NEURAL CORRELATES OF DELAY DISCOUNTING:
EFFECTS OF DOPAMINE BIOAVAILABILITY AND IMPLICATIONS FOR ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

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ERSTGUTACHTER: PROF. DR. PAUL PAULI
ZWEITGUTACHTER: PROF. DR. ANDREAS J. FALLGATTER
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Abstract

Humans and other animals share choice preference for smaller-but-sooner over later-but-larger rewards, indicating that the subjective value of a reward is discounted as a function of time. This phenomenon referred to as delay discounting (DD), represents one facet of impulsivity which is inherently connected with reward processing and, within a certain range, adaptive. Maladaptive levels, however, can lead to suboptimal decision-making and represent important characteristics of psychopathologies such as attention-deficit/hyperactivity disorder (ADHD).

In line with a proposed influence of dysregulated dopamine (DA) levels on impulsivity, neural structures involved in DD (the ventral-striatum [VS]; orbitofrontal cortex [OFC]) are highly innervated by dopaminergic neurons. However, studies explicitly testing the triadic interplay of dopaminergic neurotransmission, impulsivity and brain activation during intertemporal choice are missing.

Therefore, the first study of the thesis examined the effect of different DA-bioavailability levels, indicated by a genetic polymorphism (Val158Met) in the gene of the catechol-O-methyltransferase, on the association of delay discounting and OFC activation. OFC response to monetary rewards that varied by delay-to-delivery was recorded with functional near-infrared spectroscopy (fNIRS) in a sample of 49 healthy human subjects. The results suggest a DA-related enhancement in OFC function from low (low DA level) to partial (intermediate DA level) and full (high DA level) reward delay sensitivity. Furthermore, DA-bioavailability was shown to moderate the association of neural reward delay sensitivity and impulsivity: OFC reward delay sensitivity was strongly correlated with impulsivity at intermediate DA-levels, but not at low or high DA-levels where impulsivity was related to delay-independent OFC amplitudes. It is concluded that DA-level should be considered as a crucial factor

whenever impulsivity-related brain activation, in particular to reward delay, is examined in healthy subjects.

Dysfunctional reward processing, accompanied by a limited ability to tolerate reward delays (delay aversion), has been proposed as an important feature in ADHD putatively caused by striatal hypo-dopaminergia. Therefore, the aim of the second study of this thesis was to examine subcortical processing of reward delays and to test for neural indicators of a negative emotional response to delay periods. Using functional magnetic resonance imaging (fMRI), brain activation in adult patients with ADHD (n=14) and healthy control subjects (n=12) was recorded during the processing of immediate and delayed rewards. Compared with healthy control subjects, hyporesponsiveness of the VS reward system was evident in patients with ADHD for both immediate and delayed rewards. In contrast, delayed rewards evoked hyperactivation in the dorsal caudate nucleus and the amygdala of ADHD patients, corroborating the central predictions of the delay aversion hypothesis.

In combination both studies support the conception of a close link between delay discounting, brain activation and dopaminergic neurotransmission. The results implicate that studies on neural correlates of DD have to account for the DA-bioavailability level and for a negative emotional response to reward delays.

German Abstract (deutsche Zusammenfassung)

Menschen und andere Spezies zeigen eine Präferenz für sofortige Belohnung mit geringerem Wert gegenüber zeitlich verzögerter Belohnung mit höherem Wert. Dies weist darauf hin, dass der subjektiv empfundene Wert einer Belohnung in Abhängigkeit der Verzögerung bis zur Aushändigung abnimmt. Dieses Phänomen wird als Delay Discounting (DD) bezeichnet und stellt eine Facette von Impulsivität dar, die direkt mit Belohnungsverarbeitung verknüpft und innerhalb eines bestimmten Rahmens adaptiv ist. Ein maladaptives Ausmaß an DD hingegen kann zu suboptimaler Entscheidungsfindung führen und repräsentiert eine wichtige Eigenschaft psychischer Erkrankungen wie der Aufmerksamkeitsdefizit-/Hyperaktivitäts-störung (ADHS).

In Einklang mit der Annahme eines Zusammenhangs von dysreguliertem Dopaminhaushalt und Impulsivität sind neuronale Strukturen, die während DD aktiv sind (Ventrales Striatum [VS]; Orbitofrontaler Cortex [OFC]) stark durch dopaminerge Neurone innerviert. Bislang fehlen allerdings Studien, die explizit Interaktionen von dopaminerger Neurotransmission, Impulsivität *und* Hirnaktivierung während intertemporaler Entscheidungsaufgaben untersuchen.

Studie I der vorliegenden Promotionsschrift untersucht daher den Einfluss unterschiedlicher Dopamin (DA)-Bioverfügbarkeit (anhand eines genetischen Polymorphismus (Val158Met) im Gen der Catechol-O-Methyltransferase) auf den Zusammenhang von DD und OFC Aktivierung. Mittels funktioneller Nah-Infrarot Spektroskopie (fNIRS) wurde in einer Gruppe von 49 gesunden Versuchspersonen die OFC Aktivität bei sofortiger und verzögerter Belohnung aufgezeichnet. Die Ergebnisse zeigen eine DA-abhängige Erweiterung der Funktion des OFC von schwacher (niedrige DA Verfügbarkeit), über eine partielle (mittlere DA Verfügbarkeit) bis hin zu starker (hohe DA Verfügbarkeit) Sensitivität für Belohnungsverzögerungen. Des Weiteren konnte gezeigt werden, dass die DA-Verfügbarkeit

den Zusammenhang von neuronaler Sensitivität für Belohnungsverzögerungen und Impulsivität moderiert: ein starker Zusammenhang konnte bei mittlerer DA-Verfügbarkeit gezeigt werden, nicht aber bei niedriger oder hoher DA-Verfügbarkeit. Bei letzteren korrelierte Impulsivität mit der Höhe der OFC-Aktivität unabhängig von Belohnungsverzögerungen. Die DA-Verfügbarkeit sollte demnach als ein wichtiger Faktor berücksichtigt werden, wenn impulsivitätsabhängige Hirnaktivierung, insbesondere die Verarbeitung von Belohnungsverzögerungen betreffend, untersucht wird.

Eine dysfunktionale Belohnungsverarbeitung, verbunden mit einer eingeschränkten Toleranz von Belohnungsverzögerungen, wird als ein wichtiges Merkmal von ADHS angenommen, dessen Ursache möglicherweise eine verminderte DA-Konzentration im Striatum ist. Das Ziel von Studie II der vorliegenden Promotionsschrift ist daher, subkortikale Verarbeitung von Belohnungsverzögerung zu untersuchen und Hinweise für eine negative emotionale Reaktion auf Verzögerung zu prüfen. Mittels funktioneller Magnetresonanztomographie (fMRT) wurde die Hirnaktivierung adulter Patienten mit ADHS (n=14) und gesunder Kontrollpersonen (n=12) während der Verarbeitung von sofortiger und verzögerter Belohnung aufgezeichnet. Im Vergleich zu gesunden Kontrollpersonen zeigte sich bei Patienten mit ADHS eine Minderaktivierung auf sofortige und verzögerte Belohnung im ventral-striatalen Belohnungssystem. Im Gegensatz dazu führte verzögerte Belohnung bei Patienten mit ADHS zu einer Überaktivierung im dorsalen Nucleus Caudatum sowie in der Amygdala. Diese Ergebnisse stützen die zentrale Annahme der Verzögerungsaversions-Hypothese bei ADHS.

Gemeinsam weisen beide Studien auf eine enge Verbindung von DD, Hirnaktivierung und dopaminerger Neurotransmission hin. Die Ergebnisse implizieren, dass Untersuchungen der neuronalen Korrelate von DD sowohl die DA-Bioverfügbarkeit, als auch negative emotionale Reaktionen auf Belohnungsverzögerung berücksichtigen sollten.

General introduction

Delay discounting (DD) is a widely accepted explanation for impulsive decision-making in intertemporal choice situations wherein subjects often prefer smaller-but-sooner over later-but-larger rewards (Ainslie, 1975; Ainslie & Herrnstein, 1981; Logue & King, 1991; Logue, Pena Correal, Rodriguez, & Kabela, 1986). Accordingly, this choice preference occurs because delay reduces the subjective value of the reward. The nature of DD can be described by hyperbolic discounting functions (Green & Myerson, 2004) and the steepness of DD is an often used quantification of impulsivity (Green & Myerson, 2004; Reynolds, 2006)[1].

DD represents one facet of the broad construct impulsivity (Evenden, 1999) that is inherently connected to reward processing (Gray, 1987) and, to some degree, adaptive by involving positive effects on the individual and/or the population fitness under particular circumstances (Daly & Wilson, 2005; Dickman, 1990; Williams & Taylor, 2006). It can be life-saving to choose a smaller-but-sooner reward option (e.g. food) rather than to wait for a larger option that might, in the end, be too late. At the population level, highly impulsive members can serve as negative models for the remaining members, preventing them from danger (Williams & Taylor, 2006).

Maladaptive levels of impulsivity, however, can lead to suboptimal decision-making (Bechara & Damasio, 2002) with far-reaching negative (e.g. ecologic, economic) consequences. Furthermore, maladaptive levels of impulsivity represent cardinal characteristics of severe psychopathologies such as attention-deficit/hyperactivity disorder (ADHD).

The overlap of the neural substrate, i.e. brain structures and neurotransmitters, involved in the processing of DD and referred to as dysfunctional in ADHD, indicates DD as a putatively worthwhile concept to explore. Therefore, the present thesis examines neural correlates of

[1] Delay Discounting is often used as synonymous with impulsivity. However, some authors (Stephens, Kerr, & Fernandez-Juricic, 2004) claim that DD is only one possible *interpretation* of preference for smaller but sooner rewards. An alternative view would be an aversive reaction to delay periods.

(mal-)adaptive DD against the background of dopaminergic neurotransmission which is thought to play a significant role in the neurobiology of impulsivity. The aim of the thesis is to expand existing knowledge concerning a) dopaminergic effects on DD-related brain activation in healthy subjects and b) DD-related emotional effects in adult patients with ADHD, where a hypo-dopaminergic state is assumed (Sagvolden, Johansen, Aase, & Russell, 2005).

Neural correlates of Delay Discounting

Two distinct accounts concerning the neural basis of DD are currently discussed: a one-component and a two-component model. The classical two-component models explain DD as an evolutionary conserved "hot emotional" component counteracted by a more recently evolved "cool cognitive" system (Loewenstein, Rick, & Cohen, 2008; McClure, Laibson, Loewenstein, & Cohen, 2004; Strack & Deutsch, 2004). Functional neuroimaging studies on DD support this view by showing that deliberative processes involve the dorsolateral prefrontal cortex (DLPFC), while limbic areas (e.g. VS) and interconnected regions, particularly the orbitofrontal cortex (OFC), preferentially respond toward immediate rewards (McClure, 2007; McClure et al., 2004)[2].

Support for a one-component model of DD is given by Kable & Glimcher (2007) who replicated the identified structures involved in DD but re-interpreted their functional role. BOLD activity in the VS, medial prefrontal cortex and posterior cingulate cortex areas was found to directly track the subjective value (DD) of rewards as determined from behaviour (=steepness of DD), rather than indicating the presence of a separate system which is more impulsive than the person's choice behaviour, as predicted by two-component models. Therefore, Kable & Glimcher concluded that immediate rewards are simply more valuable

[2] Deliberative processes furthermore involved right and left intraparietal cortex, right ventrolateral prefrontal cortex, and right lateral orbitofrontal cortex. Further structures exhibiting an immediacy-bias: medial prefrontal cortex, posterior cingulate cortex, and left posterior hippocampus.

than delayed rewards, and that these areas do not encode immediacy alone nor that they mirror an impulsive signal that is "pushing" subjects to take the reward now (Joe Kable, personal communication, 10-17-2008).

In contrast to McClure et al. (2007; 2004), OFC activation was not significantly associated with DD in the study of Kable & Glimcher. This might be due to differences in the applied paradigm, due to the small sample size (n=10), or due to local susceptibility gradients that cause image distortions and signal losses especially in the orbitofrontal cortex (Deichmann, Gottfried, Hutton, & Turner, 2003). Another explanation for this inconsistency might be that neither McClure et al. nor Kable & Glimcher did account for potential dopaminergic effects. If DA bioavailability affects neural activation during DD (see section *The role of dopamine in Delay Discounting)*, the unknown sample compositions regarding the subjects' DA bioavailability may account for the different findings.

The role of dopamine in Delay Discounting

The dopaminergic neurotransmitter system has been proposed to play a significant role in the neurobiology of impulsivity (Sagvolden et al., 2005; Tripp & Wickens, 2008). Notably, the identified neural structures involved in DD are highly innervated by dopaminergic neurons. However, the influence of DA on DD is complex for several reasons: a) multiple subcomponents might be involved in DD (reward processing; foresight) implying different interacting neural systems[3]; b) the DA system itself is complex consisting of several pathways that innervate distinct brain areas; c) the DA system is highly dynamic by means of inner-structure interactions (e.g. tonic/phasic DA levels) and inter-structure interactions (e.g. PFC induced adaptive changes in subcortical DA activity) – (Cools, 2008). Therefore, the present description of DA-related effects relevant for DD will be limited to aspects which are considered as relevant for the present thesis: *subcortical/cortical and tonic/phasic DA effects*.

At the subcortical level, dopaminergic neurons are critically involved in reinforcement learning (RL) by (1) responding to unexpected reward, (2) predicting reward and (3) signalling discrepancies between reward expectation and the actual reward (Schultz, 1998; Schultz, Dayan, & Montague, 1997; Sutton & Barto, 1998). Whenever a rewarding stimulus is delivered to a subject, midbrain dopamine cells increase firing rate. If the rewarding stimulus is predicted by a preceding cue, dopamine cell firing transfers toward the earliest available cue across time while the response to the actual reward disappears. Therefore, the cue-stimulus becomes a discriminative stimulus with secondary reinforcer quality. If a predicted reward does not appear, depression of dopamine activity occurs at the time of the missing delivery.

Altered DA-predicting-signals have been proposed to account for maladaptive levels of DD in ADHD (Sagvolden et al., 2005; Tripp & Wickens, 2008)[4]. Accordingly, DA signal transfer to the reward predicting cue is diminished in patients with ADHD, narrowing the duration of the time window available for detecting coincidence of action and reward and producing a steeper delay-of-reinforcement gradient. With a hypo-dopaminergic state, successful RL occurs only if the delay between an action and its reinforcement is short. In an operant DD task this results in preference for the smaller-but-sooner option. Support for diminished anticipatory reward processing in ADHD is coming from fMRI studies demonstrating striatal hypo-responsiveness of the VS in adolescent and adult patients with ADHD (Scheres, Milham, Knutson, & Castellanos, 2007; Strohle et al., 2008). Pharmacological evidence suggests that dopamine release within the VS comprising the nucleus accumbens (Nacc) is tightly coupled to fMRI BOLD signals (Knutson & Gibbs, 2007) and therefore these results might be related to hypo-dopaminergic responses in anticipation of a reward in ADHD.

[3] A critical discussion of the involved subcomponents in DD processing with different experimental paradigms can be found in section *General Discussion.*

[4] Sagvolden et al. propose that ADHD is associated with a dysregulation of tonic/phasic dopamine comprising reduced phasic dopamine responses and low tonic DA levels.

However, in healthy subjects opposite effects have been reported: Forbes et al. (2007) as well as Hariri et al. (2006) found positive correlations of reward related VS activity and measures of trait impulsivity, which is well in line with classic personality theory of a heightened reward sensitivity in high impulsive subjects (Gray, 1987). Additionally, Forbes et al. assessed functional DA-related polymorphisms, suggesting that high striatal DA levels were associated with higher levels of impulsivity and higher VS reactivity. However, this apparent inconsistency with the model of a decreased DA state in ADHD might be related to baseline levels of DA neurotransmission which may differ markedly between patients with ADHD and healthy adults (Oswald et al., 2007).

At the prefrontal cortex (PFC) level, the most consistent finding is that DA stabilizes neural representations during PFC mediated processes (working memory; decision-making; future planning) by actively up-holding information and improving the signal-to-noise ratio (Durstewitz, Seamans, & Sejnowski, 2000; Previc, 1999). Accordingly, PFC hypo-dopaminergia is associated with faster decay of neural representations of relevant information and an increased interference with competing inputs resulting in poor target/background differentiation (Savitz, Solms, & Ramesar, 2006). An important DD-related subregion of the PFC, the OFC is responsible for (a) calculating the value of a reward outcome, (b) assessing trade-offs; (c) determining how well the outcome satisfies current needs, and (d) comparing the outcome with other potential reward outcomes (Wallis, 2007). For the OFC and other prefrontal regions DA-related effects on reward processing have been shown (Cetin, Freudenberg, Fuchtemeier, & Koch, 2004; Rossetti & Carboni, 2005; Yacubian et al., 2007), suggesting that low DA levels lead to blunted neuronal anticipatory processing of rewards.

Therefore, hypo-dopaminergic states in the PFC might represent a second mechanism to account for the preference of smaller-but-sooner rewards due to a failure to inhibit prepotent responses (=choosing immediate rewards) or due to a deficient ability for imaginative foresight (Boyer, 2008).

For the dose–response signature of prefrontal DA upon related signalling an inverted-U model has been suggested, with excessively high or low DA levels leading to altered performance (Goldman-Rakic, Muly, & Williams, 2000). In healthy subjects Egan et al. (Egan et al., 2001) demonstrated that a higher prefrontal DA level, as operationalized by the catechol-O-methyltransferase (COMT) Val158Met polymorphism, was associated with relatively better performance on a PFC-dependent task. However, administration of amphetamine which induces DA activity enhanced the efficiency of PFC function in subjects with low DA level while it caused deterioration of PFC processing during demanding tasks in subjects with high DA level (Mattay et al., 2003). Comparable to the situation at the subcortical level the opposite DA effects can be existent in patient groups (Barnett, Scoriels, & Munafò, 2008; Prata et al., in press) putatively due to distinct baseline levels of DA.

Direct prefrontal DA effects on DD have been suggested by Boettiger et al. (2007). Lower DA bioavailability was associated with a higher ratio of impulsive choices and greater immediacy-biased PFC activation. However, the sample of this study is small and heterogeneous comprising healthy subjects and abstinent alcoholics. Therefore, the results should be interpreted cautiously and need re-examination. Furthermore, the results of Drabant et al. (Drabant et al., 2006) suggest that DA-bioavailability moderates the relationship of brain activation and trait measures potentially by influencing the functional coupling of brain areas such as the OFC and the amygdala. Therefore, besides direct effects of prefrontal DA on impulsivity, which has yet not been consistently shown (Congdon & Canli, 2008), the moderating potency of DA-bioavailability on trait/brain-couplings may represent a useful approach to examine.

A close and reciprocal relationship of prefrontal and striatal DA (Bilder, Volavka, Lachman, & Grace, 2004; Grace, 1991) has been proposed: Accordingly, DA receptor stimulation in the PFC promotes goal stability and distractor resistance, while DA receptor stimulation in the striatum promotes goal flexibility. Therefore, DA-effects most likely have to be seen as

arising from the interplay of cortical and subcortical DA levels, which might be reciprocal in healthy subjects (Bilder et al., 2004). Dopamine dysregulation at both levels, PFC and striatum, may exist in the diseased brain and independently alter subcomponent processes related to DD, comprising altered reward processing at the subcortical level and diminished processing at the cortical level, for example upholding information and foresight.

In sum, DA-related effects on neural activation can be expected at the subcortical and the cortical level and both levels comprise neural structures which are involved in the processing of DD.

Decreasing positive or increasing negative emotional response?

DD is implicitly assumed to be mirrored by *decreased* neural activation to delayed versus immediate rewards. Neuroimaging studies support this view (Kable & Glimcher, 2007) by demonstrating that temporal devaluation of a reward is strongly correlated with neural activation of structures which are involved in intertemporal choice. However, in the domain of ADHD (Sonuga Barke, 2002, 2003; Sonuga Barke, Taylor, & Heptinstall, 1992; Sonuga Barke, Taylor, Sembi, & Smith, 1992; Sonuga Barke, Williams, Hall, & Saxton, 1996) it has been proposed that two different (neural) processes are engaged in an inter-temporal choice situation and that both processes contribute to the observable DD effect: (1) steeper devaluation of temporally distant rewards (discounting function) in patients with ADHD and (2) a negative emotional response to the (expected) delay period (=delay aversion). Indirect support for this two-process-model comes from fMRI studies that show neural structures preferentially responding to delayed rewards in the dorsal part of the striatum (Tanaka et al., 2004) and activation of the parahippocampal gyrus, an area which overlaps with the amygdala, during waiting periods suggesting a heightened negative emotional response to delays (Boettiger et al., 2007).

A core structure involved in emotional processing is the amygdala. Amygdala activation mirrors the arousing features of stimuli (Small et al, 2003; Anderson et al., 2003). Emotionally arousing stimuli, whether of an appetitive or aversive nature, can engage the amygdala, which in turn modulates the psychological and physiological internal milieu in order to deal adaptively with emotionally salient events (Hamann et al., 2002). This view has been recently confirmed by meta-analyses (Sergerie et al., 2008; Murphy et al., 2003; Wager et al., 2003) and provides support for the notion of the amygdala as being involved in the processing of biologically relevant information, regardless of valence (Sander et al., 2003). Potentially, spatial resolution of standard neuroimaging techniques may not be able to separate sub-regions within the amygdala that code for either positive or negative values (see Salzman et al., 2007). If the central assumption of the delay aversion hypothesis is valid (see *Study II)*, the litmus test is to detect amygdalar hyper-activation to delayed rewards.

Hypotheses

Based on existing evidence, the following hypotheses will be examined. *Study I:* In healthy subjects a positive correlation of OFC activation (immediate minus delayed rewards) and behavioral DD is expected (Hypothesis A1). It is tested whether DA-bioavailability, indicated by a genetic polymorphism (Val158Met) in the catechol-O-methyltransferase Val158Met polymorphism, moderates this correlation (Hypothesis A2) – see Figure 1. *Study II:* In adult patients with ADHD, where a hypo-dopaminergic state is assumed, VS hypo-responsiveness to delayed rewards is expected (Hypothesis B1a). Furthermore, it is tested whether VS activation to immediate rewards in adult ADHD patients is altered (Hypothesis B1b). Based on the delay aversion hypothesis, hyperactivation of the amygdala to delayed rewards is expected in adult patients with ADHD (Hypothesis B2) – see Figure 2.

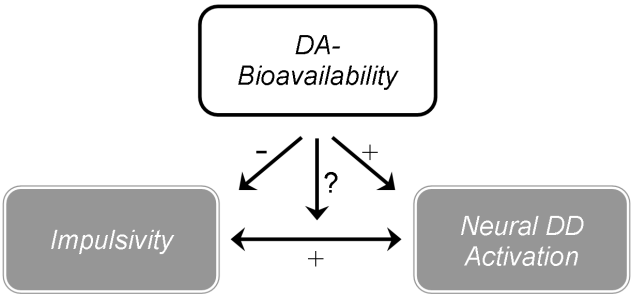

Figure 1 – Main hypothesis of *study I*. A positive correlation of impulsivity and OFC activation (immediate minus delayed) is assumed for structures such as the OFC (black-boxes). Moreover, DA-bioavailability may enhance cortical information processing and may be negatively related to impulsivity. Due to the modulating potency of DA-bioavailability on PFC functioning, it is furthermore hypothesized that DA-bioavailability moderates the general relationship. Therefore, it is tested if the general relationship holds at low to high DA levels.

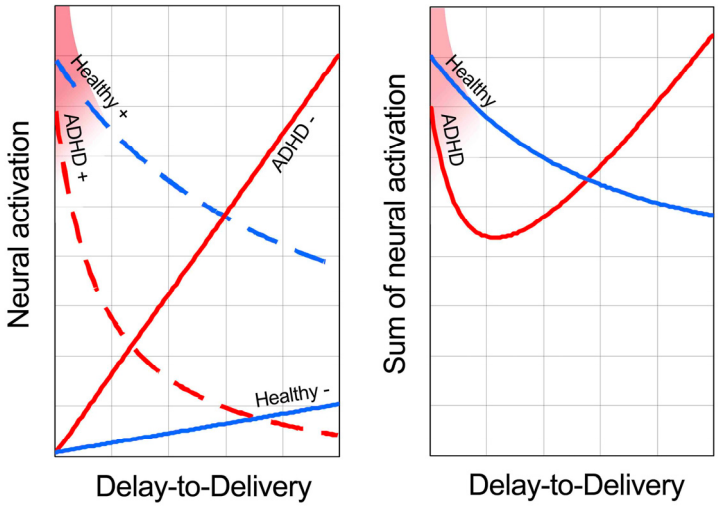

Figure 2 – Main hypotheses of *study II*. Predictions of the two-process hypothesis about neural activation in patients with ADHD and healthy controls. A steeper DD gradient in patients with ADHD compared to healthy subjects is shown in the left panel (dashed lines). This pattern is assumed for the VS which is coding the value of a reward. Furthermore, a negative emotional response to delays is assumed in patients with ADHD (solid lines) –the same but attenuated process might be assumed for healthy subjects. Since no explicit models for the delay-dependent negative emotional response are currently available, a linear relationship with delay is assumed for the sake of simplicity (a parabolic relation might prove to be more valid). Both decreasing positive as well as increasing negative emotional response is expected for the amygdala. The right panel shows *the summation* of the assumed two processes: structures such as the amygdala responding to biologically relevant information, regardless of valence, are expected to exhibit decreasing delay-dependent activation in healthy controls, while in patients with ADHD a kind of U-shaped activation pattern is expected. Red shaded areas in both panels indicate the possibility of either no group difference regarding immediate reward processing or hyper-response of the ADHD group to immediate rewards.

Organization of the thesis

The aim of this thesis is to expand existing knowledge concerning a) dopaminergic effects on DD-related brain activation in healthy subjects and b) neural correlates of DD in patients with ADHD. Therefore, the doctorial thesis comprises two experiments: *Study I* examines the effect of different DA-bioavailability levels, indicated by a genetic polymorphism (Val158Met) in the catechol-O-methyltransferase Val158Met polymorphism, on the association of delay discounting and OFC activation in healthy subjects. To obtain an adequate sample size required for the intended interaction analyses, functional near-infrared spectroscopy (fNIRS) is applied as a neuroimaging tool. Four methodological studies focusing on quality criteria of fNIRS measurements have been conducted prior to *study I* (see Appendices A-D). *Study II* investigates the predictions of the Delay Aversion hypothesis concerning subcortical activation in adult patients with ADHD. To target the relevant brain structures, i.e. the VS and amygdala, functional magnetic resonance imaging (fMRI) is applied.

Study I

Impulsivity-related prefrontal brain activation during Reward Discounting is moderated by COMT Val158Met genotype

Introduction

It has been proposed that dysregulated dopaminergic neurotransmission plays an important role for impulsivity (Tripp & Wickens, 2008). Dopamine (DA) system-related effects on DD have been reported (Boettiger et al., 2007) for the catechol-O-methyltransferase (COMT) Val158Met polymorphism which metabolizes DA in the prefrontal cortex. Compared to the Val-allele, the Met-allele causes a significant decrease in COMT activity, resulting in higher extracellular (synaptic) DA levels (Bilder et al., 2004; Chen et al., 2004). Notably, the identified neural structures involved in DD are strongly innervated by DA neurons (Kobayashi & Schultz, 2008; McClure et al., 2004) and the COMT Val158Met polymorphism has been demonstrated to moderate reactivity and connectivity in these circuits (Drabant et al., 2006).

COMT Val158Met polymorphism

Functioning of the prefrontal cortex (PFC) is strongly dependent on dopamine[5] (Seamans & Yang, 2004). The COMT enzyme is critically involved in degrading dopamine (and other catecholamines) in frontal brain areas (Grossman, Emanuel, & Budarf, 1992).

A common functional single nucleotide polymorphism (Val158Met) in COMT affects synaptic breakdown of dopamine in the PFC (Chen et al., 2004; Goldberg & Weinberger, 2004). This COMT variation is caused by a single base pair change (G → A) at amino acid position 158 (or 108 respectively). Valine (Val) is substituted by methionine (Met) (Lotta et al., 1995) which exerts a significant effect on the enzymatic activity of COMT. The Met variant results in a more thermolabile enzyme which catabolizes dopamine less rapidly than the Val variant (Lachman et al., 1996; Lotta et al., 1995). The variants exert a co-dominant effect (Chen et al., 2004). COMT has its impact on dopamine degradation primarily in prefrontal brain areas as there is a paucity of dopamine transporters (DAT) in this region

(Chen et al., 2004; Gogos et al., 1998) - but it is also found subcortically (Matsumoto et al., 2003). Existing evidence suggest that the Met allele is advantageous for prefrontally mediated processes (Bearden et al., 2004; Bilder et al., 2004; Blasi et al., 2005; de Frias et al., 2005; Diamond, Briand, Fossella, & Gehlbach, 2004; Egan et al., 2001; Gallinat et al., 2003; Goldberg & Weinberger, 2004; Malhotra et al., 2002)[6]. However, the results seem to be task dependent (Bilder et al., 2004) and influenced by the environmental context and age. Since both alleles are maintained at high levels in populations worldwide (Palmatier, Kang, & Kidd, 1999), it has been proposed that each variant has environment-specific advantages representing a trade-off between cognitive efficiency and emotional resiliency (Goldman's "warrior/worrier" model (Goldman, Oroszi, & Ducci, 2006)). Both domains, cognitive abilities and emotion regulation, may also effect neural processing of immediate vs. delayed rewards.

Aim of the study

Converging evidence (Bilder et al., 2004; Boettiger et al., 2007; Hariri et al., 2006; Kobayashi & Schultz, 2008; Loewenstein et al., 2008; McClure et al., 2004; Plichta et al., 2009; Tripp & Wickens, 2008) points to a triadic interplay of impulsivity, DA and neural RD sensitivity. However, existing studies either neglected one of the pivotal players (Hariri et al., 2006; Kable & Glimcher, 2007; Plichta et al., 2009), or did not formally test for possible interaction effects (Boettiger et al., 2007). Therefore, the goal of the present study was to examine the effect of DA bioavailability, indicated by the COMT Val158Met polymorphism, on the association of impulsivity and neural RD sensitivity. We hypothesized that impulsivity is

[5] Other neurotransmitters (noradrenaline, serotonin and acetylcholine) do also have important influence on PFC functioning (Cools & Robbins, 2004).

[6] Other studies report more favorable results for Val allele carriers (Baker, Baldeweg, Sivagnanasundaram, Scambler, & Skuse, 2005; Bellgrove et al., 2005), or cannot find an impact of COMT on executive function measures (Ho, Wassink, O'Leary, Sheffield, & Andreasen, 2005; Mills et al., 2004; Taerk et al., 2004).

correlated with neural RD sensitivity (Kable & Glimcher, 2007) and, most importantly, tested if this association is moderated by DA bioavailability.

Materials and Methods

Participants

The original sample consisted of 58 healthy subjects. The subjects were recruited for the present study, regardless of gender, handedness or hair color. Nine subjects had to be excluded from further analyses: Eight subjects due to motion artefacts during the functional measurement (evaluated by two independent observers of the recorded time series, who were blind to any subject information) and one due to insufficient fit ($R^2 < 0.3$) in the delay discounting task, resulting in a total of 49 subjects (twenty-four females; mean age: 24.9 ± 1.3 years). Excluded subjects did not differ from the included subjects regarding impulsivity ($t_{56} = 0.61$; $P > 0.54$) or COMT genotype (Fishers exact test $P = 0.72$). To exclude any history of Axis I or II pathology, neurological disorders or psychoactive medication all subjects were screened by interview and questionnaires based on DSM-IV criteria for the whole spectrum of mental diseases.

We assessed the intelligence quotient (IQ) using the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Lehrl, 2005), handedness by the Edinburgh Handedness Questionnaire as well as the financial situation of the subjects by two rating scales (Item 1: “Are you in direct need of 20€ cash?”, Item 2: “How is your current financial situation?”) to control for potential confounding variables.

All subjects gave written informed consent after detailed explanation of the protocol. The study was reviewed and approved by the Ethics Committee of the University of Würzburg. All procedures involved were in accordance with the latest version of the Declaration of Helsinki.

Delay discounting task

The subjects were confronted with a series of paired and repeated inter-temporal choices between smaller but sooner (SS) and later but larger (LL) monetary reward options (e.g., "Would you prefer to have 75€ today or 100€ in a month?"). A constant hypothetical reward of 100€ to be received in the future was compared to 29 reward amounts to be received immediately; the amounts were as follows: 100€, 99.90€, 99.50€, 99€, 96€, 92€, 85€, 80€, 75€, 70€, 65€, 60€, 55€, 50€, 45€, 40€, 35€, 30€, 25€, 20€, 15€, 10€, 8€, 6€, 4€, 2€, 1€, 0.50€, 0.10€. Delay time intervals were: 1 day, 1 week, 2 weeks, 4 weeks, 3 months, 6 months, 1 year, 5 years and 25 years.

The task procedure involved successively decreasing amounts of SS rewards each compared to the constant delayed reward (100€) in order to identify the point at which participants switch from selecting the SS to LL reward. After switching from choosing the SS option, the procedure continued for three additional monetary decrements to ensure that the switch was truly intended. Reciprocally, the switching point in the series of inter-temporal choices between successively increasing immediate rewards and the constant delayed rewards was determined. The decreasing and increasing procedure of inter-temporal choices was repeated for each of the nine delay times. The two switching points of each delay time were averaged resulting in nine points of indifference (POI) per participant. In order to derive the individual participant's degree of delay discounting, the individual POIs were employed in Mazur's hyperbolic discounting function:

$$V = A/(1 + kD)$$

where V = subjective value of the delayed reward (POI), A = full amount of the delayed reward, k = empirically determined constant proportional to the degree of delay discounting, and D = delay duration.

Higher k-values indicate more discounting and the log-transformed k-values were taken as index of impulsivity. The individual goodness of fit of the hyperbolic model was indicated by R^2, and DD task data with R^2 values below 0.30 were excluded from principal analyses[7]. Administration of the DD task took approximately 10 minutes.

Genetic analysis

The genotyping procedure has been described previously (Ehlis, Reif, Herrmann, Lesch, & Fallgatter, 2007; Hunnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007; Reif et al., 2007). All genotypes were scored by two independent readers by comparison to sequence-verified standards. Participants were classified by genotype as follows: Met/Met (n=13), Val/Met (n=23), Val/Val (n=13). There was no association of sex, age and IQ with COMT genotype (gender: chi-square = 3.1; *df* = 2; $P > 0.20$; age: $F_{2,46} = 0.86$; $P > 0.20$; IQ: $F_{2,46} = 0.70$, $P > 0.20$). According to previous work (Smolka et al., 2005), we also assessed a common serotonin transporter gene polymorphism (5-HTTLPR) which has been shown to modulate hemodynamic response to emotional stimuli (Hariri et al., 2002; Munafo, Brown, & Hariri, 2008). No association with the COMT genotype was found (Fishers exact test $P > 0.20$). Furthermore, we performed genotyping of a common variable number tandem repeat polymorphism (40bp-VNTR) in the dopamine transporter (DAT) gene for which additive effects on COMT genotype effects has been shown (Bertolino et al., 2006). Since the DAT genotype was highly correlated with gender in our sample (chi-square = 7.53; *df* = 1; $P > 0.01$), further analyses were omitted.

[7] R^2 = coefficient of determination, i.e. the proportion of variability in a data set that is accounted for by a statistical model. The 0.30 criterion is based on Eisenberg et al. (2007).

fNIRS paradigm

A validated inter-temporal choice paradigm (McClure et al., 2004) comprising a total of 40 inter-temporal choice trials was used. During each trial subjects had to choose between two monetary reward options which differed in amount and delay-to-delivery. During each trial two yellow triangles underneath the two money/time pairs indicated that a choice could be made. Responses were made by pressing one of two response buttons corresponding to the location of the options on the screen. Once the subjects made their choice, the associated yellow triangle turned red for 2 s signaling that the selection was successfully recorded.

Each trial consisted of a SS and a LL monetary reward option which were presented on the left and right side of the screen, respectively. The early money amount option was randomly selected from a Gaussian distribution with a mean of 20€ and a standard deviation of 10€ (the minimum and maximum amount were set to 5€ and 40€, respectively). The amount of the LL monetary option was randomly set to be either 1%, 3%, 5%, 10%, 15%, 25%, 35% or 50% higher than the earlier option. Delay-to-delivery of SS option was set to be either today, 2 weeks or 4 weeks. The delay between SS and LL was either 2 or 4 weeks. As in the original report (McClure et al., 2004), LL options of more than six weeks delay to reward delivery were excluded from the set of trials. Time for decision making was not constricted and it was emphasized to carefully weigh up each decision because one of the subjects choices would be randomly selected at the end of the experiment. For today choices subjects were informed that they would receive their earnings immediately after the fNIRS session in cash. This condition is referred to as (relatively) immediate reward in contrast to delayed rewards that were paid per bank transfer at their respective time.

Both the selected choices and decision times were recorded for each trial. The protocol involved an event-related design with 14.5 sec inter-trial-interval. The average duration of the experiment including training trials was about 15 minutes and varied only due to the individually different decision times.

fNIRS data acquisition

Fundamentals of fNIRS are described in detail elsewhere (Hoshi, 2007; Obrig & Villringer, 2003). Concentration changes of oxy- and deoxy-hemoglobin were measured by a continuous wave NIRS-system (ETG-4000, Hitachi Medical Co., Japan) using a 3x11 optode holder (16 photo-detectors and 17 light emitters) resulting in a total of 52 channels. Two different wavelengths (695±20nm and 830±20nm) are emitted and its frequency is modulated for wavelengths and channels to prevent crosstalk. Both wavelengths are used to solve the modified Beer-Lambert equation for highly scattering media. Emitter-detector distance was 30 mm and sampling rate was set to 10Hz.

For the present study the NIRS 52-channel probe set was adjusted to five head-surface markers according to the international EEG 10-20 system (Fpz, Fp1, Fp2, F7, F8). Channel #37 (medial, second lowest row of channels) was placed on Fpz. The line of lateral channels was adjusted to the horizontal axis defined by Fp1-F7 and Fp2-F8. Using these definitions we employed virtual registration (Tsuzuki et al., 2007) to register NIRS data to Montreal Neurological Institute (MNI) standard brain space (Okamoto & Dan, 2005). Utilizing the positional information of a particular channel relative to the international 10-20 system landmarks, this method enables the placement of a virtual probe holder on the scalp by simulating the holder's deformation and thereby registering probes and channels onto the reference brains, in place of a subject's brain, in a probabilistic manner. The NIRS probes and channels were registered onto the surface of an averaged reference brain in MNI space (Jurcak, Okamoto, Singh, & Dan, 2005; Okamoto et al., 2004; Okamoto & Dan, 2005; Singh, Okamoto, Dan, Jurcak, & Dan, 2005) and the most likely coordinates for the channels were subjected to anatomical labeling using a Matlab function (Okamoto et al., in press) (available at http://brain.job.affrc.go.jp), which reads anatomical labeling information coded in a macroanatomical brain atlas constructed by Tzourio-Mazoyer et al.(2002) and the Brodmann cytoarchitectonic area atlas available in the MRIcro program (Rorden & Brett, 2000).

Specifically, for each surface voxel of the atlas brains, the function scanned anatomical labels of surface voxels located within a sphere with a radius of 10 mm from a given voxel corresponding to a channel location, and reassigned the most frequent labels to that voxel.

Thus acquired macroanatomical and Brodmann cytoarchitectonic area labels were cross-referenced in the following manners. First, we primarily referred to Brodmann labeling. Channels primarily labeled BA9 and 46 were categorized as DLPFC candidate channels, and those primarily labeled BA11, as OFC candidate channels. In addition, channels primarily labeled BA10 were further referred to a secondary label. This is because the definition of the frontopolar region is rather arbitrary, and a looser definition seems functionally relevant. If the secondary label was BA9 or BA46, the channel was categorized as a DLPFC candidate channel. If the secondary label was BA11, the channel is categorized as an OFC candidate channel. Second, DLPFC candidate channels were referred to their macroanatomical labels. If they were primarily labeled as the middle or superior frontal gyri, they qualified as DLPFC channels. OFC candidate channels were subjected to Anatomical Automatic Labeling (AAL) (Maldjian, Laurienti, Kraft, & Burdette, 2003). If they were primarily labeled as the middle or superior orbitofrontal gyri, they qualified as OFC channels. Consequently, channels 4, 5, 14, 15 and 25 were classified as right DLPFC channels, while channels 36, 37, 38, 46, 47, 48, and 49 were classified as OFC channels – see also section *Results – fNIRS results*.

Because no a-priori hypothesis about the laterality of neural RD sensitivity was existent, we also calculated the contrast (immediate > delayed) for both hemispheres. No significant effects were found in the left OFC ($P > 0.05$).

Event-related fNIRS data analysis

A model-based analysis procedure according to the general linear model (GLM) was applied to the fNIRS data (Plichta, Heinzel, Ehlis, Pauli, & Fallgatter, 2007; Plichta et al., 2006a, 2006b). The GLM approach has been extensively described in fMRI literature (see Friston et

al., 1995; Worsley & Friston, 1995; Bullmore et al., 1996). Briefly, the data matrix Y of order (TxC) containing the functional NIRS time series T of each channel C is predicted by X consisting of a set of reasonable haemodynamic response functions (HRFs) which are convolved with the event sequence (the order of X is (TxM) where M is the number of modelled effects – see below). The functional data can be modelled as:

$$Y = X\beta + \varepsilon \qquad (1.1)$$

Where X is the design matrix and β is the parameter matrix. In the simplest case, each column M of matrix X contains the predicted hemodynamic response for one experimental condition over time (T). To address inter-individual differences regarding the HRF's latency and dispersion, the inclusion of the HRF's first and second temporal derivative has been proposed (Friston et al., 1998). The inclusion of derivative terms results in an extension of the design matrix X: for one experimental condition, X contains two (HRF + 1. derivative) or three columns (HRF + 1. derivative + 2. derivative).

The ordinary least square (OLS) estimates of β are given by

$$\beta = (X'X)^{-1}X'Y \qquad (1.2)$$

The ß-weights quantify the contribution of a predictor (e.g. HRF) for explaining the functional time series Y and serve as the parameter set for subsequent hypothesis testing. At the single subject level t-tests can be applied to an estimated beta-weight (testing e.g. H_0: β=0) or the beta weights of a sample are collected and analyzed at the group level (e.g. with paired t-tests, analysis of variance etc.). Testing the beta-weights (e.g. by one sample t-tests) gives an answer to the question whether a particular brain area is activated by the experimental condition. For models incorporating derivative term(s), the amplitudes are estimated from the

non-derivative term only (Friston et al., 1998) based on the assumption that "error" variance caused by inter-individual latency or dispersion differences will be explained by the derivative terms (see Calhoun et al., 2004 for a critique of this assumption).

The unbiased estimates of the significance of *ß*-weights (= t-values) are based on the assumption that the error term ε (see formula 1.1) is, uncorrelated (representing an identity matrix I), independent and normally distributed:

$$\varepsilon \sim \text{i.i.d. } (0, \sigma^2 I) \qquad (1.3)$$

Because several physiological processes (respiration, blood-pressure changes, heartbeat) are known to produce structured "noise" to the data (autocorrelation), a common strategy to deal with this problem is to calculate OLS estimate(s) first (see formula 1.1 and 1.2) and fit an AR(p) model (autoregressive model of the order p) to the resulting residuals (Cochrane & Orcutt, 1949). This leads to a decomposition of the error term ε into a systematic part as well as into the model conform error part. After this, the AR transformation coefficient is applied to both sides of the regression equation:

$$Y_t - \rho Y_{t-1} = \beta_0 (1 - \rho) + (X_t - \rho X_{t-1}) \beta + u_t \qquad (1.4)$$

where ρ is the estimate of the autocorrelation coefficient in an AR(1) process and $u_t = \varepsilon_{t-1} + \varepsilon$. By redefining each transformed variable as follows:

$$Y^* = Y_t - \rho Y_{t-1} \qquad X^* = X_t - \rho X_{t-1} \qquad k^* = 1 - \rho$$

one can simplify (1.4) to:

$$Y^* = \beta_0 k^* + \beta_1 X^* + u_t \qquad (1.5)$$

As a result, the serial correlation is reduced.

Prior to the GLM analyses the functional data was pre-processed by applying a low-pass filter (cut-off frequency of 0.7 Hz). Thereafter, GLM is applied by using a gauss function as HRF (peak time=6.0s; Full Width Half Maximum (FWHM) = 5.89) and its first and second temporal derivative to modulate the onset and the dispersion of the HRF.

The HRF was used for both NIRS parameters (O2Hb and HHb). Thus significant positive beta weights indicate activation in the O2Hb data while significant negative beta weights indicate activation in the HHb data. An autoregressive process of order [1] is applied by default.

The time series of each subject were modeled by three regressors, one for each delay condition. Events of one condition (today, 2 and 4 weeks) were modeled as delta functions at the respective onset times and convolved with a Gaussian hemodynamic response function. The duration of each event was set to the overall mean of decision time (3.7 s). In order to control for individual decision times, an additional decision time weighted parametric regressor was included. Control for global activation effects, potentially arising from extra-cerebral sources (Plichta et al., 2007) was realized by subtracting the condition-dependent mean of all channels from each channels' amplitude of the respective condition before entering statistical analyses.

Validity of the fNIRS recordings was verified by testing the contrasts "immediate > delayed rewards" and "all-choices > zero" in order to replicate the reported corresponding brain area (OFC and DLPFC) activation for the particular condition contrasts (McClure et al., 2004). To protect against multiple comparisons, we used Bonferroni correction including the spatial correlation among channels within the respective anatomically defined ROIs by calculating

the Dubey/Armitage-Parmar boundary for a corresponding one-tailed alpha-level of 0.05 (Plichta et al., 2006a, 2006b; Sankoh, Huque, & Dubey, 1997).

Moderator analysis

For testing the main hypotheses of the study, multiple regression analysis was applied (Cohen, 2003) comprising the within-subjects variable delay-to-delivery (immediate vs. delayed reward) and two between-subjects variables: two predictors coding for COMT and one predictor containing the individual impulsivity scores (log[k]). A subject vector containing the criterion mean (i.e. dependent variable) was included (Cohen, 2003). All predictors were centered before entering into analyses by subtracting the mean value from each data entry. Interaction terms were constructed as the particular multiplicative products of COMT, log[k] and delay-to-delivery levels. The average of significant amplitude estimators within the respective ROI was the dependent variable. Main and interaction effects were tested by means of F tests (alpha-level of 0.05, uncorrected). Significant interaction effects (second order or simple) were analyzed by post-hoc analyses on separate factor-levels. Since the design comprises unequal cell sizes, sum-of-square type III was applied for correction.

The assumption of homogeneous error variance for multiple regression analysis was tested with ALTMMR software (Aguinis, Petersen, & Pierce, 1999) – see Appendix E for descriptive statistics and assessing compliance with homogeneity assumption.

Behavioral Data analysis

Preference for SS rewards and decision times (DT) were analyzed by two separate repeated measurement ANOVAs (within-subjects factor delay-to-delivery [immediate; delayed] and between-subjects factor group COMT [Val/Val; Val/Met; Met/Met]).

Table 1. Demographic Data (mean and SD unless otherwise stated)

	Total	Val/Val	Val/Met	Met/Met	*P-Value* [a]
n	49	13	23	13	-
Age (years)	24.9 (1.3)	24.5 (0.8)	24.9 (0.9)	25.2 (2.1)	.43
Gender-Ratio (m/f)	25/24	6/7	9/14	9/4	.22[b]
IQ	118.2 (15.1)	117.2 (15.3)	119.1 (14.8)	117.7 (16.5)	.93
Lateralitiy-Score [c]	86.7 (30.8)	90.1 (30.9)	84.6 (30.8)	88.2 (35.4)	.80[b]
Financial-Status [d]	4.5 (2.1)	4.2 (2.1)	4.2 (1.8)	5.3 (2.5)	.29
Log[k] – median	-4.56 (2.5)	-4.7 (2.6)	-4.5 (2.3)	-4.6 (3.1)	.72
R^2 – median	0.92	0.96	0.90	0.93	.89

[a] p-Values obtained from one-way ANOVA if not otherwise stated
[b] results obtained from Fisher's exact test
[c] ranging from -100 to +100 – median and inter-quartile-range is shown
[d] Average of two items (Cronbachs alpha = 0.65). Range: 1-10 (1=high financial status; 10=low financial status).

Table 2. Behavioral Data.

	Total	Val/Val	Val/Met	Met/Met
DT – overall (sec)	3.7 (1.4)	3.8 (1.2)	3.7 (1.7)	3.7 (1.3)
DT – immediate	3.6 (1.5)	3.7 (1.3)	3.5 (1.7)	3.5 (1.3)
DT – delayed	3.8 (1.4)	3.8 (1.2)	3.9 (1.7)	3.8 (1.2)
Choices SS – overall (%)	44.6 (19.5)	37.3 (20.3)	51.1 (19.0)	40.5 (17.3)
Choices SS - immediate	49.4 (20.2)	43.8 (21.8)	56.3 (20.3)	42.8 (15.3)
Choices SS - delayed	42.3 (21.8)	34.1 (21.3)	48.5 (20.4)	39.4 (23.1)

Results

Sample characteristics and behavioral data are shown in Table 1 and Table 2, respectively. No significant COMT-related group differences occurred (see Table 1). While decision times (DT) during the functional task significantly varied between the delay-to-delivery levels ($F_{1,46}$ = 7.58, P < 0.01), there was no significant main effect for COMT on DT ($F_{2,46}$ = 0.02, P = 0.97) nor an interaction of COMT and DT ($F_{2,46}$ = 0.98, P = 0.38). There was a significant effect of delay-to-delivery level on choice behavior ($F_{1,46}$ = 6.60, P < 0.05), but no significant effect of COMT on choosing the smaller-but-sooner option ($F_{2,46}$ = 2.81, P = .07), nor an interaction of COMT x delay-to-delivery ($F_{2,46}$ = 0.41, P = 0.66).

fNIRS results

Figure 3 shows that the separate neural systems (DLPFC and OFC) with their characteristic response pattern as demonstrated in the original study (McClure et al., 2004) were detectable by fNIRS. Right DLPFC responded uniformly to all rewards (independent of the delay-to-delivery), whereas OFC was sensitive to RD (immediate > delayed) – see Figure 3b and 4a.

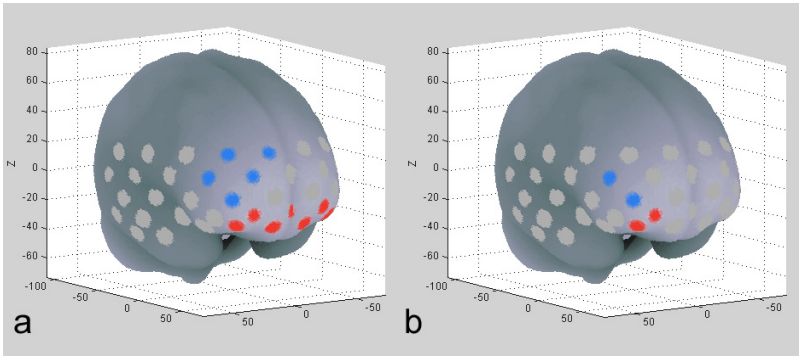

Figure 3. ROI definitions are shown in panel A (blue = rDLPFC; red = OFC). Significant brain activation elicited by the different conditions of the intertemporal choice task (p<0.05; corrected) within the pre-defined ROIs is shown in panel B (blue = all choices; red = immediate > delayed rewards).

Confirming our first hypothesis, degree of neural RD sensitivity was positively correlated with impulsivity across the whole sample ($P < 0.05$; Figure 4b). Regarding COMT genotype, the number of Met-alleles was linearly related to RD sensitivity in the OFC ($P < 0.05$ – see Figure 4c). However, a COMT genotype effect on impulsivity was entirely absent (see Table 1). Three-way interaction tests revealed COMT genotype to moderate the association of impulsivity and neural RD sensitivity (Figure 4d). Separately for immediate and delayed rewards, simple interaction-effect analyses revealed a COMT moderator-effect for delayed rewards and a marginally significant effect for immediate rewards (Figure 5).

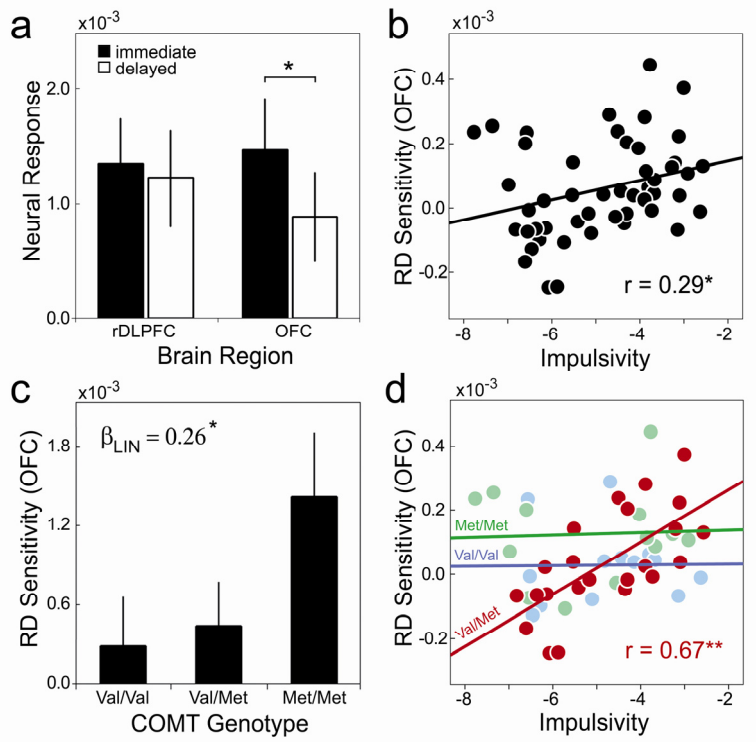

Figure 4. Summary of effects. **(a)** Independent of the reward-delay, rDLPFC uniformly responded to all rewards, while RD sensitive activation was found in the OFC ($P < 0.05$; corrected). **(b)** Across all subjects RD sensitivity was correlated with the individual impulsivity scores ($P < 0.05$). **(c)** Linear relationship of the met-allele-number with OFC RD sensitivity ($P_{LIN} < 0.05$ and $P_{QUAD} = 0.13$), **(d)** *COMT* moderated relationship between impulsivity and OFC RD sensitivity: Multiple regression analysis (Delay-to-Delivery x Impulsivity x COMT; $F_{2,43} = 4.04$; $P < 0.05$) revealed that neural sensitivity to RD (immediate minus delayed) was strongly correlated with impulsivity at intermediate DA levels (Val/Met: $r = 0.67$; $P < 0.001$), while no association was apparent at low (Val/Val) or high (Met/Met) levels ($P > 0.20$).

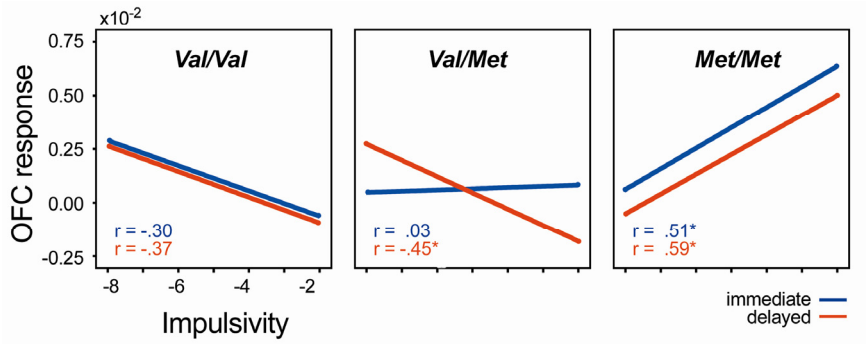

Figure 5. Separated for immediate and delayed rewards, analyses revealed a COMT moderator-effect on the association of impulsivity and OFC-activation elicit by delayed rewards (Impulsivity x COMT: $F_{2,43} = 6.71$; $P < 0.005$) while the effect toward immediate rewards was marginally significant (Impulsivity x COMT: $F_{2,43} = 2.69$; $P < 0.10$) - see also Table 1.

Sample-size effects?

In order to test potential effects arising from differing sample sizes (Val/Val=13; Val/Met=23; Met/Met = 13) the distribution of correlation coefficients (neural RD sensitivity with trait-impulsivity) resulting from all possible combinations of k=13 out of n=23 heterozygote Val/Met carriers ($N_{combinations}$ = n!/k!*(n-k)! = 1144066) was examined. The resulting mean correlation coefficient of all combinations was $r = 0.67$, $df = 11$ (std = 0.08). Correlation coefficients of $r > 0.48$ are significant at an alpha-level of 0.05 (one-tailed) with a sample-size of n = 13. The probability of obtaining correlation coefficients $r < 0.48$ was $P < 0.02$. Therefore, it is concluded that the revealed correlation of impulsivity and neural RD in the Val/Met group is not attributable to the larger sample size.

Discussion

Dopamine-related enhancement of OFC function

The results of the present study indicate that higher DA-bioavailability, as indicated by COMT Val158Met polymorphism, enhances the ability of (at least parts within) the OFC to discriminate between different RD levels. Furthermore, the association of neural RD

sensitivity and impulsivity was shown to depend on DA-bioavailability. The conditional relationship between impulsivity and OFC activation suggests that OFC sensitivity for RD reflects neural discrimination ability, rather than being synonymous with impulsivity. While lowest at decreased DA-levels (Val/Val) and entirely unrelated to impulsivity, RD discrimination ability was enhanced at intermediate DA-bioavailability level (Val/Met) and was restricted to high impulsive subjects. Neural discrimination ability was highly predictive for impulsivity score at intermediate DA levels. At high DA-level (Met/Met), neural RD discrimination ability was existent across subjects, and "decoupled" from the impulsivity dimension. Rather, independent of the delay-level OFC amplitudes were predictive for impulsivity in this group. Thus, our results indicate that high DA bioavailability extends OFC functioning by incorporating RD information. Importantly, the analyses showed that the absence of a correlation at low or high DA levels was not due to the smaller sub-sample size or other variables, but that the relationship between neural and behavioral level was qualitatively distinct.

An evolutionary perspective

Previc (1999) has hypothesized that DA is the key regulator of important cognitive abilities comprising cognitive flexibility, abstract reasoning and planning, making DA a candidate neural substrate for modification by natural selection. In Previc's schema, physiological adaptations to ecological changes promoted the expansion of dopaminergic pathways and with it cognitive abilities. While evolutionary changes in the DA neurotransmitter system might be more complex than a quantitative increase of DA bioavailability (Raghanti et al., 2008), the revealed moderator effect was shown to depend on an evolutionary recent allele variant of COMT Val158Met polymorphism that is absent in other primates (Palmatier et al., 1999). Increasing DA-bioavailability was associated with an increase of sensitivity to discriminate between rewards at different delay levels. This ability was dependent on

impulsivity at intermediate DA levels while at highest DA-levels it decoupled from the dimension of impulsivity, putatively because of its general advantage in (recent) environments with important positive but delayed events such as retirement pension, bear interest or a monthly pay-out of salary.

Limitations

The sample was not stratified according to the COMT genotype a-priori. This led to different subsample sizes, requiring corrections of the applied multiple regression analyses. Although the potential confound of different sample size was explicitly tested, future studies focusing on the impact of genetic polymorphisms should use equally sized subsamples.

Conclusions

Even though the analyses are limited to the cortical surface and reciprocal tonic-phasic effects of COMT (Bilder et al., 2004) are neglected, the present findings indicate to consider COMT Val158Met polymorphism as a crucial factor whenever impulsivity-related brain activation, in particular to RD, is examined. The COMT-related extension in OFC function from low (Val/Val) to partial (Val/Met) and full (Met/Met) RD sensitivity, is suggested as causative for the distinct impulsivity-brain couplings. Our approach to include genetic, trait and neural level and test for their joint effect enabled insight into the complex interplay for which purely bivariate analyses were blind for. Therefore, this approach may also complement classical analytic strategies as described in the imaging genetics domain (Green et al., 2008).

Study II

Neural hypo- and hyper-responsiveness during immediate and delayed reward processing in adult ADHD: an fMRI study

Introduction

Three Models on processing of delays in ADHD

The ability to tolerate delays has been demonstrated to be limited in attention-deficit/hyperactivity disorder (ADHD). Behavioral studies suggest that patients with ADHD have a steeper delay discounting gradient (Barkley, Edwards, Laneri, Fletcher, & Metevia, 2001), are unusually sensitive to delays (Tripp & Alsop, 2001) and make more impulsive choices in delay-of-gratification paradigms (Luman, Oosterlaan, & Sergeant, 2005) (but see also (Scheres et al., 2006)). Three models of ADHD, the Dopamine Transfer Deficit (DTD) theory, the Dynamical Developmental theory (DDT), and the Delay Aversion (DAv) hypothesis propose altered reinforcement mechanisms in ADHD to account for these findings (Sagvolden et al., 2005; Sonuga Barke, 2002, 2003; Tripp & Wickens, 2008). These models agree on a hypo-functioning DA system which may result in a shorter and steeper delay-of-reinforcement gradient in ADHD, and result in increased levels of impulsive behavior as indexed by relatively strong preferences for immediate rewards (Sagvolden et al., 2005). Accordingly, predictions about neural activation during the processing of rewards in ADHD refer to hypo-activation in reward-related structures either on (a) delayed rewards only (Tripp & Wickens, 2008) or (b) both immediate and delayed rewards (Sagvolden et al., 2005). Two studies provide evidence for hypo-activation within central parts of the reward system in ADHD (Scheres et al., 2007; Strohle et al., 2008). However, in these studies temporal proximity of the rewards has not been varied.

Predictions of the Delay Aversion hypothesis

In addition to the assumptions about altered reinforcement mechanisms, secondary effects in terms of interactions with environmental factors or changes during adolescence are less explicitly described by the DTD and DDT models. Specifically the development and consequences of emotional states associated with the opportunity to obtain rewards of

different temporal proximities appear important to better understand which factors contribute to maladaptive behavioral, emotional and cognitive outcomes in ADHD.

The DAv hypothesis (Sonuga Barke, 2003, 2005; Sonuga Barke, Taylor, & Heptinstall, 1992; Sonuga Barke, Taylor, Sembi et al., 1992; Sonuga Barke et al., 1996) with its core element of a delay-related negative affect acquired during development provides predictions about neural activation during immediate versus delayed reward processing specifically for emotional structures. From DAv perspective, ADHD characteristics are explained as a consequence of an aversive experience of delay periods which increases the probability of impulsive responses in inter-temporal choice situations. In situations, where no options are available, DAv theory predicts that motor activity increases, or that attention is averted from the actual situation to decrease its subjective length. In contrast to models predicting neural hypo-activation (i.e. DDT and DTD), DAv theory postulates hyper-activation toward delayed rewards in emotional core structures, especially the amygdala (Sonuga Barke, 2003). However, this has not been tested with functional brain imaging yet.

Neural correlates of motivational and emotional processing

For reward processing and reward prediction previous brain imaging studies identified the striatum as a pivotal player (Breiter, Aharon, Kahneman, Dale, & Shizgal, 2001; Haruno & Kawato, 2006; Knutson & Cooper, 2005; Koepp et al., 1998; Schultz et al., 1997). At a fine-grained level, the striatum and in particular the caudate nucleus (NC) can be functionally subdivided in a ventral-to-dorsal direction (Atallah, Lopez Paniagua, Rudy, & O'Reilly, 2007; Delgado, Locke, Stenger, & Fiez, 2003; Delgado, Nystrom, Fissell, Noll, & Fiez, 2000; Haber, 2003; Haruno & Kawato, 2006; Ito, Dalley, Robbins, & Everitt, 2002; O'Doherty et al., 2004; Tanaka et al., 2004; Volkow et al., 2006; Zink, Pagnoni, Martin, Dhamala, & Berns, 2003). In healthy subjects, a ventral-to-dorsal gradient within the striatum has been shown for decisions between rewards of different temporal proximity: Immediate rewards are mainly

processed within ventral regions while future rewards are more preferentially processed in dorsal regions of the striatum (Tanaka et al., 2004). Additionally, the dorsal caudate has been associated with the subjective experience of craving (Volkow et al., 2006), wanting and desire (Aron et al., 2005; Volkow et al., 2002). In contrast, ventral striatal (VS) activation is more likely to be correlated with a more passive receipt of reward. VS hypo-responsiveness during reward anticipation in ADHD has recently been supported by two functional magnetic resonance imaging (fMRI) studies (Scheres et al., 2007; Strohle et al., 2008).

Among other brain structures, the amygdala and the insula mediate emotional processing. Amygdala activation is evident during processing of negative (Weiskrantz, 1956) and positive emotions (Hamann & Mao, 2002; Hommer et al., 2003) and can be summarized to mirror the arousing features of stimuli (Anderson et al., 2003; Small et al., 2003). The anterior region of the insula shares close reciprocal connection with the amygdala, and is involved in processing aversive states (O'Doherty, Critchley, Deichmann, & Dolan, 2003) as well as positive emotions (Iaria et al., 2008). According to the DAv hypothesis, an aversive perception of delayed rewards should be reflected by an increased activation to delayed versus immediate rewards in emotional core structures.

Aim of the study

To investigate neural processing of rewards at different delay-to-delivery levels in adult patients with ADHD, a validated inter-temporal choice paradigm (McClure et al., 2004) was used wherein subjects make a series of choices between monetary reward options which vary by delay-to-delivery (today, 2 or 4 weeks). At the behavioral level, we investigated the frequencies of early reward choices and the decision time to index preference for immediacy. Here, however, no marked group differences were predicted because the rate of impulsive choices has been shown to decrease in adult patients with ADHD (Bjork et al., 2004; Kaplan & Stevens, 2002). Functionally, group differences in neural responses during processing of

immediate and delayed rewards were investigated within the striatal system along its ventral-to-dorsal extension, including the nucleus accumbens (NAcc) as the most ventroanterior part and reaching into the dorsal parts of the caudate nucleus body. Based on existing evidence (Scheres et al., 2007; Strohle et al., 2008), we hypothesized VS/NAcc hypo-responsiveness in adult patients with ADHD. According to the central prediction of the DAv hypothesis, we expected group differences in neural responses for the dorsal parts of the striatal system and particularly for emotional structures, specifically during the processing of delayed rewards.

Methods and Materials

Participants

Fourteen right-handed males (age: 19-32 yrs.) with a diagnosis of adult ADHD combined subtype (according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)) were recruited from outpatient clinics at the Departments of Psychiatry, University of Würzburg and University of Ulm, Germany. Diagnosis of ADHD in adulthood was evaluated with the Structured Clinical Interview for DSM-IV Axis I and II Disorders (SCID-I and SCID-II) by an experienced clinical psychiatrist (C.J.) specialized in ADHD and was re-confirmed by a second psychiatrist (either A.B-H. or M.H.). Childhood manifestation of ADHD was retrospectively assessed with the DSM-IV symptom list for ADHD and the German version of the Wender Utah Rating Scale (WURS) (Retz-Junginger et al., 2007; Ward, Wender, & Reimherr, 1993). The WURS was also used for assessing parent ratings of ADHD symptoms during childhood.

Patients diagnosed with concurrent axis I and II disorders according to DSM-IV criteria were excluded from the study following the structured clinical interview (SCID-I and -II for DSM-IV) conducted by C.J.. Further exclusion criteria were any sign of neurological disorder, manifest reading disabilities (based on patients' self-reports), IQ level below 80 (Lehrl, 2005),

regular consumption of psychoactive drugs within the last two years or singular drug use (psychoactive substances) within the last 4 months prior to the fMRI scan (based on patients' self-reports).

All patients had a history of methylphenidate treatment. At the time of the fMRI scanning, 7 patients had been off medication for at least 6 weeks, while the remaining 7 patients discontinued their MPH treatment four days prior to the scanning procedure, i.e. for at least 5 half-lives (Swanson & Volkow, 2002).

The healthy control group originally consisted of 14 right-handed males (two healthy controls were excluded due to technical artifacts) group-matched for age, IQ and handedness (see Table 3). Healthy control subjects had neither any history of Axis I or II pathology nor neurological disorder. Current ADHD symptoms were assessed in both groups by means of a validated German version of the Adult ADHD Self Report Scale (ASRS) (Kessler et al., 2005; Reuter, Kirsch, & Hennig, 2006) (for examinations of executive functioning in both groups, see Table 4).

Table 3. Sample characteristics.

	Healthy controls		ADHD patients		Group Differences [a]
	Mean	SD	Mean	SD	
Age (years) [b]	23.6	1.9	23.3	5.2	ns
Laterality Score (Edinburgh handedness questionnaire)	86.1	11.3	89.8	9.5	ns
Education (years)	12.4	1.2	11.3	1.9	ns
Verbal IQ (MWT-B) [c]	112.3	8.0	108.5	13.5	ns
Cigarette smoking [d]	(n=4)	-	(n=6)	-	ns
Wender Utah Rating Scale (WURS-k) [e]	-	-	38.3	6.8	-
Wender Utah Rating Scale (WURS-k) – parent rating	-	-	33.9	6.2	-
Adult Self Report Scale (ASRS): total score	24.7	3.1	44.6	10.7	$P < 0.001$
ASRS-Part A (inattentive)	14.4	3.3	22.8	6.8	$P < 0.001$
ASRS-Part B (hyperactive/impulsive)	10.3	2.1	21.8	6.3	$P < 0.001$

[a] group differences were tested by means of two-samples *t*-tests (alpha = 0.05; two-tailed; uncorrected); ns = not significant ($P > .05$)
[b] Age ranges: 19-26 yrs (controls); 19-32 yrs (ADHD patients)
[c] MWT-B = Mehrfachwahl-Wortschatz-Intelligenztest
[d] group differences tested by means of Fisher's exact test (chi-square = 0.24; p> .20)
[e] cut-off score >=30

The project was in accordance with the latest version of the Declaration of Helsinki and was approved by the local Institutional Review Board. All subjects were informed about the nature of the experiment in detail before giving written informed consent.

Table 4. Neuropsychological test scores.

	Healthy controls		ADHD patients		*P*
	Mean	SD	Mean	SD	
Digit Span, Forward	8.0	1.6	8.1	1.0	ns
Digit Span, Backward	6.7	2.2	6.2	1.8	ns
Block Spatial Span task, Forward	7.7	1.9	8.4	1.3	ns
Block Spatial Span task, Backward	6.3	1.7	6.4	1.5	ns
Wisconsin-Card-Sorting-Test (WCST), Perseverative Errors	0.9	0.9	1.2	1.9	ns
Wisconsin-Card-Sorting-Test (WCST), Switch-Costs (sec.)	2.6	1.5	1.9	1.6	ns
Stroop Color-Word Interference Test, RT-Difference-Effect	101.4	68.7	79.7	104.6	ns
Stroop Color-Word Interference Test, Errors total	7.7	3.7	10.0	5.1	ns

ns = not significant

fMRI paradigm

We used a validated inter-temporal choice paradigm (McClure et al., 2004) comprising a total of 40 trials. During each single trial subjects had to choose between two monetary reward options which differed in amount and delay-to-delivery. Each trial consisted of an early but smaller (always presented on the left side of the screen) and a later but larger monetary reward option (presented on the right side of the screen). A detailed description of the paradigm can be found in the section *Methods and Materials* of *study I*). Both decision times and the selected choices were recorded for each trial. The fMRI-protocol involved an event-related design with 20 seconds inter-trial-interval. Due to the individually different decision times, a total of 450 to 550 volumes were recorded per subject, which results in an average duration of about 17 minutes.

fMRI data acquisition

Functional MRI data were acquired using a 3.0 T Magnetom ALLEGRA (Siemens, Erlangen, Germany) head MRI system. T2*-weighted whole-brain images were obtained using echo-planar imaging (TR=2000ms, TE=40ms) in an orientation approximately 15 degree steeper than the AC-PC line. Image size was 64 by 64 pixels (3.6 by 3.6 mm pixels). The volume consisted of 27 slices with a slice thickness of 3.0 mm (gap of 1.0 mm). Stimuli were presented via MR-compatible LCD video goggles (Resonance Technologies, Northridge, CA). Head movement was minimized using padded ear phones. The first 8 volumes of each session were discarded to allow for equilibrium in the magnetization.

fMRI data analysis

The functional imaging data were analyzed using statistical parametric mapping (SPM5; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK). Preprocessing of the fMRI data included slice-time and motion correction, and spatial normalization into a standard stereotactic space (2 x 2 x 2 mm3) via an EPI template (MNI space). Finally, images were spatially smoothed with a 10 mm full width at half maximum (FWHM) Gaussian kernel.

The functional data of each subject were modeled by three regressors, one for each delay condition. Events of one condition (today, 2 and 4 weeks) were modeled as delta functions at the respective onset times and convolved with the canonical hemodynamic response function. The duration of each event was set to the overall mean of decision time (3.1 s). In order to control for different decision times, an additional decision time weighted parametric regressor was included. Furthermore, a set of regressors of no interest was included referring to the motion parameters as obtained from motion correction during pre-processing. Parameter estimation was corrected for temporal autocorrelations using a first-order autoregressive model.

Second level analyses were performed by an ANOVA comprising a between-subjects factor *group* (controls, ADHD) and a within-subjects factor *delay-to-delivery* (immediate, delayed reward). Likewise to the original report (McClure et al., 2004), we combined the delay levels of 2 and 4 weeks for subsequent analyses to increase statistical power. VS hypo-responsiveness in ADHD (Scheres et al., 2007) was examined by main effect analysis of factor group. Between-group differences of brain activation on different delay levels were tested by means of interaction analyses of the factors group and delay-to-delivery. Given the a priori formulated conjectures on the motivational system, these tests were constrained to an anatomically defined region of interest (ROI) comprising the NAcc, the head and body of NC. For investigation of the emotional system, ROI analyses were performed for the amygdala and the insula. For both ROI definitions, the WFU Pickatlas software (Version 2.3, Wake Forest University, School of Medicine, NC; www.ansir.wfubmc.edu) was used (Maldjian et al., 2003).

To control for multiple comparisons within the predefined ROIs, nominal alpha levels of $P < 0.05$ at each voxel were adjusted by the requirement of a predefined number of contiguously activated voxels, calculated with reference to the number of voxels within each anatomically predefined ROI (Forman et al., 1995). The principle underlying multiple comparisons is that true regions of activation will tend to show up over contiguous, rather than scattered, significant voxels. Calculation of minimum cluster sizes within regions of interest in AlphaSim for Analysis of Functional NeuroImages (AFNI) uses Monte Carlo simulations taking into account the spatial correlation of voxels, the size of the region of interest, and a pre-defined level of significance at the voxel level Ward (2000).

In order to cope with the analyses' susceptibility for potentially increased movement artifacts in the experimental group we compared translation and rotation parameters across groups. ANOVAs showed that groups did not differ in any of the translation or rotation parameters (all $P > 0.20$).

Correlation analyses of BOLD responses and ADHD symptom scales (ASRS) were performed for reasons of external validation. Significance level was set to alpha = 0.05 (uncorrected; two-tailed). To control for potential artificial correlation due to significant group differences on ASRS scores, analyses were re-run in both groups separately for confirmation (alpha = 0.10; uncorrected; one-tailed).

Behavioral data analyses

Preference for early rewards and decision times were assessed by two separate repeated measurement ANOVAs (within-subjects factor delay-to-delivery [immediate; delayed] and between-subjects factor group [controls; ADHD]) and post-hoc dependent and independent samples *t*-tests (alpha < 0.05).

Valence ratings

Valence rating of the mean reward amount (i.e., 20€) received at the three different delay-to-delivery levels (immediate, 2 weeks, 4 weeks) was assessed in a separate examination (outside the scanner) using a 9-point valence scale that ranged from 1 = extremely negative to 9 = extremely positive (5 = neutral). The individual valence-decay score was defined as the difference of immediate minus delayed rewards' valence rating scores. Valence-decay scores were analyzed for group differences (two-sample t-test, one-tailed alpha-level set to 0.05), and used for correlation analyses with differential BOLD responses in the amygdala.

Results

Behavioral Data

The different levels of delay-to-delivery did not affect preference for early rewards (factor delay-to-delivery ($F_{1,24}$ = 1.08; P = 0.31)). Preference for early rewards in ADHD did not differ significantly from healthy controls across both delay levels (group: $F_{1,24}$ = 3.40; P < 0.08 - see Figure 6A) or at any separate delay level (group x delay-to-delivery: $F_{1,24}$ = 0.75; P = 0.39). The numerical between-group difference on choosing the smaller/earlier option when delivered immediately was statistically not significant (P = 0.07; Figure 6A).

The groups' overall decision time (DT) averaged for all choices did not significantly differ (2.87 s +/- 0.69 s in the ADHD group and 3.32 +/- 1.26 s in the healthy control group - $F_{1,24}$ = 1.34; P = 0.26). The different delay-to-delivery levels did not affect DT across groups ($F_{1,24}$ = 4.06; P = 0.06). However, there was a significant group x delay-to-delivery interaction ($F_{1,24}$ = 4.36; P < 0.05). While the control group showed nearly constant mean DT across delay-to-delivery levels (t_{11} = 0.05; P = 0.96), DT on delayed rewards was significantly increased in the ADHD group when compared to immediate rewards (t_{13} = -2.87; P < 0.05) – see Figure 6B.

fMRI Data

The ADHD group showed significantly reduced VS/NAcc activation toward all choices while the order of amplitudes (immediate > delayed) was similar for both groups (Figure 7, and Table 5 for fMRI statistics).

Between-group differences of brain activation on different delay levels were revealed within the bilateral head and body of the NC (see Table 5). As shown in Figure 8A significant between-group differences extended from the ventral part (head of NC, z-plane 2) to the dorsal part of the ROI (body of NC, z-plane 22).

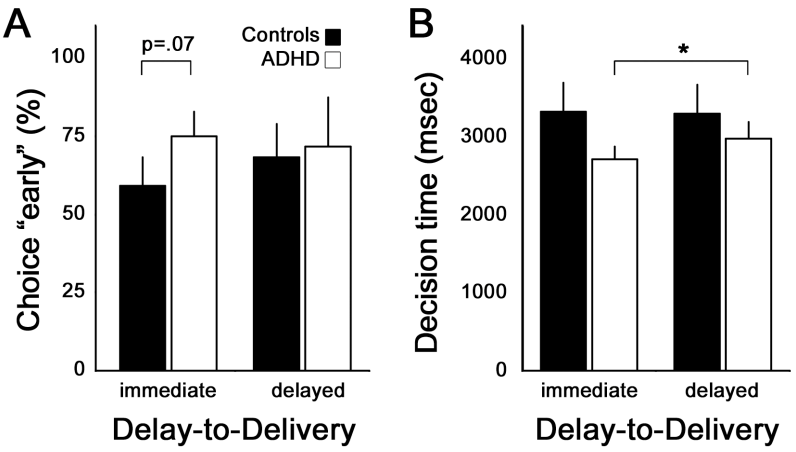

Figure 6. Frequencies of choosing the early reward option and decision times separated by the delay-to-delivery levels. (A) Healthy controls and ADHD patients did not differ significantly with respect to choices of smaller/earlier rewards. Numerically, ADHD patients chose the smaller/earlier reward option more frequently when it was delivered immediately (P = 0.07). (B) While healthy controls showed nearly invariant decision times, ADHD patients were significantly slower during delayed compared to immediate reward trials ($P < 0.05$).

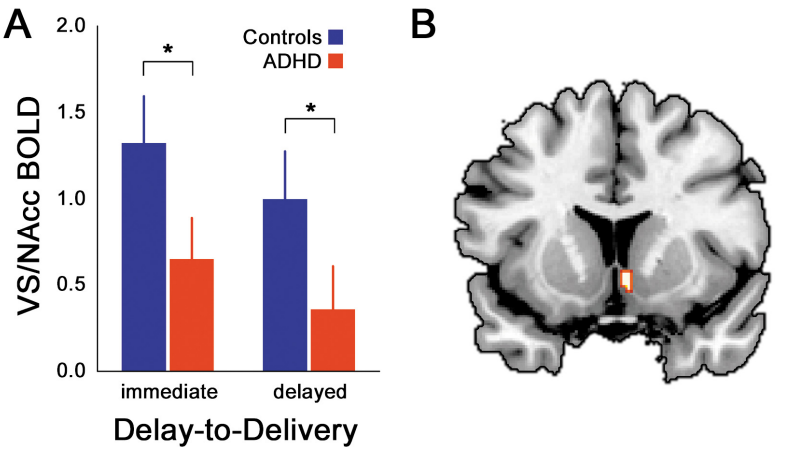

Figure 7. Ventral striatum/Nucleus accumbens hypo-responsiveness in ADHD. **(A)** Mean beta-estimates on immediate and delayed rewards (averaged over significant voxels and hemispheres). Asterisks indicate significant group differences at a level of $P < 0.05$. Within-group comparisons between immediate and delayed rewards demonstrated a significant effect for both groups separately ($P < 0.05$) **(B)** Significant ventral striatal/Nucleus accumbens hypo-responsiveness toward rewards independent of the delay-to-delivery level in the ADHD group. See appendix D for BOLD time courses.

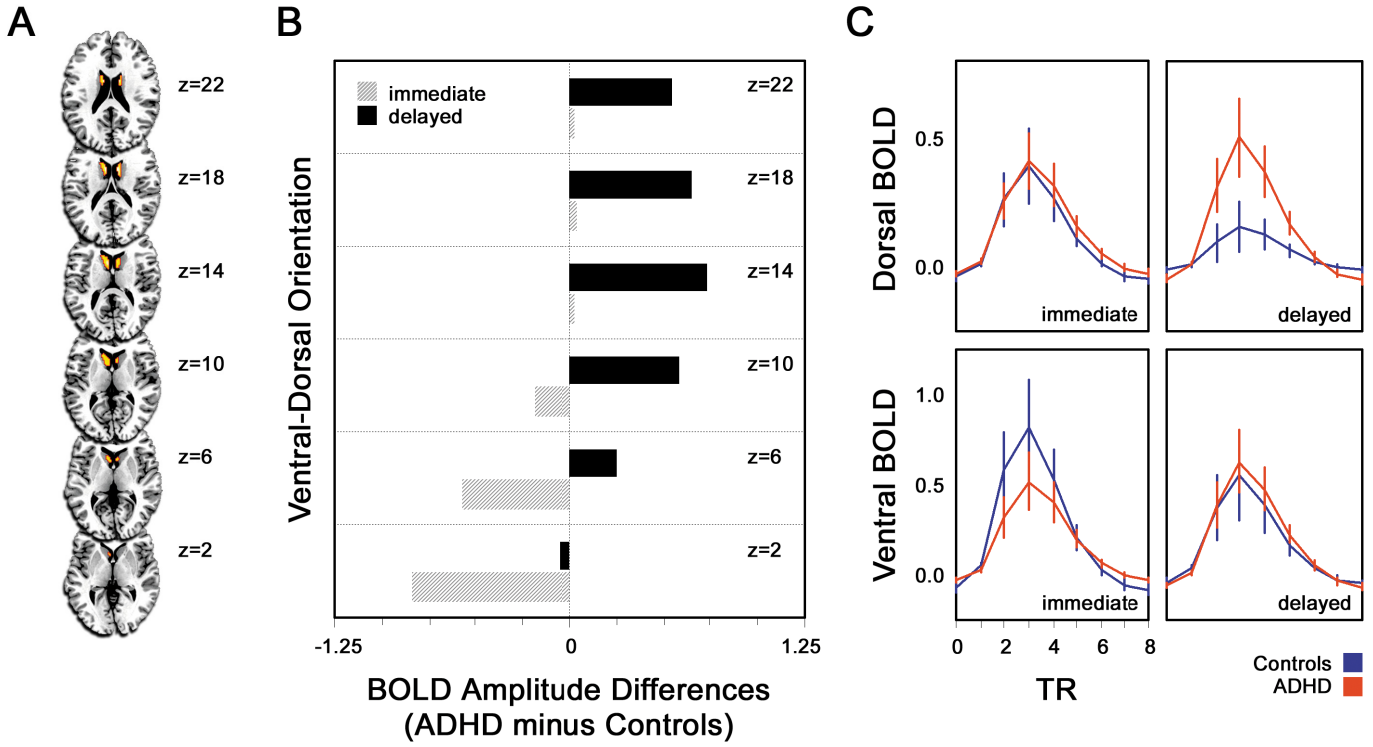

Figure 8. Graded neural responses toward different delay-to-delivery levels within the reward system. **(A)** The significant interaction cluster shown slice-wise in its ventral-to-dorsal extent. **(B)** Corresponding to each z-slice, group response differences (mean parameter estimates averaged over significant voxels of both hemispheres; ADHD minus controls) toward immediate and delayed rewards are shown. The interaction effect consists of two distinct effects: as compared to healthy controls, ADHD patients show (1) ventral-striatal hypo-activation toward immediate rewards; (2) dorsal-striatal hyper-activation toward delayed rewards. **(C)** The corresponding mean BOLD time courses.

Table 5. fMRI statistics.

Contrast	Structure	MNI coordinates	Max z	Cluster size	*P* [c]
All choices[a]	N. accumbens - right	6, 14, -10	1.77	14	.040
Interaction[b]	Caudate (body) – left	-8, 6, 18	2.54	201	.002
	Caudate (body) – right	12, 0, 22	2.56	128	.004
	Amygdala – left	-24, -8, -16	2.43	32	.026
	Amygdala – right	28, -8, -14	2.17	11	.009

[a] All choices = conjunction of group differences (controls > ADHD) for immediate and delayed delivery levels
[b] Delay x Group interaction
[c] required contiguously activated voxels: NAcc = 4; caudate nucleus (body and head) = 125; amygdala = 4; insula = 213

Two separate effects contributed to the significant interaction: Compared to healthy controls, ADHD patients showed hypo-activation toward immediate rewards in the most ventral part which attenuates in a gradient-like manner toward dorsal direction (Figure 8B). In contrast, ADHD patients showed hyper-activation toward delayed rewards in dorsal regions which attenuates toward ventral direction. The corresponding BOLD responses are shown in Figure 8C.

Emotional responses toward delays

Within the emotional core structures analyses of BOLD responses revealed significant delay x group interactions in the left and right amygdala (Figure 9). Neural activation toward immediate rewards was reduced in the ADHD group as compared to healthy controls. Furthermore, the ADHD group showed higher activation toward delayed rewards as compared to healthy controls (see Appendix D for BOLD time-courses). For the insula as the second predicted region the number of significant voxels did not pass the predefined cluster threshold.

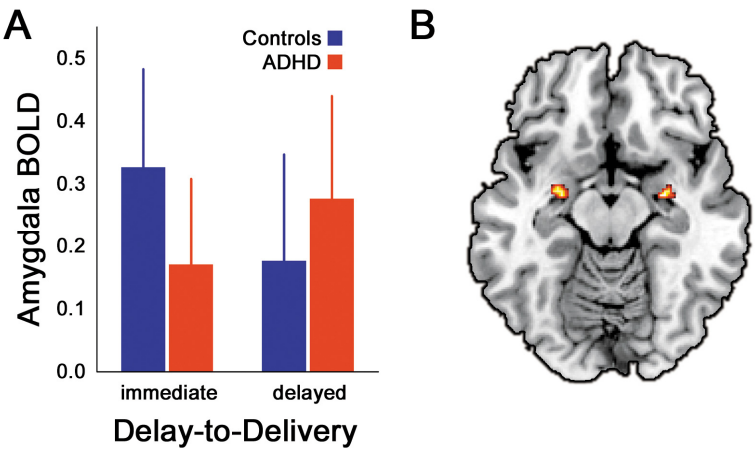

Figure 9. (A) Mean parameter estimates of BOLD responses averaged over subjects and hemispheres separated for the delay-to-delivery levels in the amygdala **(B)**. Averaged BOLD time-courses in (C) Nucleus accumbens and (D) amygdala for immediate and delayed reward trials. See appendix E for BOLD time courses.

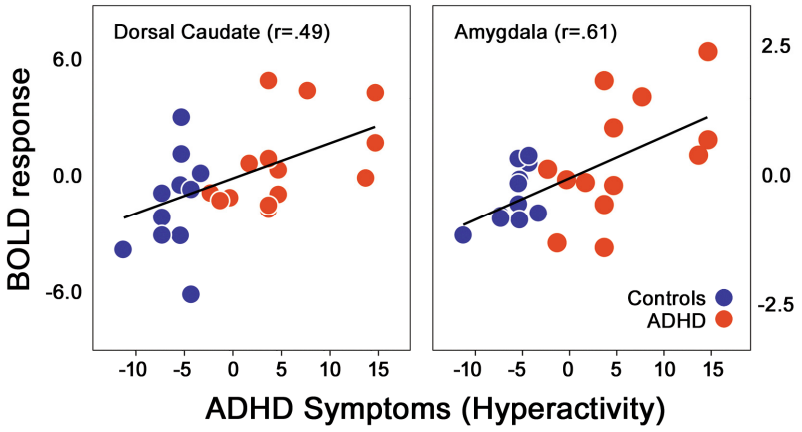

Figure 10. Correlation of ADHD symptom severity (ASRS self-ratings of hyperactivity/impulsivity; mean corrected) with differential BOLD responses (delayed minus immediate; individual mean differences of parameter estimates averaged over hemispheres; mean corrected) in the dorsal caudate (left panel) and amygdala (right panel).

Correlations

As shown in Figure 10 (left panel), the magnitude of neural activation toward delayed rewards in the dorsal part of NC was positively correlated with ASRS scores (hyperactive/impulsive) ($r = 0.50$; $P < 0.01$) and was confirmed in the ADHD group ($r = 0.49$; $P < 0.05$) but not in the control group ($r = 0.34$; $P = 0.14$). Responses of amygdala toward delayed rewards were positively correlated with ASRS scores (hyperactive/impulsive) ($r = 0.61$; $P < 0.001$) and were confirmed in both groups separately (controls: $r = 0.52$; $P = 0.05$; ADHD: $r = 0.58$; $P < 0.05$; Figure 10, right panel). No significant correlations were found for the inattention subscale of the ASRS.

Delay dependent valence rating

Subjective valence ratings for an immediately delivered 5€, 20€ and 40€ gift are shown in Figure 11A. As expected valence ratings increase with increasing money amounts ($F_{2,48} = 8.35$; $P < 0.01$). No group difference on valence rating was revealed for the pooled money amounts ($F_{1,24} = 0.21$; $P = 0.65$) and no interaction of group by amount occurred ($F_{2,48} = 0.01$; $P = .99$). Numerically, patients with ADHD (red curves) showed slightly lower valence ratings across all three amounts. Valence-decay-score was significantly influenced by amount across both groups ($F_{2,48} = 7.61$; $P < 0.01$) with no interaction effect ($F_{2,48} = 0.88$; $P = .420$) – see Figure 11B. Valence-decay-scores were higher across all amount levels in the ADHD group compared to healthy controls ($F_{1,24} = 4.41$; $P < 0.05$). Numerically, the group difference was more pronounced for smaller compared to higher amounts. Red curves: ADHD patients; blue curves: healthy controls subjects.

The delay depended valence-decay was significantly higher in the ADHD group compared to controls ($t_{24} = 1.82$; $P < 0.05$; Figure 12A). Furthermore, the valence-decay scores were significantly correlated with the differential BOLD effect (delayed minus immediate) in the right amygdala across both groups ($r = 0.51$; $P < 0.05$; see Figure 12B). Within-group

correlations were confirmed for both groups separately (controls: $r = 0.48$; $P < 0.05$; ADHD: $r = 0.54$; $P < 0.05$).

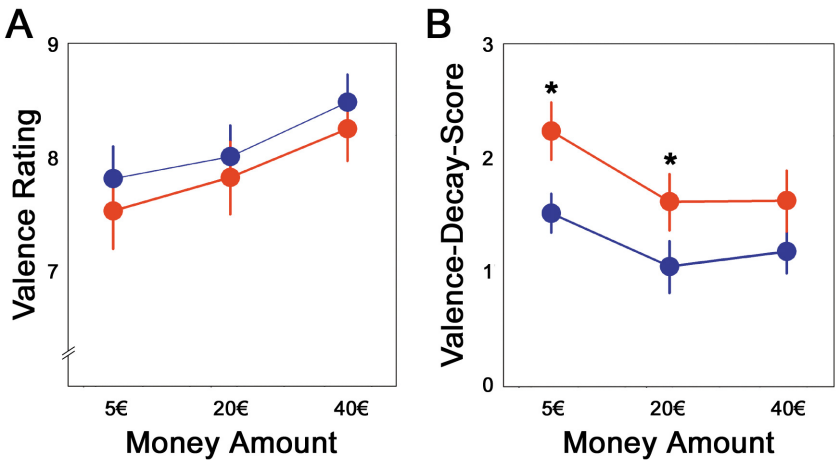

Figure 11. Subjective valence ratings (A) and Valence-decay-scores separated by the different money amounts (B).

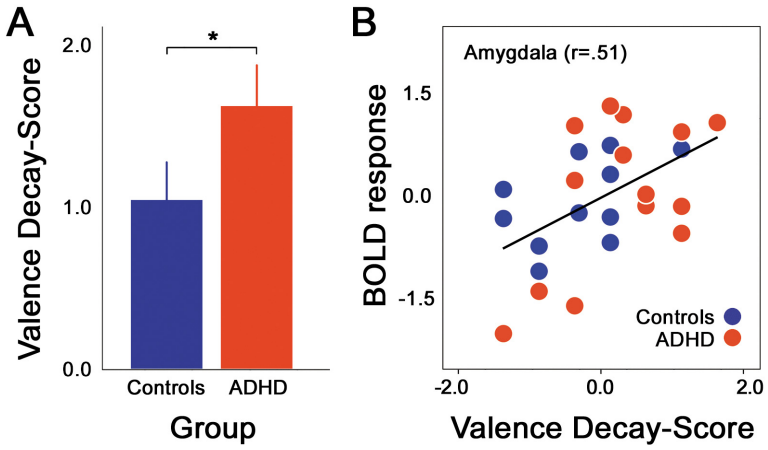

Figure 12. (A) Subjective valence ratings and Valence-decay-scores separated by the different money amounts. **(A)** Group differences on the valence-decay score (for calculation, see Methods section). (B) Correlation of individual valence-decay scores (mean corrected) with differential BOLD responses (delayed minus immediate; individual mean differences of parameter estimates averaged over hemispheres; mean corrected) in the amygdala.

Discussion

With the present study we were able to replicate the finding of hypo-responsiveness in adult ADHD patients (Scheres et al., 2007; Strohle et al., 2008) on immediate rewards in the ventral part of the striatum including the NAcc, a core structure of reward processing. The observed effect was combined with significantly reduced activation of ADHD patients in the amygdala. However, hypo-responsiveness was not confined to immediate rewards, but was present for both immediate *and* delayed rewards. Furthermore, there was a significant shift of between-group activation differences on delayed rewards along the ventral-to-dorsal direction of the caudate nucleus resulting in a significant hyper-activation of ADHD patients in the most dorsal part of the NC on delayed rewards. Dorsal hyper-activity was accompanied by significantly increased activation of the amygdala in ADHD patients. In both structures neural activity toward delayed rewards was significantly correlated with symptom severity. VS/NAcc hypo-responsiveness in ADHD patients is in accordance with predictions from the DDT and DTD theory assuming either a general hypo-dopaminergic state (Sagvolden et al., 2005) or a specific attenuation of dopamine firing in anticipation of reinforcement (Tripp & Wickens, 2008). Since in the present paradigm immediacy emerges from its relation to delayed rewards an extrapolation from the present results to truly immediate reward processing seems not feasible.

Hypo-activation and hyper-activation in ADHD

Hypo-activation and hyper-activation emerged along the ventral to dorsal extension of the NC. This region has been shown to respond in a gradient-like manner toward choices on different time scales (Tanaka et al., 2004; Wittmann, Leland, & Paulus, 2007). In particular, the dorsal part of the striatum was found to be modulated whenever immediate rewards had to be deliberatively rejected to obtain a larger future reward (Tanaka et al., 2004). Dorsal caudate hyper-activation toward delayed rewards in ADHD is in agreement here, since

missing immediate reward options (as in the delayed reward trials) may act as a "loss" and therefore increase dorsal caudate activation. Also, the caudate body has been associated with subjective experiences of craving (Volkow et al., 2006), wanting or desire (Volkow et al., 2002) and our findings of a hyper-activation in ADHD on delayed rewards may be interpreted similarly. Increased activation of the amygdala accompanying caudate hyperactivity on delayed rewards may indicate increased concomitant arousal putatively in a sense of experiencing unpleasantness and aversion associated with waiting (see below). Also, higher emotional arousal can facilitate motor preparation that would interfere with the execution of an actual task and may therefore demand deliberative inhibition to counteract arousal induced (preparatory) motor activity (Hare, Tottenham, Davidson, Glover, & Casey, 2005).

Amygdala response

Based on previous reports and the present empirical evidence from subjective ratings amygdala activation towards immediate rewards plausibly relates to an appetitive state. Decreased amygdala activation toward delayed rewards in healthy controls is in accordance with delay discounting and reinforcement theory and appears to correspond to an attenuated but still positive state. For the ADHD group, amygdala response toward immediate rewards is interpreted similar to that of controls. Its reduction compared to healthy controls is in line with the data from valence ratings and concomitant reduced NAcc/VS activation where the rewarding effect of a stimulus is thought to be coded.

The increase of amygdala activity in the ADHD group on delayed rewards is incompatible with the above mentioned theoretical predictions. Subjective ratings neither indicate increased positive emotional responses toward delayed rewards in ADHD nor do stimulus features other than delay differ. Rather, in line with the prediction of the DAv hypothesis, the valence-decay scores and their significant correlation with amygdala responses toward delayed rewards

suggest that the neural hyperactivity in ADHD most likely relates to a negative emotional response.

DA activity in ADHD and the differential BOLD responses

The identified regions of hypo- and hyper-activation in adult ADHD are strikingly similar to those reported in a recent work by Volkow and colleagues (Volkow et al., 2007). Using ^{11}C labeled raclopride positron emission tomography (PET) in combination with intravenous administration of methylphenidate (MPH) it was shown that dopamine (DA) activity in the dorsal NC of an ADHD cohort was depressed when compared to controls. In addition, DA abnormalities were also shown in the amygdala of the ADHD group. As an increased BOLD response is commonly associated with increased levels of DA (for review, see (Knutson & Gibbs, 2007)) the direction of these results appear contradictory to ours. However, PET imaging has a rather low temporal resolution and results mainly reflect tonic DA activity, while BOLD responses in an event-related paradigm most likely reflect phasic changes of DA activity. Also, the blunted DA responses to MPH in subjects with ADHD could reflect an even higher baseline DA tone that would interfere with further increase by MPH via activation of autoreceptors. Future BOLD imaging studies could incorporate the methodological advantage of MPH bolus to further elucidate the relation between an altered DA activity in ADHD and the differential BOLD responses along the ventral to dorsal extension of the NC and the amygdala.

Limitations

No significant group differences on preferences for early rewards were revealed which is in accordance with previous findings and may well be associated with adaptive strategies acquired during life-time (Bjork et al., 2004; Kaplan & Stevens, 2002). Furthermore, a shortened delay gradient is seen as a putative endophenotype (Castellanos & Tannock, 2002; Sonuga Barke, 2003), i.e. disease related changes to neural circuits exist but need not

necessarily lead to overt behavioral consequences especially in highly controlled experimental fMRI settings. Moreover, the modality of the offered rewards, i.e. secondary rewards and payment of only one choice, might have attenuated delay discounting. Since even the immediate option was associated with a delay this might have reduced impulsive choices in the patient group. Thus, the delay-associated emotional and motivational neural activity in ADHD might trigger behavior more strongly if the experience of immediacy was boosted and/or primary rewards were delivered (McClure, 2007). Also, the present paradigm differs from previous discounting and delay aversion tasks as there is no real time elapsing. In the present task, delay times are of abstract nature and span over longer timescales (weeks rather than seconds). This constitutes a difference to real-time tasks that involve more definite opportunity costs.

To validate the negative emotional response as reflected in amygdalar hyper-activation to delays future studies should incorporate psychophysiological measures such as skin conductance and/or electromyography. Furthermore, questionnaires concerning the negative emotional response to delay periods have to be constructed and involved.

Conclusions

We replicated hypo-responsiveness toward rewards in ADHD that was evident for both immediate and delayed levels of delivery, further supporting the hypothesis that the neural responses on rewards are diminished in ADHD (Scheres et al., 2007; Strohle et al., 2008). Along the ventral-dorsal extension of the caudate nucleus, activation toward delayed rewards increased resulting in a relative hyper-activation of the dorsal caudate nucleus in ADHD which was accompanied by hyper-activation within the amygdala. The ventral-striatal hypo-responsiveness is in accordance with the assumption of an altered dopamine functioning in ADHD, the hyper-responsiveness in the dorsal caudate, and especially in the amygdala corroborates the predictions of the delay-aversion hypothesis.

General Discussion

The aim of this thesis was to attain a better understanding of (a) dopaminergic effects on DD-related brain activation in healthy subjects as well as (b) DD-related emotional effects in adult patients with ADHD. For that purpose two studies have been conducted that focus on prefrontal cortical as well as subcortical brain activation elicited by immediate and delayed monetary rewards. These studies revealed complex interactions of DD-related brain activation with DA-bioavailability *(study I)* and diagnosis *(study II)*. Specifically, *study I* demonstrated that DA-bioavailability moderates the coupling of OFC reward delay sensitivity and impulsivity. *Study II* showed that processing of delayed rewards involves a negative emotional component in adult patients with ADHD. Implications will be discussed with regards to the present knowledge about neural correlates of DD against the background of dopaminergic neurotransmission.

Current findings on neural correlates of DD (Kable & Glimcher, 2007; McClure, Ericson, Laibson, Loewenstein, & Cohen, 2007; McClure et al., 2004) represent initial attempts to determine a *general relationship*, i.e. valid across subjects despite apparent differences on other variables, between the neural and behavioral level during intertemporal choice. Furthermore, these attempts rely on the implicit assumption that decreasing subjective value is associated with *decreasing neural activation*.

Based on theoretical and empirical evidence (Drabant et al., 2006), *study I*, in turn, sought to answer the question whether the assumption of a general relationship between the neural and behavioral level is valid at different DA bioavailability levels. Based on the predictions of the delay aversion hypothesis, *study II* tested whether *increased* activation to delayed rewards, putatively due to a negative emotional response, is additionally involved in DD.

Neural correlates of delay discounting: dopamine moderated relationship

In line with McClure (2007; 2004) and corroborating the important role of OFC in human reward processing (Tremblay & Schultz, 1999; Wallis, 2007), *study I* revealed OFC activation to be associated with DD across the sample. However, this correlation was weak and is in contrast to a recent fMRI study where no such association of OFC activation and DD was evident (Kable & Glimcher, 2007). Plausibly, this inconsistency might be due to the small sample obtained in the latter study, differences regarding the experimental paradigm or limitations of the fMRI recordings in detecting OFC activation. However, besides technical and design influences, the findings of *study I* provide a more fundamental explanation to account for both the weak strength of correlation and the inconsistent results: Accordingly, genetically-driven variation in dopamine bioavailability, that was not accounted for in the mentioned neuroimaging studies on DD, significantly moderates the association of OFC activity and DD. Consequently, a random dominance of the different COMT variants in a given sample will significantly affect the strength and direction of correlations between impulsivity and OFC activation. Samples predominantly consisting of homozygote carriers of the Met or Val allele will indicate zero relationship while substantial relationship of impulsivity and OFC RD sensitivity can be expected in samples predominantly consisting of heterozygote carriers (compare *study I* – Figure 4). Furthermore, significantly negative (heterozygote carriers) to positive correlations (homozygote met-carriers) can arise when the OFC response to delayed rewards is under investigation without accounting for the COMT status.

Uncovering that COMT moderates the relationship of OFC activation and DD was enabled by examining and formally testing joint effects of genetic, trait and neural measures. Such an interaction effect is naturally impossible to detect in studies that do not account for DA bioavailability (Kable & Glimcher, 2007; McClure, 2007; McClure et al., 2004) nor in studies were purely bivariate analyses are conducted despite having assessed DA, DD and imaging

data (Boettiger et al., 2007). Crucially, the latter bivariate analysis strategy is predominantly applied in the imaging genetics domain. The traditional view in imaging genetics is that difference in molecular function of polymorphic genes is assumed to be reflected in *different levels* or *localizations* of neural activity during a particular task. Accordingly, differences in neural activity are thought to be putatively reflected in differences in cognition (Green et al., 2008). In *study I,* however, the effect of differing molecular function (COMT variants) on DD-related brain activation was not associated with behavioral impulsivity measures. This contradicting result was dissolvable by identifying that differing molecular function was associated with distinct trait/brain couplings. Consequently, rather than reflected in different levels or localizations of neural activity as proposed by Green (Green et al., 2008), differences in molecular function might also be reflected in the absence or existence of a neuronal coding ability. In such a case, postulating and testing for a general relationship of traits such as impulsivity with activation in neural structures that qualitatively differ in their neuronal coding abilities seems to be limited in validity. Although the majority of neural structures involved in DD may show simple effects, i.e. neural structures closely tracking the behavioral level (Kable & Glimcher, 2007), the results of *study I* suggest to additionally test for moderating effects of DA on trait/brain couplings. Other neural structures, which were not accessible by fNIRS, and other polymorphic genes may also show interactional effects rather than simple main effects on trait measures.

Finally, an evolutionarily motivated interpretation for the revealed moderator-effect of DA on the association of OFC activation and DD is provided. This interpretational framework considers changing environmental requirements to produce ongoing selection pressure (Petter Portin, 2008) and differences in (neural) processing associated with genetic variation.

An example for such an evolutionary motivated interpretational framework is given by Goldman's "warrior/worrier" model (Goldman et al., 2006) for the COMT gene. Accordingly, COMT gene variants have particular evolutionary advantages. Val158 alleles may be

advantageous in threatening environments where maximal performance is required despite threat and pain (warrior strategy), while Met158 alleles, suggested to have evolved more recently (Palmatier et al., 1999), may be advantageous in complex environments where maximal performance is required on tasks of memory and attention (worrier strategy). Importantly, the persistence of both variants may reflect the possibility that both warrior and worrier strategies can potentially be advantageous, depending on the circumstances (Stein, Newman, Savitz, & Ramesar, 2006).

Based on this framework, one might speculate that (experimental) intertemporal choice situations which require (a) to process mental representations of secondary rewards and (b) to consider relatively extended time-scales, represent a recent and abstract demand (see also section *Neural correlates of Delay Discounting: Time scale demands*). These demands may have grown quantitatively in modern societies wherein delayed positive events such as cultivation of grain or retirement pension gain importance but are contrasted by the availability of numerous desirable products that give immediate satisfaction. Therefore, these environmental requirements may accelerate selection pressure for specific cognitive capacities, such as abstract reasoning, flexible planning or foresight which may have led to biochemical adjustments favoring them (Jacob, 1977) – for a critique on plausible 'just-so' stories on why specific genetical variations and associated traits are under selection, see Vallender (2004). Nevertheless, evolutionary motivated interpretations may provide novel and testable hypotheses and as a consequence may accelerate the number of hypothesis-driven studies in the field of imaging genetics.

Negative emotional response to delay

In line with the general notion, that both cognitive *and* affective components frame decision-making (Bechara, 2004), the aim of *study II* was to test whether DD is accompanied by a negative emotional response to delay as proposed by the delay aversion hypothesis in ADHD

(Sonuga Barke, 2002, 2003, 2005; Sonuga Barke, Taylor, & Heptinstall, 1992; Sonuga Barke, Taylor, Sembi et al., 1992; Sonuga Barke et al., 1996). The results of *study II* indicate that a negative emotional response to delay is a further process involved in DD at least in adult patients with ADHD. Here, processing of delayed rewards was associated with a relative hyper-activation of the amygdala which was correlated with symptom severity and subjective valence-ratings.

Interestingly, altered DA release in the amygdala has recently been shown with positron emission tomography (Ludolph et al., 2008; Volkow et al., 2007) and a morphological study indicates bilateral reduction of amygdala volume in young patients with ADHD (Plessen et al., 2006). The authors of the latter study postulate that the morphological disturbances in the basolateral complex amygdala may interfere with the attribution of valence to sensory stimuli, which may be associated with disrupted emotional learning. Notably, this region contains neurons that project directly to the nucleus accumbens (NAcc), a part of the ventral striatum and, in addition, is reciprocally connected to the orbital and medial prefrontal cortex (Baxter & Murray, 2002).

Importantly, even in healthy subjects, amygdala response to delayed rewards was correlated with the level of impulsivity as well as with subjective valence-decay scores. Therefore, DD might be understood as representing a trade-off between positive affect related to magnitude and/or immediacy and negative emotional response to delay. Interestingly, existing accounts focus on the tempting feature of the immediate option (Laibson, 1997; McClure et al., 2004; Tripp & Alsop, 2001) but neglect the adverse effects of delays. This finding suggests that negative emotional responses to delay should be considered in future studies on DD (Boettiger et al., 2007; Wittmann et al., 2007).

Neural correlates of Delay Discounting: Time scale demands

It has been demonstrated that molecular aspects, i.e. DA bioavailability, as well as psychological aspects, i.e. emotional response to delay, play an important role during the processing of DD. Thus, the list of neural correlates involved in DD and their particular roles has been expanded by the results of the two studies of this thesis. However, the question arises *whether* and *to which extend* neural processing during DD depends on the specific characteristics of the used DD task. One important component might be the range of delay time between choice and the rewarding outcome. Since DD paradigms vary largely by imposing delays from sub-seconds to years, distinct processes and neural correlates might be involved (Izhikevich, 2007; Nitz, Kargo, & Fleischer, 2007).

Assessing DD with operant conditioning typically involves short delays (sub-seconds - seconds). Here, the subjects are not a-priori aware of the contingencies but need to learn them by e.g. repeatedly pressing button *y* or *z*. If the elapsing time between the response (R) and its reinforcer is in a range compatible to the subjects DA-transfer ability, the subject learns the association of *R* and the rewarding outcome: *Eligibility trace* has been proposed as a putative mechanism for dealing with small delays in reinforcement learning (Raymond and Lisberger, 1998; Wickens and Kotter, 1995). According to the hypo-dopaminergia model for ADHD and impulsivity (Sagvolden et al., 2005; Tripp & Wickens, 2008), deficient DA-transfer due to striatal hypo-dopaminergia, alters the learning ability concerning delayed rewards. The occurrence of the delayed reward after pressing button *z* and the associated delay period *x* is not successfully associated with the button press but maybe perceived as accidental (Nitz et al., 2007). Therefore, deficient DA-transfer results in elevated DD as a consequence of having successfully learned the R-US associations with short delays, but not having learned associations with long delays. The requirement of PFC mediated cognitive processes in DD paradigms with short delays (sub-seconds to seconds) may be relatively low.

If the delay period between the action and the reinforcement is experimentally increased and exceeds capacities of *eligibility trace*, higher-order processes are assumed to be involved in association learning to solve the *temporal credit assignment problem* (i.e. if the phasic DA signal to a rewarding stimulus occurs later in time how does the system know which previous stimuli/actions to assign credit?). These higher-order processes involve active PFC mediated maintenance of transient input (action; stimulus) leading to reward (O'Reilly & Frank, 2006).

Delays of weeks or months as applied in the two studies of this thesis involve further processes and the delay is far beyond the effective timing range for association learning. Therefore, striatal DA signals putatively important for "bridging" time periods between CS and UCS (either by eligibility trace mechanism or by PFC mediated maintenance), may be considered as not sufficient for mediating choice preference per se. The capacity of foresight and flexible planning (Boyer, 2008; Suddendorf & Corballis, 2007) is proposed to gain relevance here but this capacity might also rely on striatal hypo-dopaminergic states: To speculate, repeated experiences of the (in-)ability to learn appropriate associations in second-range situations potentially due to variance in DA-level (see above), may (a) generalize and (b) be associated with the formation of trait-like heuristics ("A bird in the hand is worth two in the bush") which may get effective in situations involving abstract delays (Cohen, Schoene-Bake, Elger, & Weber, 2008).

To summarize, processing of DD with extended delay time scales involves subcortical processes as well as higher-order cognitive abilities predominantly mediated by prefrontal areas. Based on the close cortico-striatal connectivity (Haber, 2003), these processes are highly likely to interact during DD processing and the interplay of both processes, rather than separate processes alone, will form a particular behavioral outcome. VS and amygdala responsiveness to rewards and/or delays may be compensable by (intact) PFC mediated processes (Strohle et al., 2008). Thus, the suggested negative emotional response to delays as shown in patients with ADHD might be successfully controlled by PFC mediated processes in

healthy subjects. However, if both levels (cortical and sub-cortical) are altered, as suggested in patients with ADHD, one may expect stronger behavioral and distinct neural effects.

Final conclusions

The two presented studies described important neural structures which are involved in DD, namely the VS/NAcc, amygdala and OFC. Notably, these neural structures are proposed to build a neural network that is relevant for the integration of emotion, reward, motivation, learning, memory and attention (Cardinal, Parkinson, Hall, & Everitt, 2002; Haber, 2003; Murray, 2007; Roy et al., in press). Therefore, neural activation of these structures which have been examined separately in the present studies (due to technical and hypothetical reasons) should be considered and analyzed as a network in future studies. Nevertheless, the following general implications for future studies on neural correlates of DD can be drawn:

- At least for parts of the OFC, neural correlates of DD are heterogeneous at different DA-bioavailability levels. Therefore, it is recommended to include a quantification of DA bioavailability.
- Processing of delayed rewards involves a negative emotional response to delay periods at least in high impulsive subjects with ADHD. To a lesser degree, a negative emotional response to delays might also exist in healthy subjects. Therefore, emotional responses and structures exhibiting increasing activation to delays should be accounted for in research on DD

Limitations

The quality of "immediacy" in both studies emerged from its relation to delayed rewards. Therefore, an extrapolation from the results to truly immediate reward processing does not seem feasible. Consequently, the opposite predictions of DTD and DDT about the neural response toward *truly* immediate rewards could not be tested.

However, when using secondary rewards like money it can be argued that these reinforces evoke activity indirectly, mediated by more abstract symbolic and/or associated processes that are more susceptible to contextual framing effects (McClure, 2007). Therefore, as long as differences between different levels of delays are focused, accompanying differences in neural response are seen as valid. However, future studies should apply primary rewards (food, juice, social rewards) in situations where these rewards can instantaneously satisfy an urgent need (thirst; hunger) and compare the results with the presented evidence - see e.g. McClure 2007.

Furthermore, the delay levels (2 weeks and 4 weeks) as applied in both of the present studies are chosen on arbitrary grounds following the procedure described in the original study of McClure (2004). Neural processing during delayed rewards might differ with respect to amplitude and location when other time scales are used (see section *Neural correlates of Delay Discounting: Time scale demands)*. With regards to the negative emotional response to delays in ADHD, one might expect the negative emotional response to attenuate when delays are extended to e.g. years. Therefore, the assumed linear relationship of aversive states and delay time as depicted in the two-process hypothesis (section *general introduction*) might need revision and other functions for this relationship may prove to be more valid.

Finally, both studies of this thesis are limited with regards to the observed neural areas. In study I fNIRS was applied as a neuroimaging technique, because of its inherent advantages (Plichta et al., 2006b) and because large samples can be easily obtained which was crucial for the intended interaction analysis. However, fNIRS is limited to the cortical surface and

therefore reciprocal interactions of cortical and subcortical activation could not be analyzed. Based on theoretical grounds, *study II* focused subcortical areas while cortical activation was neglected.

Future directions

The link between a steeper delay-of-reinforcement gradient and preferences for smaller-but-sooner rewards in adult subjects as proposed by Sagvolden (2005) has not been studied yet. This could be accomplished by (1) applying an operant conditioning paradigm including experimental variation of the delay between R and US and (2) a non-learning based delay discounting paradigm with extended delay time scales of weeks. The critical test would be to test for a significant correlation of learning rate from task 1 with the steepness of DD (task 2). Due to the involvement of higher-order cognitive abilities in DD tasks with extended delay time scales, increasing prospects of the PFC to "overdrive" subcortical signaling (Barbas, Saha, Rempel-Clower, & Ghashghaei, 2003; Simpson, Snyder, Gusnard, & Raichle, 2001) and only weak associations may be expected. However, the effect of different prefrontal DA-bioavailability levels on this association should be tested.

Within a longitudinal design, patients with ADHD, a high-impulsive non-ADHD group, and healthy controls should be included to investigate the developmental theory of delay aversion in ADHD. Environmental variables (parental style; teacher behavior) should be included to test if delay aversion develops as a consequence of a steeper delay-of-reinforcement gradient interacting with suboptimal environmental effects. Positron emission tomography (PET) might help to clarify the effects of DA-levels more directly than fMRI BOLD measurements (Knutson & Gibbs, 2007).

Finally, therapeutic implications in particular with regards to a negative emotional response to delays in ADHD would comprise the need for immediate and frequent reinforcement of positive behavior and an adequate "bridging" of the delay period between action and reward

has to be included. Motivational alterations which underpin delay aversion can be modified through specific trainings comparable to that in executive and attentional training. Reorganizing delay experience in patients with ADHD, e.g. increasing tolerance for delay, thereby might reduce symptom severity (Sonuga-Barke, 2004).

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Appendix A – fNIRS experiment I

The purpose of this study was to investigate the regional specificity of multi-channel functional near-infrared spectroscopy (fNIRS) in the detection of cortical activation in humans. Therefore, brain activation evoked by a visual as well as a motor task was examined by using 52-channel fNIRS. Analyses demonstrate an isolated activation in the occipital area during visual stimulation, whereas other regions exhibit little or no activation. Analyses of the motor task data clearly identify a differential activation pattern. The observation of an extensive cortical area by multi-channel measurement during two different tasks made it possible to examine the extent to which fNIRS measurements detect regional specific activations.

In agreement with former fMRI (Agnew, Zeffiro, & Eden, 2004; Singh et al., 1998) and fNIRS studies (Franceschini, Fantini, Thompson, Culver, & Boas, 2003; Jasdzewski et al., 2003) the results show that neural activation is identifiable within the expected occipital and sensorimotor brain regions (see Figure A1), and that little to no overlap of the activation areas was identifiable as indicated by the calculated similarity/dissimilarity indices. The demonstrated regional specificity of multi-channel fNIRS in the detection of cortical activation in humans contributes to the credibility and the adequacy of interpretations drawn from fNIRS measurement. To ensure further quality factors, the reliability of fNIRS measurements (in physiological and psychological tasks) should be examined next. Already existing results obtained from fNIRS will largely benefit from these methodological studies and the role of fNIRS would be underpinned as a striking and accurate tool particularly for cognitive research.

Appendix A

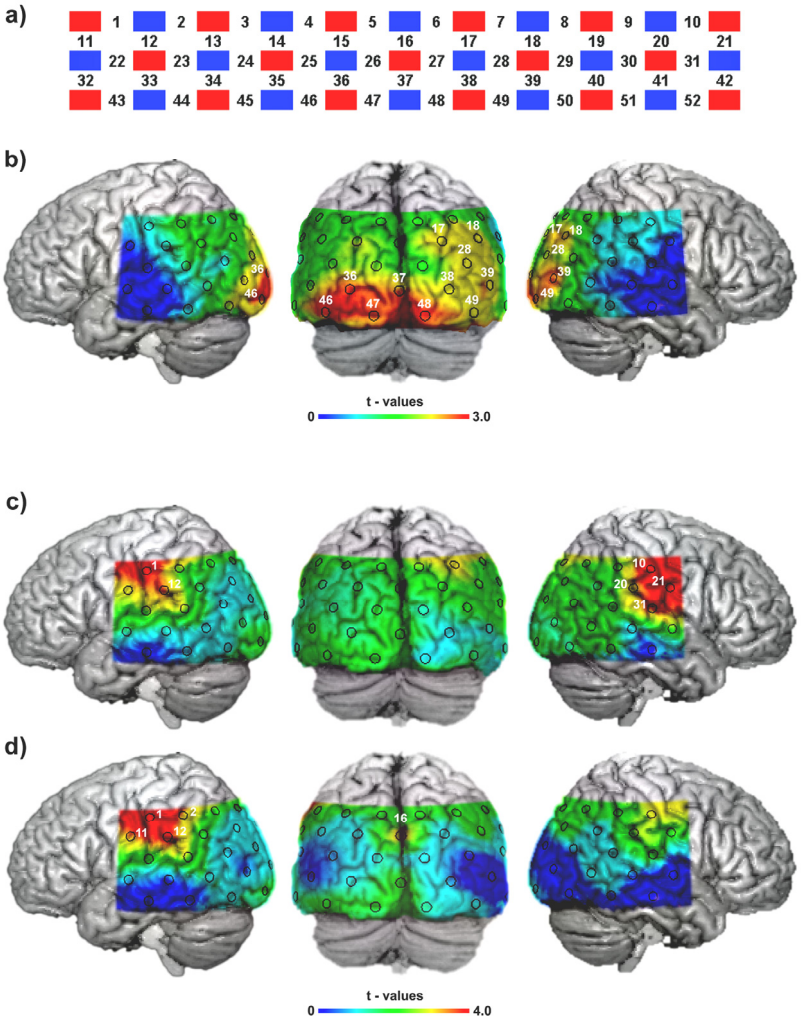

Figure A1. The 52-channel NIRS probe set is shown in (a). Statistical activation maps (t-values) of cerebral O_2Hb concentration during event-related visual stimulation compared to the baseline condition (b). (c) and (d) show cortical activation during left and right hand performance, respectively. Significant channels are labeled. Note that the activation maps are approximately superimposed on a standard anatomical brain. The scales of t-values are different for the visual and the motor task.

Appendix B – fNIRS experiment II

The purpose of this study was to investigate the retest reliability of event-related functional near infrared spectroscopy (fNIRS). Therefore, isolated functional activation was evoked in the occipital cortex by periodic checkerboard stimulation. During 52-channel fNIRS recording, 12 subjects underwent 60 trials of visual stimulation in two sessions. The retest-interval was set to 3 weeks. Linear correlations of the contrast t-values supplemented by scatter plots, channel-wise intraclass correlation coefficients (ICC) as well as reproducibility indices for the quantity of activated channels ($R_{QUANTITY}$) and the location ($R_{OVERLAP}$) of the detected activation were calculated (see Figure B1-B2).

The following conclusions can be drawn: 1) In single subjects, the degree of reproducibility is highest for O_2Hb data. The reproducibility of the location can be increased if a fixed number of channels is used as a threshold. In our study, a top-5% criterion yielded the best result for O_2Hb (mean reproducibility = 78%). An alternative arrangement of optodes could prevent channel-shifts and further increase the reproducibility. 2) Group results, in particular for O_2Hb, can be considered as highly stable over time. Regarding O_2Hb, about 96% of the channel quantity and location were reproducible in the present study if a map-wise view was applied. Considering the channel-wise view (ICCs) it can be stated that the reliability is sufficiently high especially when cluster levels are focused (ICCs for tot-Hb range from .73 to .84). As in single subjects, it seems advisable not to interpret isolated significant channels which are not framed by or adjacent to other significant channels (which implies that multi-channel instruments are preferable to single-channel systems for functional measurements). Up to now, our results are limited to the occipital lobe. Further brain areas and different paradigms should be examined. 3) Due to the lack of criteria for removing artefact trials from fNIRS datasets, criteria for excluding trials have to be established. In the present study we

proposed one criterion (signal changes of >10% in a time-window of < 2s). Future fNIRS studies should exactly report their observations of potential artefacts to establish a consensus about removing trials or even subjects.

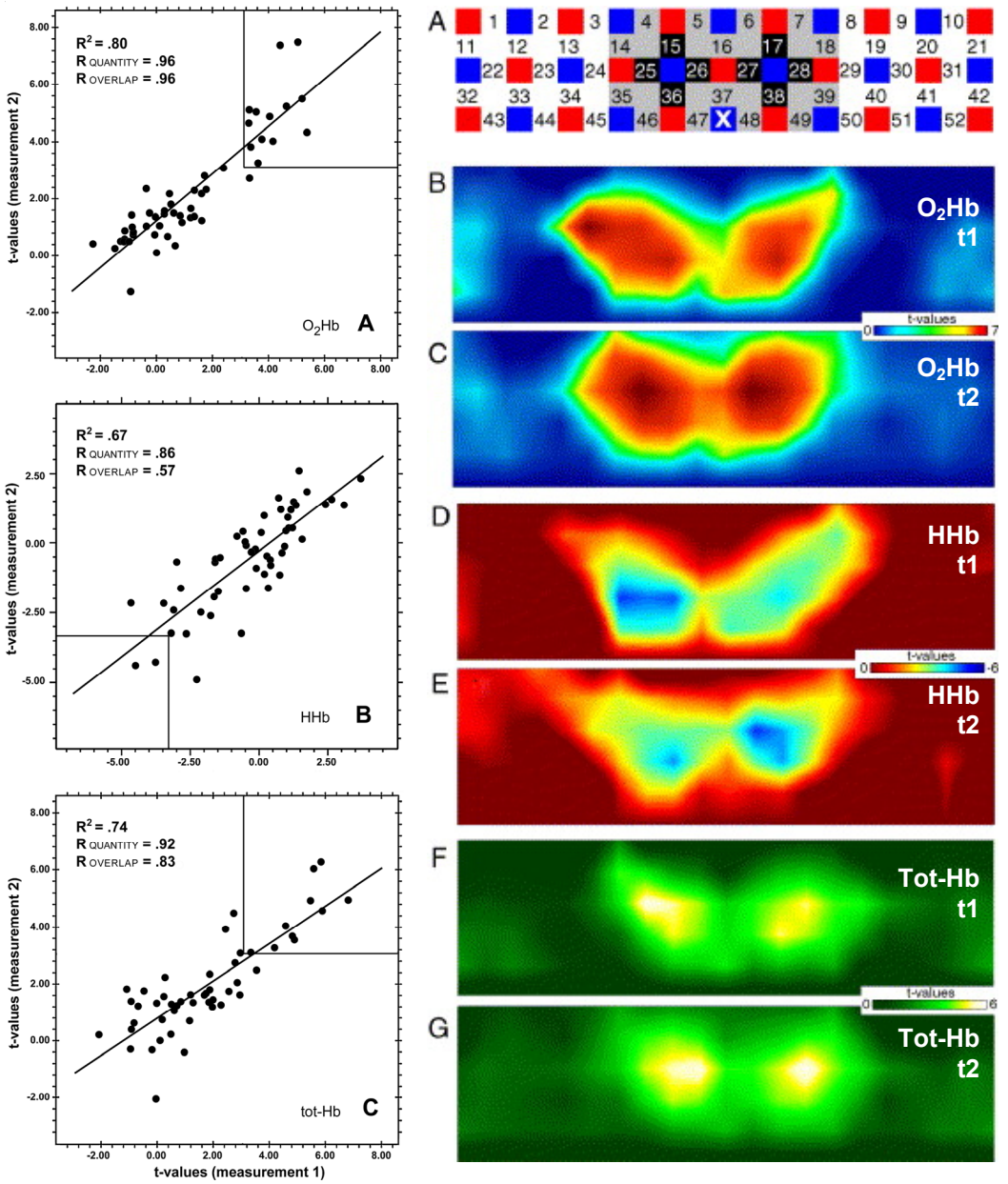

Figure B1. Right panels: Scatterplots of the t-values for O_2Hb, HHb and tot-Hb resulting from second level analysis (left panels). The squares in the corners of the scatterplots represent the area of significance ($p < .05$, corrected). All channels (O_2Hb, HHb and tot-Hb) are solely located in the predefined ROI (right panel A).

Appendix C – fNIRS experiment III

The purpose of the present study was to assess the retest reliability of cortical activation detected by event-related functional near infrared spectroscopy (fNIRS) based on cranio-cerebral correlations. Isolated functional activation was evoked in the motor cortex by a periodically performed finger tapping task. During 44-channel fNIRS recording, 12 subjects performed 30 trials of right and left index finger tapping in two sessions. The retest-interval was set to 3 weeks. Simple correlations of the contrast t-values supplemented by scatter plots, channel-wise intraclass correlation coefficients (ICC) as well as reproducibility indices for the size and the location of the detected activation were calculated. The results at the group level showed sufficient single measure ICCs (up to .80) and excellent reproducibility of the size and the location (up to 89% were reproducible). Comparisons of the intersession group amplitudes demonstrate that the fNIRS signals were stable across time in a retest study design: the number of significant differences was less than randomly occurring false positive activated channels if an alpha level of 5% is chosen. Effect size analyses indicated that the intersession amplitude differences are small (mean < 0.25). For deoxy-haemoglobin and oxy-haemoglobin distinct statistical power profiles were revealed regarding the activation vs. baseline contrast as well as the intersession amplitude differences, indicating a higher sensitivity of deoxy-haemoglobin for local haemodynamic changes.

FNIRS based on cranio-cerebral correlations is sensitive to detect significant activation in the contralateral cortex evoked by an event-related motor task. Results obtained at the single subject level suggest that the localisation of the probe sets according to anatomical or EEG marks may not be sufficiently exact in case studies. As shown by the reported results the position of the hot spot is insufficiently reproducible compared to our criterion of .80. This, in turn, could lead to a seeming signal change if numerically identical channels are compared across multiple sessions at the single subject level. Since the use of large multi-channel

systems enables to monitor the positional shift of the activation centre, a normalisation procedure could be developed based on these observations.

At the group level the detected activation is highly reproducible across 3 weeks (see Figure C1). Effect size analyses in the ROI and outside the ROI revealed distinct statistical power profiles for O2Hb and HHb, indicating locally more specific characteristics of HHb (see Figure C2).

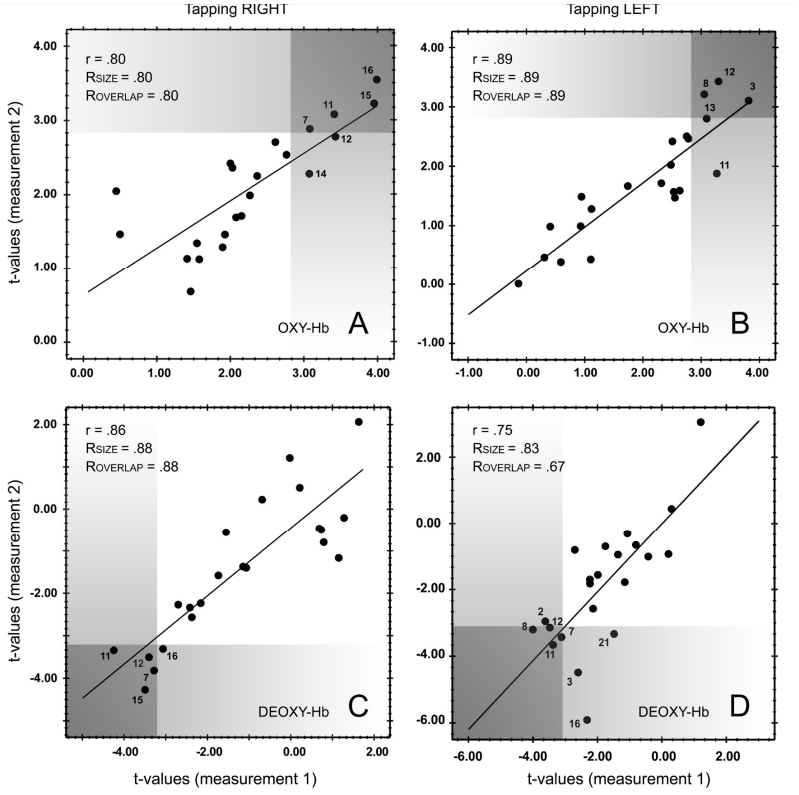

Figure C1. Scatter plots of the t-values for O2Hb are shown in 2A (right finger tapping) and 2B (left finger tapping). Similarly, the HHb data is shown in 2C (right finger tapping) and 2D (left finger tapping). The grey shaded areas represent t-value ranges of statistical significance. Reproducibility indices are shown in the upper left corner of each scatter plot. Channels which exhibit significant activation are labeled with the channel number (compare to figure 1 'probe set').

Appendix C

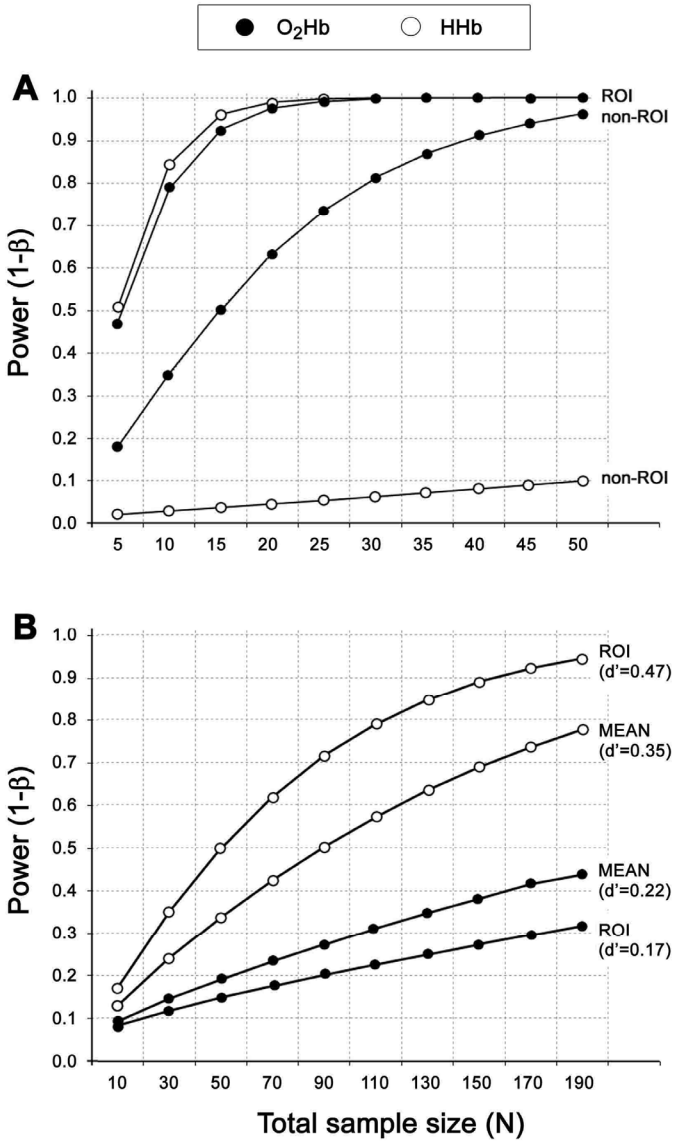

Figure C2. A-posteriori power analysis of the activation vs. baseline contrasts for O2Hb and HHb (panel A). The analyses are separated by channels of the predefined ROI and channels outside the ROI (non-ROI). Given the sample size of N=12 both NIRS parameters show sufficient statistical power (power >0.80). Note that the alpha level is corrected. Panel 2B shows the effect sizes for the intersession amplitude differences. Statistical power is plotted versus total sample size given the upper extreme effect size values at the ROI- and the non-ROI level due to randomly occurring intersession amplitude differences. Note that the power analyses for dependent samples are based on an uncorrected alpha level of 0.05.

Appendix D – fNIRS experiment IV

To validate the usefulness of a model-based analysis approach according to the general linear model (GLM) for functional near infrared spectroscopy (fNIRS) data, a rapid event-related paradigm with an unpredictable stimulus sequence was applied to 15 healthy subjects. A parametric design was chosen wherein four differently graded contrasts of a flickering checkerboard were presented, allowing directed hypotheses about the rank order of the evoked hemodynamic response amplitudes. The results indicate the validity of amplitude estimation by three main findings (a) the GLM approach is capable of identifying human brain activation in the visual cortex with inter-stimulus intervals of 4-9s (6.5s average) whereas in non-visual areas no systematic activation was detectable; (b) the different contrast level intensities lead to the hypothesized rank order of the GLM amplitude parameters: visual cortex activation evoked by highest contrast > moderate contrast > lowest contrast > no stimulation; (c) Analysis of null-events (no stimulation) did not produce any significant activation in the visual cortex or in other brain areas (see Figure D1-D2).

The present study shows that the GLM framework of statistical analyses as developed in the fMRI domain can be expanded to the fNIRS domain. The GLM approach delivers valid amplitude estimations and enables the analysis of rapid event-related data series, which is highly relevant in particular for cognitive fNIRS studies. Moreover, the effective application of a GLM based analysis in the present study may facilitate a straightforward and intuitive understanding of fNIRS results for researchers and practitioners who are familiar with fMRI interpretations.

Appendix D

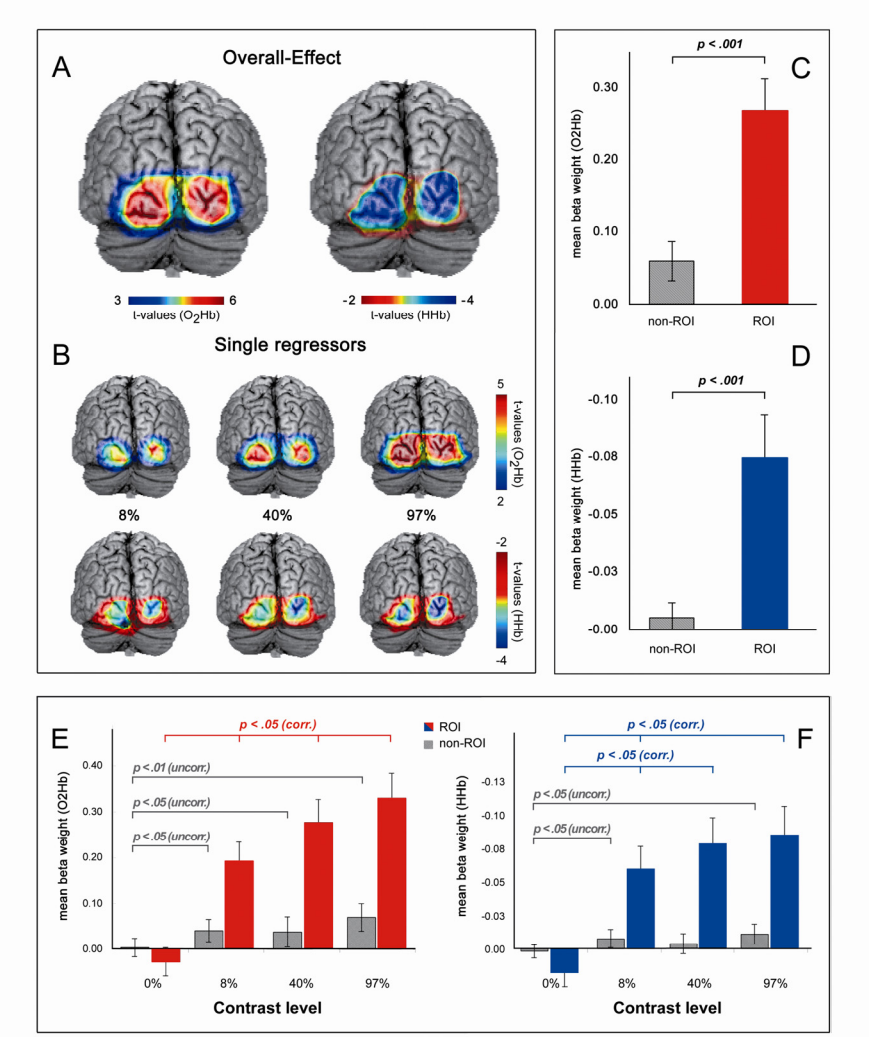

Figure D1. Panel A shows the second-level overall-effect of the checkerboard stimulation (average beta weight of 8%, 40% and 97%) overlaid on a standard brain for O2Hb and HHb, respectively. Panel B shows the second-level results evoked by the three different stimulation conditions (contrast level: 8%, 40% and 97%). Corresponding to the activation maps shown in panel B, panel E and F represents the differential activation effect due to location (REG) and stimulation intensity (CON). Note that the scale of HHb was inverted to simplify matters and that the activation maps (panel A and B) are based on interpolations from single channels.

Appendix D

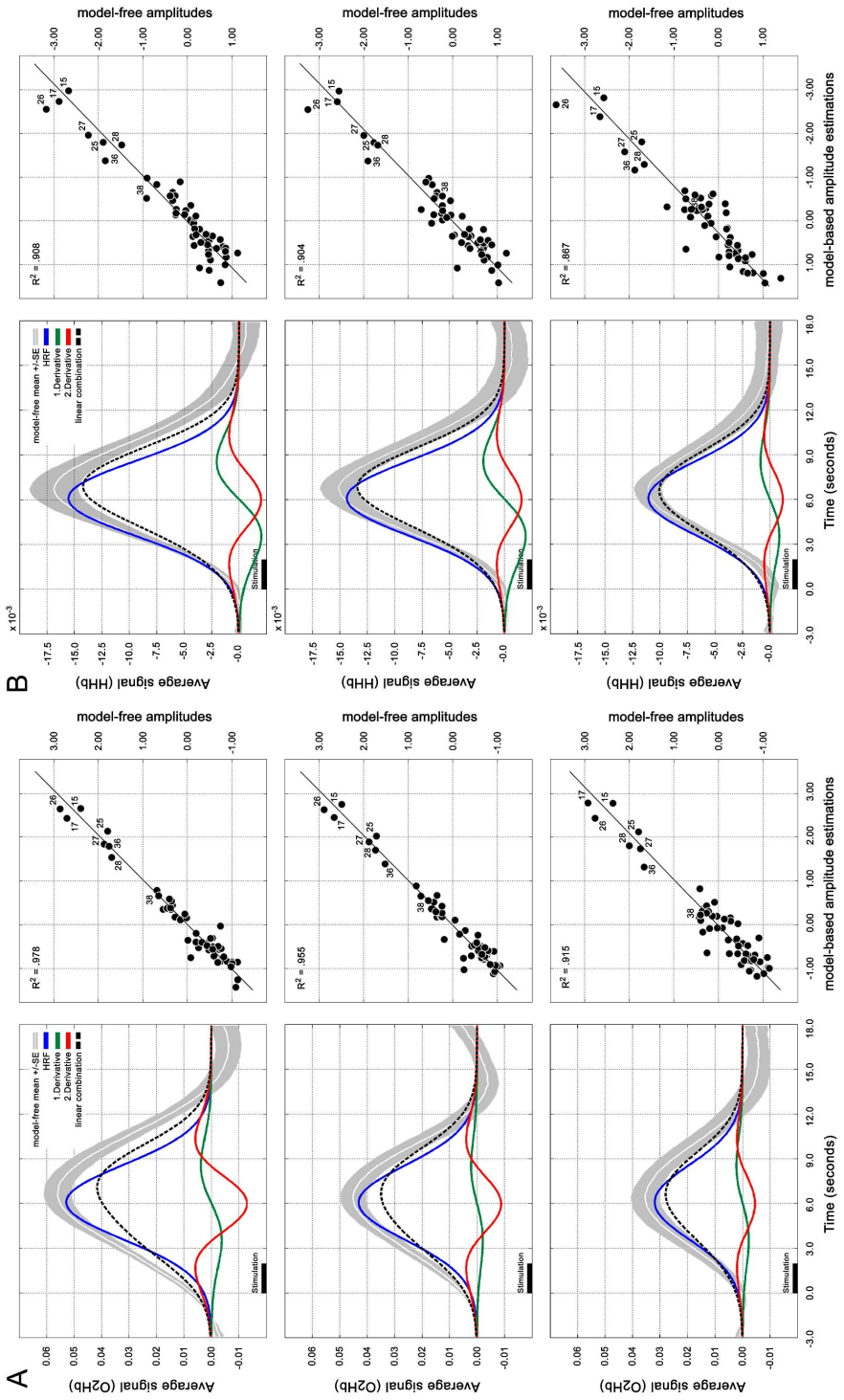

Figure D2. Event-related grand averages and model-based parameter estimations within the ROI. Panel A shows the O_2Hb data and panel B shows HHb data. On the left side of each panel the event-related average time course is shown along with the beta-weighted HRF plus its first and second (weighted) derivatives. On the right side of each panel, similarity of the model-free and the model-based amplitudes is shown with scatter-plots and determination coefficients (the amplitudes were z-transformed). ROI-channels are labelled. The scales for HHb are consistently inverted to simplify matters.

Appendix E – Homogeneity of Error Variance assumption

Criterion: OFC - DD

Sub-Group	n	sx	sy	rxy
1	13	1.33	0.0012	0.012
2	23	1.31	0.0016	0.669
3	13	1.72	0.0015	0.044

Error Variance Results:
DeShon & Alexander's rule of thumb for homogeneity is NOT met. (The highest Error Variance ratio is 1:1.59); Bartlett's Test indicates homogeneous error variance (M = 0.9872, p = 0.6104).

Alternative Differential Slopes Statistics:
James's Test indicates differential slopes. ($p < 0.05$) U = 8.3748, and U(critical) = 6.8267
Alexander's Test indicates differential slopes (A = 7.396, p = 0.0248)

Criterion: OFC - DELAY

Sub-Group	n	sx	sy	rxy
1	13	1.33	0.00215	-.37
2	23	1.31	0.00219	-.453
3	13	1.72	0.00266	0.594

Error Variance Results:
DeShon & Alexander's rule of thumb for homogeneity is met. (The highest Error Variance ratio is 1:1.2); Bartlett's Test indicates homogeneous error variance (M = 0.1351, p = 0.9347).

Alternative Differential Slopes Statistics:
James's Test indicates differential slopes. ($p < 0.05$) U = 12.5768, and U(critical) = 6.8066
Alexander's Test indicates differential slopes (A = 9.7932, p = 0.0075)

Criterion: OFC - TODAY

Sub-Group	n	sx	sy	rxy
1	13	1.33	0.00259	-.302
2	23	1.31	0.00218	0.035
3	13	1.72	0.00324	0.507

Error Variance Results:
DeShon & Alexander's rule of thumb for homogeneity is NOT met. (The highest Error Variance ratio is 1:1.64);Bartlett's Test indicates homogeneous error variance (M = 1.0052, p = 0.605).

Alternative Differential Slopes Statistics:
James's Test indicates no differential slopes. ($p > 0.05$) U = 4.5007, and U(critical) = 6.8514
Alexander's Test indicates no evidence of differential slopes (A = 3.925, p = 0.1405)

Appendix F – BOLD time courses

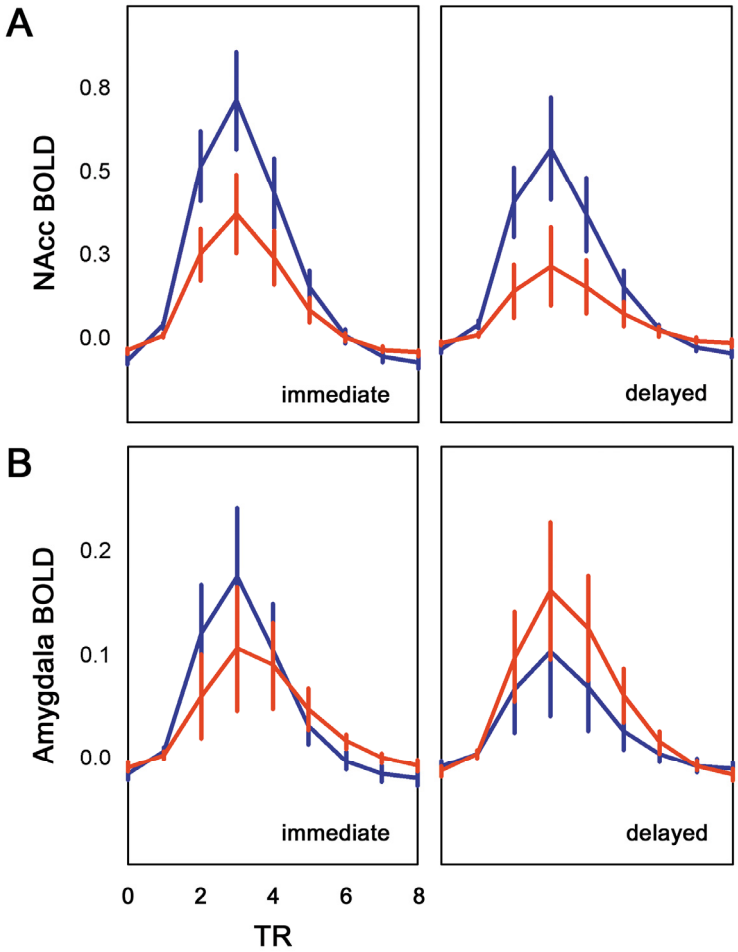

Figure E1. Averaged BOLD time-courses. **(a)** Nucleus accumbens and **(b)** amygdala for immediate and delayed rewards corresponding to the beta estimates as shown in the main text (error bars represent standard errors). Note that the group difference (controls minus ADHD) for immediate vs. delayed rewards constitutes the tested interaction contrast.

Appendix G - Publications during the period of study for PhD

1. **Plichta, MM**, Heinzel, S, Gron G, Lesch KP, Pauli P, Fallgatter AJ (submitted). Impulsivity, COMT and intertemporal choice: an fNIRS study.

2. **Plichta, MM** & Gerdes ABM (accepted). Fossil Shark-teeth of Cadzand-Bad and Groede (NL). Fossilien

3. Wolf RC, **Plichta MM**, Sambataro F, Fallgatter AJ, Jacob C, Lesch KP, Herrmann MJ, Schönfeldt-Lecuona C, Connemann BJ, Gron G, Vasic N (accepted). Regional brain activation changes and abnormal functional connectivity of the ventrolateral prefrontal cortex during working memory processing in adults with attention-deficit/hyperactivity disorder. Human Brain Mapping

4. Schecklmann, M, Ehlis, A-C, **Plichta, MM**, Romanos J, Heine M, Boreatti-Hümmer A, Jacob, C, Fallgatter AJ (in press). Diminished prefrontal oxygenation with normal and above-average verbal fluency performance in adult ADHD. Journal of Psychiatric Research

5. Baehne, CG, Ehlis, A-C, **Plichta, MM**, Conzelmann, A, Pauli, P, Jacob, C, Gutknecht, L, Lesch, K-P, Fallgatter, AJ (in press). Tph2 gene variants modulate response control processes in adult ADHD patients and healthy individuals. Molecular Psychiatry

6. **Plichta MM**, Vasic N, Wolf C, Lesch KP, Brummer D, Jacob C, Fallgatter AJ, Gron G (2009). Neural hyporesponsiveness and hyperresponsiveness during immediate and delayed reward processing in adult Attention-Deficit/Hyperactivity Disorder. Biological Psychiatry, 65(1), 7-14.

7. Schreppel, T, Egetemeir, J, Schecklmann, M, **Plichta, MM**, Pauli, P, Ellgring, H, Fallgatter, AJ, Herrmann, MJ (2008). Activation of the prefrontal cortex in working memory and interference resolution processes assessed with near-infrared spectroscopy. Neuropsychobiology, 57(4), 188-193

8. Schecklmann M, Ehlis AC, **Plichta MM**, Fallgatter AJ. (2008). Functional near-infrared spectroscopy: A long-term reliable tool for measuring brain activity during verbal fluency. NeuroImage

9. Herrmann, MJ, Huter, TJ, **Plichta, MM**, Ehlis, AC, Alpers, GW, Muehlberger, A, Fallgatter, AJ (2008). Enhancement of neural activity of the primary visual cortex during processing of emotional stimuli as measured with event-related functional near infrared spectroscopy (NIRS). Human Brain Mapping, 29(1), 28-35

10. **Plichta, MM**, Herrmann, MJ, Baehne, CG, Ehlis, AC, Richter, MM, Pauli, P, Fallgatter, AJ (2007). Event-related functional near infrared spectroscopy (fNIRS) based on cranio-cerebral correlations: Reproducibility of activation? Human Brain Mapping, 28(8), 733-741

11. **Plichta, MM**, Heinzel, S, Ehlis, AC, Pauli, P, Fallgatter, AJ (2007). Model-based analysis of rapid event-related functional near infrared spectroscopy (NIRS) data: a parametric validation study. NeuroImage, 35(2), 625-634

12. Ehlis, AC, Herrmann, MJ, **Plichta, MM**, Fallgatter, AJ (2007). Cortical activation during two verbal fluency tasks in schizophrenic patients and healthy controls as assessed by multi-channel near-infrared spectroscopy. Psychiatry Research: NeuroImaging, 156(1), 1-13

13. Richter, MM, Herrmann, MJ, Ehlis, AC, **Plichta, MM**, Fallgatter, AJ (2007). Brain activation in elderly people with and without dementia: influences of gender and medication. World Journal of Biological Psychiatry, 8(1), 23-29

14. Polak, T, Ehlis, AC, Langer, JB, **Plichta, MM**, Metzger, F, Ringel, TM, Fallgatter, AJ (2007). Non-invasive measurement of vagus activity in the brainstem - a methodological progress towards earlier diagnosis of dementias? Journal of Neural Transmission, 114(5), 613-619

15. Schecklmann M, Ehlis, AC, **Plichta, MM**, Boutter HK, Metzger FG Fallgatter, AJ (2007). Altered frontal brain oxygenation in detoxified alcohol dependent patients with unaffected verbal fluency performance. Psychiatry Research: NeuroImaging, 156(2), 129-138

16. **Plichta, MM**, Herrmann, MJ, Baehne, CG, Ehlis, AC, Richter, MM, Pauli, P, Fallgatter, AJ (2006). Event-related functional near infrared spectroscopy (fNIRS): are the measurements reliable? NeuroImage, 31(1), 116-124

17. **Plichta, MM**, Herrmann, MJ, Ehlis, AC, Baehne, CG, Richter, MM, Fallgatter, AJ (2006). Event-related visual versus blocked motor task: detection of specific cortical activation patterns with fNIRS. Neuropsychobiology, 53(2), 70-76

18. Fallgatter, AJ, Polak, T, Metzger, F, Richter, MM, Baehne, CG, **Plichta, MM**, Scheuerpflug, P, Ehlis, AC (2006). Brainstem vagus nuclei evoked potentials - New diagnostic method in neuropsychiatry? Nervenheilkunde, 25, 669-673

19. Lautsch, E, **Plichta, MM** (2005). Configural Frequency Analysis (CFA) and Latent Class Analysis (LCA): Are the outcomes complementary? Psychology Science, 47(3/4), 424-430

20. Herrmann, MJ, **Plichta, MM**, Ehlis, AC, Fallgatter, AJ (2005). Optical topography during a Go-NoGo task assessed with multi-channel near-infrared spectroscopy. Behavioral Brain Research, 160(1), 135-140

Curriculum Vitae

HOME ADDRESS	MICHAEL MARTIN PLICHTA ZINKHOF 2 97070 WÜRZBURG
PHONE NUMBER:	+49 931 201-77537
E-MAIL ADDRESS	PLICHTA_M@KLINIK.UNI-WUERZBURG.DE
DATE OF BIRTH, CITY	20.02.1975, KASSEL
CITIZENSHIP	GERMAN

04/2004	DIPLOMA IN PSYCHOLOGY
2000-2004	PSYCHOLOGY STUDIES AT THE JUSTUS LIEBIG UNIVERSITY – GIESSEN
1994-2000	STUDIES IN GERMAN LANGUAGE, INFORMATICS AND SOCIOLOGY AT THE UNIVERSITY OF KASSEL
1994	GERMAN HIGH-SCHOOL DIMPLOMA (ABITUR) AT THE GEORG-CHRISTOPH-LICHTENBERG-GYNMASIUM, KASSEL