairs).

2.2.3.4 Data analysis

Since the succession of non-reward and reward conditions in the utilized card-sorting task was not randomized (non-reward always preceded reward) we adjusted the reward effect for a practice effect. The adjustment approach has been described in chapter 2.1.3.4.1 of the previous investigation.

This study followed a placebo-controlled, counterbalanced crossover design. Crossover designs bear the potential problem that practice may confound the interpretation of drug effects (Elliott et al 1997). In an initial analysis we therefore examined how behavioral data (the numbers of total, correct and incorrect responses and the success rate) were influenced by practice across the testing sessions, and how the behavioral drug effect was influenced by the time-point of drug administration (see chapter 2.1.3.4.2 of the previous investigation for a description of the utilized analysis techniques).

Eventually, independent analyses were performed for the two testing sessions using separate two-way mixed ANOVAs with the between-subjects factor “treatment” (placebo vs. methylphenidate) and the within-subject factors “reward” (non-reward vs. reward) for behavioral data, respectively “time” (-5 vs. +70 and +120 minutes) for subjective and cardiovascular data. Given violation of

the assumption of sphericity, degrees of freedom were adjusted using the Greenhouse-Geisser correction. Significant interactions were further investigated using two-tailed Bonferroni-corrected paired sample t-tests and simple contrasts.

2.2.4 Results

2.2.4.1 Control variables

None of the participants had a current psychiatric disorder, had ever been diagnosed with ADHD or had taken methylphenidate for therapeutic purposes. Treatment groups were matched in terms of weight and sociodemographic variables (age, level of education). Also, smoking habits (number of smokers per group and number of cigarettes per smoker), habitual alcohol consumption (ADS scores) as well as recent and habitual recreational drug use (DAST scores) did not differ across treatment groups (Table 3).

Table 3. Control variables: means and standard deviations for control variables in the treatment groups. MPH 1: methylphenidate challenge in session 1; MPH 2: methylphenidate challenge in session 2.

	MPH 1	MPH 2	P
Number of participants	21	22	
Age	22.38 (2.38)	22.00 (1.77)	>.500
Weight in pounds	169.62 (24.87)	160.76 (19.48)	>.200
Number of smokers	5	4	
Cigarettes per day	2.43 (6.00)	2.24 (5.20)	>.900
Alcohol Dependence Scale score	4.95 (3.58)	5.39 (4.68)	>.700
Drug Abuse Screening Test score	1.24 (1.04)	1.28 (1.49)	>.900
Number of participants using recreational drugs within the past three months	10	9	

2.2.4.2 Behavioral variables

Justifying the calculation of two independent analyses for the two testing sessions, initial data analysis revealed a strong positive practice effect across testing sessions and a significant influence of the time-point of drug administration on methylphenidate's behavioral effect (data have been reported in chapter 2.1.4.3 of the previous investigation).

In the first testing session reward improved performance. During rewarded as compared to non-rewarded playing, the number of correct responses was increased ($F_{(1,41)}$=9.77, p=.003), whereas the number of incorrect responses was decreased ($F_{(1,41)}$=10.05, p=.003). Consequently, reward increased the success rate ($F_{(1,41)}$=17.27, p<.001). Total responding remained unchanged ($F_{(1,41)}$=1.00, p>.300) (Figure 6).

There was an interaction of reward and treatment, which was significant for the number of correct responses ($F_{(1,41)}$=8.56, p=.006) and the success rate ($F_{(1,41)}$=6.99, p=.012) and marginal for the numbers of total ($F_{(1,41)}$=2.76, p=.100) and incorrect ($F_{(1,41)}$=3.19, p=.082) responses (Figure 7). Post-hoc paired-sample t-tests (Bonferroni-corrected _-level = .025) revealed that the reward-induced performance improvement was clearly pronounced after placebo administration (main effect of reward for the number of correct responses: $t_{(21)}$=-3.61, p=.002, the success rate: $t_{(21)}$=-4.66, p<.001, the numbers of total: $t_{(21)}$=-1.67 p.=.110 and incorrect responses: $t_{(21)}$=3.91, p=.001), but inhibited after methylphenidate administration (all p's>.200). Methylphenidate thus equalized non-reward and reward-related performance.

In the second testing session, reward no longer improved performance. Contrarily, the numbers of total ($F_{(1,41)}$=4.36, p=.043) and incorrect responses ($F_{(1,41)}$=17.19, p<.001) were increased and the number of correct responses remained unchanged ($F_{(1,41)}$=0.70, p>.400), altogether

determining a decreased success rate ($F_{(1,41)}$=27.27, p<.001) in the reward as compared to the non-reward condition (Figure 6). Methylphenidate no longer influenced performance.

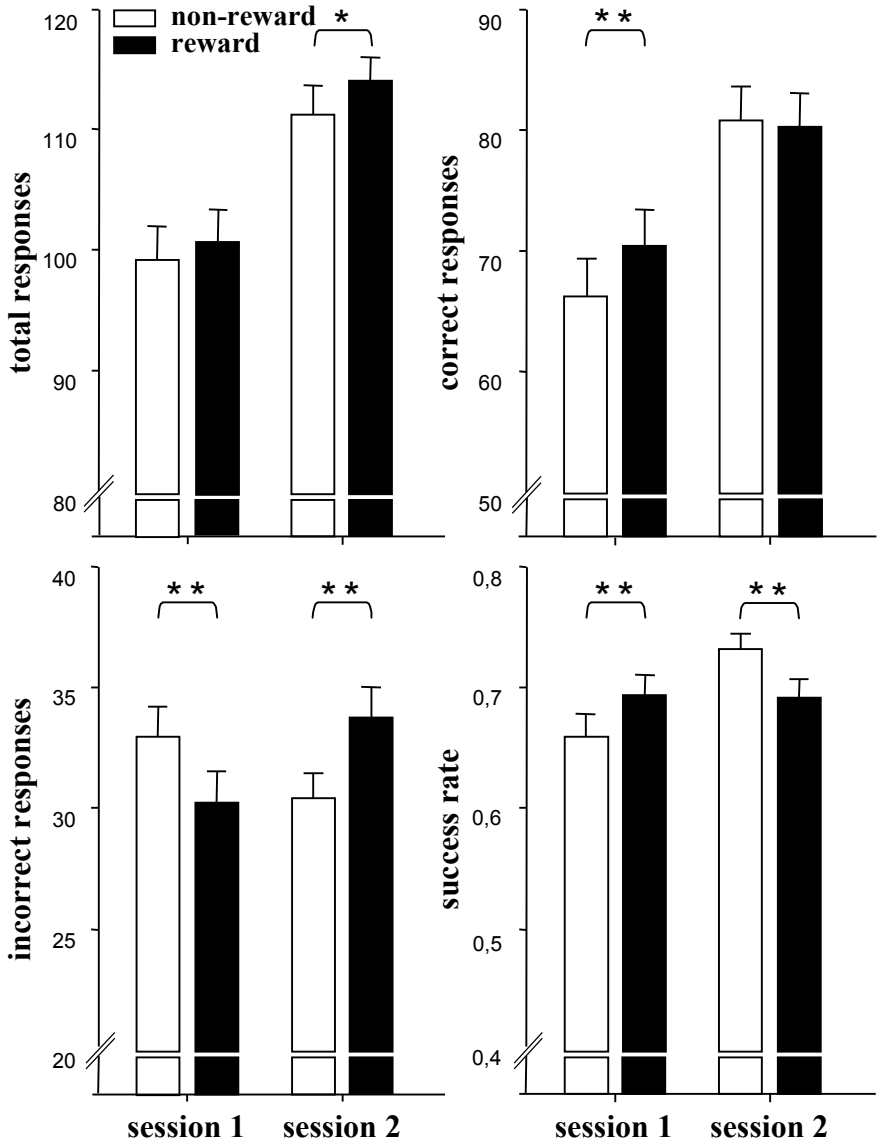

Figure 6. Means and standard errors for the main effect of reward in sessions 1 and 2. *p<.05; **p<.01.

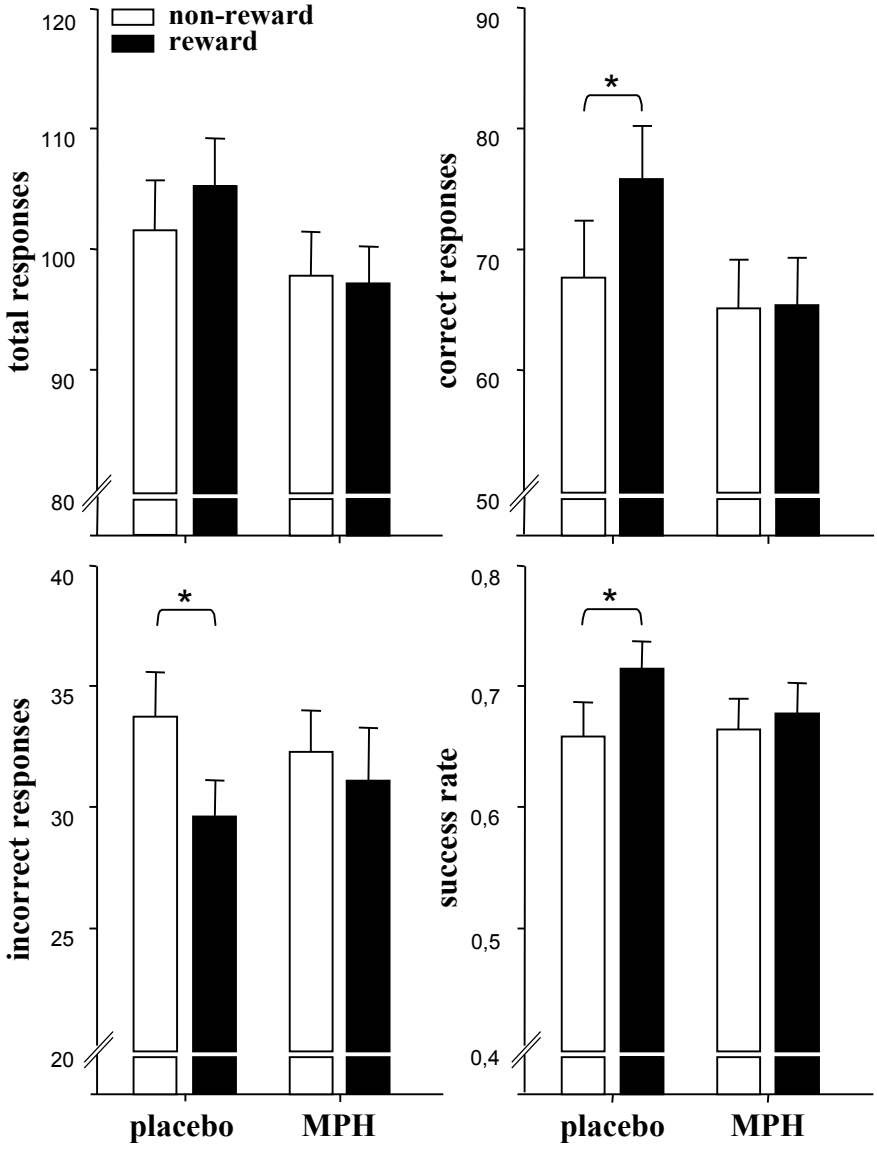

Figure 7. Means and standard errors for the interaction effect of reward and treatment in session 1. MPH: methylphenidate. *p<.025.

2.2.4.3 Subjective variables

We found a time x treatment interaction for the subjective variables "strongness" ($F_{(1.61,59.45)}$=3.62, p=.042) and "happiness" ($F_{(2,74)}$=3.46, p=.036) in the first testing session, and "alertness" ($F_{(1.74,67.92)}$=4.66, p=.016), "attention" ($F_{(1.66,64.61)}$=7.19, p=.003), "coordination" ($F_{(2,78)}$=3.44, p=.037), "energy" ($F_{(1.72,67.24)}$=3.25, p=.052) and "quick wittedness" ($F_{(1.75,68.09)}$=5.98, p=.006) in the second testing session (Figure 8). To break down this interaction, contrasts were performed comparing each of the post-treatment measurements (+70, +120 minutes) with the baseline measurement (-5 minutes) across both treatment conditions. In session 1, methylphenidate as compared to placebo effectuated an increase in feelings of strongness ($F_{(1,37)}$=4.79, p=.035) and happiness ($F_{(1,37)}$=6.54, p=.015) at +70 minutes post treatment. In session 2, methylphenidate effectuated an increase in feelings of alertness ($F_{(1,39)}$=6.82, p=.013), attention ($F_{(1,39)}$=13.64, p=.001), coordination ($F_{(1,39)}$=6.18, p=.017), energy ($F_{(1,39)}$=4.90, p=.033) and quick wittedness ($F_{(1,39)}$=8.98, p=.005) at +70 minutes post treatment.

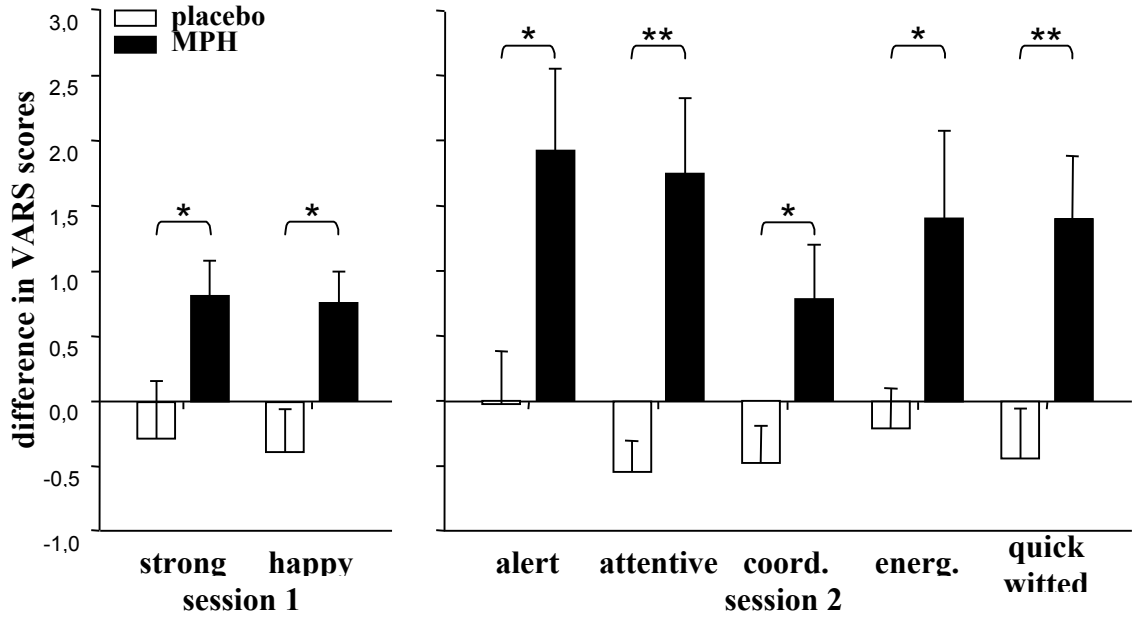

Figure 8. Means and standard errors for the interaction effect of time and treatment for subjective drug effects at +70 minutes in sessions 1 and 2. MPH: methylphenidate. *p<.05; **p<.01.

2.2.4.4 Cardiovascular variables

Restricted to the second testing session, data analysis revealed an interaction of time and treatment for systolic blood pressure ($F_{(2,80)}$=4.35, p=.016). To break down this interaction, contrasts were performed comparing each of the post-treatment measurements (+70, +120 minutes) with the baseline measurement (-5 minutes) across both treatment conditions. Whereas with methylphenidate, systolic blood pressure was significantly increased at +70 minutes post treatment ($F_{(1,40)}$=6.48, p=.015), there was no change after placebo administration. Heart rate and diastolic blood pressure were not affected by methylphenidate challenge.

2.2.5 Discussion

Main objective of the present investigation was to compare the discrete and combined behavioral influence of monetary reward and methylphenidate challenge in humans. Data analysis revealed that in the first testing session reward alone exerted a positive effect on performance by increasing the number of correct responses, decreasing the number of incorrect responses and increasing the success rate achieved in the utilized card-sorting task. A low therapeutic (20mg) oral dose of methylphenidate alone increased ratings of mood and arousal as well as systolic blood pressure, but had no performance effect. However, methylphenidate lowered the reward-induced rise in performance to the non-reward level by significantly decreasing the number of correct responses and the success rate, and marginally decreasing the number of total responses, respectively increasing the number of incorrect responses achieved with reward. Methylphenidate thus equalized non-reward and reward-related performance.

Cardiovascular and subjective drug effects as observed in this study are among methylphenidate's typical side-effects after long-time treatment (Rapport and Moffitt 2002) and

intravenous injections (Volkow and Swanson 2003), but have also been found after single oral drug doses (Chait 1994; Turner et al 2003). We have no satisfactory explanation for the fact that methylphenidate affected cardiovascular responses only in the second testing session.

At first sight, the observed pattern of behavioral drug effects correspond to neither Seeman and Madras' (2002, 1998) nor Volkow et al.'s (2005, 2002, 2004) hypotheses of methylphenidate action. We can conclude from the marginal methylphenidate effect on total and incorrect responding that the reward-induced number of correct responses was attenuated due to both a numeric decrease in total responses (reflective of activity in response to reward) and a numeric increase in the number of errors (reflective of performance accuracy in response to reward). Methylphenidate thus seems to have exerted an activity-reducing and attention-impairing effect.

Thinking back to our investigation of behavioral responsivity to monetary reward and pharmacological dopamine stimulation as a function of early parent-child relationships (see previous investigation), it becomes evident that this ambiguous drug response within the total participant sample can be attributed to diverging responsivity patterns across parental care groups. Whereas reward-induced performance in the high care group was explicitly deteriorated (correct responses were replaced by incorrect responses), the low care group exhibited a trade-off between correct and incorrect responding. As discussed in chapter 2.1.5 of the previous investigation, it is the methylphenidate responsivity pattern as found in the high care participants that does not correspond to our expectations of the drug's general cognitive effect, and which cannot be explained by either of the delineated hypotheses of methylphenidate action. In contrast, the methylphenidate responsivity pattern as found in the low care participants comprises a combination of the activity-reducing and attention-improving properties suggested by Seeman and Madras and Volkow et al., respectively. The activity-dependent methylphenidate effect

points to the relevance of differentiation between psychostimulant- and incentive-provoked neurochemical processes. In accordance with Seeman and Madras' hypothesis of biphasic methylphenidate action, elevated subcortical tonic dopamine levels following drug challenge might have acted primarily on presynaptic dopamine autoreceptors. In turn, these autoreceptors might have lowered stimulant-induced phasic dopamine release. A reward-induced increase in response-activity – determining the number of correct responses – would have consequently been inhibited. Assuming a purely activity-reducing drug effect, as proposed by Seeman and Madras, the reward-induced decrease in the number of incorrect responses should have remained unchanged across treatment conditions. However, the reward-induced decrease in the number of incorrect responses was more pronounced after methylphenidate than after placebo administration, allowing the success rate to increase (despite the parallel decrease in correct responding). Besides reducing activity in response to saliency, methylphenidate thus added to the reward-induced improvement of performance accuracy in the low care participants.

At this point it is important to note that the neurochemical mechanisms, which the authors suggest to underlie methylphenidate action, are contradictory only at first sight. Volkow et al. propose methylphenidate to amplify stimuli-induced dopamine increases in magnitude and duration. Seeman and Madras, on the other hand, argue that the phasic dopamine surge elicited by a salient stimulus should be decreased following the administration of a therapeutic methylphenidate dose. Yet, the authors emphasize that the phasic dopamine surge should be only *relatively* lower than it would have been in the absence of the drug (approximately threefold lower). The total amount of extracellular dopamine measurable in the striatum should nevertheless be highest when a stimulus-triggered neurotransmitter release succeeds methylphenidate challenge (approximately twofold higher than in the absence of the drug).

In the second testing session, behavioral responses to monetary incentive and methylphenidate challenge changed considerably. For one thing, reward no longer improved performance. Contrarily, total and incorrect responses were increased and the success rate was decreased with reward. This absence of reward-induced performance improvement was most probably due to a ceiling effect. In our task, the matching rule changed after averagely 5.5 correct responses, and a minimum of averagely 2 incorrect responses was needed to detect the mew matching rule (see chapter 2.1.3.3.3 of the previous investigation for a detailed description of the monetary reward task). Given the mean number of total responses achieved in the non-reward condition (113), participants had almost reached the maximal achievable mean number of correct responses (81). In order to further improve their performance in the reward condition, participants thus had to increase total responding, which eventually caused an increase in impulsivity. Secondly, methylphenidate no longer affected reward-related performance when administered in the second testing session. We hypothesize this lack of behavioral drug effect to originate from changes in the reward perception (see chapter 2.1.5 of the previous investigation for a discussion of this hypothesis).

Several aspects of this study need to be critically addressed. For one thing, next to increasing brain dopamine levels, methylphenidate increases levels of the neurotransmitter noradrenalin by blocking its re-uptake (Kuczenski and Segal 1997), and action involved in the drug's cognitive effects (Arnsten and Dudley 2005; Berridge et al 2006). We have discussed this point in chapter 2.1.5 of the previous investigation. Another point of criticism relates to the utilized card-sorting task. Due to the applied modifications, our task – as opposed to the WCST – did not allow the distinction between different types of errors, an information which would have allowed a more precise determination of methylphenidate's cognitive influence. Eventually, our practice effect

adjustment did not account for the possibility of a non-linear practice effect. In future applications of the task, the succession of non-reward and reward conditions should be changed systematically.

In summary, we could show that a low therapeutic (20mg) dose of oral methylphenidate enhanced mood and arousal. Moreover, the drug reduced activity in response to a monetary reward incentive. To the best of our knowledge, this is the first human study showing an activity-reducing effect of methylphenidate in healthy volunteers. The fact that in healthy volunteers methylphenidate has an activity-reducing impact only in response to a salient, activity-increasing stimulus, whereas in ADHD patients it generally reduces activity, is especially interesting in relation to the psychopathology of ADHD. Concordant with a hyperdopaminergic theory for the disorder (for a review see Solanto 2002), it suggests that individuals with ADHD may exhibit chronically increased phasic dopamine release, and hence activity levels, in response to everyday stimuli.

In contrast to the activity-related effects of methylphenidate, the quality of the cognitive-related effects was dependent on individual participant characteristics. In those individuals who experienced a behavioral benefit from methylphenidate challenge, the drug action comprised a combination of the activity-reducing and attention-improving properties suggested by Seeman and Madras (2002, 1998) and Volkow et al. (2005, 2002, 2004). Although the neurochemical mechanisms underlying these behavioral drug effects could not be verified by means of the present investigation, the fact that methylphenidate specifically affected activity in response to saliency – whereas general activity remained unchanged – can be explained most conclusively by the inhibition of phasic dopamine release in striatal brain regions, as suggested in Seeman and Madras' model of biphasic methylphenidate action. Other than suggested by Volkow et al., the

assessed cognitive performance effects were unrelated to methylphenidate's – supposedly striatally-mediated – motivational influence. For several reasons, it rather seems that the cognitive drug effects were determined by direct changes in prefrontal dopamine neurotransmission. Next to increasing subcortical dopamine availability, both reward and low methylphenidate doses increase neurotransmitter levels in the prefrontal cortex (Arnsten and Dudley 2005; Berridge et al 2006; Schultz 2002). Moreover, prefrontal dopamine efflux after low methylphenidate doses is associated with improved attention and working memory (Arnsten and Dudley 2005; Berridge et al 2006), and the utilized WCST-like card-sorting task requires prefrontal cognitive functioning (see chapter 2.1.5 of the previous investigation for the discussion of a model of prefrontal dopamine involvement in the cognitive drug effects).

2.3 Behavioral sensitivity to dopamine stimulation and vulnerability to substance abuse: Influence of Cloninger's personality dimensions

2.3.1 Summary

Cloninger's tridimensional personality theory associates high scores on the personality trait of Novelty Seeking with increased sensitivity to dopamine stimulation and increased vulnerability to substance abuse. In the present investigation, we examined the relationship between Cloninger's personality dimensions of Novelty Seeking, Harm Avoidance and Reward Dependence and behavioral sensitivity to dopamine stimulation by the means of monetary reward and a low therapeutic (20mg) dose of the indirect dopamine agonist methylphenidate. We also tested the role of the personality dimensions in accounting for abuse behavior. 39 male university students completed the Tridimensional Personality Questionnaire and accomplished a card-sorting task involving a monetary reward component in a double-blind, placebo-controlled crossover design. Data analysis revealed that Novelty Seeking determined behavioral sensitivity to reward and methylphenidate. Reward Dependence determined behavioral sensitivity to methylphenidate and was additionally associated with the cardiovascular drug response. High Novelty Seeking was found to positively correlate with abuse behavior. Regarding the trait of Novelty Seeking, our findings confirm Cloninger's theory on a behavioral level. Reward Dependence, however, seems to be related to both noradrenergic and dopaminergic functioning.

2.3.2 Introduction

Cloninger's biosocial theory of personality identifies three heritable personality dimensions, which are operationalized by the Tridimensional Personality Questionnaire (TPQ; Cloninger et al 1991): Novelty Seeking, Harm Avoidance and Reward Dependence. Each of these dimensions is

hypothesized to represent an independent behavioral response disposition and is linked to a specific neurotransmitter system. Extreme variations in the personality dimensions and underlying neurotransmitter systems are thought to be expressed in psychiatric and personality disorders (Cloninger 1987b).

The trait of Novelty Seeking, theoretically related to dopaminergic functioning in mesocorticolimbic projections, is defined as a tendency to initiate exploratory activity in response to novelty, to approach potential reward and to actively avoid monotony and punishment. Harm Avoidance is linked to serotonin activity. On a behavioral level, Harm Avoidance is described as a tendency towards behavioral inhibition in order to avoid novelty, punishment and frustrating non-reward. Reward Dependence, related to noradrenergic functioning, is understood as a tendency to respond intensely to signals of reward (particularly verbal signs of social approval) and to maintain behaviors that have previously been associated with reward or relief from punishment (Cloninger 1987b).

Cloninger hypothesized an association between the trait of Novelty Seeking and vulnerability to adolescent-onset polysubstance abuse (Cloninger 1987a). According to Cloninger (1987b) and Ruegg et al. (1997), Novelty Seeking correlates positively with the density of the dopamine transporter, higher levels of Novelty Seeking being linked to reduced basal dopaminergic tone and compensatory postsynaptic dopamine receptor upregulation. High Novelty Seekers should thus exhibit hypersensitivity to stimulation of the dopamine system, rendering them more susceptible to primary incentives and the rewarding effects of psychostimulants (Cloninger 1987a). Various patterns of abuse behavior including cigarette smoking, alcoholism, recreational drug use and gambling have indeed been shown to involve elevated mean Novelty Seeking scores

(Battaglia et al 1996; Dughiero et al 2001; Howard et al 1997; Kim and Grant 2001; Mitchell 1999).

Aiming at the validation of Cloninger's neurochemical classification with regard to the dopaminergic basis of Novelty Seeking, we examined the relationship between the three personality dimensions and behavioral sensitivity to dopamine stimulation. Performance in a card-sorting task involving a monetary reward component following a low therapeutic (20mg) oral dose of the indirect dopamine agonist methylphenidate was investigated. Reward (Knutson et al 2001a; Koepp et al 1998; Schultz 2002) and methylphenidate (Arnsten and Dudley 2005; Berridge et al 2006; Volkow et al 1998) have been shown to trigger central dopamine release. Furthermore, we tested the role of the TPQ scales in accounting for abuse behavior. Based on Cloninger's theory, we expected to find a positive association between Novelty Seeking scores and behavioral sensitivity to both monetary reward and methylphenidate, as well as between Novelty Seeking scores and abuse behavior (smoking, alcohol and recreational drug use).

2.3.3 Materials and Methods

2.3.3.1 Participants

39 male university students were enrolled in the investigation. These participants constituted a subsample of the total group initially recruited to examine the association between parental care experiences in early life and behavioral responsivity to methylphenidate challenge (see first investigation) and thus scored either high or low in self-reported parental care measured by the PBI (Parker et al 1979). Participants had a mean age of 22.2 years (SD 2.06). The study was approved by the local Research Ethics committee and all procedures were carried out with the adequate understanding and written consent of the participants.

2.3.3.2 Study design and procedure

The study design and procedure have been described in detail in chapter 2.1.3.2 of the first investigation. In summary, a double-blind, randomized placebo-controlled crossover study design was applied such that approximately one half of the group (21 participants) received methylphenidate on the first and placebo on the second testing day, the other half (18 participants) received the reciprocal order of treatments. An oral dose of 20mg (2x10mg) methylphenidate was administered. Blood pressure and heart rate were monitored at 5 minutes pre-, as well as 70 and 120 minutes post-treatment to control for methylphenidate's cardiovascular influence. Succeeding each cardiovascular measurement, participants were asked to complete a set of Visual Analogue Rating Scales to examine drug effects on mood and arousal.

2.3.3.3 Measurements

2.3.3.3.1 Measurement of personality and abuse behavior

Cloninger's personality dimensions of Novelty Seeking, Harm Avoidance and Reward Dependence were measured using the 100-item TPQ (Cloninger et al 1991). The ADS (Horn et al 1984) and the DAST (Skinner 1982) provided information on alcohol dependence and recreational drug abuse. All participants were asked to specify the last time they had used recreational drugs within the past three months, and smokers were asked to specify their daily average number of cigarettes.

2.3.3.3.2 Control variables

Next to verifying the absence of psychiatric conditions in our participant sample (see chapter 2.1.3.3.2 of the first investigation for the utilized semi-structured diagnostic interview), the distribution of the above described abuse behaviors across treatment groups was assessed.

2.3.3.3.3 The monetary reward task

A computerized monetary reward task was developed based upon the WCST (Grant and Berg 1948; Heaton et al 1993), using an Apple Macintosh™ computer and the program SuperCard (Solutions EtCetera, Pollock Pines, CA, USA) (Pruessner 2004). A detailed description of the task can be found in chapter 2.1.3.3.3 of the first investigation. Briefly, response cards depicting figures of varying forms, colors and numbers of figures had to be matched with four different stimulus cards. Participants received immediate feedback whether a match was correct, but were not told the selected matching rule. After a randomized number of (three to eight) correct responses, the rule changed, requiring the participant to develop a new matching strategy. Overall playtime amounted to 15 minutes (5 minutes of practice, non-rewarded and rewarded playing). The program determines the numbers of total, correct and incorrect responses and the ratio of correct to total responses (the success rate).

2.3.3.3.4 Visual Analogue Rating Scales

Methylphenidate-induced changes in ratings of mood and arousal were assessed using Visual Analogue Rating Scales (Norris 1971). Participants indicated the point that best represented the perception of their current state on 100mm horizontal lines anchored by word descriptors at each end (see chapter 2.1.3.3.4 of the first investigation for a list of the utilized 16 descriptor pairs).

2.3.3.4 Data analysis

Since the succession of non-reward and reward conditions in the utilized card-sorting task was not randomized (non-reward always preceded reward) we adjusted the reward effect for a practice effect. The adjustment approach has been explained in chapter 2.1.3.4.1 of the first investigation.

This study followed a placebo-controlled, counterbalanced crossover design. Crossover designs bear the potential problem that practice may confound the interpretation of drug effects (Elliott et al 1997). In an initial analysis we therefore examined how behavioral data (the numbers of total, correct and incorrect responses and the success rate) were influenced by practice across the testing sessions, and how the behavioral drug effect was influenced by the time-point of drug administration (see chapter 2.1.3.4.2 of the first investigation for a description of the utilized analysis techniques).

Eventually, independent analyses were calculated for the two testing sessions. We first assessed the effect of reward on behavioral performance using two-tailed paired sample t-tests. In subsequent analyses, the effect of methylphenidate on overall performance (non-reward plus reward performance divided by two) and reward responsivity (reward minus non-reward performance) was determined using one-way independent ANOVAs with the between-subjects factor “treatment” (placebo vs. methylphenidate). For subjective and cardiovascular data two-way mixed ANOVAs with the between-subjects factor “treatment” and the within-subjects factor “time” (-5 vs. +70 and +120 minutes) were calculated. In order to assess the influence of personality, the scales of Novelty Seeking, Harm Avoidance and Reward Dependence were individually introduced as covariates into the analyses. Pearson’s correlation method was used to examine the relation between TPQ scores and behavioral performance, and between TPQ scores

and assessments of abuse behavior. Significant interactions for subjective and cardiovascular data were further investigated using simple contrasts. We did not perform a median split on the TPQ scales, because the distribution of high and low Novelty Seekers within treatment groups was inhomogeneous.

2.3.4 Results

2.3.4.1 Control variables

As in the total group (see chapter 2.2.4.1 of the previous investigation), the assessed control variables were equally distributed across treatment groups in this participant subsample (Table 4).

Table 4. Control variables: means and standard deviations for control variables in the treatment groups. MPH 1: methylphenidate challenge in session 1; MPH 2: methylphenidate challenge in session 2.

	MPH 1	**MPH 2**	**P**
Number of participants	21	18	
Age	22.38 (2.38)	21.94 (1.66)	>.500
Weight in pounds	169.62 (24.87)	163.94 (19.82)	>.400
Number of smokers	5	4	
Cigarettes per day	2.43 (6.00)	2.61 (5.55)	>.900
Alcohol Dependence Scale score	4.95 (3.58)	5.39 (4.68)	>.700
Drug Abuse Screening Test score	1.24 (1.04)	1.28 (1.49)	>.900
Number of participants using recreational drugs (past three months)	10	6	

2.3.4.2 Abuse variables

We found significant positive correlations between Novelty Seeking and the number of daily cigarettes (r=.32, p=.044) and ADS scores (r=.33, p=.042). Neither Harm Avoidance nor Reward Dependence were associated with the assessed abuse behaviors.

2.3.4.3 Behavioral variables

Justifying the calculation of two independent analyses for the two testing sessions, initial data analysis revealed a strong positive practice effect across testing sessions and a significant influence of the time-point of drug administration on methylphenidate's behavioral effect (data have been reported in chapter 2.1.4.3 of the first investigation).

In the first testing session reward improved performance. During rewarded as compared to non-rewarded playing, the number of correct responses was enhanced ($t_{(38)}$=-2.38, p=.022), whereas the number of incorrect responses was reduced ($t_{(38)}$=2.92, p=.006). Consequently, reward raised the success rate ($t_{(38)}$=-3.45, p=.001).

Methylphenidate did not affect overall performance (non-reward plus reward performance divided by 2). Reward responsivity (reward minus non-reward performance), however, was influenced by the drug. After methylphenidate as compared to the placebo administration, the reward-mediated increase in correct responding ($F_{(1,37)}$=6.43, p=.016), the decrease in incorrect responding – although insignificantly ($F_{(1,37)}$=3.56, p=.067) – and the consequent rise in success rate ($F_{(1,37)}$=5.84, p=.021) were inhibited (Figure 9).

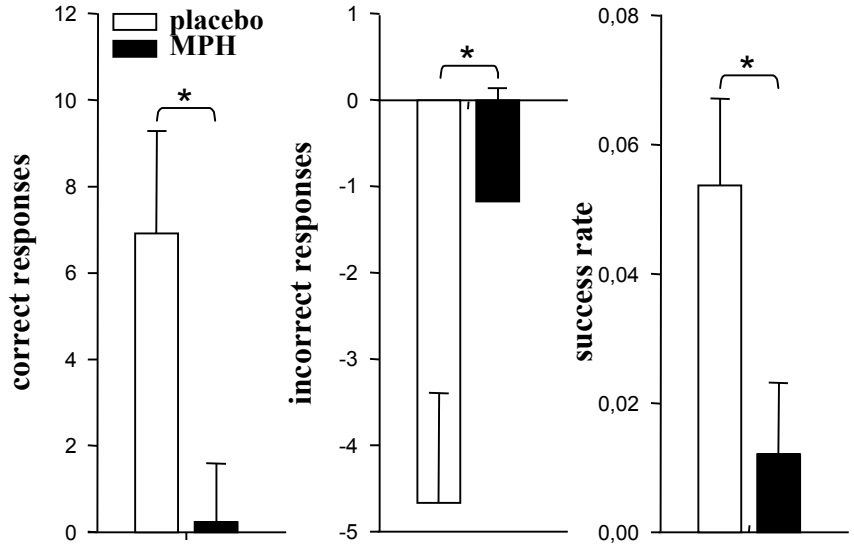

Figure 9. Means and standard errors for the main effect of treatment on reward responsivity for the numbers of correct responses, incorrect responses and the success rate in session 1. MPH: methylphenidate. *p<.05.

There was an interaction of treatment and Novelty Seeking for the reward-mediated rise in the number of total responses ($F_{(1,35)}$=5.43, p=.026), correct responses ($F_{(1,35)}$=10.93, p=.002) and the success rate ($F_{(1,35)}$=8.16, p=.007) (Figures 10a-f). Further correlation analysis revealed that in the after placebo administration, reward responsivity for the number of total responses (r=.56, p=.015), correct responses (r=.58, p=.011) and the success rate (r=.47, p=.049) was the higher, the higher participants scored on the Novelty Seeking scale. After methylphenidate administration, there was no association between Novelty Seeking and reward responsivity for the number of total (r=.00, p>.900) and correct responses (r=-.27, p>200), but a marginal negative correlation between the personality trait and success rate (r=-.40, p=.077). Methylphenidate thus inhibited increased reward responsivity in participants with high Novelty Seeking scores. To ensure that the disclosed correlations were not primarily driven by increased habitual smoking and alcohol consumption in high Novelty Seekers, we additionally assessed

correlations between the number of daily cigarettes, ADS scores and reward responsivity across treatment groups. No correlations were found.

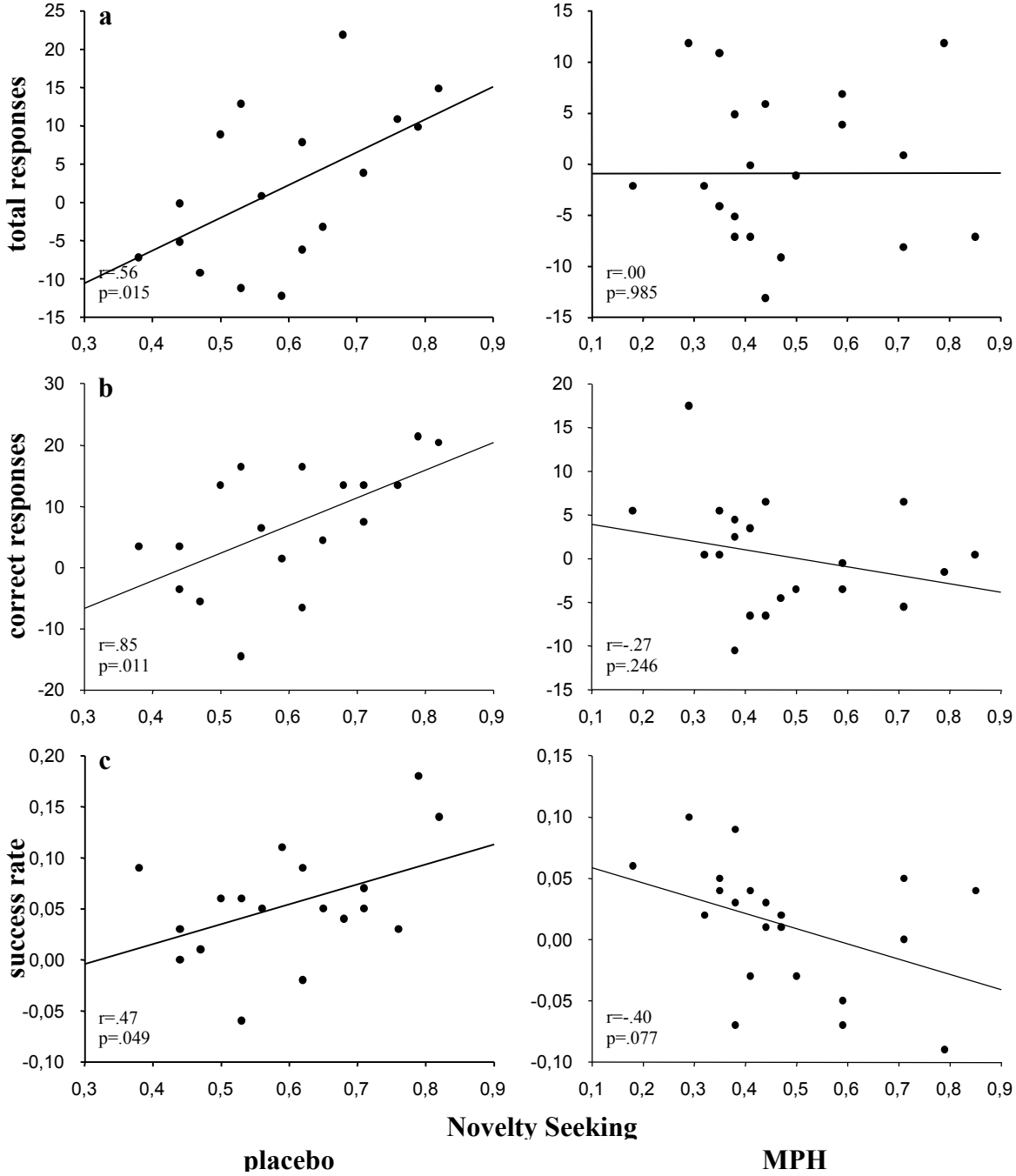

Figure 10. Scatterplots between Novelty Seeking scores and the reward-mediated rise in (a) the numbers of total responses, (b) correct responses and (c) the success rate after placebo as compared to methylphenidate (MPH) administration in session 1.

We also detected an interaction of treatment and Reward Dependence for the reward-mediated decrease in the number of incorrect responses ($F_{(1,35)}$=4.55, p=.040) (Figures 11a+b). Correlation analysis revealed that after placebo administration, the reward-mediated numeric decrease in the number of errors was the less pronounced, the lower Reward Dependence scores (r=-.37, p=.136). This relation was reversed after methylphenidate administration (r=.33, p=147) (the reward-mediated decrease in the number of errors was the more pronounced, the lower participants scored on Reward Dependence). There was no association between methylphenidate and the personality dimension of Harm Avoidance.

As in the total group, reward no longer improved overall performance in the second testing session. The numbers of total and incorrect responses were contrarily increased and the success rate decreased in the reward as compared to the non-reward condition. Methylphenidate had no influence on overall performance. Since no interaction of reward and personality emerged, and the main effect of reward as found in the total group has already been reported in chapter 2.2.4.2 of the previous investigation, data are not presented in more detail here.

2.3.4.4 Subjective variables

As in the total group, at +70 minutes post treatment, measures of mood and arousal were increased after methylphenidate as compared to placebo administration in both testing sessions. Since personality had no influence on subjective measures, and the time x treatment interaction as found in the total group has already been reported in chapter 2.2.4.3 of the previous investigation, data are not presented in more detail here.

2.3.4.5 Cardiovascular variables

Contrary to what was found in the total group, methylphenidate had no overall impact on cardiovascular measures within this participant subsample. However, in the first testing session, we found a three-way interaction of time, treatment and Reward Dependence for systolic blood pressure ($F_{(1.68,50.40)}$=5.85, p=.008; using Greenhouse-Geisser corrected degrees of freedom) and heart rate ($F_{(2,56)}$=8.12, p=.001) (Figures 11c-f). We performed contrasts to break down these interactions and found differences between cardiovascular measures at baseline and +120 minutes post-treatment (systolic blood pressure: $F_{(1,30)}$=9.57, p=.004; heart rate: $F_{(1,28)}$=17.50., p<.001) when comparing placebo and methylphenidate conditions in dependence of Reward Dependence scores. Correlation graphs plotting Reward Dependence against the discrepancy between post and pre-treatment measures (+120 minutes minus baseline) showed that in the placebo condition increases in systolic blood pressure (r=.50, p=.048) and heart rate (r=.63, p=.012) over time were the more pronounced, the higher participants scored on Reward Dependence. However, in the methylphenidate condition, the relation over time was reversed: systolic blood pressure (r=-.51, p=.020) and heart rate (r=-.57, p=.008) increased the more, the lower scores of Reward Dependence. Methylphenidate thus exerted a stronger rise in cardiovascular measures in participants with low Reward Dependence. There was no association between methylphenidate's pressor effects and the TPQ scales of Novelty Seeking and Harm Avoidance.

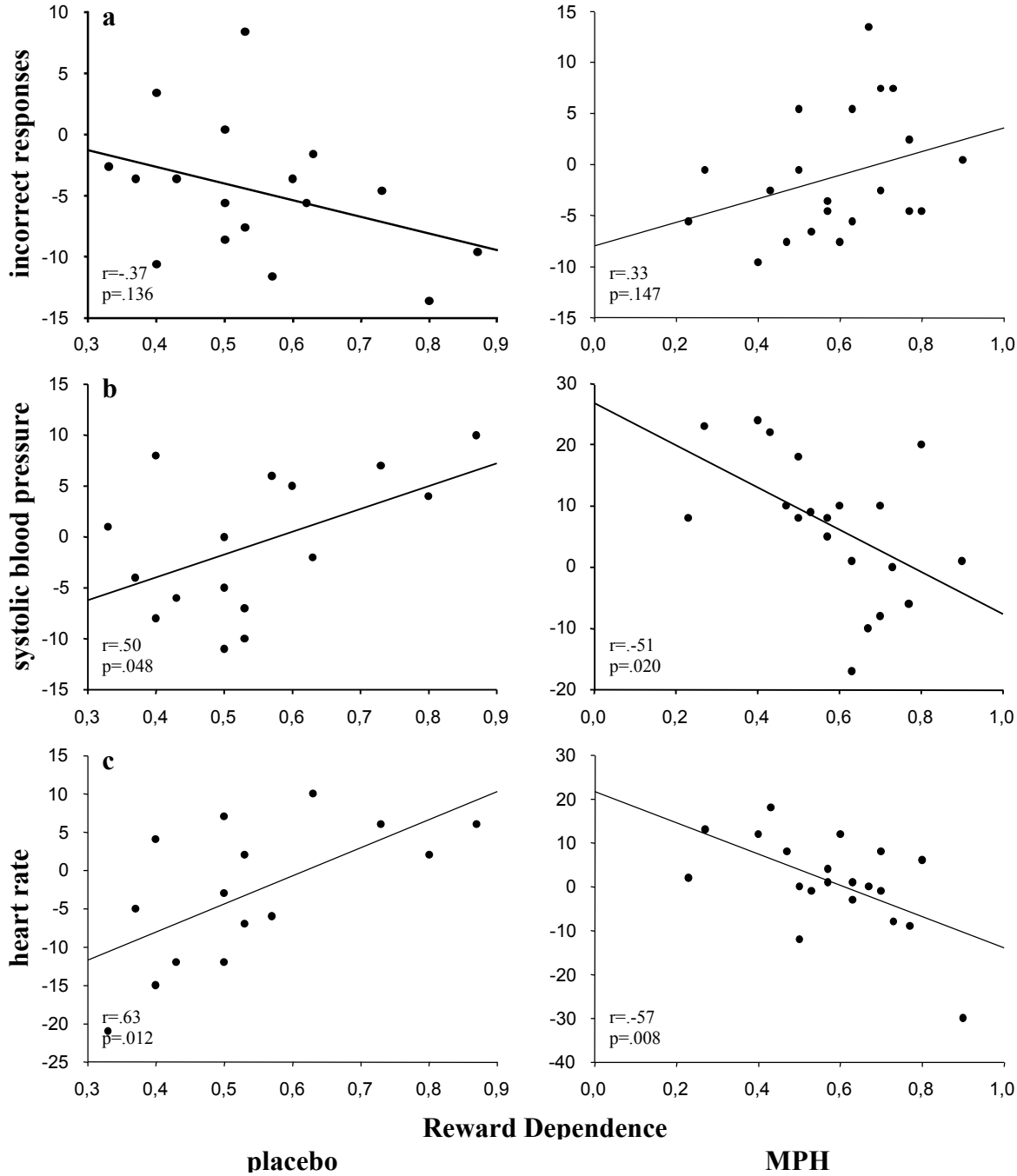

Figure 11. Scatterplots between Reward Dependence scores and (a) the reward-mediated rise in the number of incorrect responses, (b) systolic blood pressure and (c) heart rate after placebo as compared to methylphenidate (MPH) administration in session 1.

2.3.5 Discussion

In the present investigation we sought to examine the relationship between Cloninger's personality dimensions of Novelty Seeking, Harm Avoidance and Reward Dependence and behavioral sensitivity to stimulation of the dopamine system. We additionally tested the role of Novelty Seeking in accounting for abuse behavior. Based on Cloninger's theory, we expected to find a positive correlation between Novelty Seeking scores and behavioral sensitivity to both monetary reward and methylphenidate challenge, as well as between Novelty Seeking scores and abuse behavior.

Independent of personality, methylphenidate had no effect on overall performance in the utilized card-sorting task, but it affected reward responsivity in the first testing session. After methylphenidate as compared to placebo administration, the reward-mediated increase in correct responding, the decrease in incorrect responding and the consequent rise in success rate were inhibited.

Novelty Seeking – assumedly reflecting dopaminergic functioning – was a significant contributor to both reward and methylphenidate sensitivity. The higher scores of Novelty Seeking, the higher reward responsivity in the placebo condition and the more pronounced methylphenidate's reward-related inhibitory effect (which differentially affected total and correct responding and the success rate depending on Novelty Seeking scores). Since Novelty Seeking was correlated with both reward and methylphenidate sensitivity, it was not possible to ascertain whether different drug responses as a function of the personality trait were indirectly mediated by differences in reward sensitivity or due to direct differences in neurochemical methylphenidate processing (consequent to variable dopamine transporter or receptor status, as suggested by Cloninger). Despite this ambiguity, the current findings support Cloninger's theory of an association between

high Novelty Seeking scores and increased sensitivity to stimulation of the dopamine system. They also complement previous studies demonstrating increased subjective sensitivity to d-amphetamine in high Novelty Seekers (Hutchison and Swift 1999; Sax and Strakowski 1998). Likewise in support of Cloninger's theory, we found Novelty Seeking scores to positively correlate with scores on the Alcohol Dependence Scale and the number of daily cigarettes.

Other than expected, Reward Dependence – believed to reflect noradrenergic functioning – was also correlated with methylphenidate's performance effects. Whereas, after placebo administration, the reward-mediated decrease in the number of errors was the less pronounced, the lower participants scored on Reward Dependence, the reverse was true after methylphenidate administration (the reward-mediated decrease in the number of errors was the more pronounced, the lower Reward Dependence scores). Low Reward Dependence scores were thus linked to performance accuracy-improving and high Reward Dependence scores to performance accuracy-impairing drug effects. Since Reward Dependence was not correlated with reward sensitivity per se, it can be hypothesized that differences in methylphenidate sensitivity as a function of the personality trait reflect direct differences in neurochemical drug processing.

The potential neurochemical mechanisms underlying methylphenidate's behavioral effects have been discussed in chapters 2.1.5 and 2.2.5 of the previous investigations. Briefly, methylphenidate-induced reduction of activity (the number of total and correct responses) in response to reward can be explained by Seeman and Madras' hypothesis of biphasic methylphenidate action (Seeman and Madras 2002; Seeman and Madras 1998). The hypothesis suggests that in striatal brain regions, elevated tonic dopamine levels following drug challenge primarily act on presynpatic dopamine autoreceptors, which in turn lower subsequent reward-induced phasic dopamine release – and thus reduce activity. On the other hand, methylphenidate-

induced changes in performance accuracy (the number of incorrect responses and the success rate) in response to reward, can be ascribed to prefrontal brain regions. An inverted-u shaped function has been shown to describe the relationship between stimulation of prefrontal dopamine receptors and cognitive effects (Arnsten and Dudley 2005; Granon et al 2000). It is thus possible that the participants who deteriorated with methylphenidate functioned on an optimal prefrontal dopamine level in the absence of the drug. Given the combined impact of reward and the drug, the peak of optimal stimulation might have been exceeded, thus determining impaired performance accuracy. The participants who improved their performance accuracy with methylphenidate might have functioned on a relatively decreased prefrontal dopamine level in the absence of the drug. Drug challenge might have consequently raised prefrontal dopamine closer to the peak of the inverted u-function, thus determining improved performance accuracy.

Reverting to Cloninger's personality dimensions, it may be concluded that Novelty Seeking (which differentially affected total and correct responding) is linked to subcortical dopamine regulation. Reward Dependence (which differentially affected incorrect responding), however, should not be linked to prefrontal dopamine regulation. In favour of Cloninger's theory, it could be argued that the detected interaction of treatment and Reward Dependence was mediated via noradrenergic pathways. This argument is supported by the fact that, next to increasing brain dopamine levels, methylphenidate increases levels of the neurotransmitter noradrenalin (Kuczenski and Segal 1997), an action, which is especially prominent in the prefrontal cortex and was shown to be involved in the drug's cognitive effects (Arnsten and Dudley 2005; Berridge et al 2006). However, the fact that methylphenidate selectively affected reward responsivity and specifically dopamine activity has been implicated in the processing of reward (Ikemoto and Panksepp 1999; Schultz 2002) suggests a major role of dopamine neurotransmission in the drug's

performance influence. Our findings thus add to several previous investigations, which have questioned the primarily noradrenergic basis of Reward Dependence by demonstrating a link between the personality trait and dopaminergic functioning (Benjamin et al 1998; Keltikangas-Jarvinen et al 2006; Kuhn et al 1999; Noble et al 1998).

In the second testing session, behavioral responses to monetary incentive and methylphenidate challenge changed considerably. For one thing, reward no longer improved performance. Contrarily, total and incorrect responses were increased and the success rate decreased with reward. Furthermore, methylphenidate no longer affected reward-related performance. These changes in the behavioral response to dopamine stimulation most likely originate from changes in the reward perception and learning effects (see chapters 2.1.5 and 2.2.5 of the previous investigations for a discussion of this hypothesis).

Contrary to what was found in the total group, methylphenidate had no overall impact on cardiovascular measures within this participant subsample, although increases in blood pressure and heart rate are among its typical side effects after both long-time treatment and single oral drug doses (Rapport and Moffitt 2002; Turner et al 2003). However, in the first testing session, low Reward Dependence scores were correlated with decreased cardiovascular measures at 120 minutes after placebo administration and increased cardiovascular measures at 120 minutes after methylphenidate administration. Since the cardiovascular impact of methylphenidate is thought to be primarily mediated via the noradrenergic system, this finding supports Cloninger's theory of an association between the personality trait of Reward Dependence and noradrenergic functioning. We have no satisfactory explanation for the fact that methylphenidate had no overall impact on cardiovascular measures and did not differentially affect cardiovascular responses as a function of Reward Dependence in the second testing session.

Several aspects of this study need to be critically addressed. Due to the applied modifications, our task – as opposed to the WCST – did not allow the distinction between different types of errors, an information which would have allowed a more precise determination of methylphenidate's cognitive influence. Also, our practice effect adjustment did not account for the possibility of a non-linear practice effect. In future applications of the task, the succession of non-reward and reward conditions should be changed systematically.

Cloninger hypothesized a genetically determined increased number of dopamine transporters, consequently decreased basal dopaminergic tone and compensatory postsynaptic dopamine receptor upregulation in high Novelty Seekers. As a result, high Novelty Seekers are believed to be hypersensitive to stimulation of the dopamine system and thus more susceptible to primary incentives and the rewarding effects of psychostimulants (Cloninger 1987a). In summary – although the neurochemical mechanisms underlying the trait of Novelty Seeking could not be verified by the means of the present investigation – our findings confirm Cloninger's theory on a behavioral level: high Novelty Seeking was associated with increased behavioral sensitivity to reward (which triggers phasic dopamine release) and methylphenidate (which elevates tonic dopamine levels). Furthermore, high Novelty Seekers exhibited elevated measures of abuse behavior. Likewise in accordance to Cloninger's theory, Harm Avoidance, which is theoretically linked to serotonin activity, was related to none of the assessed dopamine-dependent behavioral variables. The primarily noradrenergic basis of Reward Dependence may however be questioned on the basis of our findings. Reward Dependence rather seems to be linked to both noradrenergic and dopaminergic functioning.

3 RELATIONSHIP BETWEEN PARENTAL CARE, REWARD DEPENDENCE AND BEHAVIORAL MEASURES

3.1 Introduction

In the above delineated investigations we have demonstrated an influence of both self-reported early life parental care and the personality trait of Reward Dependence on the behavioral response to reward and methylphenidate, more precisely, on the number of incorrect responses achieved in the reward as compared to the non-reward condition of a monetary reward task after methylphenidate as compared to placebo administration. The relationship among parental care and Reward Dependence, however, remained unclear. Given an association between the variables, two possible models of their relationship can be formulated. Based on Cloninger's personality theory, it might be expected that the heritable trait of Reward Dependence is the basic variable, which determines the perception of early life parental care and thus influences behavior in our study. Based on the assumption that a phenotype will emerge from the interaction of nature (gene) and nurture (environment) (Meaney et al 2002), we contrarily suggest the following, less deterministic model: parental care received during childhood and adolescence modulates the development of the genetically predetermined trait of Reward Dependence and, referring to our study, its behavioral influence.

3.2 Data analysis

In the present analysis of how parental care and personality interacted to influence behavior, „reward responsivity for the number of incorrect responses" was used as dependent variable. At this point it is essential to understand that, although we have used different analysis designs in the

above investigations to examine the influence of care versus personality on reward and methylphenidate responsivity, these designs came down to the assessment of an identical measure: the effect of methylphenidate on reward responsivity for the number of incorrect responses. In the parental care investigation we used three-way mixed ANOVA with the between subjects factors "care" (high vs. low) and "treatment" (placebo vs. methylphenidate), the within subjects factor "reward" (non-reward vs. reward) and the dependent variable "number of incorrect responses". In order to simplify the design for the personality investigation, we used one-way ANCOVA with the between subjects factor "treatment", the covariate "Reward Dependence" and the dependent variable "reward responsivity for the number of incorrect responses" (calculated as reward minus non-reward performance). It may thus seem as if different measures were assessed in the two investigations. Fact is, however, that except for the within subjects main effect of reward, the calculated between subjects effects of the one-way ANCOVA were identical with what would have been the within subjects effects in a two-way mixed ANOVA design. We have once more made use of our artificial data set to illustrate this point in the first part of the results section (see chapter 2.1.3.4.1 of the first investigation for a description of the artificial data set).

Importantly, we have not found the variables parental care and Reward Dependence *per se* to influence reward responsivity for the number of incorrect responses. Rather, the behavioral measure was influenced by the *interactions* between the variables treatment and parental care and treatment and Reward Dependence, respectively. We thus had to determine, which of the two interactions (treatment x parental care or treatment x Reward Dependence) exerted the basic influence. Given our hypothesis, the analysis followed the following three steps. First, we showed that the interaction of parental care and treatment was associated with the outcome variable.

Therefore, “parental care” as well as “treatment” were used as the independent variables in a two-way independent ANOVA with the dependent variable “reward responsivity for the number of incorrect responses”. In the second step, we examined the association between parental care and Reward Dependence. “Parental care” was therefore used as the independent variable in a one-way independent ANOVA with the dependent variable “Reward Dependence”. In the third step, we examined the association between the treatment x Reward Dependence interaction and the outcome variable after controlling for the effect of the treatment x parental care interaction. “Parental care” and “treatment” were used as independent variables and “Reward Dependence” as covariate in a two-way independent ANOVA. “Reward responsivity for the number of incorrect responses” was again used as the dependent variable.

3.3 Results

3.3.1 Artificial data set

Calculation of a two-way mixed ANOVA with the between subjects factor “group”, the within subjects factor “reward” (non-reward vs. reward) and the dependent variable “practice-corrected number of total responses” resulted in a main effect of group ($F_{(1,198)}$=13.33, p<.000) and an interaction of reward and group ($F_{(1,198)}$= 7.98, p=.005). Calculation of a one-way independent ANOVA with the between subjects factor “group” and the dependent variable “reward responsivity for the corrected number of total responses” (calculated as reward minus non-reward performance) resulted in a group main effect ($F_{(1,198)}$= 7.98, p=.005), which was identical to the reward x group interaction in the two-way mixed ANOVA (Figure 3).

3.3.2 Relationship between parental care, Reward Dependence and behavioral measures

Unsurprisingly, the first step of the analysis showed that the interaction of treatment and parental care had a significant effect on reward responsivity for the number of incorrect responses ($F_{(1,39)}$=9.28, p=.004) (importantly, this effect is identical with the one reported in chapter 2.1.4.3.1 of the first investigation). In the second step of the analysis, it was found that parental care had a marginal effect on Reward Dependence ($F_{(1,37)}$=4.00, p=.053). In the third step it was found that when the interaction of treatment and parental care was controlled for, the interaction of treatment and Reward Dependence no longer influenced reward responsivity for the number of incorrect responses (p>.180). However, the interaction of treatment and parental care kept a significant influence on the behavioral measure ($F_{(1,33)}$=5.55, p=.025).

3.4 Discussion

The delineated results indicate that it was early life parental care rather than Reward Dependence that determined the basic influence on our behavioral measure. This finding is especially interesting in reference to the above mentioned nature/nurture debate. Contrasting the models that life emerged as a function of either nature or nurture, or both nature and nurture in equal terms, Meaney (2001) argues that it is the interaction of the two from which a phenotype derives. Our results provide a confirmation of this model.

This data analysis followed the three steps of a mediation analysis as established by Baron and Kenny (1986). However, Baron and Kenny's mediation analysis refers to the examination of a causal relationship between single variables. In our case, the relationship between interactions was examined. Therefore, in the second step of the analysis, it would have been necessary to

assess the association between the interactions treatment x parental care and treatment x Reward Dependence, not the association between the single variables parental care and Reward Dependence. This was, of course, not possible. Considering the complexity of this data set, the given approach was the best possible approximation of Kenny and Baron's mediation analysis.

4 GENERAL DISCUSSION

The basic objective of this work was the design of an indirect measurement method, which would allow assessing behavioral correlates of dopamine regulation. We thus investigated how performance in a card-sorting task involving a monetary reward component was influenced by methylphenidate challenge. With regard to the interactions of reward and methylphenidate, we examined whether methylphenidate would have an additive, attention-improving or inhibiting, activity-reducing, impact on behavioral performance in response to saliency. This research question aimed at an enhanced general understanding of the therapeutic effects of methylphenidate, which would further allow analyzing differences in drug responsivity patterns as a function of two selected, dopamine-associated variables: parental bonding experiences in early life and the personality dimension of Novelty Seeking. With regard to parental bonding experiences, we examined whether opposite scores on the care scale of the PBI (Parker et al 1979) were associated with differential behavioral responsivity to methylphenidate challenge, a finding which would indicate an influence of early life parental care on the regulation of the dopamine system. Eventually, aiming at the verification of Cloninger's biosocial personality theory (Cloninger 1987b), we examined whether high scores on the trait of Novelty Seeking were associated with increased measures of abuse behavior as well as increased sensitivity to both reward and pharmacological stimulation of the dopamine system.

4.1 Overview of results

The utilized paradigm, which allowed observing the combined behavioral influence of a monetary incentive and the indirect dopamine agonist methylphenidate, provided us with numerous answers to the above formulated research questions. The comparison of parental care

groups revealed inverse cognitive responsivity patterns to methylphenidate challenge, whereby performance accuracy was impaired in the high care participants and improved in the low care participants. Other than expected, activity in response to methylphenidate was not influenced by parental care. We conclude the drug responsivity patterns across parental care groups to be mediated by differences in the regulation of prefrontal (cognitive) rather than striatal (activity-related) brain regions. The behavioral effect of methylphenidate on reward responsivity was shown to be neither additive nor inhibiting per se. More precisely, in those individuals, in which the drug altogether improved performance, it reduced activity in response to reward (by reducing the number of correct responses achieved in the reward condition) and it added to the reward-induced increase in performance accuracy (by reducing the number of incorrect responses achieved in the reward condition relatively more than the number of correct responses). High Novelty Seeking was positively correlated with sensitivity to reward, methylphenidate challenge and abuse behavior. Cloninger's theory concerning the trait of Novelty Seeking was thus confirmed. Given the additional finding of an inverse correlation of Reward Dependence and performance accuracy-improving drug effects, the biochemical theory of personality was however challenged concerning the neurochemical basis of Reward Dependence. Eventually, we could show that parental care rather than Reward Dependence determined the basic influence of methylphenidate's effect on performance accuracy.

4.2 Implications for the functionality of methylphenidate

Beyond the above-discussed implications, several further deductions with regard to the dopamine agonist methylphenidate can be drawn from these findings. For one thing, the data exemplify the extremely high variability of methylphenidate's behavioral impact. Not the intensity, but the

quality of the drug action was influenced by both early life parental care and personality traits. Thus, the successful therapeutic administration of methylphenidate in ADHD may be strongly determined by factors, which are generally unknown and altogether unrelated to the disorder. This completely unexpected observation might contribute to the discussion of a more careful therapeutic application of the drug. Since we tested healthy young adults and not ADHD patients, the present finding might be even more significant for the target group of methylphenidate users without medical prescription. Effectively, recent studies have shown that the annual prevalence of illicit methylphenidate use was 4% within a nationally representative U.S. sample of 8th, 10th and 12th graders (McCabe et al 2004) and 3% within a sample of 2250 undergraduate students (Teter et al 2003). Given that students typically use the drug with the aim of improving their cognitive performance in examination periods, it would certainly be of importance to inform the public about potential deteriorating drug effects.

Moreover, the different drug responsivity patterns, which emerged as a function of early life parental care and personality traits, indicate that behavioral drug effects on activity and performance accuracy are not necessarily interrelated, but may actually be mediated by different – namely, striatal and prefrontal – brain regions. This indication is especially relevant in reference to a hypothesis of dopamine dysfunction in ADHD, which suggests basal ganglia and prefrontal cortex to be differentially involved in the motor and cognitive symptoms of the disorder (Diamond et al 2002; Grace et al 2001; Solanto et al 2002) On the basis of his tonic/phasic model of dopamine system regulation, (Grace et al 2001) thus argues that reduced stimulation from the prefrontal cortex determines low tonic dopamine activity in subcortical regions. Low tonic stimulation of inhibitory autoreceptors may in turn trigger increased phasic activity, which may again result in dysregulated motor and impulse control in ADHD patients.

Aiming at the validation of this hypothesis, it would be of interest to repeat our test paradigm in a sample of ADHD patients with and without symptoms of hyperactivity, and to examine whether the achieved responsivity patterns dissociate between both types of the disorder.

Finally, the consistency with which methylphenidate induced changes in mood and arousal, independent of parental care or personality throughout both testing sessions, indicates a relatively strong subjective drug effect (although apparently not strong enough to unveil potential differences in dopamine responsivity between high and low parental care or Novelty Seeking participants). This finding is astonishing in consideration of the low therapeutic (20mg) oral drug dose administered. According to Volkow and Swanson (Volkow and Swanson 2003), the oral administration of methylphenidate – compared to intravenous injection, smoking and insufflation – does not reliably trigger reinforcing effects. Volkow and Swanson explain their argument based on the temporal course of methylphenidate's pharmacokinetics after different routes of administration. Injection, smoking and sniffling of methylphenidate produce a relatively fast peak brain uptake (within 6-10 minutes), which is thought to mimic the short and strong increase of phasic dopamine release and is held responsible for the drug's reinforcing properties. Oral administration, which produces peak brain uptake after 60-120 minutes, is thought to mimic the slow, steady state increase of tonic dopamine, and underlie the drug's therapeutic potential. The authors further hypothesize that the lack of reinforcing effects after oral methylphenidate administration accounts for the drug's low abuse potential (for a review see Volkow and Swanson 2003). Our findings challenge the reasoning of Volkow and Swanson's model. It may consequently be questioned whether the abuse potential of oral methylphenidate is actually as small as the authors suggest.

4.3 Remaining questions

The price of finding answers is typically the generation of new questions. The most prominent question emerging from our data concerns the methylphenidate-induced deterioration of the reward response in the high parental care group (we limit the following discussion to the care variable, because care, not Reward Dependence, was our main selection criterion). As mentioned above, many studies in healthy adults observed positive behavioral drug effects after therapeutic methylphenidate doses (Camp-Bruno and Herting 1994; Cooper et al 2005; Mehta et al 2000). Few reported the lack of behavioral effects (Bray et al 2004; Turner et al 2003) and still less reported deteriorating effects (Elliott et al 1997). What could be the reason for drug-induced performance decrement? Considering the inverted-u shaped relationship between stimulation of prefrontal dopamine receptors and cognitive performance (Arnsten and Dudley 2005; Berridge et al 2006; Granon et al 2000), we have suggested that high care participants functioned on an optimal prefrontal dopamine level in the absence of methylphenidate. Given the combined impact of reward and the drug, the peak of optimal stimulation might have been exceeded, thus determining impaired performance accuracy. Although this is the most obvious explanation for our finding, one question remains unanswered. The administered 20mg methylphenidate dose lies at the low end of the drug's therapeutic dose range (0.3-0.6 mg/kg). Should it have nevertheless effectuated overstimulation in combination with monetary reward, why was no drug effect – whatsoever – measurable in the non-reward condition?

Based on the animal model, our investigation focused on behavioral indicators of (relative to the mean) decreased prefrontal dopamine levels in participants with low parental care. However, the question arises, whether different responsivity patterns across care groups might rather have been driven by (relative to the mean) increased prefrontal dopamine levels in participants with high

parental care. Taken together, the definite answer to any question regarding the neurochemical mechanisms, which contributed to methylphenidate's behavioral effects in the presented paradigm can only be found with the help of imaging techniques. It accordingly remains to be discussed, whether our monetary reward task is qualified for application in the context of imaging procedures.

4.4 The monetary reward task

The influence of the indirect dopamine agonist methylphenidate on behavioral performance has been examined in various cognitive tasks. The special characteristic of our task was the combination of methylphenidate challenge with a monetary reward component. The drug-induced increase in tonic dopamine levels thus interacted with a phasic dopamine surge. Eventually, it was this combination of two different dopamine stimulators that allowed to detect a behavioral drug effect. Methylphenidate challenge alone failed to influence performance.

4.4.1 Task disadvantages

Several problematic aspects of the task became apparent during testing. For one thing, task performance was subjected to a strong learning effect. Sooner or later, performance in any cognitive task will reach a maximum level. This specific WCST-like task, however, involves an "Eureka component", which means that there is a moment where participants realize the task's basic principle. As of this turning point, difficulty decreases significantly. Although our task was programmed to be clearly more difficult than the original WCST, the level of mean performance in the second testing session indicated that the "Eureka moment" had been achieved in the majority of participants. Consequently, ceiling performance was reached and reward could no

longer trigger improvement. Parallel to this drastic change in the behavioral reward response, results indicate a change in the neurochemical reward response, since no interaction of reward and methylphenidate, and thus, no drug effect on performance emerged in the second testing session. A potential explanation for the lacking interaction can be derived from Schultz' exemplary primate studies. Unit recordings in fully awake monkeys identified that only initial contact with primary appetitive stimuli activated phasic firing of midbrain dopamine neurons. With repeated exposure, neural responses to food reward habituated, revealing unpredictability to be an important feature of midbrain dopamine responses. Dopamine neurons are thus activated during the learning phase but stop responding after full acquisition of various reward-delivering tasks (for a review see Schultz 2002). If we transfer the same logic to our task, reward most probably ceased to trigger a phasic dopamine release in the second testing session, in which participants were familiar with the testing situation, had discovered the basic principle of the task and knew the approximate amount they would be able to win. By this means, the lack of phasic dopamine release with reward may have inevitably yielded to the lack of a drug effect on performance. It altogether becomes evident that the utilized monetary reward task is not suitable for repeated measures designs.

Another problem relates to the poor comparability between the utilized monetary reward task and the WCST. It is a major advantage of the WCST to differentiate between types of errors (failure to maintain set, perseverative and non-perseverative errors), and to consequently allow the interpretation of potential cognitive deficits underlying these errors. Due to the applied modifications (faster change and randomized sequence of matching rules), our task did not allow defining different types of errors. This information loss was however accepted in favor of a higher task difficulty. Results from the second testing session, in which ceiling effects masked

any variability between participants, confirm this approach. It would nevertheless be of interest to examine performance in a rewarded version of the otherwise original WCST.

Unfortunately, in the present investigation, the succession of non-reward and reward conditions was not randomized (non-reward always preceded reward). As a result, the reward effect was confounded with a practice effect (whereas, importantly, any interactions between reward and treatment were not influenced by the practice effect). Although it was possible to partially correct for this "confoundation", our correction method did not account for the possibility of a non-linear practice effect. Therefore, in future applications of the task, the succession of non-reward and reward conditions should be changed systematically.

4.4.2 Task advantages

Despite the delineated disadvantages, the monetary reward task came up to our primary expectations. It exemplified how tonic dopamine stimulation modulates phasic neurotransmitter release on a behavioral level. It further revealed methylphenidate's activity- and attention-specific effects independent of each other. Also, it was sensitive for behavioral differences as a function of the examined dopamine-dependent variables. On the basis of this task, it was thus possible to ascertain the significance of our neurochemical hypotheses and gather preliminary evidence for their validation.

4.5 Conclusion

With regard to the basic objective of the present work – establishing an indirect measurement method to investigate relations between behavior and dopamine regulation – we have clearly achieved our primary goal. We have further gathered important information about the potential therapeutic action of methylphenidate, the influence of early life parental care on dopamine regulation as well as personality-dependent differences in sensitivity to stimulation of the dopamine system. After adjustment for the above-discussed shortcomings, our test paradigm can be applied in the context of imaging techniques. If, eventually, the neurochemical mechanisms underlying task performance have been disclosed, the paradigm can be used to assess deviances in dopamine regulation in a standardized manner.

References

Abercrombie ED, Bonatz AE, Zigmond MJ (1990): Effects of L-dopa on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats. *Brain Res* 525:36-44.

Arnsten AF, Dudley AG (2005): Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct* 1:2.

Balldin J, Berggren U, Lindstedt G, Sundkler A (1993): Further neuroendocrine evidence for reduced D2 dopamine receptor function in alcoholism. *Drug Alcohol Depend* 32:159-162.

Baron RM, Kenny AK (1986): The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology* 51:1173-1182.

Battaglia M, Przybeck TR, Bellodi L, Cloninger CR (1996): Temperament dimensions explain the comorbidity of psychiatric disorders. *Compr Psychiatry* 37:292-298.

Beck AT (1987): *Beck depression inventory manual*. New York: Harcourt, Brace, Jovanovich, Inc.

Benjamin J, Ebstein RP, Lesch KP (1998): Genes for personality traits: implications for psychopathology. *Int J Neuropsychopharmcol* 1:153-168.

Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelley AE, Schmeichel B, et al (2006): Methylphenidate Preferentially Increases Catecholamine Neurotransmission within the Prefrontal Cortex at Low Doses That Enhance Cognitive Function. *Biol Psychiatry*.

Bifulco A, Brown GW, Adler Z (1991): Early sexual abuse and clinical depression in adult life. *Br J Psychiatry* 159:115-122.

Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, et al (2000): Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. *J Psychoactive Drugs* 32 Suppl:i-iv, 1-112.

Bouyer JJ, Park DH, Joh TH, Pickel VM (1984): Chemical and structural analysis of the relation between cortical inputs and tyrosine hydroxylase-containing terminals in rat neostriatum. *Brain Res* 302:267-275.

Brake WG, Zhang TY, Diorio J, Meaney MJ, Gratton A (2004): Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci* 19:1863-1874.

Bray CL, Cahill KS, Oshier JT, Peden CS, Theriaque DW, Flotte TR, Stacpoole PW (2004): Methylphenidate does not improve cognitive function in healthy sleep-deprived young adults. *J Investig Med* 52:192-201.

Brown GR, Anderson B (1991): Psychiatric morbidity in adult inpatients with childhood histories of sexual and physical abuse. *Am J Psychiatry* 148:55-61.

Camp-Bruno JA, Herting RL (1994): Cognitive effects of milacemide and methylphenidate in healthy young adults. *Psychopharmacology (Berl)* 115:46-52.

Carter MM, Sbrocco T, Lewis EL, Friedman EK (2001): Parental bonding and anxiety: differences between African American and European American college students. *J Anxiety Disord* 15:555-569.

Chait LD (1994): Reinforcing and subjective effects of methylphenidate in humans. *Behav Pharmacol* 5:281-288.

Church WH, Justice JB, Jr., Neill DB (1987): Detecting behaviorally relevant changes in extracellular dopamine with microdialysis. *Brain Res* 412:397-399.

Cloninger CR (1987a): Neurogenetic adaptive mechanisms in alcoholism. *Science* 236:410-416.
Cloninger CR (1987b): A systematic method for clinical description and classification of personality variants. A proposal. *Arch Gen Psychiatry* 44:573-588.
Cloninger CR, Przybeck TR, Svrakic DM (1991): The Tridimensional Personality Questionnaire: U.S. normative data. *Psychol Rep* 69:1047-1057.
Cohen J (1988): *Statistical power analysis for the behavioral sciences*, 2nd ed. Hillsdale, NJ: Erlbaum.
Conners CK (2002): Forty years of methylphenidate treatment in Attention-Deficit/ Hyperactivity Disorder. *J Atten Disord* 6 Suppl 1:S17-30.
Cooper NJ, Keage H, Hermens D, Williams LM, Debrota D, Clark CR, Gordon E (2005): The dose-dependent effect of methylphenidate on performance, cognition and psychophysiology. *J Integr Neurosci* 4:123-144.
Davidson ES, Finch JF, Schenk S (1993): Variability in subjective responses to cocaine: initial experiences of college students. *Addict Behav* 18:445-453.
Deminiere JM, Piazza PV, Le Moal M, Simon H (1989): Experimental approach to individual vulnerability to psychostimulant addiction. *Neurosci Biobehav Rev* 13:141-147.
Diamond A (2002): Attention-deficit disorder (attention-deficit/hyperactivity disorder without hpyeractivity): a neuroiologically and behaviorally distinct disorder from attention-deficit/hyperactivity disorder (with hyperactivity). *Dev Psychopath* 17:807-825.
Di Chiara G, Imperato A (1988): Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A* 85:5274-5278.
Di Chiara G, Loddo P, Tanda G (1999): Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biol Psychiatry* 46:1624-1633.
Diamond A (2002): Attention-deficit disorder (attention-deficit/hyperactivity disorder without hpyeractivity): a neuroiologically and behaviorally distinct disorder from attention-deficit/hyperactivity disorder (with hyperactivity). *Dev Psychopath* 17:807-825.
Dughiero G, Schifano F, Forza G (2001): Personality dimensions and psychopathological profiles of Ecstasy users. *Hum Psychopharmacol* 16:635-639.
Elliott R, Sahakian BJ, Matthews K, Bannerjea A, Rimmer J, Robbins TW (1997): Effects of methylphenidate on spatial working memory and planning in healthy young adults. *Psychopharmacology (Berl)* 131:196-206.
Enns MW, Cox BJ, Clara I (2002): Parental bonding and adult psychopathology: results from the US National Comorbidity Survey. *Psychol Med* 32:997-1008.
Enns MW, Cox BJ, Larsen DK (2000): Perceptions of parental bonding and symptom severity in adults with depression: mediation by personality dimensions. *Can J Psychiatry* 45:263-268.
Faraone SV, Spencer T, Aleardi M, Pagano C, Biederman J (2004): Meta-analysis of the efficacy of methylphenidate for treating adult attention-deficit/hyperactivity disorder. *J Clin Psychopharmacol* 24:24-29.
Farnebo LO, Hamberger B (1971): Drug-induced changes in the release of (3 H)-noradrenaline from field stimulated rat iris. *Br J Pharmacol* 43:97-106.
Fibiger HC, LePiane FG, Jakubovic A, Phillips AG (1987): The role of dopamine in intracranial self-stimulation of the ventral tegmental area. *J Neurosci* 7:3888-3896.
First MB, Gibbon M, Williams JBW, Spitzer RL (1990): *Mini-SCID*. Toronto, ON: Multi Health Systems Inc.

Freund TF, Powell JF, Smith AD (1984): Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. *Neuroscience* 13:1189-1215.

Fulford AJ, Marsden CA (1998): Effect of isolation-rearing on conditioned dopamine release in vivo in the nucleus accumbens of the rat. *J Neurochem* 70:384-390.

Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS (1996): Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sci* 58:231-239.

Gerra G, Angioni L, Zaimovic A, Moi G, Bussandri M, Bertacca S, et al (2004): Substance use among high-school students: relationships with temperament, personality traits, and parental care perception. *Subst Use Misuse* 39:345-367.

Grace AA (1991): Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41:1-24.

Grace AA (1995): The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function. *Drug Alcohol Depend* 37:111-129.

Grace AA (2001): Psychostimulant actions on dopamine and limbic system function: relevance to the pathophysiology and treatment of ADHD. In Solanto MV, Arnsten AFT editors. *Stimulant drugs and ADHD: basic and clinical neuroscience.* New York: Oxford University Press, pp134–55.

Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW (2000): Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci* 20:1208-1215.

Grant DA, Berg EA (1948): A behavioral analysis of degree of reinforcement and ease of shifting to new responses in a Weigl-type card-sorting problem. *Journal of Experimental Psychology* 34:404-411.

Gratton A, Hoffer BJ, Gerhardt GA (1988): Effects of electrical stimulation of brain reward sites on release of dopamine in rat: an in vivo electrochemical study. *Brain Res Bull* 21:319-324.

Hall FS, Wilkinson LS, Humby T, Inglis W, Kendall DA, Marsden CA, Robbins TW (1998): Isolation rearing in rats: pre- and postsynaptic changes in striatal dopaminergic systems. *Pharmacol Biochem Behav* 59:859-872.

Hall FS, Wilkinson LS, Humby T, Robbins TW (1999a): Maternal deprivation of neonatal rats produces enduring changes in dopamine function. *Synapse* 32:37-43.

Hall H, Halldin C, Dijkstra D, Wikstrom H, Wise LD, Pugsley TA, et al (1996): Autoradiographic localisation of D3-dopamine receptors in the human brain using the selective D3-dopamine receptor agonist (+)-[3H]PD 128907. *Psychopharmacology (Berl)* 128:240-247.

Hall H, Halldin C, Guilloteau D, Chalon S, Emond P, Besnard J, et al (1999b): Visualization of the dopamine transporter in the human brain postmortem with the new selective ligand [125I]PE2I. *Neuroimage* 9:108-116.

Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L (1994): Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain. *Neuropsychopharmacology* 11:245-256.

Heaton RK, Chelune GJ, Talley JL, Kay GG, Curtiss G (1993): *Wisconsin Card Sorting Test manual: Revised and expanded*. Odessa, FL: Psychological Assessment Resources.

Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G, et al (2000): Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. *Neuroscience* 100:749-768.

Hoffman BJ (1994): Molecular biology of dopamine transporters. In: Niznik HB editor. *Dopamine Receptors and Transporters*. New York: Marcel Dekker Publisher, pp 645–668.

Horn JL, Skinner HA, Wanberg K, Foster FM (1984): *The Alcohol Use Questionnaire*. Toronto, ON: Addiction Research Foundation.

Howard MO, Kivlahan D, Walker RD (1997): Cloninger's tridimensional theory of personality and psychopathology: applications to substance use disorders. *J Stud Alcohol* 58:48-66.

Hughes RN, Greig AM (1976): Effects of caffeine, methamphetamine and methylphenidate on reactions to novelty and activity in rats. *Neuropharmacology* 15:673-676.

Hutchison KE, Swift R (1999): Effect of d-amphetamine on prepulse inhibition of the startle reflex in humans. *Psychopharmacology (Berl)* 143:394-400.

Ikemoto S, Panksepp J (1999): The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Res Brain Res Rev* 31:6-41.

Jerusalem M, Schwarzer R (1992): Self-efficacy as a resource factor in stress appraisal processes. *Self-Efficacy: Thought Control of Action*. Washington DC: Hemisphere.

Jones GH, Marsden CA, Robbins TW (1990): Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: possible disruption of dopamine-dependent mechanisms of the nucleus accumbens. *Psychopharmacology (Berl)* 102:364-372.

Joyce JN, Murray A (1994): Distribution of D1- and D2-like dopamine receptors in human brain. In: Niznik HB editor. *Dopamine Receptors and Transporters*. New York: Marcel Dekker Publisher, pp 345–399.

Kehoe P, Shoemaker WJ, Arons C, Triano L, Suresh G (1998): Repeated isolation stress in the neonatal rat: relation to brain dopamine systems in the 10-day-old rat. *Behav Neurosci* 112:1466-1474.

Kehr W, Carlsson A, Lindqvist M, Magnusson T, Atack C (1972): Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. *J Pharm Pharmacol* 24:744-747.

Keltikangas-Jarvinen L, Puttonen S, Kivimaki M, Rontu R, Lehtimaki T (2006): Cloninger's temperament dimensions and epidermal growth factor A61G polymorphism in Finnish adults. *Genes Brain Behav* 5:11-18.

Kikusui T, Isaka Y, Mori Y (2005): Early weaning deprives mouse pups of maternal care and decreases their maternal behavior in adulthood. *Behav Brain Res* 162:200-206.

Kim SW, Grant JE (2001): Personality dimensions in pathological gambling disorder and obsessive-compulsive disorder. *Psychiatry Res* 104:205-212.

Knutson B, Adams CM, Fong GW, Hommer D (2001a): Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci* 21:RC159.

Knutson B, Fong GW, Adams CM, Varner JL, Hommer D (2001b): Dissociation of reward anticipation and outcome with event-related fMRI. *Neuroreport* 12:3683-3687.

Koepp MJ, Gunn RN, Lawrence AD, Cunningham VJ, Dagher A, Jones T, et al (1998): Evidence for striatal dopamine release during a video game. *Nature* 393:266-268.

Korf J, Venema K (1985): Amino acids in rat striatal dialysates: methodological aspects and changes after electroconvulsive shock. *J Neurochem* 45:1341-1348.

Kuczenski R, Segal DS (1997): Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *J Neurochem* 68:2032-2037.

Kuhn KU, Meyer K, Nothen MM, Gansicke M, Papassotiropoulos A, Maier W (1999): Allelic variants of dopamine receptor D4 (DRD4) and serotonin receptor 5HT2c (HTR2c) and temperament factors: replication tests. *Am J Med Genet* 88:168-172.

Lizardi H, Klein DN (2005): Long-term stability of parental representations in depressed outpatients utilizing the Parental Bonding Instrument. *J Nerv Ment Dis* 193:183-188.

Luthman J, Fredriksson A, Lewander T, Jonsson G, Archer T (1989): Effects of d-amphetamine and methylphenidate on hyperactivity produced by neonatal 6-hydroxydopamine treatment. *Psychopharmacology (Berl)* 99:550-557.

Matthews K, Dalley JW, Matthews C, Tsai TH, Robbins TW (2001): Periodic maternal separation of neonatal rats produces region- and gender-specific effects on biogenic amine content in postmortem adult brain. *Synapse* 40:1-10.

Matthews K, Hall FS, Wilkinson LS, Robbins TW (1996a): Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: effects of prefeeding, d-amphetamine, dopamine antagonists and clonidine. *Psychopharmacology (Berl)* 126:75-84.

Matthews K, Wilkinson LS, Robbins TW (1996b): Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. *Physiol Behav* 59:99-107.

May LJ, Kuhr WG, Wightman RM (1988): Differentiation of dopamine overflow and uptake processes in the extracellular fluid of the rat caudate nucleus with fast-scan in vivo voltammetry. *J Neurochem* 51:1060-1069.

McCabe SE, Teter CJ, Boyd CJ, Guthrie SK (2004): Prevalence and correlates of illicit methylphenidate use among 8th, 10th, and 12th grade students in the United States, 2001. *J Adolesc Health* 35:501-504.

Meaney MJ (2001): Nature, nurture, and the disunity of knowledge. *Ann N Y Acad Sci* 935:50-61.

Mehta MA, Goodyer IM, Sahakian BJ (2004): Methylphenidate improves working memory and set-shifting in AD/HD: relationships to baseline memory capacity. *J Child Psychol Psychiatry* 45:293-305.

Mehta MA, Owen AM, Sahakian BJ, Mavaddat N, Pickard JD, Robbins TW (2000): Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. *J Neurosci* 20:RC65.

Miller JD, Sanghera MK, German DC (1981): Mesencephalic dopaminergic unit activity in the behaviorally conditioned rat. *Life Sci* 29:1255-1263.

Mitchell SH (1999): Measures of impulsivity in cigarette smokers and non-smokers. *Psychopharmacology (Berl)* 146:455-464.

Nigg JT (2005): Neuropsychologic theory and findings in attention-deficit/hyperactivity disorder: the state of the field and salient challenges for the coming decade. *Biol Psychiatry* 57:1424-1435.

Noble EP, Ozkaragoz TZ, Ritchie TL, Zhang X, Belin TR, Sparkes RS (1998): D2 and D4 dopamine receptor polymorphisms and personality. *Am J Med Genet* 81:257-267.

Norris H (1971): The action of sedatives on brain stem oculomotor systems in man. *Neuropsychopharmacology* 10:233-239.

O'Driscoll GA, Depatie L, Holahan AL, Savion-Lemieux T, Barr RG, Jolicoeur C, Douglas VI (2005): Executive functions and methylphenidate response in subtypes of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57:1452-1460.

Olds J, Milner P (1954): Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 47:419-427.

Overbeek G, Vollebergh W, Meeus W, de Graaf R, Engels RC (2004): Young adults' recollections of parental bonds--does satisfaction with partner relationships mediate the

longitudinal association with mental disorders? *Soc Psychiatry Psychiatr Epidemiol* 39:703-710.

Parker G, Hadzi-Pavlovic D, Greenwald S, Weissman M (1995): Low parental care as a risk factor to lifetime depression in a community sample. *J Affect Disord* 33:173-180.

Parker G, Tupling H, Brown LB (1979): a parental bonding instrument. *British Journal of Medical Psychology* 52:1-10.

Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003): Subsecond dopamine release promotes cocaine seeking. *Nature* 422:614-618.

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003): Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28:916-931.

Pruessner JC (2004): *WCST-like monetary reward task.* 1st edition, Montreal.

Pruessner JC, Champagne F, Meaney MJ, Dagher A (2004): Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. *J Neurosci* 24:2825-2831.

Rapport MD, Moffitt C (2002): Attention deficit/hyperactivity disorder and methylphenidate. A review of height/weight, cardiovascular, and somatic complaint side effects. *Clin Psychol Rev* 22:1107-1131.

Robinson TE, Whishaw IQ (1988): Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. *Brain Res* 450:209-224.

Rosenberg M (1989): *Society and the adolescent self-image*. Middleton, CT: Wesleyan University Press.

Ruegg RG, Gilmore J, Ekstrom RD, Corrigan M, Knight B, Tancer M, et al (1997): Clomipramine challenge responses covary with Tridimensional Personality Questionnaire scores in healthy subjects. *Biol Psychiatry* 42:1123-1129.

Russek LG, Schwartz GE (1997): Perceptions of parental caring predict health status in midlife: a 35-year follow-up of the Harvard Mastery of Stress Study. *Psychosom Med* 59:144-149.

Sands SB, Barish ME (1989): A quantitative description of excitatory amino acid neurotransmitter responses on cultured embryonic Xenopus spinal neurons. *Brain Res* 502:375-386.

Sax KW, Strakowski SM (1998): Enhanced behavioral response to repeated d-amphetamine and personality traits in humans. *Biol Psychiatry* 44:1192-1195.

Scheel-Kruger J (1971): Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. *Eur J Pharmacol* 14:47-59.

Schultz W (1986): Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. *J Neurophysiol* 56:1439-1461.

Schultz W (2002): Getting formal with dopamine and reward. *Neuron* 36:241-263.

Schultz W, Romo R (1990): Dopamine neurons of the monkey midbrain: contingencies of responses to stimuli eliciting immediate behavioral reactions. *J Neurophysiol* 63:607-624.

Seeman P, Madras B (2002): Methylphenidate elevates resting dopamine which lowers the impulse-triggered release of dopamine: a hypothesis. *Behav Brain Res* 130:79-83.

Seeman P, Madras BK (1998): Anti-hyperactivity medication: methylphenidate and amphetamine. *Mol Psychiatry* 3:386-396.

Self D (2003): Neurobiology: Dopamine as chicken and egg. *Nature* 422:573-574.

Sharp T, Zetterstrom T, Ungerstedt U (1986): An in vivo study of dopamine release and metabolism in rat brain regions using intracerebral dialysis. *J Neurochem* 47:113-122.

Shaywitz SE, Hunt RD, Jatlow P, Cohen DJ, Young JG, Pierce RN, et al (1982): Psychopharmacology of attention deficit disorder: pharmacokinetic, neuroendocrine, and behavioral measures following acute and chronic treatment with methylphenidate. *Pediatrics* 69:688-694.

Silove D, Parker G, Hadzi-Pavlovic D, Manicavasagar V, Blaszczynski A (1991): Parental representations of patients with panic disorder and generalised anxiety disorder. *Br J Psychiatry* 159:835-841.

Skinner HA (1982): The drug abuse screening test. *Addict Behav* 7:363-371.

Sokoloff P, Schwartz JC (1995): Novel dopamine receptors half a decade later. *Trends Pharmacol Sci* 16:270-275.

Solanto MV (1984): Neuropharmacological basis of stimulant drug action in attention deficit disorder with hyperactivity: a review and synthesis. *Psychol Bull* 95:387-409.

Solanto MV (1986): Behavioral effects of low-dose methylphenidate in childhood attention deficit disorder: implications for a mechanism of stimulant drug action. *J Am Acad Child Psychiatry* 25:96-101.

Solanto MV (2002): Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behav Brain Res* 130:65-71.

Sonuga-Barke EJ (2005): Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. *Biol Psychiatry* 57:1231-1238.

Spielberger CD (1983): *Manual of the State-Trait Anxiety Inventory (STAI)*. Palo Alto, CA: Consulting Psychologists Press.

Stanwood GD, Zigmond MJ (2000): Dopamine, Central. In: Fink G editor. *Encyclopedia of stress*, Vol 1: Academic Press, pp 739-746.

Steiner NO, Delay ER, Isaac W (1986): Effects of drugs, age and illumination on response speed of squirrel monkeys. *Pharmacol Biochem Behav* 24:503-506.

Swanson JM, Sergeant JA, Taylor E, Sonuga-Barke EJ, Jensen PS, Cantwell DP (1998): Attention-deficit hyperactivity disorder and hyperkinetic disorder. *Lancet* 351:429-433.

Teter CJ, McCabe SE, Boyd CJ, Guthrie SK (2003): Illicit methylphenidate use in an undergraduate student sample: prevalence and risk factors. *Pharmacotherapy* 23:609-617.

Tupala E, Hall H, Bergstrom K, Sarkioja T, Rasanen P, Mantere T, et al (2001a): Dopamine D(2)/D(3)-receptor and transporter densities in nucleus accumbens and amygdala of type 1 and 2 alcoholics. *Mol Psychiatry* 6:261-267.

Tupala E, Kuikka JT, Hall H, Bergstrom K, Sarkioja T, Rasanen P, et al (2001b): Measurement of the striatal dopamine transporter density and heterogeneity in type 1 alcoholics using human whole hemisphere autoradiography. *Neuroimage* 14:87-94.

Tupala E, Tiihonen J (2004): Dopamine and alcoholism: neurobiological basis of ethanol abuse. *Prog Neuropsychopharmacol Biol Psychiatry* 28:1221-1247.

Turner DC, Robbins TW, Clark L, Aron AR, Dowson J, Sahakian BJ (2003): Relative lack of cognitive effects of methylphenidate in elderly male volunteers. *Psychopharmacology (Berl)* 168:455-464.

Udo de Haes JI, Kortekaas R, Van Waarde A, Maguire RP, Pruim J, den Boer JA (2005): Assessment of methylphenidate-induced changes in binding of continuously infused [(11)C]-raclopride in healthy human subjects: correlation with subjective effects. *Psychopharmacology (Berl)* 183:322-330.

Unis AS, Dawson TM, Gehlert DR, Wamsley JK (1985): Autoradiographic localization of [3H]methylphenidate binding sites in rat brain. *Eur J Pharmacol* 113:155-157.

Usiello A, Baik JH, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, et al (2000): Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* 408:199-203.

Volkow ND, Swanson JM (2003): Variables that affect the clinical use and abuse of methylphenidate in the treatment of ADHD. *Am J Psychiatry* 160:1909-1918.

Volkow ND, Wang GJ, Fowler JS, Ding YS (2005): Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57:1410-1415.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS, et al (1998): Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. *Am J Psychiatry* 155:1325-1331.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Wong C, et al (1999): Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. *J Pharmacol Exp Ther* 291:409-415.

Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, et al (2002): "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. *Synapse* 44:175-180.

Volkow ND, Wang GJ, Fowler JS, Molina PE, Logan J, Gatley SJ, et al (2003): Cardiovascular effects of methylphenidate in humans are associated with increases of dopamine in brain and of epinephrine in plasma. *Psychopharmacology (Berl)* 166:264-270.

Volkow ND, Wang GJ, Fowler JS, Telang F, Maynard L, Logan J, et al (2004): Evidence that methylphenidate enhances the saliency of a mathematical task by increasing dopamine in the human brain. *Am J Psychiatry* 161:1173-1180.

Wilhelm K, Niven H, Parker G, Hadzi-Pavlovic D (2005): The stability of the Parental Bonding Instrument over a 20-year period. *Psychol Med* 35:387-393.

Zhang TY, Chretien P, Meaney MJ, Gratton A (2005): Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. *J Neurosci* 25:1493-1502.