Cholinergic modulation of antisaccade performance

The role of *CHRFAM7A* polymorphisms and differential effects of nicotine as a function of baseline performance

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Contents

Abstract

Objectives. The cognitive domain that is most consistently improved by smoking and nicotine application is attentional performance. However, there are still a number of unanswered questions concerning the possible procognitive effects of nicotine and nicotine-like substances on executive functioning. Moreover, more insight is needed into what predicts the effectiveness of cholinergic treatment. Therefore, in the present investigations the antisaccade task, a paradigm of executive control, was chosen to examine cholinergic effects. *Methods*. The aim of Study 1 was to investigate whether genetic polymorphisms in the cholinergic system, the *CHRFAM7A* copy number and 2bp deletion polymorphisms, were associated with antisaccade performance. Study 2 tested the hypothesis that baseline performance level may be a behavioral predictor of the effects of nicotine on antisaccade performance. *Results*. In Study 1, no significant associations were observed of 2-bp deletion or *CHRFAM7A* copy number with antisaccade performance. Study 2 demonstrated that the administration of nicotine enhanced antisaccade performance in low-performing subjects, whereas it had no effect in high-performing subjects. *Conclusions*. The failure to observe an association between antisaccade performance and polymorphisms in the *CHRFAM7A* gene in Study 1 provides evidence of the specificity of the effects of the *CHRFAM7A* gene on hippocampal and memory functions. The results from Study 2 suggest that stimulation of the nicotinic acetylcholine receptor (nAChR) system might be an effective way of improving executive functioning in people with poor baseline performance, such as patients with dementia, ADHD, or schizophrenia.

1. Introduction

1.1 Smoking behavior and nicotine

It is estimated that 29% of the world's population aged 15 years and over smoke cigarettes daily (Rigbi et al., 2011). In Germany, the population's daily smoking rate in adults aged 15 and over is 27% (German Federal Statistical Office, 2005), in the UK, 21 % of adults aged 16 or over smoke cigarettes (UK Office for National Statistics, 2009), and in the USA the smoking prevalence in adults 18 years of age and over is 21% (US Department of Health and Human Services, Centers for Disease Control and Prevention, 2009). Motives for smoking are diverse: People smoke to reduce tension, relax or stimulate themselves, and for social reasons (i.e. to feel more confident and find it easier to talk and interact with people) (Berlin et al., 2003). Other reasons for smoking identified by factor analysis include "pleasure from smoking," "habit/automatism," "addictive smoking," and "handling/need to hold something in hands" (i.e. handling a cigarette, lighting up, watching exhaled smoke) (Berlin et al., 2003). The subjective effects of nicotine intake are as multifaceted as the motives for smoking. The key findings from a study by Kalman (2002) were that nicotine can induce positive effects, such as a drug high (which manifests itself in a head rush and euphoria), but also negative effects, such as an increase in tension. A more recent meta-analysis by Kalman and Smith (2005) further suggests that nicotine produces an increase in vigor in smokers and an increase of fatigue in non-smokers and, contrary to expectations, nicotine decreases relaxation and increases tension/jitteriness in both smokers and non-smokers. The authors suggest that stronger effects of nicotine on mood emerge when different individual variables (e.g., neuroticism) and situational contingencies (e.g., exposure to stressful stimuli) are examined (Kalman, 2002). These variables (such as personality variables) could play an

important role in the subjective effect of nicotine on mood, as there is considerable variability in the effects of nicotine across studies for a given nicotine dose and route of administration (Kalman & Smith, 2005).

Although statistics show that about three quarters of today's population in high-income countries are non-smokers, cigarette smoking remains a significant health problem. Approximately half of the smoking population dies from a disease associated with smoking (Ortells & Arias, 2010). The health consequences causally linked to smoking and exposure to second-hand smoke include (amongst others) respiratory diseases, lung cancer and other forms of cancer, cardiovascular diseases, reproductive effects in women such as reduced fertility and low-birth weight in newborns. Despite the health hazards caused by smoking, smokers' attempts to quit are mostly unsuccessful as cigarette smoking is highly addictive. Nicotine is so powerful that adolescent smokers already present the first symptoms of nicotine dependence such as withdrawal, craving, and relapse, within the first weeks of smoking (DiFranza, 2008; Ortells & Arias, 2010).

Though cigarette smoke contains more than 4000 ingredients, nicotine is the substance that causes addiction to tobacco (Greenbaum & Lerer, 2009). Nicotine reaches the brain 10-60 seconds after a puff on a cigarette, making cigarettes an ideal drug delivery system, enabling smokers to titrate brain nicotine levels each time they smoke (Greenbaum & Lerer, 2009). After inhalation, a peak of around 0.3 μM nicotine can be attained in the brain (Ortells & Arias, 2010; Picciotto et al., 2008), a concentration sufficient to activate nicotinic acetylcholine receptors (nAChRs), mostly the α4β2 nAChR sub-type and the α7 nAChR sub-type. Over the course of a day of smoking, the accumulation of nicotine is enough to produce nAChR desensitization (Ortells & Arias, 2011). Furthermore, there is a second desensitization process at work: the so-called "high-affinity desensitization," that is, even low agonist

concentrations can induce desensitization without nAChR activation (Giniatullin et al., 2005). Desensitization further triggers nAChR upregulation, mainly upregulation of the α4β2 nAChR (Picciotto et al., 2008). The combined effects of receptor activation, desensitization and upregulation by regular nicotine intake finally modulate dopamine release in the mesocorticolimbic system, and thus the rewarding properties of nicotine (Ortells & Arias, 2010), which eventually leads to the development of nicotine addiction. The mesocorticolimbic pathway is part of the "brain reward circuitry" and projects from the ventral tegmental area to the nucleus accumbens. Normally, these sites in the brain mediate the pleasurable effects of natural rewards (e.g. food, water, and sex), but they are also responsible for the motivating and rewarding properties of drugs (Pinel, 1999). As with most other drugs, dopamine release in the nucleus accumbens in particular accounts for the rewarding effect of nicotine (Dani, 2003). The most important nAChRs expressed in the mesocorticolimbic pathway are α4α5β2 and α7 nAChR in the ventral tegmental area as well as α4β2 and α6β2β3 nAChRs in the nucleus accumbens – particularly the α4β2 nAChR seems to play a major role in the development of nicotine addiction (Buisson & Bertrand, 2002). For an elaborate neuronal and molecular model of nicotine addiction, please refer to the review by Ortells and Arias (2010).

The highly addictive properties of nicotine are also reflected in the poor quit rates: In 2008, 45% of smokers in the US tried to quit smoking, but only 4-7% were successful (Heishman et al., 2010). In most smokers trying to quit, withdrawal symptoms and various non-pharmacological factors (e.g., cigarette availability) typically lead to relapse within a few days or weeks (Heishman et al., 2010). Smoking withdrawal symptoms include irritability, restlessness, anxiety, increased appetite or weight gain, depressed mood, and difficulty concentrating. The difficulties in concentration are regarded not only as a relapse factor but also as a factor in the maintenance of smoking in tobacco-dependent individuals who are not

attempting to quit (Heishman et al., 1994). Furthermore, smokers report that one of the reasons they smoke is the perceived cognitive benefit of nicotine (West, 1993). Nicotine's ability to enhance cognition has been the subject of several experimental studies in recent decades which showed that nicotine has positive effects on some aspects of attention, such as alerting attention and orienting attention, on fine motor abilities, on short-term episodic memory, and on working memory (Heishman et al., 2010). The cognitive effects of nicotine are likely to be mediated by the action of nicotinic acetylcholine receptors (nAChR) in the brain and the subsequent reactions at these receptor sites.

1.2 The nicotinic acetylcholine receptor (nAChR)

Nicotine binds to acetylcholine receptors in the brain. Acetylcholine receptors (AChRs) are usually classified according to their "pharmacology," that is to say according to their relative affinities and sensitivities to different substances apart from the endogenous neurotransmitter acetylcholine, the substance naturally produced in the body. Human AChRs are classified into two main sub-types: muscarinic acetylcholine receptors (mAChRs), which are particularly responsive to muscarine (the natural and poisonous substance in the fly agaric mushroom Amanita muscaria var. Muscaria) and nicotinic acetylcholine receptors (nAChRs), which are specifically responsive to nicotine (an alkaloid found in the tobacco plant and in smaller amounts in other plants of the nightshade family). The nAChRs are ionotropic receptors; this means they form ligand-gated ion-channels in the plasma membranes of neurons (neuronal-type nAChRs) and at the neuromuscular junction (muscle-type nAChRs). The neuronal-type nAChRs are a structurally and functionally diverse group of receptors, in each case composed of five sub-units of polypeptide chains arranged symmetrically around an axis perpendicular to the cell membrane (Le Novere et al., 2002). Neuronal or brain nAChRs can be composed of either five identical (homopentamers) or different (heteropentamers) sub-

units. An example of a homopentameric nAChR in the brain is the α7 nAChR which is composed of five identical α7 sub-units with five identical acetylcholine binding sites (Court et al., 2000). The α4β2 nAChR is an example for a heteropentameric nAChR consisting of two α4 sub-units and three β2 sub-units.

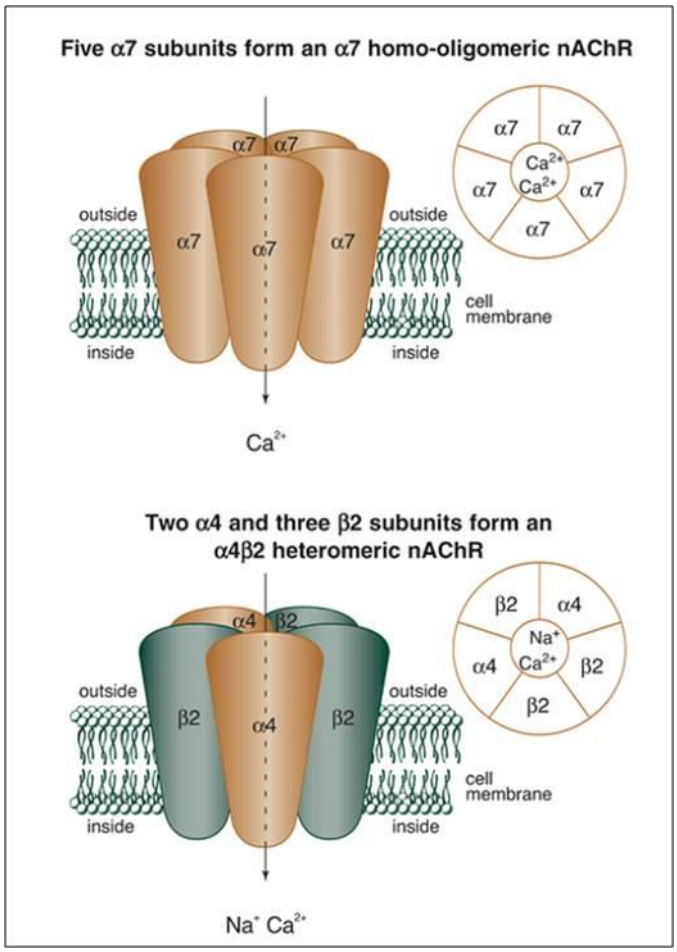

Figure 1-1
Schematic representation of the α7 nAChR and the α4β2 nAChR (NIAAA, 2011). (Graphics Gallery of the National Institute on Alcohol Abuse and Alcoholism, USA. Retrieved July 15, 2011, from http://www.niaaa.nih.gov/Resources/GraphicsGallery/Neuroscience/Pages/default.aspx).

In the human brain, the α7 nAChR and the α4β2 nAChR are the two most common sub-types of nAChRs (Boess et al., 2007). As shown in Figure 1-1, in both nAChRs, the sub-units are arranged around a central pore (= the ion-channel) that opens when acetylcholine or nicotine bind to the nAChR, allowing positively charged ions (cations) to flow through the channel into the cell. The α7 nAChR allows passage of calcium (Ca^{2+}) ions, whereas the α4β2 nAChR allows passage of both calcium and sodium (Na^{+}) (Court et al., 2000; Le Novere et al., 2002). Because some neuronal nAChRs like the α7 nAChR and the α4β2 nAChR are permeable to Ca^{2+}, they can affect the release of other neurotransmitters (Itier & Bertrand, 2001). This might be how nicotine facilitates the release of other neurotransmitters such as acetylcholine, dopamine, serotonin, and glutamate (Di Matteo et al., 2007). It is possible that nicotine's ability to enhance cognitive processing is mediated by this subsequent release of other neurotransmitters (Di Matteo et al., 2007, Heishman et al., 2010).

1.3 A link between smoking and cognition?

The idea that there might be a link between smoking and cognition originally stems from several observations within neuropsychiatric disease states, especially from schizophrenia spectrum disorders. Eventually, clinical observation led to the systematic study of the effects of smoking and nicotine on cognitive functioning both in psychiatric patients and in healthy populations.

1.3.1 The self-medication hypothesis in schizophrenia

One well-replicated clinical observation in schizophrenia is the elevated smoking rate in this disorder: The prevalence of smoking in schizophrenia is about 60-80%, which is two- to four-fold the rate of smoking in the general population (Dalack et al., 1998). Moreover, the

smoking rate in schizophrenia is also higher in comparison to other psychiatric disease states: The prevalence of smoking in major depression is about 49% and it is about 45% in anxiety disorders (de Leon et al., 2002; de Leon & Diaz 2005). There is also some evidence that schizophrenia patients who smoke consume more cigarettes per day than normal smokers (Kumari & Postma 2005, Ucok et al., 2004). Smokers with schizophrenia also favor stronger cigarettes (Olincy et al., 1997) and extract more nicotine from their cigarettes than normal smokers (Kumari & Postma, 2005; Olincy et al., 1997; Strand & Nyback, 2005).

What are the possible reasons for this heavy smoking behavior in schizophrenia patients? One reason might be that smoking reduces some of the psychiatric symptoms in patients with schizophrenia. Evidence for this notion comes from self-reports of schizophrenia patients (Glynn & Sussman, 1990), yet there are few empirical studies supporting this claim. In one study by Smith and colleagues (2002), smoking high-nicotine cigarettes compared to smoking de-nicotinized cigarettes was found to reduce negative symptoms (such as alogia and affective flattening) without affecting positive symptoms (such as delusions and hallucinations) (Smith et al., 2002, Kumari & Postma, 2005). Perhaps the reduction in negative symptoms is achieved via nicotine's ability to raise dopamine levels in the nucleus accumbens and in the prefrontal cortex (Kumari & Postma, 2005).

Another possible reason for heavy smoking in schizophrenia might be that smoking may help to reduce unpleasant side effects of neuroleptic medication, specifically the Parkinsonian symptoms (Goff et al., 1992; Kumari & Postma, 2005). Further evidence for this hypothesis stems from studies demonstrating that neuroleptic-induced akathisia is reduced by nicotine administration via patches (Anfang & Pope, 1997; Yang et al., 2002). Typical antipsychotic drugs such as haloperidol have a strong dopamine blocking action, and it is

thought that smoking can provide relief from the related side effects because it stimulates a release of dopamine (Kumari & Postma, 2005).

Finally, there is strong empirical evidence that nicotine ameliorates cognitive deficits in schizophrenia; thus, schizophrenia patients might also smoke heavily to self-medicate their cognitive impairments (Kumari & Postma, 2005). The wide range of cognitive deficits reliably associated with schizophrenia include deficits in prepulse inhibition (PPI) of the startle response, in gating of the acoustically evoked P50 wave, in antisaccade eye movements, in the Continuous Performance Test (CPT), in spatial working memory, in declarative memory, and in other neuropsychological measures of attention and memory such as in the Wisconsin Card Sorting Test, a widely applied measure of executive functioning (Allen et al., 2009; Reichenberg & Harvey, 2007). To date, several studies have shown that nicotine improves (at least some of) these cognitive deficits in schizophrenia patients. For instance, Depatie and colleagues (2002) found that nicotine improves antisaccade eye movements and performance on the CPT. Harris and coworkers (2004) found that nicotine improved measures of attention from the RBANS (Repeatable Battery for the Assessment of Neuropsychological Status). In another study by Smith et al. (2006), performance on the CPT and spatial working memory were improved by nicotine. Finally, Hong and colleagues (2008) showed that nicotine improved PPI.

One drawback of these studies on the effects of nicotine on schizophrenia is that they were all conducted on schizophrenia patients who smoked and healthy controls who smoked. Studying smoking subjects is disadvantageous because in such a study design, one has to deprive subjects of cigarettes and/or smoking for a certain amount of time in order to be able to apply a sufficient dose of nicotine. Studying satiated smokers instead is not recommendable, for in satiated smokers, nicotinic acetylcholine receptors (nAChRs) are very

likely to be occupied and an additional dose of nicotine would unlikely show an effect. It is possible that the majority of the studies on smokers only demonstrated that nicotine application reversed withdrawal-induced performance deficits (Heishman et al., 2010). Therefore, it is recommended to study the effects of nicotine in minimally deprived smokers (less than 2 hours of withdrawal) or in non-smoking subjects. Studying non-smokers offers the further advantage that they are free of long term-induced neuronal changes that could be caused by nicotine dependence.

There are, however, a few studies which tested the effects of nicotine in non-smoking schizophrenia patients and non-smoking controls. For example, a study by Barr and colleagues (2008) showed that nicotine delivered by a nicotine patch improved performance on the CPT in non-smoking schizophrenia patients and non-smoking controls. The study by Sherr and colleagues (2002) tested the effect of nicotine nasal spray on specific eye tracking measures in smoking and non-smoking schizophrenia patients and in smoking and non-smoking control subjects. Nicotine significantly improved eye acceleration during smooth pursuit initiation in both smoking and non-smoking patients but had no effects on healthy subjects (Sherr et al., 2002). Avila and colleagues (2003) conducted a similar eye tracking study with smoking and non-smoking schizophrenia patients and controls. They found that nicotine reduced the abnormal elevated number of leading saccades during a smooth pursuit eye movement (SPEM) task in schizophrenia patients. Particularly, the beneficial effects of nicotine were not restricted to smoking patients, as non-smoking patients exhibited the greatest number of leading saccades in a drug-free state and the most pronounced improvements after nicotine administration (Avila et al., 2003). Another study that demonstrated that effects of nicotine are not dependent on whether someone smokes or not is a study by Ettinger et al. (2009). Ettinger and colleagues (2009) administered nicotine and placebo on two separate occasions and tested healthy light-to-moderate smokers and healthy

non-smokers with the antisaccade task. Nicotine significantly reduced antisaccade latencies in both smokers and non-smokers (Ettinger et al., 2009). In addition, the amount of the nicotine-induced reduction in antisaccade latency in smokers was comparable to the reduction in antisaccade latency caused by nicotine in non-smokers (Ettinger et al., 2009).

Taken together, the evidence from the experimental nicotine application studies in schizophrenia patients indicates that nicotine improves some of the cognitive deficits (i.e. mainly measures of attention) in schizophrenia and that smoking might represent an attempt by patients with schizophrenia to self-medicate these cognitive deficits (Kumari & Postma, 2005). Analogous to nicotine's beneficial effects on psychiatric symptoms and on antipsychotic-induced side-effects, it seems that nicotine acts as a cognitive enhancer via its interaction with dopaminergic as well as glutamatergic transmitter systems (Kumari & Postma, 2005). Moreover, the observations that both typical (Mishara & Goldberg, 2004) and atypical antipsychotics (Hill et al., 2010) do not sufficiently ameliorate the cognitive deficits in schizophrenia further strengthen the self-medication hypothesis. Finally, Dolan et al. (2004) found that those smokers with schizophrenia who exhibited pronounced neuropsychological deficits in an executive task (i.e. the Wisconsin Card Sorting Test) and in a visuo-spatial working memory task before they took part in a smoking cessation program were less able to quit smoking than schizophrenia patients without these cognitive deficits (Dolan et al., 2004). The results from that study can be interpreted as indirect evidence for the notion that smoking might be a way of self-medicating cognitive deficits in schizophrenia patients. Indeed, the authors conclude that schizophrenia patients may continue to smoke cigarettes because of specific neuropsychological benefits they may receive from smoking (Dolan et al., 2004), and thus interventions aimed at remediating prefrontal cortex-related neuropsychological deficits may lead to improved smoking cessation outcomes in schizophrenia patients (Dolan et al., 2004).

1.3.2 The self-medication hypothesis in ADHD

Studies of the effects of nicotine on cognition showed that attentional performance is the most likely candidate to be positively influenced by nicotine (Heishman et al., 2010; Newhouse et al., 2004; Rezvani & Levin, 2001). Therefore, it is tempting to adapt the self-medication hypothesis to other pathological disease states with attentional deficits besides schizophrenia. Attention deficit/hyperactivity disorder (ADHD) is one neuropsychiatric disorder of interest as it is characterized by inappropriate levels of inattention, hyperactivity, and impulsivity (McClernon & Kollins, 2008). A recent review on smoking and ADHD suggests that the smoking rate is increased both in adolescents and adults with ADHD (McClernon & Kollins, 2008). Prevalence of smoking in ADHD is about twofold higher than in non-ADHD individuals (41-42% for ADHD adolescents vs. 26% for non-ADHD, 19-46% for ADHD adults vs. 10-24% for non-ADHD) (McClernon & Kollins, 2008). Furthermore, there is evidence that individuals with ADHD start smoking at an earlier age and are more likely to progress to regular smoking (McClernon & Kollins, 2008). In addition, the self-reported number of ADHD symptoms, independent of clinical diagnosis, is associated with greater cigarette consumption and higher levels of nicotine dependence. Individuals with ADHD or elevated ADHD symptoms retrospectively report greater difficulty quitting smoking and exhibit greater problems with inhibitory control following quitting (McClernon & Kollins, 2008). Together, these findings speak in favor of increased comorbidity of severe smoking and ADHD.

One reason for severe smoking behavior in ADHD might be that nicotine improves ADHD symptomatology. A study employing a single dose of transdermal nicotine (7 mg) showed that nicotine improved ADHD symptoms measured with the Clinical Global Impressions (CGI) scale (Levin et al., 1996; McClernon & Kollins, 2008). A more recent

study by the same research group confirmed that nicotine administration over four weeks of treatment reduced clinician ratings of ADHD symptoms (measured with the CGI) (Levin et al., 2001). Furthermore, self-reported symptoms of depression (measured with the Profile of Mood States test, POMS) were decreased by nicotine (Levin et al., 2001). Two other studies with novel nAChR agonists, ABT-418 and ABT-089, also demonstrated that these compounds displayed efficacy in reducing both inattentive and hyperactive-impulsive symptoms in adults with ADHD (McClernon & Kollins, 2008; Wilens et al., 1999; Wilens et al., 2006).

Besides these therapeutic effects of nicotinic stimulation on the symptoms of ADHD, there is also strong evidence that nicotine and nicotine-like substances have a positive effect on cognition in ADHD. The cognitive deficits in attention in ADHD are mainly failures of cognitive/behavioral inhibition (Barkley, 1997; Newhouse et al., 2004). In the aforementioned study by Levin and colleagues (2001) it was also examined how nicotine treatment over four weeks affected performance on an attention task. Acute and chronic nicotine treatment significantly attenuated the rise in hit reaction time standard error over session blocks on the Continuous Performance Test (CPT) (Levin et al., 2001). This result also underlines that nicotine affects measures of intra-subject variability – a parameter which discriminates well between ADHD-individuals and non-ADHD-individuals and which might even reflect a unitary construct in ADHD (Klein et al., 2006). Another study on nicotine and cognition in ADHD found that both nicotine and methylphenidate treatment improved stop signal reaction time (an estimate of the speed of inhibiting a response) in non-smoking adolescents with ADHD aged 13-17 years (Potter & Newhouse, 2004). A similar study by the same researchers showed that nicotine also had the same significant positive effect on the stop signal reaction time measure in non-smoking young adults with ADHD (Potter & Newhouse, 2008). Furthermore, there was also a trend ($p=.06$) for nicotine to improve recognition memory in a

verbal recognition memory task (Potter & Newhouse, 2008). The study by Wilens and coworkers (2006) which evaluated the effectiveness of ABT-089 in treating ADHD also included neuropsychological test measures. Researchers found that ABT-089 improved spatial working memory, ABT-089 improved numeric working memory by trend, and ABT-089 reduced errors of commission in the CPT (Wilens et al., 2006). In summary, nicotine and nicotinic compounds show a procognitive effect in ADHD, speaking in favor of the notion that heavy smoking in ADHD might be (at least partially) explained by its remediating effects on cognitive deficits in ADHD.

1.3.3 Procognitive effects of nicotine in Parkinson's and Alzheimer's diseases

In addition to schizophrenia and ADHD, Parkinson's disease (PD) and Alzheimer's disease (AD) are two disorders in which treatment with nicotinic compounds might help to improve cognitive deficits. PD is primarily considered a movement disorder with a typical onset after age 55 (Levin et al., 2006). The main symptoms of PD include shaking, rigidity, slowness of movement and difficulty walking (Jankovic, 2008). Patients with PD might also exhibit cognitive (dementia-like) symptoms, sleep disorders and sensory abnormalities (Jankovic, 2008). AD is the most common form of dementia, characterized by a late onset, typically after the age of 60, with the main symptoms being long-term memory loss, the inability to acquire new memories, confusion, irritability and aggression, mood swings, and a breakdown of language abilities (Waldemar et al., 2007).

In PD and AD there is no strong evidence for smoking being an attempt to self-medicate and treat cognitive symptoms (Levin et al., 2006). Further, there is no evidence that individuals with AD or PD are at a higher risk for smoking (Levin et al., 2006). Nevertheless, nicotinic receptors might play a role in the pathophysiology of both diseases (Levin et al.,

2006). That is why nicotine-like substances might also be useful in these conditions as an adjunctive treatment for attenuating cognitive deficits. Indeed, there is some experimental evidence that nicotine administration improves measures of attention in AD (Newhouse et al., 1988; Sahakian & Coull, 1994; White & Levin, 1999). However, there is no evidence for nicotine-induced improvement in memory functions in AD; so far, four published studies demonstrated negative findings (Sahakian & Coull, 1994; Snaedal et al., 1996; White & Levin, 1999; Wilson et al., 1995). The review by Levin et al. (2006) nicely summarizes these findings on AD. It also offers an overview about the findings on the possible procognitive effects of nicotine in PD. For example, a study by Kelton and colleagues (2000) showed that patients with PD exhibited improvements in measures of attention after nicotine was administered intravenously. In summary, the evidence in AD and PD further underlines the notion that nicotine mainly affects the attentional domain. Therefore, nicotine-like compounds might be of interest whenever the treatment of attentional deficits is not successful with traditional medication.

1.3.4 Self-medication in a sub-population of "normal" smokers?

The idea that patients with various disease states smoke to self-medicate their cognitive impairments can be extended to the hypothesis that even in so-called "normal" smokers, there might be a sub-population of smokers who also attempt to self-medicate (subclinical) attentional deficits. Indeed, in recent years, this hypothesis has received growing interest. For instance, a research group from Israel published several papers on the question "Why do young women smoke?" addressing the question why smoking rates in women from high income countries increase although global trends indicate an overall decline in cigarette smoking (Yakir et al., 2007). In the third paper of this series of publications by Yakir and colleagues (2007) tested female current smokers, occasional smokers, past smokers, and non-

smokers with a computerized neurocognitive battery, which tested the domains of attention, memory, impulsivity, planning, information processing, and motor performance. Current and occasional smokers were not in a withdrawal state, as all smoking subjects smoked their last cigarette less than 90 min before testing (Yakir et al., 2007). Results from this study showed that current smokers made significantly more errors than non-smokers on the Continuous Performance Task (CPT), Matching Familiar Figures Test (MFFT), and Tower of London (TOL) test (Yakir et al., 2007). Interestingly, past smokers did not differ significantly from current smokers on any test (Yakir et al., 2007). Furthermore, there was no association between duration of smoking and neurocognitive performance (Yakir et al., 2007). All subjects in this study were young adults, between 20 and 30 years of age who had been smoking for a few years, but not for many years (mean number of smoking years was 5.56 years (SD=2.32 years)). Therefore, these results suggest that poorer cognitive performance in current and past smokers might not be a consequence of the neurotoxic and deleterious long-term effects of the consumption of nicotine. Instead, these results indicate that a priori neurocognitive deficits may be one of several factors that influence young women to smoke (Yakir et al., 2007). Indeed, the authors further concluded from their study that individuals who have attentional and impulse control difficulties may find nicotine consumption beneficial and use this substance as a form of self-medication that eventually leads to addiction (Yakir et al., 2007).

The authors also acknowledge that these effects of nicotine on cognitive performance may be subtle and covert in comparison to other factors that influence the individual when he or she initially starts to smoke (e.g. social factors). Once the habit is established, the cognitive effects of nicotine may become more salient and contribute both to smoking maintenance and difficulties with quitting (Yakir et al., 2007). Conducting longitudinal studies including non-smokers and smokers would be a plausible approach to disentangle the issue whether

cognitive deficits already exist before the onset of smoking or whether cognitive deficits are acquired over the course of time after decades of smoking. However, such a project would require repeated testing of the study subjects for a time period of about 30 or 40 years and would, of course, be a very costly and challenging project. Another (and more feasible) research approach to test the hypothesis that a priori cognitive deficits might be one predisposing factor to initiative smoking behavior is genetic studies.

1.4 Genetic evidence for the role of cholinergic neurotransmission in cognition

Genetic variation (i.e. polymorphisms) within the nicotinic acetylcholine receptor genes might be (partially) responsible for inter-individual variation in attentional performance. Moreover, it is likely that carriers of certain polymorphisms in the nAChR system react differently to nicotinic stimulation than individuals who do not carry these polymorphisms. It is possible that carriers of a "disadvantageous" polymorphism of the nAChR (e.g. the receptor function is suboptimal) benefit from nicotine intake to a greater extent than non-carriers of such a polymorphism. Therefore, it is likely that some polymorphisms in the nAChR system increase the vulnerability of developing nicotine addiction. In recent years, several candidate gene association studies of nAChR genes and nicotine addiction have been published. Furthermore, association studies of nAChR genes and attentional performance (or other cognitive phenotypes) have underlined the possible link between nicotine addiction and attentional functioning.

1.4.1 Polymorphisms in the *CHRNA4* gene

To date, the nAChR sub-unit genes *CHRNA4* (which codes for the α4 component of the α4β2 receptor) and *CHRNA7* (which codes for the α7 sub-unit of the α7 nAChR) are the most frequently studied genes in connection with nicotine addiction and cognitive functioning. So far, the *CHRNA4* single nucleotide polymorphism (SNP) rs1044396 (C/T) has received a lot of attention, as it has been repeatedly associated with nicotine addiction in Chinese and European-American subjects (Li et al., 2005, Feng et al., 2004, Breitling et al., 2009). However, there is also one negative finding of rs1044396 and nicotine addiction (Etter et al., 2009). The *CHRNA4* gene is located on chromosome 20q13.3 and consists of six exons. SNP rs1044396 is a synonymous SNP located in exon 5; the SNP has not been functionally characterized. This SNP has also been associated with various aspects of attention in several studies. Therefore, the SNP rs1044396 is likely to play a role in the triad of nicotine addiction, attentional deficits and nAChR variation.

Parasuraman and colleagues (2005) studied the association of rs1044396 with performance on a cued visuospatial attention task in healthy adults. An increasing "gene dose" of the C allele (i.e., no C alleles, one C allele, and two C alleles) was associated with larger benefits of valid attentional cuing (Parasuraman et al., 2005). The researchers also tested their subjects on a working memory task, but did not find an association of rs1044396 with working memory performance (Parasuraman et al., 2005). A similar study by the same research group replicated the finding that visuospatial task performance increased with the number of C alleles in a slightly different paradigm (Greenwood et al., 2005). In a more recent study, this research group also examined the combined or epistatic effects of *CHRNA4* and *APOE* (*APOE* being a major susceptibility gene for late-onset Alzheimer's disease) (Espeseth et al., 2006). (An epistatic gene effect – or epistasis – decribes the nonadditive

interaction between genes at different loci. The effect of one gene depends on that of another (Plomin et al., 2001).) Subjects were healthy middle aged (53-64 years) and older (65-75 years) adults. Carriers of the *APOE*-ε4 allele (the risk allele for Alzheimer's disease) who were also TT homozygotes for the rs1044396 *CHRNA4* SNP showed poorer performance on a visuospatial attention task involving letter discrimination (Espeseth et al., 2006). In a follow-up study, the research group demonstrated further epistatic effects of *APOE* and *CHRNA4*. Being an *APOE*- ε4/*CHRNA4* TT carrier was associated with slower and less efficient neuropsychological test performance, with steeper decline in speed tasks and in delayed recall (Reinvang et al., 2010). Age dependent genetic effects were found for both *APOE* and *CHRNA4* , where elderly participants (60–79 years) showed a negative influence of TT carrier status on initial memory performance, but a tendency for steeper memory decline in ε4 carriers (Reinvang et al., 2010).

There is also evidence for that rs1044396 is associated with physiological phenotypes of attention. Espeseth and colleagues (2007) genotyped their subjects for rs1044396 and conducted a study with auditory and visual oddball paradigms measuring event-related potentials. Results showed that TT homozygotes displayed increased amplitudes in the auditory N1 and visual P1 components (both components being present 100-150 ms after stimulus onset) (Espeseth et al., 2007). Later ERP components peaking 300-500 ms post-stimulus appeared to be unrelated to this *CHRNA4* polymorphism (Espeseth et al., 2007).

Finally, there is evidence from imaging data that the SNP rs1044396 affects attentional processing. Winterer et al. (2007) assessed attentional network function in healthy subjects with functional magnetic resonance imaging (fMRI) during an attention-requiring visual oddball task. The SNP rs1044396 showed genotype effects on attentional network function in both the supplementary motor area/anterior cingulate and the parietal cortex in the

absence of overt behavioral effects. In the parietal cortex, a gene-dosage effect was seen: Stronger BOLD (blood oxygen level-dependent) response was seen with an increasing number of A alleles (which corresponds to an increasing number of T alleles) (Winterer et al., 2007).

An association study by Todd and coworkers (2003) provides further genetic evidence for a possible connection of the nAChR system and attentional functioning: The researchers found that an intronic *CHRNA4* polymorphism was associated with attention problems. Todd et al. (2003) divided ADHD subjects into two ADHD sub-types: combined ADHD and inattentive ADHD. They demonstrated a significant association of the G allele of their so-called marker "SNP3" (located in Intron 2 of *CHRNA4*) with inattentive ADHD. The possible functionality of this intronic SNP has not been determined yet, however, the authors speculate about the SNP3 polymorphism affecting pre-mRNA stability and/or splicing (Todd et al., 2003).

1.4.2 Polymorphisms in the *CHRNA3* gene

There is also evidence that polymorphisms within the α3/α5 nAChR sub-unit (*CHRNA3/CHRNA5*) gene cluster on chromosome 15q25.1 are associated with (pre-)attentional functioning. Research from our own laboratory demonstrated that two common *CHRNA3* polymorphisms (rs1051730/rs1317286) influenced prepulse inhibition (PPI) of the acoustic startle response (Petrovsky et al., 2010). PPI is a measure of inhibitory function and time-linked information processing by which a weak sensory stimulus (the prepulse) inhibits the elicitation of the startle response caused by a sudden intense stimulus (the pulse) (Braff et al., 2001). PPI is commonly viewed as an operational measure of a process called "sensorimotor gating," by which excess or trivial stimuli are screened or "gated out" of

awareness so that an individual can focus attention on the most salient aspects of the stimulus-laden environment (Braff et al., 2001). In our study, the TT genotype of rs1051730 and the GG genotype of rs1317286 were associated with decreased PPI levels in both healthy volunteers and schizophrenia patients (Petrovsky et al., 2010). Our findings from this study further support the view that the cholinergic system plays a key role in pre-attentional and attentional mechanisms. Interestingly, these SNPs (the *CHRNA3* rs1051730 T allele and rs1317286 G allele) have been firmly established as risk alleles for nicotine dependence (Berrettini et al., 2008, Bierut et al., 2008, Caporaso et al., 2009, Saccone et al., 2009). For an elaborate review on the involvement of nAChR genes in nicotine dependence, please refer to Greenbaum and Lerer (2009): They also stress the pivotal role of α3 and α5 sub-unit-encoding genes for nicotine dependence.

1.4.3 Polymorphisms in the *CHRNA7* gene

Regarding polymorphisms within the *CHRNA7* gene, most evidence for a possible connection between smoking behavior and attentional functioning is provided by studies that test subjects with schizophrenia. The *CHRNA7* gene is located on chromosome 15q13-q14 which is also a presumed susceptibility locus for schizophrenia. A microsatellite polymorphism (D15S1360) in intron 2 of the *CHRNA7* gene is associated with P50 sensory gating deficits in schizophrenia patients and in their first-degree relatives (Freedman et al., 1997). The P50 wave is an EEG-based averaged event-related potential (ERP) that can be elicited in the context of the auditory "paired click" paradigm (Potter et al., 2006). In the paired click paradigm, two auditory clicks are presented within 500 ms of each other. P50 sensory gating or P50 suppression describes the phenomenon by which the occurrence of the first sound click inhibits the P50 response to the second sound click (Potter et al., 2006). Therefore, P50 suppression reflects the inhibition of responsiveness to repetitive stimulation

and provides the individual with the ability to negotiate a sensory-laden environment by blocking out redundant stimuli (Potter et al., 2006).

Recently, the D15S1360 microsatellite polymorphism was also associated with smoking in schizophrenia (De Luca et al., 2004). However, a similar study by Stassen et al. (2000) did not find the D15S1360 polymorphisms to be associated with smoking. Furthermore, polymorphisms in the promoter of *CHRNA7* are associated with schizophrenia and P50 sensory gating (Leonard et al., 2002). The -86T variant of the -86C→T promoter polymorphism was associated with schizophrenia and with P50 gating deficits in schizophrenia patients and controls (Leonard et al., 2002). Carrying the -86T variant causes a 20% reduction in promoter activity (Leonard et al., 2002), indicating that transcription is reduced. Moreover, the prevalence of other promoter polymorphisms was also greater in schizophrenia patients than in controls (Leonard et al., 2002). Another study by Houy and colleagues (2004) exploring the association of promoter polymorphisms within the *CHRNA7* gene and P50 gating in schizophrenia could not replicate the results by Leonard et al. (2002). However, they found a protective effect of the -194C variant for the sensory gating deficit (Houy et al., 2004). The authors suggest that such conflicting results can be reconciled if one considers that the -194C polymorphism has no causative effect, but is in linkage disequilibrium with other causal variations for the P50 sensory gating deficit, and that different alleles are in disequilibrium in different populations (Houy et al., 2004). A study by Faraone et al. (2004) tested the involvement of 16 nAChR genes as risk factors for smoking in schizophrenia families. The *CHRNA2* gene and the *CHRNB2* gene were significant in this study (Faraone et al., 2004). In addition, the *CHRNA7* gene and the *CHRNA1* gene were marginally significant (Faraone et al., 2004). The results from the study by Faraone et al. (2004) further underline the notion that nAChR dysfunction may mediate susceptibility to smoking in schizophrenia.

There is also evidence in the aforementioned "Why do young women smoke?" (WDYWS) sample that polymorphisms of the *CHRNA7* gene influence attentional processing in healthy subjects. Rigbi and colleagues (2008) demonstrated that impulsivity/response inhibition as measured with the Matching Familiar Figures Test (MFFT) in the WDYWS sample was associated with polymorphisms in the nAChR system. The C allele of SNP rs891398 in *CHRNA2* was associated with significantly increased errors (poorer response inhibition) in both smokers and non-smokers. However, SNP rs2337980 in *CHRNA7* had the opposite effects on smokers and non-smokers: The T allele was associated with decreased errors (better response inhibition) in smokers, while in non-smokers it was associated with increased errors (poorer response inhibition) (Rigbi et al., 2008). In a more recent study by Rigbi et al. (2011), the research group extended their findings to a pharmacogenetic study: placebo or nicotine (4mg as gum) serving as the within factor and genetic profile (SNP rs2337980 in *CHRNA7*) as the between factor. In the MFFT task carriers of the CC variant of SNP, rs2337980 benefited more from nicotine than CT/TT carriers as predicted by their previous findings (i.e. Rigbi et al., 2008) in which CC carriers manifested poorer MFFT performance (Rigbi et al., 2011).

Finally, the hybrid gene *CHRFAM7A* is an interesting genetic marker to be investigated in connection with attentional phenotypes. The gene *CHRFAM7A* is a hybrid gene of *CHRNA7* which contains exons 5-10 of *CHRNA7* and four exons of an unrelated gene, *FAM7A*. Most individuals carry the *CHRFAM7A* duplicon; an individual can carry no copy, one copy, or two copies of *CHRFAM7A*, i.e. the *CHRFAM7A* polymorphism is a so-called copy number variation (CNV) polymorphism (Flomen et al., 2006). In addition, the *CHRFAM7A* gene contains a 2bp deletion polymorphism located in exon 6 (Raux et al., 2002). That means carriers of the *CHRFAM7A* gene might also be carriers of no deletion, one deletion, or two deletions.

In an association study by Raux et al. (2002), carrying at least one *CHRFAM7A* 2bp deletion polymorphism was associated with deficient P50 sensory gating both in schizophrenia and in control subjects. Moreover, most of the effect detected in the entire group was contributed by the non-schizophrenic sub-group (Raux et al., 2002). Furthermore, there is a weak but significant association of psychosis with reduced *CHRFAM7A* copy number (Flomen et al., 2006). Flomen et al. (2006) found that for a combined psychosis phenotype (i.e. for a sample consisting of individuals with the diagnoses of either schizophrenia, bipolar affective disorder, schizoaffective or other psychotic disorder) there was an association with only one copy of *CHRFAM7A*, regardless of presence or absence of the 2bp deletion. Dempster and colleagues (2006) also found an association of the 2bp deletion with episodic memory: The presence of the deletion predicted poorer performance. The functional consequences of *CHRFAM7A* remain to be investigated; currently, it is unknown how *CHRFAM7A* affects P50 gating and episodic memory. Flomen et al. (2008) hypothesize that the translation products of *CHRFAM7A* may interact with those of *CHRNA7* e.g. via the competition for transcriptional factors.

1.5 Clinical trials searching for nicotinic agonists to treat attentional impairments

Evidence from experimental studies with nicotine administration suggests that nicotine enhances some aspects of cognition with the attentional domain being most consistently positively influenced by nicotine (Heishman et al., 2010; Levin et al., 2006; Newhouse et al., 2004,). This holds true both for patient populations (Levin et al., 2006; Newhouse et al., 2004) with clinically relevant attentional problems and for healthy subjects (Heishman et al., 2010). These findings, along with the evidence from genetic studies indicating that the nAChR system plays a major role in attentional processing, led to the (ongoing) search for

new nicotinic substances for the treatment of attentional and other cognitive deficits. Currently, there are several nicotinic compounds under investigation which might be useful in the adjunctive treatment in various disease states. So far, three α4β2 nAChR agonists (ABT-418, AZD3480, varenicline) and the α7 nAChR agonist DMXB-A have been employed in clinical trials testing their efficacy on cognitive functioning (see Table 1-1). Moreover, the effectiveness of the acetylcholinesterase inhibitors (AChEIs, licensed for the treatment of Alzheimer's diseases) galantamine, donepezil, and rivastigmine have been tested as adjunctive treatment in schizophrenia (Ribeiz et al., 2010) (see Table 1-1). Galantamine has also been tested in Mild Cognitive Impairment (MCI) (Koontz & Baskys, 2005) (see Table 1-1).

While α4β2 agonists showed effectiveness in healthy subjects (Dunbar et al., 2007; Loughead et al., 2010), in patients with Alzheimer's disease (Potter et al., 1999), in patients with ADHD (Wilens et al., 1999), and in patients with schizophrenia (Hong et al., 2011), the results for the partial α7 nAChR agonist DMXB-A is more ambiguous. Although there is evidence for specific α7 nAChR pathophysiology in schizophrenia, the evidence on adjunctive DMXB-A treatment in schizophrenia remains ambiguous. While DMXB-A improved some aspects of attention and memory in healthy subjects (Kitagawa et al., 2003) and in patients with schizophrenia (Olincy et al., 2006), it showed no effects in another extensive study with schizophrenia patients (Freedman et al., 2008). Given that in a novel study by Hong et al. (2011) the partial α4β2 nAChR agonist varenicline demonstrated beneficial effects on antisaccade performance, P50 sensory gating and startle reactivity in patients with schizophrenia or schizoaffective disorder, the stimulation of α4β2 subunits (beside the α7 subunits) might also be responsible for beneficial nicotinic effects in schizophrenia patients. It is reasonable to assume that there a several nicotinic mechanisms which are responsible for procognitive effects in patients and in healthy subjects. This also

Compound	Mechanism of Action	Study Population	Cognitive Domain	Effects	Reference
ABT-418	α4β2 agonist	Alzheimer's Disease	Learning/Memory	+	Potter et al., 1999
ABT-418	α4β2 agonist	ADHD	Attention	+	Wilens et al., 1999
AZD3480 (TC-1734)	α4β2 agonist	Healthy subjects	Attention	+	Dunbar et al., 2007
			EEG/MMN	+	
			Episodic memory	+	
Varenicline	partial α4β2 agonist	Healthy subjects	N-back working memory task	+	Loughead et al., 2010
Varenicline	partial α4β2 agonist	Schizophrenia	Antisaccades	+	Hong et al., 2011
			Attention	0	
			P50 sensory gating	+	
			Prepulse inhibition	0	
			Startle reactivity	+	
			SPEM	0	
			Speed of processing	0	
			Working memory	0	
DMXB-A	partial α7 agonist	Healthy subjects	Attention	+	Kitagawa et al., 2003
			Episodic memory	+	
			Working memory	+	
DMXB-A	partial α7 agonist	Schizophrenia	P50 sensory gating	+	Olincy et al., 2006
			RBANS total score	+	
DMXB-A	partial α7 agonist	Schizophrenia	Attention	0	Freedman et al., 2008
			Learning	0	
			Reasoning/Problem solving	0	
			Speed of processing	0	
			Working memory	0	
Galantamine	AChEI	MCI	Executive functions	+	Koontz & Baskys, 2005
			Learning/Memory	+	
Galantamine, Donepezil, Rivastigmine	AChEI	Schizophrenia	Attention	+	Ribeiz et al., 2010 (meta-analysis)
			Executive functions	0	
			Memory	+	
			Language	0	

Table 1-1
The efficacy of nAChR agonists on cognition in human subjects.
Legend: AChEI = acetylcholinesterase inhibitor, ADHD = attention deficit/hyperactivity disorder, SPEM = smooth pursuit eye movement, MCI = Mild Cognitive Impairment, "+" = positive effect on cognitive performance i.e. cognitive improvement, "0" = no effect on cognitive performance, AChEI = acetylcholinesterase inhibitor.

becomes apparent when looking at other drugs which are able to increase acetylcholine levels: acetylcholinesterase inhibitors and serotonergic drugs that act indirectly on the acetylcholine system.

The acetylcholinesterase inhibitor galantamine improved learning and memory as well as executive functioning in patients with Mild Cognitive Impairment (MCI) (Koontz & Baskys, 2005). A recent meta-analysis by Ribeiz and coworkers (2010) also showed that the adjunctive treatment with acetylcholinesterase inhibitors might be an effective way to treat cognitive deficits in schizophrenia. Amelioration of cognitive deficits by acetylcholinesterase inhibitors was found for memory and attentional functions (Ribeiz et al., 2010). However, confirmatory studies are needed to determine the clinical utility of this treatment strategy (Ribeiz et al., 2010). This also holds true for the nAChR agonistic compounds currently being tested. Two compounds that have been assessed in humans have used alternative strategies involving the use of nicotinic agonists to increase the endogenous release of acetylcholine (Olincy & Stevens, 2007): Ondansetron and Tropisetron (both are licensed antiemetics). Ondansetron increases acetylcholine levels via 5-HT_3 receptors antagonism and enhances P50 auditory suppression in patients with schizophrenia (Adler et al., 2005).

Ondansetron also reduced negative symptoms and some adverse side effects of antipsychotic therapy (such as Parkinsonism and akathisia) (Zhang et al., 2006) and enhanced memory functioning in schizophrenia patients (Akhondzadeh et al., 2009). Although ondansetron was well tolerated in a study with patients with Alzheimer's disease, the study failed to demonstrate any significant cognitive improvement (Dysken et al., 2002). Tropisetron, also a 5-HT_3 receptor antagonist, also acts as a partial α7 nAChR agonist (Macor et al., 2001; Papke et al., 2005). In a study by Koike and colleagues (2005), tropisetron improved P50 sensory gating in schizophrenia patients.

In summary, the existing evidence on nAChR agonists indicates that these compounds have some positive effects on attention and memory both in healthy and in clinical populations. However, more research is needed to clarify which nAChR sub-types should be targeted. At present, no nAChR agonist is ready to be released for marketing. Therefore, more clinical trials are needed, especially to develop adjunctive nicotinic treatment for the severe cognitive impairments seen in schizophrenia.

1.6 Cholinergic modulation of executive control

Limitations of the existing studies on the effect of nicotine on cognitive functioning include the fact that there are only a few studies on nicotine and executive control (Heishman et al., 2010). Moreover, very few studies on the efficacy of new nAChR agonists on cognition assessed executive functioning and those that did, did not show an effect (Ribeiz et al., 2010). However, it would be important to further test whether cholinergic substances are able to improve executive functioning, as a treatment is needed for executive control deficits in schizophrenia. Moreover, in schizophrenia, deficits in executive control have the most substantial impact on the outcome of the illness (Friedman et al., 1999). Executive deficits prevent the schizophrenia patient from retaining or relearning skills that are necessary in order for them to function within and be re-integrated into the community. Therefore, it is hypothesized that improvement of these executive deficits would lead to an improved outcome (Friedman et al., 1999). Thus, it is of particular relevance to find new ways of treating executive dysfunctions. The following paragraphs illustrate the involvement of cholinergic neurotransmission in executive functions and why the cholinergic system might be a target system for enhancing executive functioning. The terms “executive control” and “cognitive control” describe the same phenomenon and will be used interchangeably.

Executive control involves the activation of internal representations that correspond to the goals of a behavior and the rules for achieving it (Miller & Cohen, 2001). Various cognitive functions are engaged in carrying out executive control: top-down selective attention (biasing in favor of task-relevant information and inhibiting irrelevant information), working memory (maintaining information online, i.e. in an activated state), and inhibitory control (inhibit a prepotent response, i.e. impulsive, inappropriate, or disorganized behavior) (Miller & Cohen, 2001). The executive control system is assumed to be represented in the prefrontal cortex (PFC) (Miller & Cohen, 2001). In addition, Miller and Cohen (2001) propose that cognitive control stems from the active maintenance of patterns of activity in the PFC that represent goals and the means needed to achieve them. However, cognitive control no doubt involves neural circuitry that extends over much of the brain. The PFC is a collection of interconnected neocortical areas that sends and receives projections from virtually all cortical sensory systems, motor systems, and many subcortical structures, such as limbic areas. These wide-ranging inputs and intrinsic connections provide a substrate for synthesizing and representing diverse forms of information needed to guide performance in complex tasks (Miller & Cohen, 2001).

Currently, it remains largely unknown which role the cholinergic system plays in executive control. The review by Sarter and Parikh (2005) offers the intriguing idea that the prefrontal cortex might be able to systematically recruit cholinergic transmission in order to initiate executive functioning. Initially, the cortical cholinergic input system has been described as a diffuse, modulatory input system that innervates the entire cortical mantle and is primarily designed to enhance sensory input processing (Sarter & Parikh, 2005). However, more recent evidence and conceptualizations indicate that the cortical cholinergic input system consists of modules that target specific cortical areas and have individual afferent organizations, and therefore have the potential to regulate cortical functions in a region-

specific manner (Sarter & Parikh, 2005). This hypothesis further predicts that individual modules of the cholinergic system can be recruited by the prefrontal cortex, for example, to enhance the detection and processing of stimuli of a particular modality (Sarter & Parikh, 2005). As cholinergic inputs to the prefrontal cortex are predicted to contribute to the initiation of such executive functions, increases in prefrontal cholinergic transmission, particularly during conditions that tax attentional resources, might trigger complex patterns of recruitment of cholinergic modules that project to sensory and sensory-associational cortical regions (Sarter & Parikh, 2005).

1.7 The antisaccade task as a measure of executive control

In the laboratory, executive functioning can be measured with several tasks. The Stroop task and the Wisconsin card sort test (WCST) are two classic examples (Miller & Cohen, 2001). In the Stroop task, subjects either read words or name the color in which they are written. To perform this task, subjects must pay attention to one attribute (Miller & Cohen, 2001). This is especially so when naming the color of a conflict stimulus (e.g. the word GREEN displayed in red), because there is a strong prepotent tendency to read the word ("green"), which competes with the response to the color ("red") (Miller & Cohen, 2001). This illustrates one of the most fundamental aspects of cognitive control and goal-directed behavior: the ability to select a weaker, task relevant response (or source of information) in the face of competition from an otherwise stronger, but task-irrelevant one. Patients with frontal impairment have difficulty with this task, which suggests that they have difficulty adhering to the goal of the task or its rules in the face of a competing stronger (i.e. more salient or habitual) response (Miller & Cohen, 2001). Furthermore, when the instructions in the Stroop test vary frequently, patients with frontal lesions also exhibit pronounced

difficulties with this task (Miller & Cohen, 2001) – they have difficulties with "task switching," another aspect of cognitive control.

Similar findings are evident in the WCST. Subjects are instructed to sort cards according to the shape, color, or the number of symbols that appear on them, and the sorting rule varies periodically. Thus, any given card can be associated with several possible actions, no single stimulus-response mapping will work, and the correct one changes and is dictated by whichever rule is currently in effect (Miller & Cohen, 2011). Humans with PFC damage show stereotyped deficits in the WCST. They are able to acquire the initial mapping without much difficulty but are unable to adapt their behavior when the rule varies, which results in perseverative behavior (Miller & Cohen, 2011).

The Stroop task and the WCST constitute relative complex tasks in contrast to the antisaccade task which represents a relatively simple paradigm, which serves as a model system for executive control of oculomotor responses (Hutton & Ettinger, 2006; Reuter & Kathmann, 2004). The ability to control behavior flexibly, responding automatically to stimuli in one situation and suppressing this automatic response in favor of an alternative response in a different situation, is one of the key components of executive control. The saccadic eye movement system provides an excellent model for investigating this ability of the brain because eye movements are easy to measure in the laboratory and because we have considerable knowledge of the neural networks that participate in controlling gaze (Klein & Ettinger, 2008; Munoz & Everling, 2004).

The instruction of the antisaccade task for a subject is that, after presentation of a peripheral target, he or she must look away from it to its mirror image position. Correct performance on the antisaccade task requires two steps. The subject must first suppress the automatic response to look at the target (that is making a prosaccade) and then transform the

location of the stimulus into a voluntary motor command to look away from the target (i.e. conducting an antisaccade). Thus, performance on the antisaccade task can be contrasted with performance on the prosaccade task in which the location of the sensory stimulus and the goal of the saccade are compatible (see Figure 1-2, left), requiring a direct sensory-motor transformation. In the antisaccade task (see Figure 1-2, right), however, stimulus location and saccade goal are decoupled: The direct response must be suppressed and the stimulus vector must be inverted into the saccade vector (Munoz & Everling, 2004). Whenever the subject fails to suppress the automatic prosaccade (i.e. the subjects looks towards the target) in an antisaccade trial, he or she makes an "antisaccade direction error" or "antisaccade error." Figure 1-3 schematically displays a recorded antisaccade error (on the left side of the figure) and a recorded correct antisaccade (on the right side of the figure). Figure 1-3 illustrates that subjects often correct their antisaccade error (see left side of the figure) and that the performance of a correct antisaccade involves a longer latency than the erroneous gaze at the target (i.e. performing a prosaccade) (see right side of the figure).

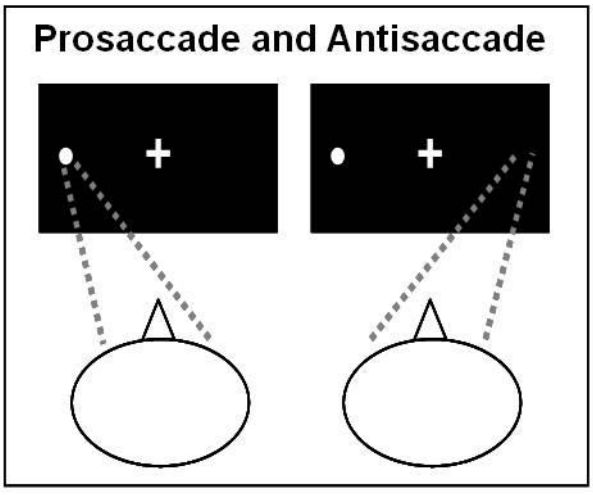

Figure 1-2
Pro- and antisaccade task. The instruction of the task prompts the subject to either look at the peripheral target and generate a prosaccade towards the target (left) or to look away and make an antisaccade towards the mirror position of the target (right).

A serial model of antisaccade function assumes that the sudden appearance of the peripheral target in an antisaccade task automatically triggers a motor program for a prosaccade in its direction, and that antisaccade errors occur when subsequent endogenous processes fail to inhibit or cancel this program (Hutton & Ettinger, 2006). More recent competition models of antisaccade performance emphasize the parallel nature of motor programming and suggest that whether an antisaccade error is made or not is determined by the relative strength of activation in neural systems supporting the pro- and antisaccade (Hutton & Ettinger, 2006; Massen, 2004; Reuter & Kathmann, 2004). More precisely, with the onset of the peripheral target, a "competition" ensues between neural processes underlying the exogenously triggered prosaccade and the endogenously initiated antisaccade – that is two clusters of parallel processes are racing towards threshold (Hutton & Ettinger, 2006; Massen, 2004; Munoz & Everling, 2004).

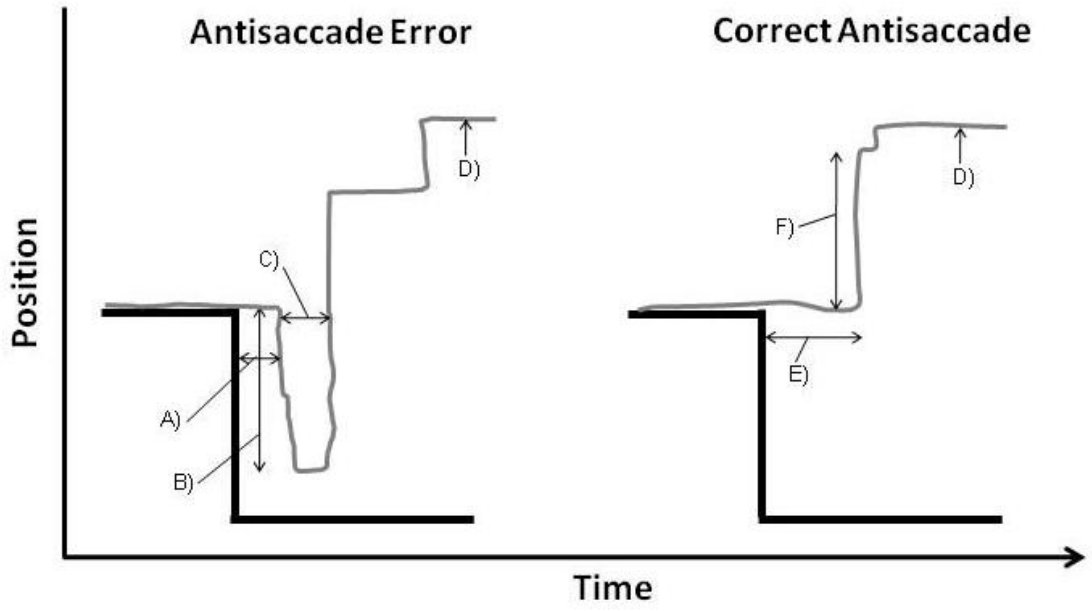

Figure 1-3
Schematic diagram of an antisaccade error (left) and a correct antisaccade (right). Stimulus display = black line, recorded gaze of the subject = grey line. Legend: A) Error latency B) Error amplitude C) Time to correct D) Final eye position E) Correct antisaccade latency F) correct antisaccade amplitude. Schematic diagram modified in accordance with Hutton and Ettinger (2006).

If activation in the neural systems supporting the antisaccade reaches threshold fast enough, the correct antisaccade is initiated, and the reflexive saccade is cancelled. Alternatively, if activation in the neural systems supporting the prosaccade reaches threshold first, an erroneous saccade towards the target is made, and the correct antisaccade follows (Hutton & Ettinger, 2006). As prosaccade latencies are significantly shorter than antisaccade latencies, activation in the neural systems supporting the prosaccade must be somehow reduced in order to allow activation in neural systems supporting the more complex antisaccade program time to reach threshold first (Hutton & Ettinger, 2006).

Neurophysiological findings in monkeys as well as fMRI and EEG studies in humans demonstrated that antisaccade performance recruits a fronto-parieto-subcortical network. Brain areas primarily involved in the antisaccade task include the frontal eye fields (FEF), supplementary eye fields, dorsolateral prefrontal cortex (DLPFC), anterior cingulate, posterior parietal cortex, thalamus, and striatum (Ettinger et al., 2008a; Munoz & Everling, 2004). The FEF plays a crucial role in executing voluntary saccades and the preparation of antisaccade eye movements. The DLPFC is important for working memory and suppressing automatic, reflexive responses and is involved in the suppression of unwanted reflexive prosaccades in the antisaccade task (Pierrot-Deseilligny et al., 2005), for it is believed that the DLPFC provides important top-down signals to the FEF and perhaps the superior colliculus to inhibit the automatic prosaccade (Munoz & Everling, 2004).

The top-down control of the DLPFC is particularly important for correctly performing the antisaccade task, for studies in patients with discrete lesions of the DLPFC have difficulty in suppressing the automatic prosaccade (Pierrot-Deseilligny et al., 2002) and a fMRI study in patients with schizophrenia and control subjects revealed that schizophrenia subjects did not demonstrate the increased BOLD contrast in the right DLPFC during antisaccade

performance that was apparent in the healthy subjects (McDowell et al., 2002). Interestingly, there is also a correlation between antisaccade direction errors and performance on the WCST (Crawford et al., 1995; Rosse et al., 1993); this further underlines the executive control component of the antisaccade task. Furthermore, the correlation between antisaccade and WCST performance indicates that solving these tasks recruits the same or similar prefrontal brain areas, including the DLPFC.

1.7.1 The antisaccade task as a useful tool for investigating cholinergic effects

The antisaccade task is also particularly interesting in connection with the need for treatment of executive control deficits in schizophrenia. Several sources of evidence indicate that the antisaccade task might be a useful laboratory test for investigating cholinergic effects on executive control mechanisms.

First, the task has several general advantages: the measurement of antisaccades is highly reliable, the antisaccade task is easy to administer, the instructions are simple, making failure to comprehend unlikely, and, with the prosaccade and fixation conditions, there are suitable oculomotor control conditions available (Ettinger et al., 2003a; Hutton & Ettinger, 2006).

Second, the neural correlates of the oculomotor system are well-known; the brain areas engaged in the antisaccade task include the frontal eye fields, supplementary eye fields, dorsolateral prefrontal cortex, anterior cingulate, posterior parietal cortex, thalamus, and striatum (Munoz & Everling, 2004).

Third, in the case of schizophrenia, antisaccades are particularly interesting as antisaccade performance deficits are considered to be schizophrenia endophenotypes, i.e. antisaccade performance deficits mark genetic liability for schizophrenia (Calkins et al., 2008; Hutton & Ettinger, 2006).

Finally, it has been shown previously that antisaccade performance is sensitive to cholinergic manipulation. Nicotine enhanced antisaccade performance in studies with schizophrenia patients (Depatie et al., 2002; Larrison-Faucher et al., 2004) and in healthy subjects (Bowling & Donnelly 2010; Dawkins et al., 2007; Depatie et al., 2002; Ettinger et al., 2009; Rycroft et al., 2006; Rycroft et al., 2007), while the anticholinergic substance procyclidine worsened antisaccade performance in schizophrenia patients (Ettinger et al., 2003b).

1.8 Hypotheses and aims of the present studies

As outlined in the previous paragraphs, the abundance of evidence from studies investigating nicotine and other cholinergic substances suggests that cholinergic stimulation affects mainly the attentional domain. Genetic studies demonstrated the involvement of cholinergic polymorphisms in attention and pharmacological studies with nicotinic substances showed positive effects on this cognitive domain. However, the possible procognitive effects of nicotine and nicotine-like substances on executive functioning needs to be investigated. Especially in connection with schizophrenia, possible cholinergic effects on executive functions are interesting, as a treatment is needed for executive dysfunction in this disorder. In addition, more insight is needed into what predicts the effectiveness of cholinergic treatment. Therefore, in the present two investigations, the antisaccade task was chosen, a paradigm of executive control and a schizophrenia endophenotype, to examine cholinergic effects. Two

research strategies were employed: a molecular genetic strategy and a pharmacological strategy. Specifically, the aim of Study 1, the genetic study, was to investigate whether genetic polymorphisms in the cholinergic system, the *CHRFAM7A* copy number and 2bp deletion polymorphisms, were associated with antisaccade performance. Study 2, the nicotine study, tested the hypothesis that baseline performance level may be a behavioral predictor of the effects of nicotine on antisaccade performance.

2. Empirical Studies

2.1 Study 1: Genetic study: *CHRFAM7A* copy number and 2bp deletion polymorphisms and antisaccade performance (Petrovsky et al., 2009)

2.1.1 Abstract

Chromosome 15q13-q14 harbors the gene for the alpha-7 nicotinic acetylcholine receptor subunit (*CHRNA7*) and a related gene (*CHRFAM7A*) which arises from a partly duplicated portion of *CHRNA7*. Recent evidence suggests that *CHRFAM7A* is a locus with a possible role in schizophrenia and cognitive functioning. We studied an antisaccade task as a fronto-parietal measure of executive function that reflects risk for schizophrenia. Association of the *CHRFAM7A* genotype with antisaccade performance was assessed in 103 healthy Caucasian individuals. No significant associations of 2bp deletion or *CHRFAM7A* copy number with antisaccade performance parameters were observed. The failure to observe an association between antisaccade performance and polymorphisms in the *CHRFAM7A* gene is consistent with specificity of the gene effects on hippocampal and memory functions as previously demonstrated.

2.1.2 Keywords

CHRNA7, *CHRFAM7A*, antisaccade, schizophrenia, alpha-7 nicotinic acetylcholine receptor

2.1.3 Introduction

Convergent findings from a number of approaches suggest that the cholinergic system may play a role in schizophrenia. For example, an altered neuronal nicotinic acetylcholine receptor system may contribute to the pathophysiology of schizophrenia (Freedman et al., 1995) and the nicotinic acetylcholine receptor α7 sub-unit has been implicated in the genetics of this condition. Further, nicotine consumption through cigarette smoking is increased in schizophrenia patients and their biological relatives (Lyons et al., 2002) compared with the general population. Given that cognitive deficits in schizophrenia benefit from nicotine administration (Barr et al., 2008; Depatie et al., 2002), smoking might represent a form of self-medication (Kumari & Postma, 2005).

The antisaccade task is a fronto-parietal measure of executive function that reflects risk for schizophrenia (Hutton & Ettinger, 2006). In this task, the subject first fixates a central stimulus and then makes a saccade away from a peripheral target to its mirror position. Correct performance on this task requires suppression of the reflexive saccade towards the target and transformation of the stimulus location into a volitional motor command mediated by frontal cortex, posterior parietal cortex, basal ganglia and superior colliculus (SC) (Hutton & Ettinger, 2006). Patients with schizophrenia, their relatives, and individuals at risk for psychosis show impaired antisaccade performance (Nieman et al., 2007; Petrovsky et al., 2008), supporting the status of the task as a schizophrenia endophenotype. Endophenotypes are biological markers thought to represent a simpler and more direct reflection of genetic risk for an illness than the heterogeneous illness phenotype itself.

So far, little is known about the genetics of antisaccades, but it can be hypothesized that genetic polymorphisms relating to the cholinergic system might play a role in inter-individual differences in performance. It has been shown that antisaccades are influenced by

cholinergic modulation. Nicotine improves performance in schizophrenia patients (Depatie et al., 2002) and healthy subjects (Rycroft et al., 2006), effects which are similar to those seen on other cognitive tasks (Barr et al., 2008). At present, little is known about the molecular mechanisms by which cholinergic agents influence antisaccade performance. Agonists of the nicotinic acetylcholine receptors (nAChRs) stimulate the activity of these receptors, thereby perhaps directly allowing enhanced attention or functioning on this task. It is also possible, however, that stimulation of nAChRs evokes the release of other neurotransmitters, such as dopamine, which in turn might lead to altered performance. One way of investigating whether nAChRs play a role in antisaccade performance would be to search for functional nAChR genes which might be responsible for at least some of the differences between individuals in antisaccade behavior. To our knowledge, this has not yet been investigated.

The α7 nicotinic acetylcholine receptor sub-unit gene (*CHRNA7*) represents a promising starting point for this work. *CHRNA7* is widely expressed in the central nervous system and maps to 15q13-q14. Markers at or near *CHRNA7* are strongly linked to another endophenotype of schizophrenia, the electrophysiological measure P50 (Freedman et al., 1997; Leonard et al., 2002). Deficits in P50 sensory gating are normalized by nicotine (Freedman et al., 1997) and have been shown to be jointly linked with antisaccades to a locus on chromosome 22q11-12 (Myles-Worsley et al., 1999). Weaker linkage to schizophrenia itself was demonstrated by Freedman et al. (1997), while the prevalence of functional *CHRNA7* promoter polymorphisms was also greater in schizophrenia (Leonard et al., 2002). Most recently, Stefansson and colleagues (2008) found a significant association of schizophrenia with a deletion in chromosome region 15q13.3, between breakpoints BP4 and 5, which removes *CHRNA7* and several other genes, underlining the importance of this region in schizophrenia.

In most individuals, the *CHRNA7* gene is partially duplicated, giving rise to a hybrid gene, *CHRFAM7A*, which contains exons 5 to 10 of *CHRNA7* joined to four exons from an unrelated gene, *FAM7A*. Chromosomes both with and without the *CHRFAM7A* duplicon have been identified, indicating a copy number variation (CNV) with respect to exons 5 to 10 (Flomen et al., 2006). Reduced copy number has previously shown weak association with psychosis (Flomen et al., 2006). Where present, *CHRFAM7A* exists as a polymorphic inversion in either direct or inverted orientation with respect to *CHRNA7* (Flomen et al., 2008). In addition, it contains a polymorphic 2bp deletion within exon 6, which has been associated with deficits in P50 sensory gating (Raux et al., 2002) and episodic memory performance (Dempster et al., 2006). The 2bp deletion is in strong linkage disequilibrium with the direct orientation of *CHRFAM7A* with respect to *CHRNA7* (Flomen et al., 2008), which may therefore be the actual variant responsible for the above associations.

It is unclear how variants of *CHRFAM7A* might affect these psychosis endophenotypes, as it is unknown whether it is translated or whether it is expressed in the same neurons as *CHRNA7*. Its translation products may interact with those of *CHRNA7*, acting in a dominant negative manner, which would be prevented by the 2bp deletion polymorphism truncating the *CHRFAM7A* product. Alternatively, *CHRNA7* expression may be modulated by *CHRFAM7A* expression (e.g. by competition for transcriptional factors), which might be influenced by its orientation (Flomen et al., 2008). Interestingly, the direct orientation of *CHRFAM7A* with respect to *CHRNA7* is likely to predispose to the microdeletions at 15q13.3 associated with schizophrenia (Stefansson et al., 2008) by non-allelic homologous recombination between the duplicated segments (Makoff & Flomen, 2009).

While both P50 and episodic memory are functions thought to rely on hippocampal integrity, associations of the *CHRFAM7A* genotype with tests of fronto-parietal cognition have not been studied. The aim of this study was to explore whether variants in the nicotinic receptor gene account for variance in a fronto-parietal schizophrenia endophenotype. We therefore investigated the possible association between the *CHRFAM7A* copy number variant with its associated 2bp deletion / inversion polymorphism and antisaccades. We restricted this preliminary investigation to healthy individuals, as this allows the study of gene-cognition relationships in the absence of clinical and treatment confounds. It also takes into account the previous observation of stronger *CHRFAM7A* genotype effects on cognition in healthy compared to schizophrenic individuals (Raux et al., 2002).

2.1.4 Method

Subjects

Healthy volunteers were recruited through advertisements at the university and around the local community. Participants provided information regarding age, gender, ethnicity, handedness, smoking status (smoker, non-smoker), years spent in full time education, and paternal as well as maternal socio-economic status (SES), which was measured on a 1-4 scale (1=elementary, 4=professional). Volunteers were screened for the exclusion criteria of DSM-IV Axis I disorders using the Structured Clinical Interview for DSM-IV Disorders (SCID-I). Additional exclusion criteria were a history of head injuries with loss of consciousness of more than one minute, any known neurological abnormalities or systemic illness with known neurological complication, a first-degree relative with psychosis, a history of substance abuse or dependence, or visual impairments. Participants were given a health questionnaire in which to provide information pertaining to their general physical health. In addition, participants were asked to complete four standardized personality questionnaires: the Rust Inventory of

Schizotypal Cognitions (RISC) (Rust, 1988), the World Health Organization (WHO) Adult ADHD Self-Report Scale (ASRS) (Kessler et al., 2005), the Obsessive-Compulsive Inventory (OCI) (Foa et al., 2002), and the neuroticism scale of the Eysenck Personality Questionnaire – Revised (EPQ-R) (Eysenck & Eysenck, 1991) to ascertain possible effects of genotype on personality traits. Ethical approval by the local ethics committee was obtained and volunteers provided written informed consent.

Genotyping

DNA was extracted from venous blood. The 2bp deletion genotype and copy number of *CHRFAM7A* were determined as described previously (Flomen et al., 2006).

The genotyping yielded 5 groups, consisting of subjects with 1 copy with 0 deletions (1C0D), 1 copy with 1 deletion (1C1D), 2 copies with 0 deletion (2C0D), 2 copies with 1 deletion (2C1D), and 2 copies with 2 deletions (2C2D). We did not observe any examples of the rare 6th genotype (subjects having 0 copies of *CHRFAM7A*).

Oculography

Eye movements were recorded using infrared oculography (IRIS Skalar 6500) of the left eye and sampled at 500Hz as described previously (Ettinger et al., 2003a). Participants were seated 57cm from a 17-in monitor with their heads on a chinrest. The target was a white dot (0.3º diameter) presented on a black background. A 3-point calibration (0º, ±12º) was carried out, followed by 60 antisaccade trials. A trial consisted of the target in the center of the monitor for a random duration of 1000-2000ms and, subsequently, in one of four peripheral locations (±6º, ±12º) for 1000ms. Participants were instructed to look at the target while in the center and to the exact mirror image location when it jumped to the side.

Data analysis (Eyemap, AMTech GmbH) involved automatic detection of saccades using criteria of minimum amplitude (1°), velocity (30°/s), and latency to target (100ms), and

individual categorization into directional correct antisaccades and reflexive error saccades. Antisaccade latency (ms), reflexive saccade error rate (% reflexive saccades over total number of valid trials), antisaccade gain (% saccade amplitude over target amplitude), and antisaccade spatial error were calculated. Spatial error was obtained by subtracting the target amplitude from saccade amplitude and dividing the result by the target amplitude. The absolute value of this term reflects the residual error and was then averaged across all saccades and multiplied by 100.

Statistical Analyses

Statistical analyses were conducted using SPSS15.0. Genotype (1C0D, 1C1D, 2C0D, 2C1D, 2C2D) was used as an independent variable and the socio-demographic (age, education, paternal and maternal SES) and antisaccade (error rate, latency, gain, and spatial error) variables were used as dependent variables in separate univariate analyses of variance (ANOVA). We also examined the relationship between genotype and gender using χ^2 test. Finally, we included smoking status (smoker, non-smoker) as an additional independent variable in the ANOVA model and investigated whether smoking status was associated with genotype (using χ^2 test).

2.1.5 Results

Socio-demographic and antisaccade variables are summarized in Table 2-1. 111 participants completed the study. There were 8 genotyping failures, leaving a final sample size of N=103 (57 males; 25.87±5.50 years of age; 17.64±3.36 years of education; paternal SES=3.12±90 maternal SES=2.83±1.02; 27 smokers). All participants were Caucasian. The genotype groups did not differ in any socio-demographic variable or smoking status (all

p>0.20). The genotype distribution did not significantly differ from Hardy-Weinberg-equilibrium (χ^2=4.51, df=3, p=0.21).

Analyses of antisaccade variables revealed no association with genotype for the combined copy number / 2bp deletion assay (all p>0.37). Grouping subjects by 2bp deletion alone (N=31 without deletions; N=72 with at least one deletion) and by copy number alone (N=28 with one copy; N=75 with two copies) in separate analyses did not yield any significant effects (p>0.34 and p>0.59, respectively).

	1C0D (N=16)	1C1D (N=12)	2C0D (N=15)	2C1D (N=42)	2C2D (N=18)
Age (years)	24.88 (4.86)	26.17 (8.18)	25.60 (5.21)	26.17 (5.46)	26.11 (4.66)
Gender (N male)	9	5	8	28	7
Education (years)	17.87 (3.58)	17.25 (4.56)	17.27 (3.35)	18.05 (3.25)	17.06 (2.71)
Paternal SES	2.92 (0.86)	3.00 (0.87)	3.64 (0.67)	2.97 (1.00)	3.33 (0.78)
Maternal SES	3.00 (0.82)	2.89 (1.17)	3.09 (0.94)	2.69 (1.15)	2.75 (0.87)
Smoker (N, %)	3 (18.75%)	3 (25%)	5 (33.33%)	13 (30.95%)	3 (16.66%)
AS Gain (%)	-111.79 (22.05)	-102.70 (23.50)	-105.37 (15.35)	-106.15 (25.02)	-112.64 (24.66)
AS Spatial Error (%)	39.54 (11.53)	36.69 (5.35)	33.96 (7.08)	38.97 (10.16)	39.97 (10.93)
AS Latency (ms)	272.61 (44.47)	291.78 (40.78)	276.27 (41.73)	280.22 (44.39)	284.89 (47.88)
AS Error Rate (%)	25.91 (15.36)	20.75 (13.33)	27.77 (24.16)	23.84 (20.52)	29.47 (20.49)
RISC	24.19 (11.86)	21.33 (10.25)	22.87 (6.79)	21.50 (7.14)	21.56 (8.13)
ASRS (N = 78)	21.15 (7.55) (N = 13)	22.11 (11.36) (N = 9)	24.64 (10.28) (N = 11)	20.19 (7.04) (N = 32)	21.15 (6.94) (N = 13)
OCI (N = 78)	8.77 (9.49) (N = 13)	9.89 (6.90) (N = 9)	8.91 (5.26) (N = 11)	8.03 (7.60) (N = 32)	7.15 (6.30) (N = 13)
EPQ-R N (N = 77)	7.31 (5.09) (N = 13)	8.67 (6.16) (N = 9)	7.30 (6.41) (N = 10)	5.47 (4.75) (N = 32)	7.38 (5.17) (N = 13)

Table 2-1

Socio-demographic, antisaccade and personality variables by genotype. Note: Data represent means (standard deviation) unless stated otherwise (total N=103). SES = socio-economic status; AS = antisaccade; 1C0D = 1 copy 0 deletion; 1C1D = 1 copy 1 deletion; 2C0D = 2 copies 0 deletion; 2C2D = 2 copies 2 deletions. The RISC data was available for the total N=103, the data of the other questionnaires was available for the Ns given in the table. RISC = Rust Inventory of Schizotypal Cognitions; ASRS = Adult ADHD Self-report Scale; OCI = Obsessive-Compulsive Inventory; EPQ-R N = Neuroticism scale of the Eysenck Personality Questionnaire Revised.

The effect sizes for the analyses of antisaccade variables are presented in Table 2-2. Finally, given the known effects of smoking on antisaccade performance (Rycroft et al., 2006) and the possibility of an interaction between an acetylcholine-related genotype and nicotine consumption, smoking status was added into the model as an independent variable. Genotype effects remained unchanged, and there were no significant main or interaction effects involving smoking status (all $p>0.18$).

Data from the personality questionnaires are presented in Table 2-1. There were no associations with genotype for the combined copy number / 2bp deletion assay (all $p>0.48$). Grouping subjects by 2bp deletion alone and by copy number alone also did not reveal any significant effects ($p>0.25$ and $p>0.22$ respectively). Finally, there were no effects of smoking status or interactions of smoking status with genotype on questionnaire variables (all $p>0.05$).

	CHRFAM7A copy number/deletion			*CHRFAM7A* copy number			*CHRFAM7A* deletion		
	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2
AS Gain (%)	.554	.697	.022	.004	.947	.00004420	.090	.765	.001
AS Spatial Error (%)	1.075	.373	.042	.003	.959	.00002678	.908	.343	.009
AS Latency (ms)	.401	.808	.016	.001	.978	.00000781	.903	.344	.009
AS Error Rate (%)	.482	.749	.019	.277	.599	.00273733	.244	.622	.002

Table 2-2
Effect sizes for the analyses of antisaccade variables. Note: The partial Eta squared (η_p^2) estimates the proportion of variance in the dependent variable that is attributable to each effect. *CHRFAM7A* copy number/deletion = ANOVA with the combined copy number/deletion groups (1C0D, 1C1D, 2C0D, 2C1D, 2C2D). *CHRFAM7A* copy number = ANOVA with copy number only as a factor (one copy, two copies). *CHRFAM7A* deletion = ANOVA with deletion only as a factor (no deletion, at least one deletion).

2.1.6 Discussion

This is, to our knowledge, the first study to explore the association between *CHRFAM7A* and measures of executive function. We selected the antisaccade task as it represents a marker of risk for schizophrenia with well-defined fronto-parietal neural correlates and because performance can be modulated by cholinergic agents (Hutton & Ettinger, 2006). The *CHRFAM7A* gene was selected as it is a promising locus in the cholinergic system with regard to schizophrenia and cognition (Dempster et al., 2006; Raux et al., 2002).

In this study, no significant association of *CHRFAM7A* copy number or 2bp deletion / inversion polymorphism with antisaccade performance parameters was observed. It should be noted, however, that given the relatively small sample size of the present study, we can only exclude a large effect size. It is possible that *CHRFAM7A* does in fact impact aspects of executive function and future studies with larger samples are required to address this question. A power calculation found that >600 subjects are required to detect effects of d=0.2 with >80% power.

Failure to detect association may actually be consistent with the neurophysiological and cognitive specificity of the effects of this genotype, given that previous studies have shown evidence for an association with episodic memory and P50 suppression (Dempster et al., 2006; Raux et al., 2002). Although episodic memory and P50 suppression involve widespread neural correlates and *CHRFAM7A* is not expressed exclusively during hippocampal formation, the results suggest a more specific effect on hippocampally mediated memory and inhibitory functions. In this context it is important to note that the hippocampus primarily mediates long-term memory processes, while working memory relies more heavily

on fronto-parietal networks (however, see Weinberger et al., 1992 for evidence of an association between hippocampus and working memory).

We did not observe any significant associations of the *CHRFAM7A* polymorphisms with questionnaire measures of personality traits indexing variation in schizotypy, neuroticism, attention deficit hyperactivity disorder, and obsessive-compulsive disorder. The lack of association with personality traits is unlikely to be due to measuring error, as the questionnaires used here have established reliability. The same applies to the antisaccade task, for which high test-retest reliabilities and internal consistencies have been reported (Ettinger et al., 2003a).

Both smokers and non-smokers were included in the present study. Smoking status was not associated with genotype and did not affect antisaccade or personality variables, nor did it mediate genotype associations with these measures.

Given that the antisaccade task is a schizophrenia endophenotype with good heritability, future investigations of the specific molecular genetic mechanisms underlying inter-individual differences are important. There is strong evidence for cholinergic influences on antisaccades, so in addition to assessing the *CHRFAM7A* CNV and 2bp deletion / inversion polymorphism genotype in a much larger sample, it would be worthwhile to investigate other polymorphisms in cholinergic system genes associated with schizophrenia or frontal lobe functioning, including *CHRFAM7A* and *CHRNA.*

A related issue concerns the possible role of *CHRFAM7A* and other cholinergic genotypes in the effects of cholinergic manipulation on neurocognitive performance. Even in the absence of a main effect of genotype on behavioral performance as in this study, it is possible that there are modulating pharmacogenetic effects of this polymorphism that become

apparent in pharmacological challenge studies. Similarly, it would be interesting to investigate whether this polymorphism mediates inter-individual differences in the effects of smoking withdrawal on cognitive performance (Powell et al., 2002).

The main limitation of the present study is the small sample size. Given the power calculations presented here, multi-center collaborative efforts will be required to demonstrate the operation of small gene effects on performance. Future studies may also wish to examine possible associations of *CHRFAM7A* on standard neuropsychological tests of different domains of executive function and memory.

2.2 Study 2: Nicotine study: Nicotine differentially modulates antisaccade performance in healthy male non-smoking volunteers stratified for low and high accuracy (Petrovsky et al., in press)

2.2.1 Abstract

Rationale: Nicotinergic agents are currently being examined as possible pro-cognitive drugs for a variety of clinical conditions marked by cognitive deficits, such as attention deficit hyperactivity disorder (ADHD) or schizophrenia. The response to acute nicotine is heterogeneous across subjects and samples; however, only a few reliable predictors of response have been identified. *Objectives:* We tested the hypothesis that baseline performance level in cognitive control may be a predictor of the cognitive effects of nicotine. *Methods:* We tested 28 healthy Caucasian, male, non-smoking volunteers with the antisaccade task, an oculomotor measure of cognitive control. Participants were given a 7-mg nicotine patch in a double-blind, placebo-controlled, counterbalanced, within-subjects design. Subjects were stratified into high and low performers based on their antisaccade error rate in the placebo condition (median-split). *Results:* Nicotine tended to reduce response time variability of prosaccade latency ($p=0.06$). There was no main effect of nicotine on antisaccade error rate ($p=0.31$). However, nicotine significantly reduced antisaccade error rate in the low-accuracy probands while leaving performance of the high-accuracy probands unaffected (interaction $p<0.05$). Furthermore, we found a nicotine-induced reduction of response time variability of antisaccade latency at one target location in the low-performing group (interaction $p<0.05$). *Conclusions:* The present results demonstrate the importance of baseline performance differences for the effectiveness of pharmacological enhancement of cognitive control. More generally, the results suggest that stimulation of the nicotinic acetylcholine receptor (nAChR)

system might be an effective way of improving cognition in people with poor cognitive performance, such as patients with ADHD or schizophrenia.

2.2.2 Keywords

Nicotine, acetylcholine, executive function, antisaccade, oculomotor control, attention

2.2.3 Introduction

In the last decades, growing interest in the pro-cognitive effects of nicotine has emerged. Earlier research studied mostly the effects of nicotine or smoking in deprived smokers (Heishman et al., 1994). A limitation of studying nicotine effects in smokers is that putative genuine cognitive enhancing effects of the compound cannot be disentangled from the reversal of withdrawal-induced performance deficits (Heishman et al., 1994). More recent studies have taken this methodological problem into account by testing effects of nicotine in non-deprived smokers, minimally deprived smokers (deprivation for less than 2 hours), or non-smokers (Heishman et al., 2010). A recent meta-analysis by Heishman et al. (2010) found significant positive effects of nicotine on six cognitive domains: fine motor, alerting attention-accuracy and response time (RT), orienting attention-RT, short-term episodic memory-accuracy, and working memory-RT. Some performance domains could not be included in the meta-analysis as there were not sufficient numbers of studies available in these domains, which included reasoning, arithmetic and executive function (Heishman et al., 2010).

Therefore, more studies on the effects of nicotine on executive functioning are needed, especially as there is an unmet need for satisfactory treatment of attention and executive control deficits in psychiatric disorders. Particularly in schizophrenia, cognitive symptoms such as deficits in executive control have the most substantial impact on the outcome of the

illness (Friedman et al., 1999) and the adjunctive treatment with cholinergic substances might be useful for remediation (Ribeiz et al., 2010).

Another aspect in nicotine research that has mostly been disregarded concerns inter-individual differences in treatment response. These inter-individual differences might partially explain why some studies showed beneficial effects of nicotine but others did not. Parallel to earlier findings regarding the dopamine system, Newhouse et al. (2004) suggest an inverted-U shaped function of baseline differences in performance and nicotinic stimulation. Depending on the baseline level of cognitive performance, an equivalent degree of nicotinic stimulation can either enhance or impair performance. Figure 2-1(a) illustrates the presumed positive effect of nicotine intake in low-performing subjects: Nicotine improves performance and brings performance closer to the optimum. Figure 2-1(b) shows the presumed disadvantageous effect of nicotine administration: Already high-performing subjects are impaired by nicotine intake.

Therefore, in studies of the effects of nicotinic agonists, it would be important to systematically consider the role of baseline performance levels in order to explain inter-individual variability in drug response. So far, there are only a few psychopharmacological studies in performance-stratified samples, and only one published study which investigated nicotine. Vollenweider et al. (2006) demonstrated that the antipsychotic clozapine significantly increased PPI levels in low PPI performers but showed no effect in high PPI performers. Likewise, Csomor et al. (2008) found that haloperidol failed to increase PPI in low PPI performers, but attenuated PPI in high PPI performers. Moreover, haloperidol increased P50 gating in low suppressors and disrupted P50 gating in high suppressors (Csomor et al., 2008). A recent study by Knott et al. (2010) demonstrated that nicotine reduced P50 gating in high suppressors while P50 in low suppressors remained unaffected by nicotine. Allman et al. (2010) found that low antisaccade performers with long-latency

antisaccades exhibited shorter antisaccade latencies on D-amphetamine while high-performing subjects with short-latency antisaccades had longer latencies on D-amphetamine (Allman et al., 2010). To our knowledge, there is no study on inter-individual effects of nicotine employing the antisaccade task.

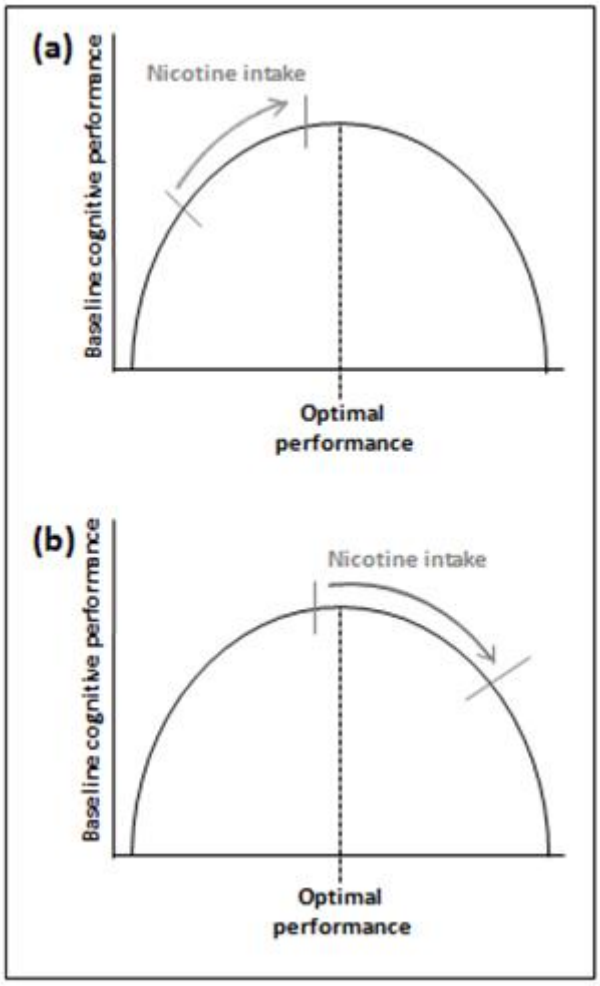

Figure 2-1
Presumed effect of nicotinic stimulation in (a) low-performing individuals and in (b) high-performing individuals: Baseline performance level may be a determinant of the cognitive effects of nicotine.

Therefore, the present study aimed to investigate the effects of nicotine on the antisaccade task in performance-stratified sub-groups of healthy, non-smoking volunteers. We

chose the antisaccade task because this paradigm addresses a relatively simple response system, which serves as a particularly useful model system for executive control of oculomotor responses (Reuter & Kathmann, 2004). Moreover, the antisaccade task recruits a well-defined fronto-parieto-subcortical network (Ettinger et al., 2008a; Munoz & Everling, 2004) and is considered a schizophrenia endophenotype (Calkins et al., 2008) with high test-retest reliability (Ettinger et al., 2003a). Furthermore, previous studies demonstrated the sensitivity of the antisaccade task to nicotine administration in schizophrenia patients (Depatie et al., 2002; Larrison-Faucher et al., 2004) and in healthy subjects (Bowling & Donnelly, 2010; Dawkins et al., 2007; Depatie et al., 2002; Ettinger et al., 2009; Rycroft et al., 2006; Rycroft et al., 2007). Based on the model by Newhouse et al. (2004), we hypothesized that participants showing low performance on the antisaccade task would benefit from nicotine administration while already high-performing participants would be impaired by nicotinic stimulation.

2.2.4 Methods and materials

Subjects

Thirty healthy Caucasian, non-smoking, male volunteers were recruited from the local community by advertisement at the university and by contacting a random sample of the inhabitants of Bonn based on a list from the city registry. Non-smokers in our sample were defined as individuals who had smoked no more than 100 cigarettes during their lifetime and had not smoked in the past year. The volunteers were required to be between 18 and 55 years old and were screened for the exclusion criteria and interviewed with the Structured Clinical Interview for DSM-IV (SCID-I, German version: Wittchen et al., 1997). Exclusion criteria were a current or lifetime Axis I disorder, a first-degree relative with psychosis, a history of neurological illness or another severe medical condition, head injury with loss of

consciousness of >5 min, lifetime history of alcohol or substance abuse or dependence, visual impairments, obesity (body mass index (BMI) > 30), intake of any medications which effect the CNS. Furthermore, the following exclusion criteria regarding the application of nicotine were employed: cardiovascular disease, hypertension, atopic or eczematous dermatitis (due to localized patch sensitivity), severe renal or hepatic impairment or active peptic ulcers, hyperthyroidism, pheochromocytoma, insulin-dependent diabetes, hypersensitivity to patches, hypersensitivity to nicotine or to any of the excipients of the patches. Subjects were allowed to drink their usual amount of coffee, tea or other caffeinated beverages in the morning. Caffeine consumption was documented for both testing sessions. Verbal IQ was estimated with a standardized German vocabulary test, the MWT-B (Mehrfachwahl-Wortschatz-Intelligenztest, Lehrl, 1989). Approval of the local ethics committee and the German Federal Institute for Drugs and Medical Devices (BfArM) was obtained and the study was registered with http://www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT01315002). Participants provided written informed consent before inclusion.

Experimental design and nicotine application

Each subject underwent a telephone-screening for a first evaluation regarding the inclusion and exclusion criteria of the present study. Subsequently, subjects were invited to the laboratory for two testing sessions, preferably with a time interval of one week between the two sessions. Before Session 1, researchers measured subjects' blood pressure to ensure that no subject suffered from undetected hypertension (diastolic value no greater than 90, World Health Organization, Whitworth et al., 2003). On both testing days, a urine drug screening test was conducted before application of the patch to ensure that subjects had abstained from amphetamine, benzodiazepine, cocaine, THC cannabinoides, and opiate/morphine (nal von minden, Moers, Germany).

Nicotine was applied in a double-blind, placebo-controlled, counterbalanced within-subjects design. Subjects received nicotine via a patch (NiQuitin Clear 7 mg, GlaxoSmithKline, Germany) and were given a placebo patch (Fink and Walter GmbH, Germany) of similar appearance. Both patches were applied to the upper back of the subject by a research assistant who was not running the test sessions in order to ensure double-blindness. Testing with the antisaccade paradigm commenced 3 hours after patch application. Nicotine administration using the NiQuitin patch generates a fast-rising nicotine plasma level (a nicotine plateau level is achieved after 2 to 4 hours after the patch has been applied according to the Summary of Product Characteristics of NiQuitin Clear). The 7 mg nicotine dosage was chosen as prior studies employed similar dosages and found cognitive effects in non-smokers in the absence of significant side effects (see Barr et al., 2008; Levin et al., 1998; Poltavski & Petros, 2006). Therefore, only a low drop-out rate due to side-effects was expected. At the end of each testing session, the patch was removed by the research assistant and participants were asked which patch they thought they had received. Mood ratings and physical symptom ratings were assessed with visual analogue scales (VAS). In each case, there was an item beside a horizontal line of 100 mm in length, ranging from "strongly disagree" at the one end (0) to "strongly agree" at the other end (100). The participants had to indicate their perception of their current state by marking the point on the horizontal line that they thought was most appropriate. The following items were included: "relaxed," "alert," "nervous," "drowsy," "comfortable," "fidgety," "concentrated," "dizzy," "excited," "attentive," "I like the substance," "I am in a bad mood," "I feel nauseous," and "I am in a good mood." After completing the second session, participants were debriefed and compensated with € 80 for their participation. A schematic overview of the timeline is depicted in Table 2-3.

Before patch application:	Blood pressure measurement
	Visit study physician and give informed consent (at Session 1)
	Urine drug screening test
	Assessment of mood and physical symptoms with visual analogue scales
Patch application (7mg nicotine or placebo) and beginning of 3-hour-waiting time	
During waiting period:	Collect demographic data, verbal IQ testing and SCID-I interview
	Have a light lunch
	Allowed to read
After 3 hours:	Collect data on caffeine intake
	Assessment of mood and physical symptoms with visual analogue scales
	Antisaccade testing
	Patch removal
	Let participant make a guess about which patch he was given
	Debriefing and financial compensation (at Session 2)

Table 2-3
Timeline of the testing sessions.

Saccadic tasks

Participants were seated 41 cm from a 17-inch monitor, head movements were minimized using a chinrest. The testing room was quiet and dimly lit. Experimental stimuli were presented using ERTS® (BeriSoft Corporation, Frankfurt, Germany). Participants performed one block of prosaccade trials and one block of antisaccade trials. The order was fixed beginning with the prosaccade trials. For both tasks, subjects fixated on a white central fixation cross on a black background. The fixation cross appeared for 1000, 1500, 2000, or 2500 ms at random. A peripheral target (a white dot) then appeared at 6° or 12° either to the left or to the right of the central fixation cross for a duration of 1000 ms. The central fixation cross was extinguished whenever the peripheral target appeared (step paradigm). Altogether, there were 96 trials (48 prosaccade and 48 antisaccade trials), in each case consisting of 12

trials×4 target positions. The sequence of peripheral target presentations was pseudorandomised. There were five practice trials before each block which were not included in the analysis. The prosaccade instruction was to look toward the peripheral target as quickly and as accurately as possible (serving as an easy control task for the antisaccade task). In the antisaccade task, subjects were instructed not to look toward the target but to look away from the peripheral target to the mirror position on the opposite side of the computer screen as quickly and as accurately as possible.

Eye movement recording and analysis

Eye movements were recorded using electrooculography (EOG). Five nonpolarizable Ag/AgCl electrodes (Easycap GmbH, Herrsching-Breitbrunn, Germany) were employed. Two electrodes recorded the horizontal electrooculogram (HEOG) from the outer canthi of the eyes, and another two electrodes recorded the vertical electrooculogram (VEOG) from supra- to suborbital sites of the right eye to detect eye blinks. A ground electrode was placed on the glabella. The electrolyte gel Abralyte® 2000 (Easycap GmbH, Herrsching-Breitbrunn, Germany) was used as an abrasive paste to minimize skin impedance level and as a conducting agent between skin and electrode. The impedance was kept below 5 kΩ at all electrode locations and checked at the beginning of each recording session. The EOG was recorded using Neuro Scan Labs™ with a Synamps® 5083 amplifier controlled by Acquire® software package (Neurosoft Inc., Sterling USA). EOG data were digitized at 250 Hz and stored on hard disk for later analysis. Simultaneously with each presentation of the target dot, a trigger marker (indicating at which position the dot was shown) was recorded. Trigger markers were stored together with the EOG data for later segmentation and analysis of the eye movement data.

The analysis of the EOG data was performed with Brain Vision Analyzer and Matlab. At first, the raw data were pre-processed with Brain Vision Analyzer. Sampling rate was set

to 250 Hz, and the raw data were segmented relative to the trigger marker positions. That is, a segment started 200 ms before the onset of a trigger marker and ended 800 ms after a trigger marker (segment length= 1000 ms). Next, the data were filtered with a high cut-off filter of 30,000 Hz and a notch filter of 50 Hz and baseline correction was employed.

After initial processing, the data were analyzed by a Brain Vision Analyzer macro-program searching for the saccadic eye movements in the HEOG channel. The automatic detection of saccades used.the criteria of amplitude (1°) and velocity (30°/s). Whenever there was such a deviation from the baseline, the onset and offset of the saccade was marked with markers categorizing the saccade into directional correct prosaccade, prosaccade direction error, directional correct antisaccade, or antisaccade direction error. Subsequently, the data were visually inspected by one of two raters blind to experimental condition (placebo/nicotine). The rater verified whether saccades were correctly identified by the program and changed markers categorizing the saccade where applicable. In addition, the rater rejected segments (=trials) if the subject's latency to respond was below 80 ms (=anticipatory response), if the subject did not respond (amplitude less than 3°), or if the subject blinked immediately before the target appearance or during the saccade. For the low-accuracy probands, 47.50±0.81 (98.96±1.68%) of prosaccade trials and 46.82±1.66 (97.54±3.46%) of antisaccade trials were valid trials. For the high-accuracy probands, 47.71±0.47 (99.40±0.98%) of prosaccade trials and 47.64±0.46 (99.26±0.95%) of antisaccade trials were valid trials. Low- and high-accuracy probands did not differ regarding the number of valid trials both for prosaccade trials ($F(1,25)=0.90$, $p=.77$) and for antisaccade trials ($F(1,25)=1.14$, $p=.30$). In addition, there were no main or interaction effects regarding nicotine treatment on number of valid trials (all $p>.32$).

The dependent variables were percentage saccade errors (an amplitude of the first saccade after target appearance greater than 3° in the wrong direction), saccade latencies (time

between target appearance and saccade initiation of correct trials), and intra-individual coefficient of variation (ICV=standard deviation of saccade latency/mean saccade latency; ICV provides a measure of response variability, adjusted for the influence of response speed (Nandam et al., 2011)). A prosaccade (direction) error was counted when the first saccade after target appearance was away from the target; an antisaccade (direction) error was detected when the first saccade after appearance of the peripheral target was performed towards the target. The error rate is calculated as the percentage of error trials over the total number of valid saccade trials (excluding e.g., eye-blink trials). In addition, the proportion of corrected antisaccade errors was collected to control whether the subjects understood the task and made an effort to correctly perform the task. A corrected antisaccade error was scored when a corrective saccade away from the target was made after the subject had made an antisaccade error.

Statistical analyses

Statistical analyses were conducted using the software PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). To test whether nicotine had a differential effect on subjects with high antisaccade error rates (i.e. low accuracy) versus subjects with low antisaccade error rates (i.e. high accuracy), subjects were divided by a median-split procedure into low- and high-accuracy probands. This median-split was based on the mean antisaccade error rate of both eccentricities (6° and 12°) of the placebo session. For the statistical analysis of nicotine effects on saccadic variables 2×2×2×2 repeated-measures analyses of covariance (ANCOVA) with verbal IQ as a covariate were calculated with Treatment (placebo, nicotine) and Eccentricity (6° eccentricity, 12° eccentricity) as within-subjects factors and Group (low-accuracy probands, high-accuracy probands) and Order (nicotine first, placebo first) as between-subjects factors. Verbal IQ was entered as a covariate in all analyses as the high- and low-accuracy groups differed on this variable (see below). Assessment of the participants'

blindness for patch treatment was evaluated with chi-squared tests. Data from the visual analogue scales assessing physical symptoms and mood ratings were analyzed with 2×2×2 repeated-measures analyses of variance (ANOVA) with Treatment (placebo, nicotine) as within-subjects factor, and Time (first assessment, second assessment) and Group (low-accuracy probands, high-accuracy probands) as a between-subjects factors. The significance level of all statistical tests was set at $p<.05$.

Power analyses

Before conducting our study, we performed an a priori power analysis using G*Power 3.1.2 (Faul et al., 2007). We chose to perform the power calculation on antisaccade error rate as the dependent variable because this is the most frequently studied measure of this task. We chose a medium effect size of Cohen's f=0.15 (Cohen, 1988) and took the correlation among repeated measures from empirical evidence of a test-retest reliability study (Ettinger et al., 2003a). The required minimum sample size was N=22 (see Table 2-4). Therefore, we aimed to measure approximately 30 subjects to ensure a sufficiently large sample size. We also calculated a post-hoc power analysis (see Table 2-5). Our test-retest correlation for antisaccade error rate was 0.75 and therefore not quite as high as 0.89 as in the study by Ettinger et al. (2003a). However, our effect size was quite large; thus, we achieved very good statistical power of 0.99. Regarding ICV of antisaccade latency, the test-retest correlation was relatively small with 0.54. Nevertheless, due to a large effect size, we also achieved adequate statistical power of 0.99.

	Antisaccade error rate: within-between interaction Treatment×Group
Cohen's effect size f (assumed)	0.15
Alpha error (assumed)	0.05
Power (1- beta error)	0.80
Critical F	4.35
Number of groups	2
Number of measurements	2
Correlation among repeated measures	0.89
Total sample size	22

Table 2-4
A priori power analysis to compute the required sample size in order to detect a within-between interaction effect of nicotine treatment and group status on antisaccade error rate.

	Antisaccade error rate: within-between interaction Treatment×Group	ICV of antisaccade latency: within-between interaction Treatment×Eccentricity×Group
Cohen's effect size f (measured)	0.40	0.48
Alpha error (assumed)	0.05	0.05
Power (1- beta error)	0.99	0.99
Sample size	28	28
Critical F	4.23	4.23
Number of groups	2	2
Number of measurements	2	2
Correlation among repeated measures	0.75	0.54

Table 2-5
Post hoc-power analyses. Computation of achieved power for significant within-between interaction effects on antisaccade variables (Treatment: Placebo/Nicotine, Eccentricity: 6° condition/12°condition, Group: low-performing probands/high-performing probands).

2.2.5 Results

Sample characteristics

Two subjects dropped out of the study due to nausea, one of these subjects also experienced vomiting. Unblinding showed that in both cases the subjects had been administered nicotine. We replaced the two drop-outs by recruiting two additional subjects. Thus, data of 30 subjects were analyzed. Exploratory data analysis identified one subject as an outlier on the antisaccade error rate (more than three times the interquartile range of the boxplot); this subject was excluded from further analyses. The final sample therefore included 29 subjects. The mean time interval between the two testing sessions was 9.28 days (SD=5.18, MIN=4, MAX=28). The median antisaccade error rate was 25.91 %. Removal of the median subject led to two groups of n=14 each. The mean age of these 28 subjects was 28.11 (SD=9.22) years, the median age was 25 years; age ranged from 20 to 55 years. Sample characteristics are summarized in Table 2-6. The two groups did not differ in age, years of education, or BMI. The two groups differed regarding verbal IQ, indicating lower verbal IQ in high-accuracy probands. Therefore, verbal IQ was entered as a covariate in the subsequent analyses of variance. Thirteen of the 28 subjects received nicotine first and fifteen received placebo first. The frequencies of patch order in the low- and high-accuracy groups did not significantly differ from the expected patch order ($\chi^2(1)=1.29$; p=.26).

Blindness for patch treatment and mood/physical symptoms ratings

Participants were able to correctly identify the nicotine patch. In Session 1, participants correctly guessed on the nicotine patch with a probability of 69.2 %, which was significantly above the level of chance ($\chi^2(1)=4.14$; p=.042). For Session 2, participants correctly guessed they had nicotine with a probability of 92.3% ($\chi^2(1)=16.45$; p=.0001). These results reveal that despite employing a double-blind design, the participants could tell the difference between administration of nicotine and placebo, especially after Session 2 when

participants were able to compare both sessions. Correct guessing was not associated with group status: There was no difference in correct guessing rate in low- and high-accuracy probands (Session 1: $\chi^2(1)=0.16$; p=.69; Session 2: $\chi^2(1)=0.47$; p=.50).

	Low-accuracy probands (*N*=14)	High-accuracy probands (*N*=14)
Age (years)	30.50 (11.71)	25.71 (5.21)
Education (years)	16.21 (2.75)	16.29 (0.73)
Verbal IQ	126.07 (18.21)	111.57 (15.41)
BMI	24.04 (3.68)	23.97 (1.53)
Daily caffeine intake (mg)	31.07 (46.93)	39.00 (68.20)
Order of patch (*N* nicotine first / *N* placebo first)	5/ 9	8/6

Table 2-6
Sample characteristics by Group. Note: Data represent means (standard deviations) unless otherwise specified. The two groups did not differ in age, education, BMI, daily caffeine intake and order of patch (all p>.17), but they differed regarding verbal IQ (p=.03), indicating a lower verbal IQ in the high-accuracy group. Abbreviations: IQ, intelligence quotient; BMI, body mass index.

Results from the visual analogue scales (VAS) on mood and physical symptoms demonstrated that probands experienced side effects from the nicotine administration. Significant interaction effects of Time×Treatment indicated that, compared to the first assessment without a patch, for the second assessment (i.e after three hours of nicotine patch application) probands felt more fidgety (F(1,26)=6.46 p=.017 η_p^2=.20; placebo: VAS mean=1.15, SD=1.54; nicotine: VAS mean=1.69, SD=1.89), more nauseous (F(1,26)=5.04 p=.034 η_p^2=.16; placebo: VAS mean=0.19, SD=0.28; nicotine: VAS mean=1.14, SD=2.32), and, by trend, the probands felt less comfortable (F(1,26)=4.25 p=.050 η_p^2=.14; placebo: VAS

mean=7.91, SD=1.41; nicotine: VAS mean=6.65, SD=2.35). Therefore, it is very likely that probands correctly identified the nicotine patch on the basis of side effects caused by the nicotine treatment.

There were also some main effects of Time, revealing lower ratings at the time of the second assessment – probably reflecting adaptation to the testing situation: Probands felt less alert ($F(1,26)=13.55$ $p=.001$ $\eta_p^2=.34$; first assessment: VAS mean=7.81, SD=1.67; second assessment: VAS mean=6.89, SD=2.30), they were less nervous ($F(1,26)=6.76$ $p=.015$ $\eta_p^2=.21$; first assessment: VAS mean=1.79, SD=2.09; second assessment: VAS mean=1.25, SD=1.46), and they felt less attentive $F(1,26)=9.67$ $p=.005$ $\eta_p^2=.28$; first assessment: VAS mean=7.61, SD=1.66; second assessment: VAS mean=6.82 SD=2.31). Moreover, probands felt more drowsy at the time of the second assessment than at the time of first assessment ($F(1,26)=4.90$ $p=.036$ $\eta_p^2=.16$; first assessment: VAS mean=2.80, SD=2.58; second assessment: VAS mean=3.75, SD=2.81).

Furthermore, there was one interaction effect of Group×Time ($F(1,26)=4.51$ $p=.043$ $\eta_p^2=.15$): For Time 1, the low-accuracy probands gave higher ratings of feeling relaxed (VAS mean=7.95, SD=2.64) than the high-accuracy probands (VAS mean=6.77, SD=2.60), at Time 2 there was no difference between the groups (low-accuracy probands: VAS mean=7.08, SD=2.93; high-accuracy probands VAS mean=7.11, SD=1.89). There were no further main or interaction effects (all $p>.11$).

Reliabilities of saccadic variables

Saccades were rated by two raters. To assess the consistency of performance in one rater (intrarater reliability), internal consistency was assessed using Cronbach's coefficient alpha. Interrater reliability of the two raters was assessed by computing intraclass correlations (ICC) with ICC (3,2) (two-way mixed average measures, absolute agreement). All reliability analyses were performed on 12 randomly chosen subjects. Raters were blind to group and

treatment status. Both intrarater and interrater reliabilities were high (all coefficients, >0.97, for all coefficients see Table 2-7).

Dependent variable	Internal consistency of Rater A (Cronbach's alpha)	Internal consistency of Rater B (Cronbach's alpha)	Intraclass correlations (ICC) of the two raters
Antisaccade error rate 6° eccentricity	0.97	0.98	0.98
Antisaccade error rate 12° eccentricity	0.99	0.99	0.99
Antisaccade latency 6° eccentricity	0.98	0.99	0.98
Antisaccade latency 12° eccentricity	0.99	0.99	0.99

Table 2-7
Intrarater and interrater reliabilities of the two raters analyzing the saccadic eye movements.

Saccadic performance: Correction of antisaccade errors

The average correction rate of antisaccade errors was high (placebo-induced condition: mean 95.64%, SD=7.62; nicotine-induced condition: mean 90.82%, SD=17.51). For the low-accuracy probands, mean correction rate under placebo was 92.97% (SD=9.66); under nicotine, it was 89.90% (SD=17.92). The high-accuracy probands exhibited mean correction rates of 98.30% (SD=3.46) in the placebo-induced condition and 91.74% (SD=17.72) in the nicotine-induced condition. These high proportions of corrected antisaccade errors indicate that subjects understood the task and were willing to perform the task. Groups did not differ in antisaccade correction rates ($F(1,23)=0.80$, $p=.78$), and there were no further main or interaction effects for this variable (all $p>.35$).

Saccadic performance: Effects of eccentricity and nicotine

Exploratory data analysis revealed that there was almost no variance in prosaccade error rate, indicating that subjects made virtually no prosaccade errors. Therefore, this variable was excluded from further analyses. Antisaccade error rate, pro- and antisaccade latencies as well as ICV of pro- and antisaccade latencies were normally distributed (all Kolmogorov–Smirnov tests $p>.11$). Table 2-8 displays means and standard deviations for all saccadic variables. In the repeated-measures ANCOVAs, there was neither a significant main effect of Order (nicotine first, placebo first) nor interactions of Order with any of the variables (all $p>.14$).

For prosaccade mean latency, there were no main or interaction effects (all $p>.18$). For ICV of prosaccade latency, there was a trend for a main effect of Treatment ($F(1,23)=3.78$, $p=.064$, $\eta_p^2=.14$), indicating lower prosaccade response time variability in the nicotine condition. No further main or interaction effects were observed for this variable (all $p>.15$).

For antisaccade error rate, there was no main effect of Treatment ($F(1,23)=1.06$, $p=.31$). As expected, due to the median-split procedure, the two groups differed in antisaccade error rates ($F(1,23)=34.93$, $p=5\times10^{-7}$, $\eta_p^2=.60$), indicating the low-accuracy probands performed worse than the high-accuracy probands (Figure 2-2). There was an interaction effect of Treatment×Group ($F(1,23)=6.45$, $p=.018$, $\eta_p^2=.14$), indicating that low-accuracy probands made fewer antisaccade errors in the nicotine-induced condition than in the placebo-induced condition, whereas the high-accuracy probands' performance did not differ between placebo and nicotine (Figure 2-2). Post hoc comparisons confirmed that nicotine decreased antisaccade errors in low-accuracy probands ($F(1,12)=6.83$, $p=.023$, $\eta_p^2=.36$) but not in high-accuracy probands ($F(1,12)=0.30$, $p=.596$, $\eta_p^2=.02$). There were no other main or interaction effects for this variable (all $p>.08$).

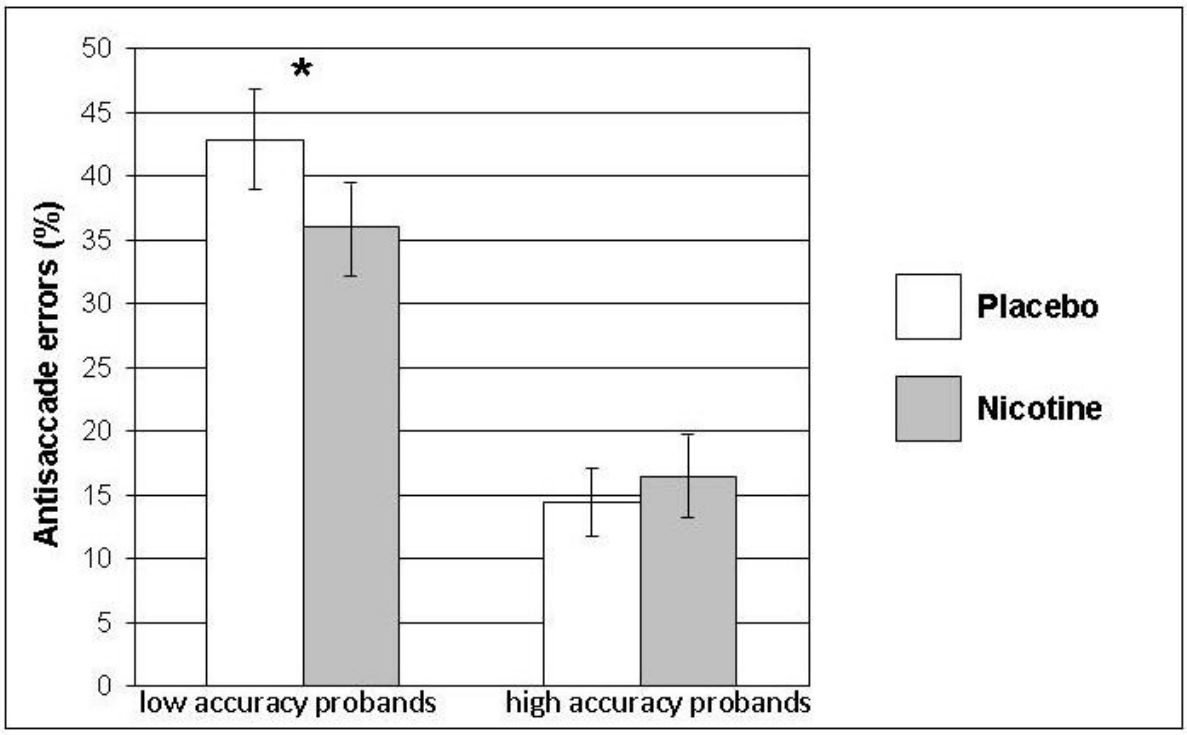

Figure 2-2
Percentage antisaccade errors in the low- and high-accuracy subgroups during placebo and nicotine treatment. Error bars refer to ±SE. Nicotine significantly reduced antisaccade error rate in the low-accuracy probands (p=.023), but not in the high-accuracy probands (p=.596).

For antisaccade latency, there were no main or interaction effects (all p>.09). For ICV of antisaccade latency, there was no main effect of Treatment ($F(1,23)=0.04$, $p=.84$); however there was a trend for a Treatment×Eccentricity interaction ($F(1,23)=4.07$, $p=.056$, $\eta_p^2=.15$). Post-hoc comparisons showed a decrease in ICV of antisaccade latency under nicotine for the 12° eccentricity condition ($F(1,27)=4.52$, $p=.043$, $\eta_p^2=.14$) but not for the 6° eccentricity condition ($F(1,27)=0.03$, $p=.866$, $\eta_p^2=.001$). Moreover, there was a significant triple interaction of Treatment×Eccentricity×Group ($F(1,23)=5.39$, $p=.029$, $\eta_p^2=.19$), demonstrating that the interaction of Treatment×Eccentricity depended on the factor Group. Post hoc

comparisons revealed that the interaction of Treatment×Eccentricity was significant in the low-accuracy probands ($F(1,11)=4.97$, $p=.048$, $\eta_p^2=.31$) but not in the high-accuracy probands ($F(1,11)=0.10$, $p=.755$, $\eta_p^2=.009$) (see also Figure 2.-3). There were no further main or interaction effects (all $p>.23$).

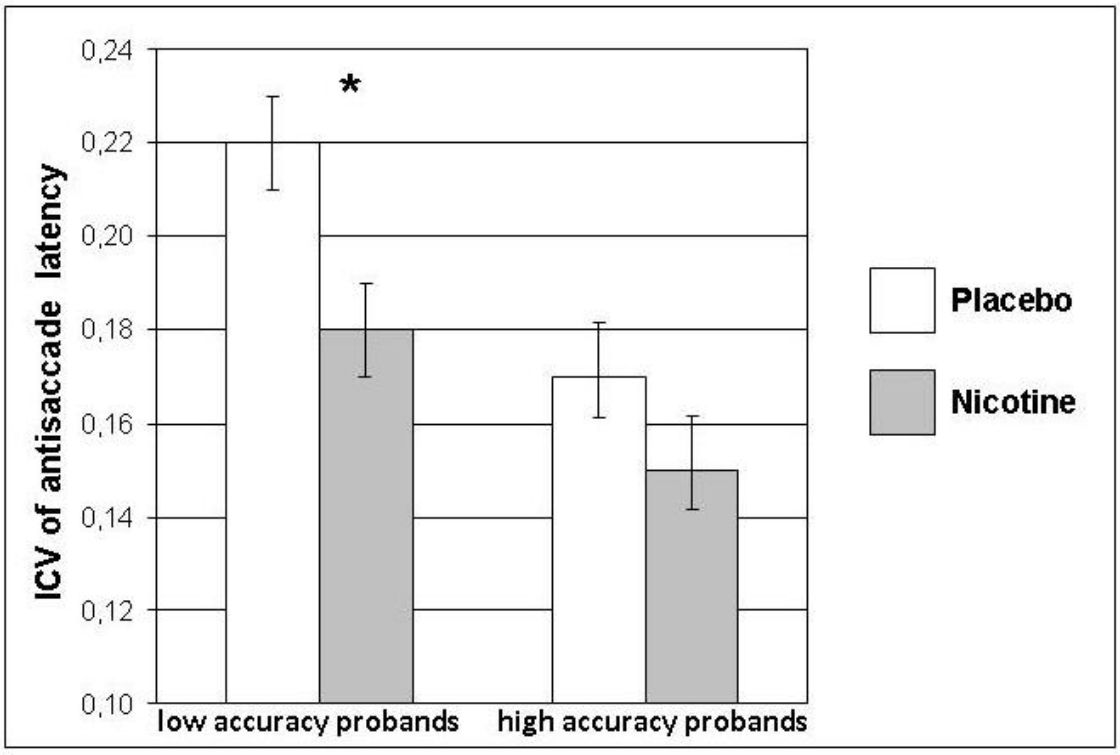

Figure 2-3
Intra-individual coefficient of variation (ICV) of antisaccade latency for the 12° eccentricity condition in the low- and high-accuracy sub-groups during placebo and nicotine treatment. Error bars refer to ±SE. Nicotine significantly decreased ICV of antisaccade latency in the low-accuracy probands ($p=.048$), but not in the high-accuracy probands ($p=.755$).

	Low-accuracy probands (*N*=14)				High-accuracy probands (*N*=14)				
	Placebo		Nicotine		Placebo		Nicotine		
	6° eccentricity	12° eccentricity	6° eccentricity	12° eccentricity	6° eccentricity	12° eccentricity	6° eccentricity	12° eccentricity	p
Prosaccade error rate (%)	0.00 (0.00)	0.00 (0.00)	0.30 (1.11)	0.60 (1.51)	0.00 (0.00)	0.00 (0.00)	0.30 (1.11)	0.00 (0.00)	n/a
Prosaccade latency (ms)	168.51 (25.76)	182.77 (27.56)	171.35 (24.20)	187.79 (34.05)	173.27 (28.45)	190.84 (28.95)	170.39 (31.71)	189.75 (37.48)	n.s.
ICV of prosaccade latency	.20 (.07)	.21 (.06)	.21 (.07)	.22 (.07)	.22 (.07)	.25 (.10)	.21 (.09)	.22 (.11)	.064[a]
Antisaccade error rate (%)	49.30 (14.65)	36.36 (14.73)	45.88 (17.17)	26.00 (10.88)	19.87 (12.60)	8.93 (7.10)	21.70 (17.17)	11.02 (6.65)	.018[b]
Antisaccade latency (ms)	302.78 (33.64)	294.66 (43.25)	304.66 (30.37)	288.25 (31.36)	288.94 (44.34)	282.92 (49.27)	283.36 (44.81)	280.67 (45.19)	n.s.
ICV of antisaccade latency	.21 (.06)	.22 (.05)	.23 (.07)	.18 (.07)	.20 (.06)	.17 (.04)	.17 (.04)	.15 (.05)	.029[c]

Table 2-8

Descriptive statistics of saccadic variables by Group, Treatment and Eccentricity.

Note: Table displays means (standard deviation) of all saccadic variables by Group (low-accuracy probands, high-accuracy probands), Treatment (placebo, nicotine), and Eccentricity (6° eccentricity, 12° eccentricity). n/a=not applicable, n.s.=not significant.

[a] Trend for a main effect of Treatment: placebo > nicotine

[b] Treatment×Group interaction: Nicotine decreased antisaccade error rate in the low-accuracy group (p=.023), but not in the high-accuracy group (p=.596)

[c] Treatment×Eccentricity×Group interaction: Nicotine decreased ICV of antisaccade latency in the 12° eccentricity condition (p=.043), but not in the 6° eccentricity condition (p=.866). Above interaction of Treatment×Eccentricity was significant in the low-accuracy probands (p=.048), but not in the high-accuracy probands (p=.755).

2.2.6 Discussion

The present study investigated the influence of nicotine on prosaccade and antisaccade eye movements in healthy, male, non-smoking volunteers stratified for low and high antisaccade performance. We did not detect a main effect of nicotine on antisaccade performance. However, nicotine enhanced antisaccade performance in low-performing subjects, whereas it had no effect in high-performing subjects.

Concerning antisaccade error rate, we found an interaction effect of nicotine and group status: nicotine reduced antisaccade error rate in the low-performing group while leaving the performance of the high-performing group unaffected. This finding is in agreement with the notion that baseline performance level may be a determinant of the cognitive effects of nicotine (Newhouse et al. 2004). However, we did not observe a significant detrimental effect of nicotine intake in the already high-performing subjects as proposed by the inverted U-shaped model by Newhouse et al. (2004). Possibly, our dose of nicotine was comparatively low; a larger dose of nicotine might have induced a performance decline in the high-accuracy probands and might have led to an even greater performance improvement in the low-accuracy group. It is also possible that our "high-accuracy participants" exhibiting an average antisaccade error rate of about 15% in the placebo condition did not exhibit peak performance in this task and for that reason we did not observe a nicotine-induced performance decline in these participants. Psychometrically defined, an antisaccade error rate of 0% would represent optimal performance (i.e. 100% accuracy) and thus a floor effect leaving no further room for improvement by a substance such as nicotine. Therefore, it would be interesting to conduct a nicotine study in participants who might actually exhibit peak performance in the antisaccade task (i.e. who exhibit a very low error rate of less than 5%, ideally 0%). Possibly, in those participants a performance decline with nicotine administration will be more readily observable.

We also checked our data for a possible statistical phenomenon which might be a trivial explanation of the present data. In a repeated-measurement design in which subjects are tested twice, their scores tend to regress towards the mean. This regression to the mean (also known as the law of initial value) describes the statistical phenomenon that if a variable is extreme on its first measurement, it will tend to be closer to the average on a second measurement, and if it is extreme on a second measurement, it will tend to have been closer to the average on the first measurement (Bland and Altman 1994, Wilder 1958). This statistical phenomenon might also explain why initially low-performing subjects improve on the second testing session and high-performing subjects performing less well on the second testing session. We checked the present antisaccade error data for this issue by breaking down the three-way interaction of Treatment (placebo, nicotine)×Group (low-accuracy probands, high-accuracy probands)×Order (placebo/nicotine, nicotine/placebo). This interaction was not significant ($F(1,23)=3.36$, $p=.08$), though one could argue there is a trend towards significance. However, mean values revealed that probands with low accuracy always exhibited a lower antisaccade error rate in the nicotine condition regardless whether they received nicotine first or placebo first. Those probands with lower accuracy who had received nicotine first exhibited a mean error rate of 47.22% under placebo and a mean error rate of 40.01% under nicotine. Those probands with lower accuracy who had received placebo first exhibited a mean error rate of 40.45% under placebo and a mean error rate of 32.85% under nicotine. Mean values from the probands from the group with high accuracy revealed that they showed a practice effect from Session 1 to Session 2: Those who received placebo at Session 1 showed poorer performance during the placebo session (mean error rate = 15.12%) than during the nicotine session (mean error rate = 10.45%) and those who received nicotine at Session 1 showed poorer performance during the nicotine session (mean error rate = 21.74%) compared with the placebo session (mean error rate = 13.79%). These result patterns argue against a regression to the mean and in favor of the notions that the performance

enhancement of the probands with lower accuracy can be attributed to the nicotinic treatment, whereas the pattern of mean values in the probands with high accuracy revealed that they displayed a practice effect which cannot be connected with the nicotine administration. In addition, the pattern of results regarding the response time variability of antisaccade latency (i.e. ICV of antisaccade latency) also argues against a regression to the mean: The interaction Treatment × Eccentricity × Group × Order was also not significant ($F(1,23)=1.31$ $p=.27$).

We did not observe any effects of nicotine on mean antisaccade latency, contrary to a few previous studies (Ettinger et al., 2009; Larrison-Faucher et al., 2004; Rycroft et al., 2007). However, for ICV of antisaccade latency, we did find a trend for an interaction of nicotine and eccentricity condition indicating a nicotine-induced decrease in variability of antisaccade latency for only the 12° eccentricity condition. There was a significant three-way interaction of nicotine treatment, eccentricity condition and group for ICV of antisaccade latency. This three-way interaction indicated that the simple interaction of nicotine and 12° eccentricity condition was influenced by the factor group. Post hoc comparisons showed that the two-way interaction of nicotine and 12° eccentricity condition was only significant in the low-performing group but not in the high-performing group. There are two published studies examining the effects of stimulus eccentricity on antisaccade latencies in human subjects. In a study by Fischer and Weber (1997), a decrease in antisaccade latencies was seen with increasing stimulus eccentricity (ranging from 1° to 12° stimulus eccentricity). Fischer and Weber also found that antisaccade error rate increased with increasing eccentricity (Fischer & Weber, 1997). On the contrary, Dafoe et al. (2007) did not find a significant effect of eccentricity on antisaccade latencies. However, Dafoe et al. (2007) found that a near stimulus eccentricity provoked more antisaccade errors than a far eccentricity condition. Given these contradictory findings on effects of eccentricity on antisaccade performance, we can only speculate why we found a specific effect of nicotine on a more distant 12° stimulus

eccentricity condition. In the present study, there were no significant effects of eccentricity on antisaccade error rate and antisaccade latency, although mean values indicate that the 6° condition tended to provoke more antisaccade errors and tended to lead to longer antisaccade latencies. Thus, we would have expected that nicotine effects will emerge on the (presumably) more difficult 6° condition. Therefore, the present finding of a decrease in variability of antisaccade latency for the 12° eccentricity condition only is somewhat unexpected. It is possible that the 12° condition is more sensitive to nicotine effects than the 6° condition. If one inspects the mean values of antisaccade error rate under placebo and nicotine in the low-performing group, it becomes obvious that nicotine led to a reduction in antisaccade error rate of about 3% in the 6° condition, while there was a nicotine-induced reduction of antisaccade errors of about 10% in the 12° condition. Analogous to this tendency of a more pronounced effect of nicotine in the 12° condition for antisaccade errors, variability of antisaccade latencies was reduced with nicotine treatment to a greater extent in the 12° condition.

We did not find nicotine effects on mean prosaccade latency indicating that there was no general speeding in reaction time of saccadic eye movements by nicotine in our study. There was, however, a trend for a main effect of nicotine on intra-individual coefficient of variation (ICV) of prosaccade latencies revealing a tendency for a reduction in reaction time variability under nicotine. Possibly, this intra-subject reaction time variability or ICV might be a sensitive measure to detect nicotine effects in a similar way as measures of intra-subject variability were particularly impaired in patients with ADHD (Klein et al. 2006). The increased intra-subject variabilty in ADHD has been replicated consistently and is not part of a general performance decrement; rather, increased intra-subject variability seems to represent a specific deficit (Klein et al. 2006). Hence, parameters of intra-subject variability should be recognized in future drug-challenge studies beside the traditional measures of central tendency like the arithmetic mean. The notion that parameters of intra-subject variability

might be worthwhile to investigate in drug-challenge studies is further supported by recent findings in a study testing the effects of methylphenidate in a stop-signal reaction time (SSRT) task in 24 healthy young men (Nandam et al., 2011). The SSRT is a task measuring response inhibition as this task requires subjects to cancel a prepotent "go" response upon presentation of an infrequent "stop" signal (Nandam et al., 2011). In that study, methylphenidate did not affect mean reaction time to go-stimuli, rather methylphenidate decreased response time variability (as measured by the ICV) of the go-reaction (Nandam et al., 2011). Similar to the effects of nicotine we found on ICV of prosaccade latency, the results by Nandam et al. (2011) argue against a simple enhancement of motor or processing speed but indicate that the stimulant methylphenidate reduced behavioral variability.

The influence of baseline performance on subsequent response to a drug-challenge has been previously discussed by a number of other authors (Kimberg et al. 1997; Mattay et al. 2000; Mehta, 2002; Mehta et al. 2000; Robbins and Sahakian, 1979) demonstrating that despite the absence of an overall effect of a drug, the drug might still be beneficial to a subgroup of individuals. Our results indicate that it might be useful to stratify probands in clinical trials according to their performance level in order to test the efficacy of a compound. Future clinical trials might stratify patients into subgroups with and without cognitive deficits or with more pronounced versus less pronounced deficits. Especially in disease states involving attentional and executive functioning impairments such as schizophrenia and ADHD, those patients exhibiting persistent and severe attentional deficits might benefit from adjunctive treatment with nAChR agonists. While acetylcholinesterase inhibitors have been found to ameliorate deficits in memory and attention in schizophrenia patients (Ribeiz et al. 2010), the evidence on the partial α7 nAChR agonist DMXB-A is more ambiguous. DMXB-A improved some aspects of attention and memory in healthy subjects (Kitagawa et al. 2003) and in schizophrenia (Olincy et al. 2006), although it showed no effects on cognitive

performance in another extensive study with schizophrenia patients (Freedman et al. 2008). Future studies might consider subdividing schizophrenia patients into a group of individuals with severe impairment and a group with only slight or no impairment. Results that further support this idea come from a study by Larrison-Faucher et al. (2004) which investigated the effects of nicotine on antisaccade performance in schizophrenia patients. Nicotine treatment significantly decreased antisaccade errors in task-impaired schizophrenia patients, whereas no nicotine effects were demonstrated for non-impaired schizophrenic subjects or controls (Larrison-Faucher et al. 2004). Although there is evidence for specific α7 nAChR pathophysiology in schizophrenia (De Luca et al. 2006; Freedman et al. 1995; Severance and Yolken, 2008), future clinical trials should also target at α3 nAChR as polymorphisms of the α3 subunits are associated with prepulse inhibition – another schizophrenia endophenotype and a measure of early attentional gating (Petrovsky et al. 2010).

Limitations of the present study include that double-blindness in our design was partially uncovered by the participants as they could correctly guess which patch they had received above chance level, especially after session two. Secondly, we appreciate the limitations of the median split approach. With turning a continuous variable into a categorical one there is reduced power to detect interaction effects due to loss of information in contrast to a regression approach. In addition, median splits are sample-dependent. Although the present study was adequately powered, future studies might want to opt for a regression model when analyzing what predicts a nicotine effect. Thirdly, we did not measure nicotine plasma levels, but we chose our nicotine dosages in line with previous studies. Fourthly, a larger dose of nicotine might have produced larger performance changes in the subjects; therefore future studies might use higher nicotine doses preferably in combination with a nausea-preventing substance such as domperidone. Finally, it would also be of interest to conduct a multi-dose study in a stratified study population, similar to the study with repeated

nicotine administration by Heishman et al. (2000): tolerance to the aversive effects of nicotine might develop with repeated exposure and performance changes might be more readily observed.

In conclusion, the present study showed that nicotine significantly enhanced antisaccade performance in the low-accuracy probands while leaving performance of the high-accuracy probands unaffected. The results are in favour of the notion that baseline cognitive performance influences the effect of acute nicotine administration in healthy non-smokers. Additionally, the findings suggest that stimulation of the nAChR system might be an effective way to treat deficits in executive control. Future studies on nicotine and nicotine-like drugs should account for inter-individual differences in task performance as this appears to be an important predictor of treatment effectiveness. Further research is needed to clarify other predictors of response to nicotinic stimulation such as genetic variation in nicotinic receptors.

3. Discussion

The discussion will review molecular genetic studies of antisaccade performance and will illustrate how genetic findings might contribute to future pharmacogenetic investigations with the antisaccade paradigm. Subsequently, the dopaminergic system will receive special emphasis, as it will be discussed whether cholinergic modulation of antisaccade performance is mediated by enhanced dopaminergic neurotransmission. A theory regarding the effects of nicotinic receptor stimulation on neurotransmitter release and attentional function will try to integrate the beneficial effects of nicotine on antisaccade performance and on other cognitive tasks. Finally, methodological issues, strength and limitations of the present studies will be discussed and some concluding remarks will be made.

3.1 Molecular genetic studies of antisaccade performance and suggestions for future pharmacogenetic investigations

To date, there are a number of studies which have investigated the association of a genetic polymorphism in the cholinergic system with cognitive performance and other studies which tested the effect of a single dose of nicotine on cognition. However, studies which will combine these two research approaches is still needed. In recent years, only a few studies have started to examine these pharmacogenetic interactions, that is, examine the role of genetic polymorphisms in modulating individual response to nicotine administration. So far, there are only a few published pharmacogenetic studies on the procognitive effects of nicotine and not a single pharmacogenetic study related to nicotine and antisaccade performance. Since there are no published pharmacogenetic investigations using the antisaccade task, the following sections will combine the findings of molecular genetic studies of antisaccade performance with the data of pharmacogenetic studies which employed other cognitive

paradigms. Accordingly, suggestions for future pharmacogenetic investigations using the antisaccade task will be made based on this data. First, findings with cholinergic polymorphisms will be discussed, followed by findings regarding the dopaminergic and serotonergic polymorphisms. Finally, data stemming from genetic polymorphisms in candidate risk genes for schizophrenia (such as the Neuregulin gene) will be introduced.

3.1.1 Cholinergic polymorphisms

Study 1 investigated the association of *CHRFAM7A* genotype and antisaccade performance measures. No significant associations of 2bp deletion or *CHRFAM7* copy number with antisaccade performance were found. However, given that the stimulation of the cholinergic system via nicotine administration influences antisaccade performance (e.g. see Study 2 in the present work; Depatie et al., 2002; Ettinger et al., 2009; Rycroft et al., 2006), it is still likely that genetic polymorphisms of the cholinergic system are associated with antisaccades. So far, very little is known about the genetics of antisaccade performance in general, and even less is known about how genetic polymorphisms in the cholinergic system might affect antisaccade performance. A recent analysis of 94 candidate genes and 12 endophenotypes of schizophrenia from the "Consortium on the Genetics of Schizophrenia (COGS)" in 534 subjects revealed that antisaccades were not associated with the *CHRNA7* gene (Greenwood et al., 2011). These findings indicate that polymorphisms in the *CHRNA7* and *CHRFAM7A* genes are unlikely to be associated with antisaccade performance. However, these findings need to be replicated and extended. Future studies should further investigate a possible association between *CHRNA7* polymorphisms and antisaccade performance. Perhaps there are functionally relevant genetic polymorphisms in the *CHRNA7* gene that have an impact on antisaccade performance.

Other cholinergic genes, such as the *CHRNA4*, *CHRNB2* and *CHRNA3* genes, should also be investigated in connection with antisaccades. Polymorphisms in the *CHRNA4* and *CHRNB2* genes might be meaningful candidates to test, because these genes encode the α4β2 nAChR – the most abundant nicotinic receptor sub-type in the brain beside the α7 nAChR sub-type. The *CHRNA4* rs1044396 SNP is a candidate that could be tested in connection with antisaccade performance, as this SNP has been repeatedly linked to visuospatial attention and functioning (Espeseth et al., 2006; Espeseth et al., 2007; Greenwood et al., 2005; Parasuraman et al., 2005; Reinvang et al., 2010; Winterer et al., 2007).

A recent pharmacogenetic study by Rigbi et al. (2011) tested the effect of the *CHRNA7* SNP rs2337980 on three different cognitive tests and its modulation by nicotine. Twenty-four female smokers performed the Matching Familiar Figures Test (MFFT), the Tower of London Test, and the Continuous Performance Task (CPT) – three tests that measure impulsivity or response inhibition. Eight subjects were rs2337980 CC homozygotes and 16 subjects were T-allele carriers (CT: N=10 and TT: N=6). Results showed that CC homozygotes benefited from nicotine in the MFFT task (that is, they made fewer errors compared to placebo) whereas the CT/TT group was not affected by treatment with nicotine. The paper by Rigbi et al. (2011) does not report on the main effect of genotype, that is, whether CC homozygotes performed significantly poorer than the T-allele carriers when given the placebo. However, the authors refer to a previous paper in which CC homozygotes manifested poorer MFFT performance (Rigbi et al., 2008). This was also the rationale for choosing the *CHRNA7* rs2337980 SNP: It was one of the SNPs which remained significant in the regression models applied by Rigbi et al. (2008) that significantly predicted cognitive performance on the MFFT, Tower of London, and CPT. It would be interesting to extend the findings by Rigbi et al. (2011) to a sample of non-smokers, especially as Rigbi et al. (2008) found that the rs2337980 genotype had opposite effects in smokers and non-smokers: The T

(minor) allele was associated with decreased MFFT errors (better response inhibition) in smokers, while it was associated with increased errors (poorer response inhibition) in non-smokers (Rigbi et al., 2008).

The *CHRNA7* rs2337980 SNP would be an interesting candidate for a pharmacogenetic study investigating antisaccade performance and thereby extending the present findings of Study 1 and Study 2, for in the study by Rigbi et al. (2011), this SNP modulated the response to nicotine in a response inhibition paradigm. The antisaccade paradigm also involves the inhibition of a prepotent response (the automatic prosaccade). Thus, one hypothesis would be that the rs2337980 genotype might modulate antisaccade error rate. Furthermore, the SNP is also associated with schizophrenia (Peng et al., 2008), which fits to the notion that antisaccades are considered to be an endophenotype of schizophrenia. Finally, rs2337980 is also associated with the severity of nicotine dependence (Greenbaum et al., 2006), which is in line with the assumed triad of nicotine addiction, cognitive deficits, and nAChR variation.

3.1.2 Dopaminergic polymorphisms

A recent study by Haraldsson et al. (2010) found that carriers of the Val allele of the *COMT* rs4680 SNP (*COMT* Val158Met polymorphism) displayed shorter and less variable antisaccade latency and tended to exhibit less antisaccade errors that carriers of the Met allele. Haraldsson et al. (2010) tested schizophrenia patients (N=105) and healthy controls (N=95); however, they did not find any group-by-genotype interactions. The *COMT* gene is also considered a candidate gene for schizophrenia. Interestingly, the *COMT* Val allele is considered to be the risk allele for schizophrenia and carriers of the Val allele are more likely to develop a schizophreniform disorder if they used cannabis during adolescence (Caspi et al., 2005). The authors of the study by Haraldsson et al. (2010) suggest that their results may be

reconciled with a recent theory suggesting that the *COMT* Val allele is associated with better performance on tasks involving cognitive plasticity while the Met allele is hypothesized to be beneficial on tasks requiring cognitive stability (see also Bilder et al., 2004), an assumption supported further at the neural level by Ettinger et al. (2008b). The antisaccade task can be conceptualized as a measure of cognitive plasticity, such as inhibition of inappropriate responses, online monitoring of errors, and rapid generation of corrections (Haraldsson et al., 2010). The authors also acknowledge that the antisaccade task, like most complex cognitive tasks, also entails elements of cognitive stability because constant alertness and sustained attention is necessary for adequate performance (Haraldsson et al., 2010). In addition, the authors point out that there are indications that genotype-phenotype relationships in single gene association studies may be complicated by factors such as undetected copy number variations, epigenetic phenomena, and epistasis between several genes (Haraldsson et al., 2010).

The COGS study by Greenwood et al. (2011) also genotyped the *COMT* Val158Met polymorphism and did not find an association of the *COMT* gene with antisaccade performance. Therefore, further studies are needed on the *COMT* Val158Met polymorphism in connection with antisaccade performance in large samples. It would be particularly useful to account for epistatic gene effects as the role of the *COMT* gene in schizophrenia and antisaccade performance seems to be a complex one. A pharmacogenetic study of the possible modulating role of the *COMT* gene on the procognitive effects of nicotine in the antisaccade paradigm would be also of interest. One hypothesis, based on the findings by Haraldsson et al. (2010) and Bilder et al. (2004), would be that carriers of the Val allele display better antisaccade performance and will therefore be unlikely to benefit from nicotine, while carriers of the Met allele display impaired antisaccade performance and will therefore exhibit a nicotine-induced improvement of antisaccade performance.

A pharmacogenetic study by Jacobsen and colleagues (2006) tested 36 subjects (including smokers and non-smokers) genotyped for the *DRD2* gene SNP rs6277 (C957T) with nicotine administration on working memory performance. C957T is a synonymous substitution polymorphism in the *DRD2* gene, which has been shown to affect mRNA stability in vitro and striatal *DRD2* binding in vivo (Duan et al., 2003; Hirvonen et al., 2004; Jacobsen et al., 2006). In vitro, the T allele has been associated with decreased translation of *DRD2* mRNA and decreased *DRD2* mRNA stability (Duan et al., 2003). However, a human positron emission tomography (PET) study demonstrated that the binding availability of *DRD2* increases with each T allele, suggesting either increased numbers of *DRD2* receptors or increased *DRD2* binding affinity with each T allele (Hirvonen et al., 2004). Furthermore, a clinical human study showed that smokers homozygous for the T allele are significantly more likely to stop smoking in response to treatment with a nicotine patch than are carriers of the C allele (Lerman et al., 2006). The study by Jacobsen et al. (2006) suggests that during performance of a verbal working memory task with high working memory load, nicotine administration worsened performance in carriers of the T allele (N=15) while performance in the CC homozygotes (N=21) remained unaffected. In addition, the fMRI data acquired during the performance of the working memory task indicated that the activation of a network of regions, including left anterior insula, increased during nicotine administration among T allele carriers and decreased during nicotine administration among CC homozygotes (Jacobsen et al., 2006). The authors consider the inverted U model of the relationship between dopamine levels in the brain and working memory performance in order to explain their findings. They suggest that dopamine release induced by nicotine administration pushed the dopaminergic stimulation of the neurocircuits supporting working memory beyond optimal levels in the T allele carriers, leading to worsened performance and reduced efficiency in regions of the brain that support phonological rehearsal. The authors acknowledge that due to the small number of TT homozygotes (N=4), it was necessary to combine TT homozygotes

with the 11 CT heterozygotes. In a future study, it would be interesting to analyze all three genotype groups in order to test whether the genetic load of the T allele has an effect (hypothesizing that with an increasing number of T alleles, performance worsens with nicotinic stimulation). Furthermore, overall sample size was relatively small and did not allow for a separate assessment of the effects of genotype in smokers and non-smokers; this would also be of interest to be addressed in future studies in order to extend the findings. In sum, the study by Jacobsen et al. (2006) provides an apt example of the pharmacogenetic approach and shows how a genetic polymorphism contributes to the prediction of response to nicotine administration.

Regarding possible pharmacogenetic studies on antisaccade performance, it would be beneficial to test the aforementioned C957T SNP in the *DRD2* gene (as in the Jacobsen et al., 2006 study), especially as a recent association study by the Consortium on the Genetics of Schizophrenia (COGS) showed that the *DRD2* gene was significantly associated with antisaccades in 130 families with a case of schizophrenia (Greenwood et al., 2011). One hypothesis could be that T-carriers of SNP rs6277 (C957T) will exhibit poorer antisaccade performance after nicotine administration similar to the findings by Jacobsen et al. (2006) who found declined working memory performance in T-carriers.

Another recently published pharmacogenetic study by Millar et al. (2011) investigated the role of a polymorphism in the DAT (dopamine transporter) gene on P50 sensory gating and its modulation by nicotine. The authors chose the *DAT1* SLC6A3 VNTR (variable number of tandem repeats) polymorphism. Alleles ranging from 3 to 13 copies of the 40-bp repeats have been described, though alleles with 9 (9R) and 10 repeats (10R) are the most common (Kang et al., 1999; Millar et al., 2011; Mitchell et al., 2000). Dopaminergic neurotransmission is initiated by presynaptic release of dopamine and terminated largely by its reuptake of dopamine transporter (DAT) molecules. The 9R allele (lower gene expression

allele, less DAT available) is therefore associated with greater tonic striatal dopamine levels, whereas the 10R allele (higher gene expression allele, more DAT available) is associated with decreased striatal dopamine tone. Millar et al. (2011) tested 24 healthy non-smokers and found that individuals carrying the 9R allele tended to exhibit greater gating under placebo than carriers of the 10R allele. Furthermore, acute nicotine administration reduced gating in the 9R carriers while gating in the 10R carriers was not affected by nicotine. The authors demonstrated, consistent with an inverted U model of gating performance and nicotine's ability to increase dopamine levels, that 9R carriers tended to exhibit higher gating performance than the 10R carriers. Moreover, the already high-performing 9R carriers were "overstimulated" with the acute nicotine administration, that is, nicotine led to putative "overdosing" of dopamine levels and therefore had detrimental effects on gating performance. Gating in the 10R carriers was not influenced by nicotine, although one would predict a nicotine-induced enhancement of gating in the lower performing 10R carriers. The authors suggest that gating improvements in healthy individuals might require a greater dose of nicotine that required disruption in healthy volunteers (Millar et al., 2011). Further, they suggest that future studies should implement a more systematic dosing regimen that can produce dose-response curves for gating (Millar et al., 2011). A minor limitation of the study is the small sample size and the fact that the authors only offered genotype distribution of the sample in means of frequencies (60.86% 10/10 R homozygotes and 39.14% 9R carriers), but they did not offer the absolute numbers of the genotypes. Therefore, it remains unclear whether they tested 9/10 R and 9/9 R carriers or only 9/10 R carriers. It might be interesting to know, as there might be an effect of "genetic load," that is, homozygous 9/9 R carriers might exhibit even higher gating performance than 9/10 R carriers.

Polymorphisms in the *DAT1* gene, as in the study by Millar et al. (2011), might also be interesting to investigate with respect to antisaccade performance. A recent review by Barnes

et al. (2011) on the molecular genetics of executive function suggests that variants of the *DAT1* gene and the *DRD4* gene show promise for explaining significant variance in individual differences in both behavioral and neural measures of inhibitory control (Barnes et al., 2011). In addition, this review suggests that functional variants of the *DRD2* gene are reliably associated with performance monitoring, error processing, and reinforcement learning (Barnes et al., 2011). Thus, dopamine transporter and dopamine receptor genes might be promising candidate genes in connection with antisaccade performance, as this task involves both inhibitory control (i.e. suppressing the reflexive prosaccade) and performance monitoring/error processing (i.e. detecting and correcting antisaccade errors).

3.1.3 Serotonergic polymorphisms

In addition to pharmacogenetic studies testing cholinergic and dopaminergic genetic variants, there is one recent study which examined the interaction between a serotonergic polymorphism and the effects of nicotine on spatial working memory (Carlson et al., 2009). Carlson et al. (2009) administered nicotine and placebo to 64 deprived smokers genotyped for the serotonin transporter-linked polymorphic region (5-HTTLPR), a region in the *SLC6A4* gene which codes for the serotonin transporter. A deletion/insertion polymorphism in the 5-HTTLPR results in a short (S) allele and a long (L) allele. Individuals that are homozygous for the L allele are thought to have greater serotonin reuptake and potentially lower synaptic serotonin levels than carriers of the S allele (Heils et al., 1997; Lesch et al., 1996). Carlson et al. (2009) employed a computerized dot recall spatial working memory task and found that nicotine enhanced spatial working memory (SWM) in S allele carriers relative to those with two L alleles. Moreover, this enhancement in S allele carriers was greater for individuals with higher levels of depressive symptoms (Carlson et al., 2009). Carlson et al. (2009) offer several interpretations for their findings. First, 5-HTT S allele carriers with high depressive symptoms

might benefit to a greater extent from nicotine after abstaining from using nicotine because S allele carriers experience greater negative affect-related symptoms during nicotine withdrawal and might be more responsive to stressors in general (Carlson et al., 2009). Second, the authors suggest that the hippocampal system might be a substrate in which nicotine influences SWM: Depression may be associated with a dysregulated hypothalamic-pituitary-adrenal (HPA) axis response, which could contribute to depressive symptoms and hippocampal volume loss (Carlson et al., 2009). Furthermore, the hippocampus is strongly involved in spatial memory tasks and animal studies demonstrated that the hippocampus is a site of nicotine action on the 5-HT system (Carlson et al., 2009). Finally, the authors point out that 5-HTT S allele carriers who are prone to depression do not respond well to stressful situations and experience greater negative affect during nicotine withdrawal. Thus, they may be especially susceptible to self-medicate cognitive/SWM deficits and associated stress/frustration (Carlson et al., 2009). Carlson et al. (2009) acknowledge that a limitation of their study is that the observed SWM deficits under placebo might simply reflect more severe withdrawal symptoms in those individuals with low cognitive reserves. Therefore, it would be desirable to extend their findings to a sample of non-smokers.

With regard to antisaccade performance, there are no published molecular genetic association studies with serotonergic polymorphisms. However, the serotonergic system might also be involved in antisaccade behavior since there is evidence that 5-HT_{2A} receptor antagonists, such as risperidone, ameliorate antisaccade error rate in patients with schizophrenia (Burke & Reveley, 2002). The authors argue that the distribution of the 5-HT_{2A} receptor type shows high concentrations in the prefrontal cortex, an area which has been identified as the focus of antisaccade errors, which could help to explain the effectiveness of risperidone in correcting such antisaccade abnormalities (Burke & Reveley, 2002). However, the atypical antipsychotic drug risperidone also blocks the D2 dopaminergic receptor (Uchida

et al., 2009). D2 receptor blockage might actually be detrimental to cognitive functioning, as dopamine levels are reduced via dopamine receptor blockage. The study by Uchida et al. (2009) demonstrated that D2 receptor blockage by risperidone correlated with attention deficits in late-life schizophrenia. Although a causal attribution cannot be made, the study suggests that under certain constraints risperidone's dopaminergic mechanism of action might be unfavorable for cognition. Nevertheless, other authors argue that it is the preponderance of 5-HT_{2A} receptor antagonism over dopamine D2 blockage exerted by atypical antipsychotics which contributes to their cognitive-enhancing effects (Reuter et al., 2007a).

Evidence that serotonergic polymorphisms influence executive functioning also stems from a molecular genetic investigation by Reuter et al. (2007a). The authors employed the attention network test (ANT) which is a task designed to measure alerting, orienting, and executive control, all of which are regarded as sub-components of attention according to the model by Posner and Peterson (1990). Reuter et al. (2007a) investigated the possible association between ANT performance and a polymorphism in the *TPH2* gene. According to Reuter et al. (2007a), the tryptophan hydroxylase (TPH) gene is a promising candidate gene for cognitive functioning because it is the rate-limiting enzyme of 5-HT synthesis. Reuter et al. (2007a) chose the -703 G/T SNP (rs4570625), a promoter polymorphism, because this polymorphism might be a functional polymorphism, as it has been previously to modulates amygdala responsiveness to emotional stimuli (Canli et al., 2005).

In the ANT, alerting is defined as achieving and maintaining an alert state, orienting is the selection of information from sensory input, and executive control is defined as resolving conflict among responses (Fan et al., 2002). The ANT is a combination of the cued reaction time task by Posner (1980) and the Eriksen flanker task (Eriksen & Eriksen, 1974). To assess executive control, a central arrow is flanked by either two arrows on each side pointing in the same direction as the central arrow (congruent condition), or the central arrow is flanked by

arrows pointing in the opposite direction (incongruent condition). The subject's task is to indicate the direction of the central arrow. The incongruent condition represents a conflict between central arrow and the flanking arrows, as the flankers are distracting to the subjects. Therefore, solving the incongruent condition involves an increase in mean reaction time. The efficacy of the executive control network is assessed by subtracting the mean reaction time in congruent flanking conditions from the mean reaction time in incongruent flanking conditions (Fan et al., 2002); higher differences in reaction time (i.e. higher conflict scores) indicate poorer performance.

Reuter and colleagues (2007a) found that TT carriers of the *TPH2* -703 G/T polymorphism (SNP rs4570625) exhibited higher conflict scores in the ANT compared to carriers of the GT or GG genotypes. Moreover, TT carriers made significantly more errors than GT or GG carriers (Reuter et al., 2007a). The effect sizes for both results were quite large, approximately 11 and 12% explained variance, respectively (Reuter et al., 2007a). Reuter et al. (2007a) conclude that their results indicate the relevance of the 5-HT system for impulse control processes. However, the authors acknowledge that further studies have to replicate the findings and other studies need to demonstrate that a variation in rs4570625 is indeed associated with altered rates of 5-HT synthesis, an indicator of functionality of this promoter SNP (Reuter et al., 2007a).

Assuming that the 5-HT system is important for executive control and especially for inhibitory control processes, polymorphisms in the serotonin system should also have an impact on antisaccade performance. Since there are no published molecular genetic association studies with serotonergic polymorphisms and the antisaccade paradigm, one new hypothesis to be tested would be the assumption that the *TPH2* -703 G/T polymorphism has an effect on antisaccade parameters. Based on the findings by Reuter et al. (2007a), one could hypothesize that TT carriers of the *TPH2* -703 G/T polymorphism exhibit an increased

antisaccade error rate compared to GT and GG carriers. On the basis of this hypothesis, a pharmacogentic study might aim to test whether the effects of nicotine on antisaccade performance are modulated by the *TPH2* -703 G/T polymorphism. Since there is some evidence that smoking behavior is associated with the *TPH2* -703 G/T polymorphism (Reuter et al., 2007b), this polymorphism might also affect acute nicotine administration. However, in the study investigating the role of the *TPH1* and *TPH2* genes for nicotine dependence, GG carriers of the *TPH2* -703 G/T SNP started smoking significantly earlier than carriers with a T allele (Reuter et al., 2007b). This result argues against a simple interrelationship between *TPH2* genotype, executive control, and smoking; the assumption that the T allele is the "disadvantageous" allele and TT carriers will benefit most from nicotine administration is oversimplified since the G allele seems to play a role in nicotine dependence. Thus, in a future study, one might postulate a unidirectional rather than a directional hypothesis about the possible modulating effect of the *TPH2* -703 G/T genotype on the effects of nicotine on antisaccade performance.

3.1.4 Polymorphisms in risk genes for schizophrenia

Since antisaccade performance is a well-founded endophenotype of schizophrenia (Calkins et al., 2008; Hutton & Ettinger, 2006; Turetsky et al., 2007), the risk genes for schizophrenia are also of interest in connection with antisaccade performance. Some of the aforementioned genes encoding for neurotransmitter receptors (such as *CHRNA7*, *COMT* and *DRD2*) are also considered to be risk genes of schizophrenia. As their possible modulating role on antisaccade performance has already been discussed in the previous paragraphs, the following section will address other risk genes of schizophrenia.

A recent paper by Schmechtig et al. (2010) reported that antisaccade performance was associated with a polymorphism in the Neuregulin 1 gene *NRG1 – NRG1* being one of the

leading candidate genes for schizophrenia (Harrison & Law, 2006). Schmechtig et al. (2010) tested a sample of 114 healthy volunteers and showed that the A allele of the *NRG1* rs3924999 SNP is associated with impaired spatial accuracy on the antisaccade task (i.e. hypermetric performance).

Neuregulins are a family of proteins which are involved in neural development, Schwann cell and oligodendrocyte differentiation, the formation of neuromuscular synapses, and, interestingly, acetylcholine receptor synthesis (Burden & Yarden, 1997). There are three major forms of *NRG1* (Types I-III) in addition to Types IV-VI (Steinthorsdottir et al., 2004). Type I *NRG1* plays a role in synapse development by influencing the upregulation of acetylcholine receptor genes and is important in nAChRs' post-synaptic expression (Sandrock et al., 1997). Therefore, Type I *NRG1* is also known as ARIA (= Acetylcholine Receptor Inducing Activity) (Li et al., 2006). The upregulation of nAChRs by neuregulins has been demonstrated at the neuromuscular junction, the developing interneuron synapse, and in the hippocampus (Liu et al., 2001; Usdin & Fischbach, 1986; Yang et al., 1998). Moreover, the human postmortem brain study by Mathew et al. (2007) showed that the schizophrenia-associated allelic variations within the *NRG1* gene (SNP8NRG221132 and rs6994992) were associated with α7 nAChR mRNA expression and receptor density in the DLPFC, but not in the hippocampus.

The findings by Mathew et al. (2007) also fit nicely to the idea that neuregulins, in connection with cholinergic neurotransmissions, might play a role for antisaccade performance, for the antisaccade task partially relies on DLPFC function. Indeed, the authors conclude that functional relationships between *NRG1* and nAChR neurotransmission may explain some of the intermediate phenotypes associated with schizophrenia (Mathew et al., 2007), and, that within the DLPFC, nAChR α7 receptors are located on pyramidal neurons and GABAergic interneurons (Mathew et al., 2007). However, the precise mechanism by

which specific *NRG1* isoforms influence α7 nAChR mRNA and protein levels is unknown and requires additional investigation (Mathew et al., 2007). Since there are functional relationships on the neural level between *NRG1* and α7 nAChRs in the DLPFC, an antisaccade study with the pharmacogenetic approach involving polymorphisms within *NRG1* and nicotine would be necessary. As a starting point, based on the findings by Schmechtig et al. (2010), one could hypothesize that the deficient spatial accuracy of antisaccades in carriers of the A allele of the *NRG1* rs3924999 SNP will be improved by nicotine administration.

Another risk gene of schizophrenia that might be of interest is the *GRIK4*, a gene encoding for a glutamate receptor sub-type. The study by Greenwood et al. (2011) within the COGS research program revealed a highly significant association between *GRIK4* and antisaccade performance, explaining 5.4% of the genetic variation in antisaccade performance. Besides the *GRIK4* result for antisaccades (which was one of the strongest associations), the COGS study found several other risk genes of schizophrenia to be associated with antisaccade performance in their sample: *DISC1*, *ERBB4*, *RELN*, *SLC18A1*, *DRD2*, *HTR2A*, *CRHR1*, and *PRODH* (Greenwood et al., 2011). Since the association with *GRIK4* was of such large effect size, it would be feasible to be able to replicate the finding. Furthermore, nicotine intake also triggers the release of glutamate and antisaccade performance is probably sensitive to the modulation of several neurotransmitter systems. Therefore, it is possible that one might find an association between *GRIK4* polymorphisms and nicotine administration in connection with the antisaccade task.

3.2 Cholinergic modulation of antisaccade performance – mediated by the effects of dopamine?

In the following paragraphs, it will be discussed whether cholinergic modulation of antisaccade performance is mediated by enhanced dopaminergic neurotransmission. Nicotine

intake stimulates not only the release of acetylcholine, but also the release of several other neurotransmitters such as dopamine, noradrenalin, serotonin, GABA, and glutamate (Barazangi & Role, 2001; Fu et al., 1998; Li et al., 1998; Lopez et al., 2001; Rowell et al., 1987; Summers & Giacobini, 1995). The rewarding and addictive properties of nicotine are modulated by release of dopamine, mainly in the nucleus accumbens (Fu et al., 2000). In addition, glutamate secretion in the ventral tegmental area (VTA) is also involved in the nicotine-stimulated dopamine secretion within the nucleus accumbens (Fu et al., 2000). Animal studies in rats demonstrated that self-administered nicotine activates the mesolimbic dopamine system through the VTA and that the effect of nicotine in the VTA initiates processes which are critical to the reinforcing properties of the substance (Corrigall et al., 1994; Fu et al., 2000). Moreover, nicotine induces the upregulation of dopamine D1 and D2 receptors in the nucleus accumbens, in the caudate-putamen region, and in the olfactory tubercle as shown by another study in rats by Bahk et al. (2002). Finally, both β2 nAChRs and α7 nAChRs can modulate dopamine release in the rat prefrontal cortex in vitro and in vivo (Livingstone et al., 2009). Hence, the interaction of the cholinergic and the dopaminergic systems seems to be crucial to the rewarding properties of nicotine.

Are the cognitive-enhancing properties of nicotine also ultimately mediated by the effects of dopamine? The interaction between the nAChR system and the dopaminergic system with respect to cognitive functioning has not been very well studied so far. However, there is some evidence that nicotine-induced enhanced dopaminergic neurotransmission seems to play a role in improving cognitive performance.

An animal study involving DAT knockout mice showed that these knockout mice exhibited deficits in the elevated plus maze task, that is, spatial learning and memory (Weiss et al., 2007). Interestingly, acute and chronic nicotine (administered via the drinking water) improved these spatial learning and memory deficits without eliciting tolerance (Weiss et al.,

2007). The authors speculate that the procognitive effects of nicotine in DAT knockout mice are related to the upregulation of α7 nAChRs (Weiss et al., 2007). A recent review by Herman and Sofuoglu (2010) which also reviewed this animal study also suggests that the improvement in the DAT knockout mice might have occurred through nicotine-induced up-regulation of DAT mRNA, as this process has been demonstrated by Li et al. (2004) in the two mid-brain structures of the rat brain, the substantia nigra and the ventral tegmental area (Herman & Sofuoglu, 2010).

The aforementioned study in humans by Millar et al. (2011) of the moderating role of the *DAT1* SLC6A3 VNTR polymorphism on the effects of nicotine on P50 sensory gating also stresses the possible direct connection of dopamine transporter expression and cognitive effects of nicotine. Indeed, the authors interpreted their findings that the 9R carriers of the *DAT1* polymorphism (exhibiting less available DAT and therefore greater levels of tonic dopamine) were impaired by nicotine due to further nicotine-induced release of dopamine leading to an "overstimulation" of dopamine or an "overdosing" of dopamine levels (Millar et al., 2011). However, it remains speculative whether nicotine directly modifies dopamine transporter mRNA expression in humans and is therebyresponsible for the procognitive effect of nicotine administration. Similar to the findings on the effects of nicotine and polymorphisms on the DAT gene, Jacobsen et al. (2006) also interpreted their findings on the *DRD2* rs6277 SNP (C957T polymorphism) in a way that carriers of the T allele already exhibit optimal or near-to-optimum levels of dopamine; thus,further stimulation of dopaminergic neurotransmission via nicotine leads to an overdose of dopamine and therefore poorer working memory performance.

A recent gene-gene interaction study by Markett et al. (2010) investigating the association between three *DRD2* SNPs and one *CHRNA4* SNP and working memory performance also provides indirect evidence for the role of dopamine in the nicotine-induced

cognitive improvement. Markett et al. (2010) tested 101 healthy subjects with a visuospatial working memory task (i.e. a brief visual array task) in which working memory load was systematically varied. They genotyped their subjects for the *DRD2* SNPs rs1800497, rs6277, and rs2283265 and for the *CHRNA4* SNP rs1044396. The authors constructed haplotypes on the *DRD2* gene and tested these haplotype blocks on an interaction with the *CHRNA4* SNP. Markett et al. (2010) found that carriers of the *DRD2* TCT+ haplotype who were also homozygous T/T allele carriers of the *CHRNA4* rs1044396 SNP exhibited better performance on the working memory task than non-carriers. Moreover, the gene effects were only visible when the working memory load was high (Markett et al., 2010). Markett et al. (2010) chose functional *DRD2* SNPs in their study: All three SNPs are associated with altered or reduced *DRD2* density in the striatum. The authors speculate that TCT+ carriers have generally reduced receptor availability with an additionally shifted proportion of presynaptic to postsynaptic receptors. Moreover, the authors suggest that the *CHRNA4* polymorphism alters the affinity of presynaptical nAChRs on dopaminergic neurons, thereby affecting dopaminergic neurotransmission. This and the decreased D2 receptor density might lead to an optimal saturation of D2 receptors (Markett et al., 2010), thereby providing an optimal dopaminergic tone for working memory function.

With regard to the antisaccade task, there is a pharmacological study by Rycroft et al. (2007) comparing the effects of the wakefulness-promoting agent modafinil and the effects of nicotine. Modafinil is a drug designed for the treatment of narcolepsy and other sleep and arousal-related disorders; however, modafinil's mechanism of action is largely unclear (Dopheide et al., 2007). On the one hand, modafinil is believed to serve as a selective α1-adrenergic receptor agonist (Milgram et al., 1999); on the other hand modafinil has shown to increase the levels of several monoamines. Animal studies in rats demonstrated that modafinil enhances extracellular levels of dopamine in the nucleus accumbens (Murillo-Rodriguez et

al., 2007) and evokes dopamine release from striatal neurons (Dopheide et al., 2007). Modafinil also triggers the release of noradrenalin in the hypothalamus and the release of serotonin in the prefrontal cortex (de Saint Hilaire et al., 2001).

Rycroft et al. (2007) tested 44 male non-smokers and employed a double-blind between-subjects design: 15 participants received a modafinil capsule (200 mg) and placebo spray, 15 received a placebo capsule and a nicotine nasal spray (1 mg), and 14 received a placebo capsule and a placebo spray before antisaccade testing. In this study, both modafinil and nicotine reduced antisaccade latencies. However, contrary to previous research (Depatie et al., 2002; Larrison-Faucher et al., 2004; Rycroft et al., 2006), nicotine did not lead to a reduction in antisaccade errors. Modafinil also failed to reduce antisaccade errors. The lack of an effect of both compounds on antisaccade error rate could not be attributed to ceiling effects. There was, however, a clear practice effect on antisaccade errors, with all three groups demonstrating reduced errors at post-test compared to baseline.

Unfortunately, other studies investigating the effects of dopaminergic drugs on antisaccade error rate provide ambiguous results: While both the dopaminergic agonists levodopa (Duka & Lupp, 1997) and amphetamine (Dursun et al., 1999) lead to increased antisaccade errors in healthy volunteers, the antipsychotic drugs risperidone and chlorpromazine that reduce dopaminergic neurotransmission also induced a reduction in antisaccade error rate in healthy probands (Barrett et al., 2004). Based on these results from other studies, Rycroft et al. (2007) conclude that at present there are insufficient data to determine whether these conflicting findings are due to differences in methodology, or whether the results suggest that there is an optimal level of dopamine required for successful antisaccade performance.

Further studies are needed to determine whether dopaminergic compounds act on antisaccade error rate and whether nicotine leads to a reduction in antisaccade error rate via the modulation of dopamine levels. However, both modafinil and nicotine reduced antisaccade latencies in the study by Rycroft et al. (2007). Based on these findings, the authors suggest that the reduction in antisaccade latencies achieved by both compounds might be mediated by common actions on a single neurotransmitter system, e.g. the dopamine system (Rycroft et al., 2007). Further, the authors acknowledge that this explanation might be "parsimonious" – both nicotine and modafinil also increase the release of noradrenalin (Rycroft et al., 2007). The release of noradrenalin leads to increased arousal and such a heightened state of alertness may reduce reaction times in general (Rycroft et al., 2007).

Another study on nicotine and antisaccades did not find that nicotine affected prosaccade latencies (Larrison-Faucher et al., 2004), a more recent study by Bowling and Donnelly (2010) found reduced prosaccade latencies after the administration of nicotine. Therefore, it cannot be ruled out completely that the modulation by noradrenalin is the common ground for the effects of nicotine and modafinil on antisaccade latencies. In summary, the findings by Rycroft et al. (2007) indicate that the improvements in antisaccade performance that have previously been demonstrated with nicotine are not necessarily mediated exclusively by the cholinergic system (Rycroft et al., 2007) and that the dopaminergic, the noradrenergic, or another common neurotransmitter system might be stimulated by both nicotine and modafinil.

3.3 Cognitive effects of nicotine: Do nicotinic acetylcholine receptors act as moderator variables in complex cortical networks?

As outlined in the previous chapter, it remains to be shown which neurotransmitter systems mediate the nicotine-induced improvements in antisaccade performance and in other cognitive functions. Perhaps neuronal nicotinic acetylcholine receptors play a modulatory role in neuronal circuits that are engaged in cognitive processes. The following paragraphs will attempt to integrate the beneficial effects of nicotine on antisaccade performance and on other cognitive tasks in a theory regarding the effects of nAChRs stimulation on neurotransmitter and attentional function.

Newhouse and colleagues (2004) proposed such a model for testing the effects of nicotinic receptor stimulation on neurotransmitter release and attentional function (see Figure 3-1). In their model, stimulation of nAChRs leads to enhanced release of various neurotransmitters (dopamine, noradrenalin, glutamate, serotonin, GABA, and acetylcholine). This enhanced neurotransmission takes place in various areas of the brain which are relevant to focused attention, arousal, inhibition, and processing/motor speed. The parallel activation of these areas of the brain induces sensory selectivity and acts on the central executive component of working memory (Baddeley, 2003), which eventually improves the acquisition of information. This conceptual model by Newhouse is a comprehensive description of how nicotine might exert its influence on attentional processing; however, it only offers minor explanatory value. For instance, it does not explain in which particular areas of the brain nAChR activation is initiated and how it is regulated. If one tries to transfer the idea of nAChRs acting as “modulator variables” or “adjustable screws” in cortical networks to the antisaccade paradigm, one could hypothesize that cholinergic neurotransmission in the prefrontal cortex is crucial for antisaccade performance. The review by Sarter and Parik (2005) suggests that increases in prefrontal cholinergic transmission triggers complex patterns

of recruitment of other cholinergic modules in the cortex, for example, to enhance the detection and processing of stimuli of a particular modality (Sarter & Parikh, 2005). Sarter and Parikh (2005) argue against the idea of the cholinergic system being a diffuse modulatory input system that innervates the entire cortical mantle and is primarily designed to enhance sensory input processing. Rather, they point out that the cortical cholinergic input system consists of modules that target specific cortical areas and have individual afferent organizations; thus they have the potential for region-specific regulation of cortical functions (Sarter & Parikh, 2005). Therefore, the prefrontal cortex might be able to systematically recruit cholinergic transmission in order to initiate executive functioning (Sarter & Parikh, 2005).

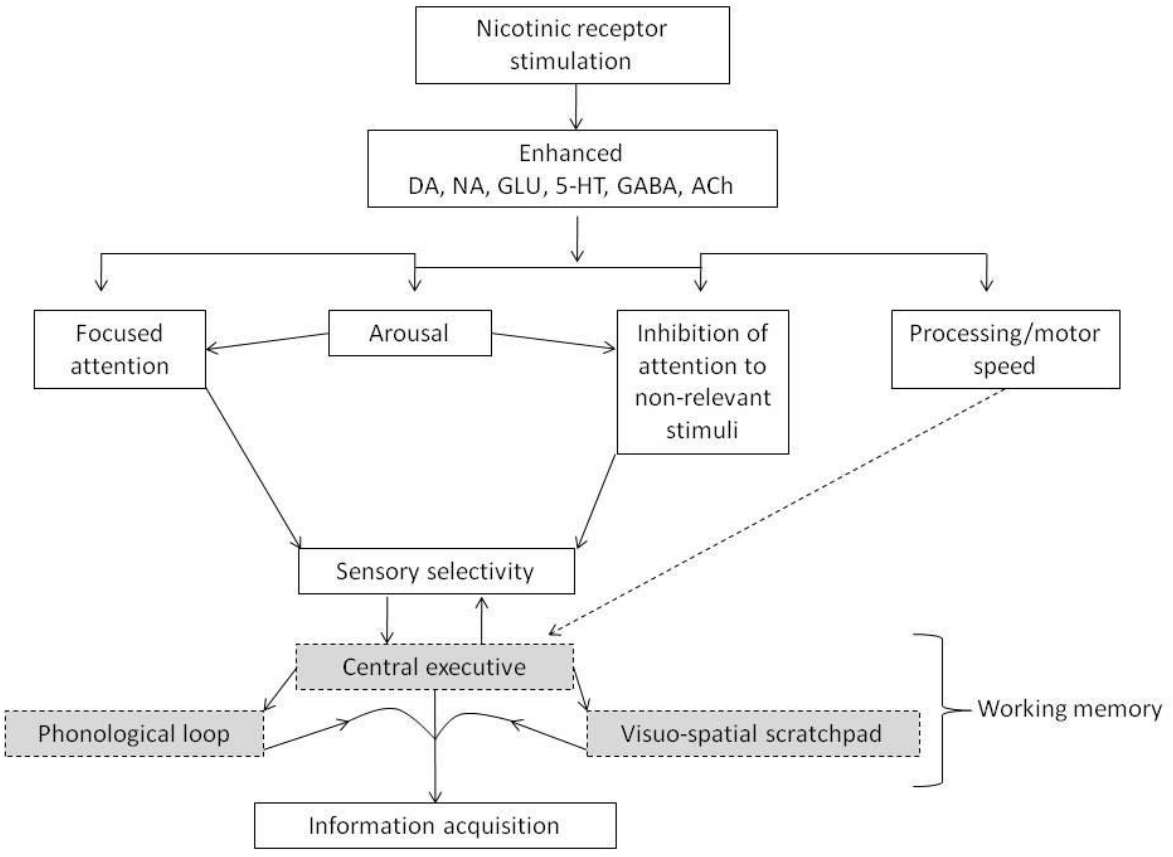

Figure 3-1
Proposed model for the effects of nAChRs stimulation on neurotransmitter function and attentional functioning, modified by Newhouse et al. (2004). DA=dopamine, NA=noradrenalin, GLU=glutamate, 5-HT=serotonin, GABA=gamma-aminobutyric acid, ACh=acetylcholine.

In a hypothetical model for antisaccade performance, based on the suggestions by Newhouse et al. (2004) and Sarter and Parikh (2005), using nicotine to stimulate nAChRs in the dorsolateral prefrontal cortex (DLPFC) could lead to enhanced suppression of prosaccades, thereby improving antisaccade performance. By cholinergic top-down signaling of the DLPFC to the frontal eye fields and the superior colliculi, the preparation and execution of voluntary antisaccade eye movements might be further enhanced. Cholinergic signaling in the DLPFC likely also leads to the activation of other neurotransmitters, such as dopamine and noradrenalin. After the nAChRs have been activated by nicotine, the subsequent release of other neurotransmitters such as dopamine, noradrenalin, and serotonin might further support antisaccade performance. Dopamine release in the DLPFC should be beneficial for working memory processes, such as maintaining and updating information about a current antisaccade trial. Noradrenalin might increase arousal; however, noradrenalin release might also increase processing/motor speed, thereby leading to faster saccadic eye movements. Since there are high concentrations of serotonin receptors in the prefrontal cortex, nicotine-induced serotonin release in the DLPFC might also support DLPFC functioning in suppressing antisaccade errors.

The idea that nAChRs act as “modulator variables” or “adjustable screws” in cortical networks is an interesting one and should initiate further research. How does nicotinic stimulation exert its influence on the neural level and how does it induce long-term changes in synapses? A recent review by Mansvelder et al. (2009) reports on the idea that nicotinic stimulation alters synaptic transmission, which in turn can induce synaptic plasticity, thereby causing long-term changes in cortical circuits. Mansvelder et al. (2009) suggest that rather than turning neuronal systems on and off, it appears that cholinergic tuning involves changes in the balance between inhibitory and excitatory inputs. Synaptic plasticity in the prefrontal cortex has been linked to attention and working memory (Laroche et al., 2000). In mice,

nicotine strongly affects synaptic plasticity in the prefrontal cortex (PFC): Nicotine influences the relative timing of action potentials in pre- and postsynaptic neurons (Mansvelder et al., 2009). This coordinated neuronal firing can induce long-term potentiation (LTP) or long-term depression (LTD). The phenomenon of timing-dependent synaptic plasticity is also called spike-timing-dependent plasticity (STDP) (Mansvelder et al., 2009). GABAergic interneurons in the PFC layer 5 express nAChR sub-units on their soma that activate these neurons when nicotine is present (Mansvelder et al., 2009). In mice, nicotine both directly activates somato-dendritic nAChRs of GABAergic interneurons and indirectly activates these GABAergic interneurons by presynaptic glutamatergic input. Thus, inhibitory GABA interneurons are activated via nicotine, leading to increased inhibitory tone. The authors acknowledge that it is somewhat counterintuitive that nicotine decreases the likelihood of LTP induction in the PFC. They suggest that nicotine alters the rules for synaptic plasticity by increasing the threshold for STDP which is supposed to enhance the signal-to-noise ratio in PFC information processing, thereby improving cognitive performance (Mansvelder et al., 2009).

In summary, cholinergic tone can regulate neuronal circuit activity, yet we are only beginning to understand the exact mechanisms by which nicotine alters the code of synaptic plasticity. Given that (1) nAChRs are located on different cell types, (2) there are various sub-types of nAChRs, and (3) the different nAChR sub-types differ in their affinity and desensitization for nicotine, there are a variety of possible ways in which nicotine regulates neuronal activity. There is some recent evidence that both α7 nAChRs and β2 nAChRs play a pivotal role in modulating neurotransmitter release and in initiating calcium (Ca^{2+}) signaling, a precondition for inducing persistent neuronal changes (Dickinson et al., 2008; Mansvelder et al., 2009). In conclusion, the concept of nAChRs as “moderators” or “modulating entities” in cortical networks is an interesting one, yet further in vitro and in vivo studies are needed to

clarify how nicotine induces long-term changes in synapses and how nAChRs modulate synaptic plasticity.

3.4 Methodological issues, strengths and limitations of the present studies

In the present two investigations, the possible cholinergic modulation of antisaccade performance was examined. A molecular genetic approach and pharmacological study accounted for inter-individual differences in the response to nicotine in order to gain more insight regarding what predicts the effectiveness of cholinergic treatment. Study 1 investigated whether *CHRFAM7A* copy number and 2bp deletion polymorphisms were associated with antisaccade performance. Study 2 tested the hypothesis that baseline performance level may be a behavioral predictor of the effects of nicotine on antisaccade performance. No association was found between *CHRFAM7A* polymorphisms and antisaccade performance (Study 1), while the effects of nicotine on antisaccade error rate and response variability depended on inter-individual differences in baseline antisaccade performance (Study 2).

One of the strengths of Study 1 lies in the investigation of the relationship of *CHRFAM7A* polymorphisms and antisaccade performance in the absence of clinical and treatment confounds. Moreover, to the knowledge of our research group, this is the first investigation to explore the association of *CHRFAM7A* and measures of executive functioning. A limitation of Study 1 is the sample size: 103 healthy volunteers were tested and genotyped for the *CHRFAM7A* polymorphisms. A power calculation revealed that it would have been necessary to assess more than 600 subjects to be able to detect small effects of d=0.2 with a power of greater than 80%. Therefore, it cannot be ruled out completely that *CHRFAM7A* genotype does in fact impact on antisaccade performance, but we were not able

to detect it due to relatively small sample size. A possible solution could be to conduct a multicenter study in order to be able to recruit a very large sample of subjects similar to the COGS study (Greenwood et al., 2011; Radant et al., 2010). The fact that antisaccades can be measured accurately across multiple study sites was demonstrated in another COGS study by Radant et al. (2007). This further supports the concept of a multicentered approach, provided that in doing so one uses standardized equipment, training, tasks, and test procedures (Radant et al., 2007).

Study 2 has its strong point in the fact that it is the first investigation to explore the effect of nicotine in a sample which was stratified according to performance level, i.e. in a sample which was divided into high- and low-performing groups. A minor limitation is the employed-median split procedure. With turning a continuous variable (i.e. antisaccade error rate) into a categorical one there is reduced power to detect interaction effects due to loss of information in contrast to a regression approach. In addition, median splits are sample-dependent. Although Study 2 was adequately powered, future studies might want to opt for a regression model when analyzing what predicts a nicotine effect. A strength of Study 2 is that a 6° and a 12° eccentricity condition was employed in the antisaccade paradigm. Thus, we made task demands difficult and provoked a sufficiently large antisaccade error rate in our sample, thereby ensuring we could detect subjects who would exhibit difficulties with the task. Hence, by making task demands difficult, we were able to mimic executive control impairment in the low-performing group as it is observed in different clinical populations such as schizophrenia or ADHD. At the same time, we were able to investigate the effect of nicotinic stimulation in a sample free of psychiatric disorder and other confounding variables, such as estrous cycle and medication, which could also have affected antisaccade performance. Thus, we studied an unbiased sample while maximizing the probability that the

observed performance enhancement was truly caused by nicotine and was not the effect of any other variable.

One strength and limitation at the same time pertains to the use of a repeated-measurement design. One the one hand, a repeated-measurement design offers higher statistical power to find an effect, as each subject serves as its own control, thereby keeping the variance down. In addition, fewer subjects are needed than in a between-subjects design. On the other hand, practice effects are always an issue in repeated-measurement designs. A limitation of Study 2 is the fact that we did not employ baseline testing before the two testing sessions. While some drug-challenge studies employed a baseline session in order to minimize practice effects between drug and placebo sessions (Allmann et al., 2010; Vollenweider et al., 2006), other drug-challenge studies did not (Csomor et al., 2008; Knott et al., 2010). As between-session practice effects have been observed for the antisaccade task (Ettinger et al., 2003a), an initial baseline session is recommended in future drug-challenge studies using the antisaccade paradigm. Nevertheless, we addressed the problem of practice effects in several ways. First, by making task demands sufficiently difficult, we ensured that our subjects did not perform at ceiling during testing Session 2. Second, we counter-balanced the order of the patches in our low- and high-performing groups; the groups did not differ regarding order of patch. Third, we included order of patch (nicotine first, placebo first) as between-subjects factors in all of our statistical analyses. There was neither a significant main effect of order nor interactions of order with any of the variables. Fourth, in a repeated-measurement design in which subjects are tested twice, their scores tend to regress towards the mean. This statistical phenomenon is known as the "regression to the mean" (also referred to as: "the law of initial value") (Bland & Altman, 1994; Wilder, 1958) (see also paragraph 2.2.6.). We checked the present antisaccade error data and our data regarding the variability in

antisaccade latency for this issue and concluded that our results are not simply explainable in terms of a regression to the mean (see also paragraph 2.2.6.).

Finally, an important strength of Study 2 lies in the assessment of blindness. At each testing session, the participants were asked to make a guess about which patch they had received. Results indicated that double-blindness was partially uncovered as participants were able to correctly guess which patch they had received, especially after Session 2, revealing a limitation of Study 2. Perhaps the participants could tell the difference between the placebo and the nicotine patch because they experienced physical side effects such as itching and mild nausea from the nicotine treatment but not from the placebo condition. In the whole sample, there was no significant association of experienced adverse side effects with correct guessing (testing Session 1: Fisher's exact test p=.21, testing Session 2: Fisher's exact test p=.57). However, it might be better to test the association between the side effects that were experienced and correct guessing of patch identity only in those probands who reported side effects during the nicotine session but not during the placebo session, as they should be the ones whose discomfort was actually caused by nicotine and they should reliably attribute their experienced discomfort to the nicotine treatment. Nineteen of the twenty-eight probands reported experiencing side effects during the nicotine session and no side effects during the placebo session. These 19 probands were the ones who guessed correctly 68.42% of the time when asked about patch identity at testing Session 1 and 100% of the time when asked about patch identity at testing Session 2. Therefore, probands who exhibited side effects from nicotine administration were those probands who uncovered double-blindness of the experiment, as they were all able to correctly identify the patches they received at Session 2. One solution to better mask the identity of the patches could be to additionally use capsaicin cream in combination with the placebo patch, as capsaicin mimics the itching and tingling sensations evoked by a nicotine patch. In fact, a recent study by Wignall and de Wit (2011)

employed this strategy. Like in Study 2, they used a 7 mg nicotine patch and a placebo patch in healthy non-smokers. The placebo patch additionally contained a small amount of capsaicin cream (Wignall & de Wit, 2011). Unfortunately, Wignall and de Wit (2011) did not ask their probands about patch identity. However, the participants' ratings on perceived drug effects indicate that the participants experienced some aversive side effects of the nicotine treatment, as nicotine significantly increased ratings on the question whether the participants "are currently feeling any drug effects" and nicotine significantly decreased ratings on the question whether participants "like the effects they feel" (Wignall & de Wit, 2011). Thus, it is possible that double-blindness was uncovered, as probands probably attributed their experienced discomfort to the nicotine treatment. Cancelling out all aversive side effects caused by nicotine will be difficult in subjects who do not usually consume nicotine; however, one possible solution might be to administer an additional nausea-preventing drug (e.g. a tablet of domperidone) with nicotine treatment and an analogous placebo tablet with placebo treatment.

3.5 Conclusions

In summary, the present work did not find an association between *CHRFAM7A* polymorphisms and antisaccade performance (Study 1) and demonstrated that effects of nicotine on antisaccade error rate and response variability depend on inter-individual differences in baseline antisaccade performance (Study 2). To date, little is known about the genetics of antisaccade performance, therefore future studies should continue to investigate genetic effects on antisaccade parameters. In addition, future clinical studies should account for inter-individual differences in baseline performance when testing a new cholinergic compound for the remediation of cognitive deficits. The results from Study 2 indicate that a

possible cognitive enhancer does not necessarily have to be effective for everyone. Instead, inter-individual differences in performance form one important predictor of drug response.

Previous studies have focused on the strategy "one medication for the whole patient group," e.g. the partial α7 nAChR agonist DMXB-A for schizophrenia patients (Freedman et al., 2008). That study, however, was not effective in enhancing the cognitive function in the schizophrenia patients. Hence, future studies might want to stratify their study populations in probands with and without cognitive deficits or in patients with only mild cognitive impairment and patients with severe cognitive impairment. It is possible that the substance DMXB-A might only be effective in a sub-population of schizophrenia patients; that is, in those patients with more severe cognitive deficits.

It is also possible that the effectiveness of a cholinergic substance highly depends on the patient's genetic make-up. Patients exhibiting "disadvantageous" cholinergic polymorphisms (i.e. cholinergic polymorphism which are associated with poorer cognitive performance) might be those patients who are equipped with a suboptimal cholinergic system and will benefit from further cholinergic stimulation. Indeed, this pharmacogenetic research strategy of taking inter-individual differences in the genetic makeup into account when giving medications has received growing interest in recent years. Pharmacogenetic drug therapy can be related to the "personalized medicine" approach – personalizing treatment in general with all decisions in healthcare being tailored to the individual whenever possible. However, pharmacogenetic treatment strategies are still in a very experimental stage in basic science and are not being routinely applied in clinical practice. It would be very helpful for clinicians to predict which patient will benefit from which medication and which medication is likely to be ineffective in the patient. This is especially true for the treatment of psychiatric diseases. Today, trying out several psychotropic drugs is the routine and quite often it takes a few weeks for the drug to unfold its desired effects. Avoiding this "trying out procedure" would

help to cut down the exposure of patients to ineffective drugs, provide them with more effective and efficient treatment of their symptoms, and would also help to reduce costs.

In conclusion, future studies should continue to investigate the effectiveness of new cholinergic substances on executive function. The antisaccade task might serve as a useful laboratory tool to test the effectiveness of such new compounds. Further, future studies should account for the notion that it is not disease status per se which predicts poorer cognitive performance and the response to a cholinergic substance. Rather, other predictors of response such as baseline performance and genetic differences should also be considered in future investigations.

German Summary (Deutsche Zusammenfassung)

Cholinerge Modulation der Antisakkadenleistung

Die Bedeutung von CHRFAM7A-Polymorphismen und differentielle Effekte von Nikotin in Abhängigkeit vom Ausgangsleistungsniveau

Einleitung. Die vorliegende Arbeit untersuchte inwiefern das nikotinerge Acetylcholinrezeptor-(nAChR)System eine Rolle für die Antisakkadenleistung spielt. Insbesondere die Bedeutung von interindividuellen Unterschieden bezüglich der Reaktivität auf eine cholinerge Stimulation wurde untersucht. Zwei Forschungsstrategien wurden dabei verfolgt: ein molekulargenetischer Ansatz, bei dem untersucht wurde, ob Polymorphismen im *CHRFAM7A*-Gen interindividuelle Varianz in der Antisakkadenleistung erklären können, sowie ein verhaltensexperimenteller und pharmakologischer Ansatz, bei dem geprüft wurde, ob interindividuelle Unterschiede im anfänglichen Antisakkadenleistungsniveau die Reaktion auf eine cholinerge Stimulation durch Nikotin beeinflussen.

Patienten mit Schizophrenie, ihre Angehörigen und Individuen mit einem erhöhten Psychoserisiko zeigen Antisakkadendefizite (Hutton & Ettinger, 2006; Nieman et al., 2007; Petrovsky et al., 2008) – dies spricht dafür, dass die Antisakkadenleistung einen Endophänotyp der Schizophrenie darstellt. Bisher ist sehr wenig über die Genetik der Antisakkadenleistung bekannt. Cholinerge Polymorphismen könnten eine Rolle spielen, da ein verändertes nAChR-System möglicherweise zur Pathophysiologie der Schizophrenie beiträgt (Freedman et al., 1995). Außerdem kann Nikotin (ein Agonist des nAChRs) die Antisakkadenleistung positiv beeinflussen, die Antisakkadenleistung wird sowohl in Schizophreniepatienten (Depatie et al., 2002), als auch in gesunden Probanden (Rycroft et al., 2006) durch Nikotin verbessert. Ein Forschungsansatz, um herauszufinden, ob nAChRs eine Rolle für die Antisakkadenleistung spielen, stellt die Suche nach nAChR-Genen dar, die

zumindest teilweise die interindividuellen Unterschiede in der Antisakkadenleistung erklären könnten.

Als Startpunkt für die vorliegende Arbeit wurde das *CHRNA7*-Gen gewählt. *CHRNA7* steht nachweislich mit der P50-Suppression, einem anderen Schizophrenie-Endophänotyp, in Verbindung (Freedman et al., 1997; Leonard et al., 2002). Ein schwacher Zusammenhang direkt zur Erkrankung Schizophrenie besteht ebenfalls (Freedman et al., 1997) und das häufigere Vorkommen von funktionalen Polymorphismen im *CHRNA7*-Promoter ist mit Schizophrenie assoziiert (Leonard et al., 2002). In den meisten Individuen ist das *CHRNA7*-Gen teilweise doppelt vorhanden, was zur Folge hat, dass ein Hybridgen entsteht, *CHRFAM7A*, welches die Exons 5-10 von *CHRNA7* enthält zusammen mit vier Exons des unverwandten *FAM7A*-Gens. Es sind Chromosomen mit und ohne das *CHRFAM7A*-Duplicon identifiziert worden, es besteht also ein Genkopienpolymorphismus (englisch: copy number variation, CNV) hinsichtlich der Exons 5-10 (Flomen et al., 2006). Reduzierte Genkopienanzahl ist schwach aber signifikant mit Psychose assoziiert (Flomen et al., 2006). Wenn das *CHRFAM7A*-Gen vorhanden ist, dann existiert es als polymorphe Inversion entweder in derselben oder in der entgegengesetzten Ausrichtung wie das *CHRNA7*-Gen (Flomen et al., 2008). Zusätzlich enthält das *CHRFAM7A*-Gen eine 2-bp Deletion in Exon 6, welche mit Defiziten in der P50-Suppression (Raux et al., 2002) und mit episodischer Gedächtnisleistung (Dempster et al., 2006) assoziiert ist. Die 2-bp Deletion steht im starken Linkage-Disequilibrium mit der Ausrichtung des *CHRFAM7A*-Gens in Bezug auf das *CHRNA7*-Gen. Daher könnte auch die Ausrichtung des *CHRFAM7A*-Gens die eigentliche Variante sein, die für die oben erwähnte Assoziation verantwortlich ist. Wie diese *CHRFAM7A*-Varianten die Schizophrenie-Endophänotypen beeinflussen, ist noch nicht erforscht. Möglicherweise beeinflusst die *CHRFAM7A*-Genexpression die Genexpression von

CHRNA7, indem ein Wettbewerb um Transkriptionsfaktoren besteht; dies könnte durch die Ausrichtung des *CHRFAM7A*-Gens beeinflusst sein (Flomen et al., 2008).

Das Ziel von Studie 1 war es deshalb, herauszufinden, ob Varianten im nAChR Gen Varianz in einem fronto-parietalen Schizophrenie-Endophänotyp erklären können. Daher wurde die mögliche Assoziation von *CHRFAM7A*-Genkopienanzahl-/2-bp Deletionspolymorphismen und Antisakkadenleistung untersucht. Studie 1 war hierbei auf gesunde Probanden beschränkt, um Gen-Kognition Beziehungen ohne klinische und medikamentöse Störfaktoren zu testen. Auch konnte auf diese Weise ein stärkerer Effekt erwartet werden, da vorherige Studien gezeigt haben, dass *CHRFAM7A*-Genotypeffekte auf Kognition stärker in gesunden Probanden als in Patienten mit Schizophrenie auftreten (Raux et al., 2002).

Studie 2 beschäftigte sich mit der möglichen Beeinflussung der Antisakkadenleistung durch ein cholinerges Pharmakon, nämlich mit der Wirkung von Nikotin, einem bekannten nAChR-Agonisten. Eine Meta-Analyse von Heishman et al. (2010) hat bereits gezeigt, dass Nikotin auf eine Reihe von kognitiven Funktionen positive Wirkungen hat, unter anderem verbessert Nikotin Aufmerksamkeits- und Arbeitsgedächtnisleistungen. Manche kognitiven Domänen konnten allerdings nicht in diese Meta-Analyse einfließen, weil zu ihnen noch zu wenige Studien durchgeführt wurden, u.a. der Bereich der vorliegend untersuchten exekutiven Funktionen. (Unter dem Begriff exekutive Funktionen bzw. exekutiver Kontrolle fasst man verschiedene höhere kognitive Prozesse zusammen, u.a. inhibitorische Kontrollprozesse wie z.B. die Hemmung einer vorherrschenden Reaktion, zielgerichtetes Initiieren und Sequenzieren von Handlungen, sowie die Beobachtung von Handlungsergebnisse und die evtl. erforderliche Selbstkorrektur.) Besonders im Zusammenhang mit Schizophrenie ist die Wirkung von Nikotin auf exekutive Funktionen interessant, da die Behandlung mit den bisherigen Medikamenten hier kaum zu Verbesserungen führt und diese kognitiven Störungen einen ungünstigen Einfluss auf den Verlauf der Erkrankung haben (Friedman et al., 1999).

Zudem gibt es Hinweise darauf, dass cholinerge Substanzen nützlich bei der Behandlung von kognitiven Störungen bei Schizophrenie-Patienten sein könnten (Ribeiz et al., 2010). Daher könnten cholinerge Substanzen möglicherweise die Störungen exekutiver Kontrolle bei Patienten mit Schizophrenie verbessern und so einen neuen, zusätzlichen Behandlungsansatz darstellen für die bisher nicht ausreichend behandelbaren exekutiven Störungen. Bisherige Studien berücksichtigten aber kaum den Aspekt interindividueller Unterschiede bei der Reaktion auf die Behandlung mit einer cholinergen Substanz. Diese interindividuellen Unterschiede können wahrscheinlich zumindest teilweise erklären, warum manche Studien positive Effekte einer Nikotingabe zeigten und andere Studien nicht. Parallel zu Theorien, die sich auf das dopaminerge System beziehen, haben Newhouse und Kollegen (2004) eine umgekehrte U-Funktion des Ausgangsleistungsniveaus und der nikotinergen Stimulation postuliert. Abhängig vom Ausgangsleistungsniveau kann eine äquivalente nikotinerge Stimulation entweder die Leistung steigern oder beeinträchtigen. In Probanden mit einem niedrigen anfänglichen Leistungsniveau führt die Nikotingabe zu einer Leistungssteigerung, das heißt die Probanden werden näher an das optimale Leistungsniveau herangebracht. Bei Probanden mit einem hohen anfänglichen Leistungsniveau, das bereits nahe am Optimum liegt, führt die Nikotingabe zu einer „Überstimulation“ des Systems, die Probanden verschlechtern sich in ihrer Leistung (Newhouse et al., 2004). In Studien, die die Effekte von cholinergen Agonisten wie Nikotin auf kognitive Leistungen untersuchen, könnte es daher wichtig sein, Unterschiede im Ausgangsleistungsniveau systematisch zu beachten, um in der Lage zu sein, mögliche Effekte der Substanz zu beurteilen.

In Studie 2 wurde hierfür das Antisakkadenparadigma gewählt, um zu untersuchen, ob anfängliche Leistungsunterschiede in exekutiven Funktionen die Effekte von Nikotin modulieren können. Das Antisakkadenparadigma wurde gewählt, weil es ein relativ simples Modell exekutiver Kontrolle okulomotorischer Reaktionen darstellt (Reuter & Kathmann,

2004). Außerdem wird die Antisakkadenaufgabe durch ein gut erforschtes neuronales Netzwerk vermittelt, das frontale, parietale und subkortikale Strukturen umfasst (Ettinger et al., 2008a; Munoz & Everling, 2004). Des Weiteren stellt die Antisakkadenleistung einen bekannten Schizophrenie-Endophänotyp dar (Calkins et al., 2008), der eine hohe Retest-Reliabilität aufweist (Ettinger et al., 2003a). Schließlich zeigten schon einige wenige Studien, dass die Antisakkadenaufgabe auf eine Stimulation durch Nikotin anspricht; Nikotineffekte fanden sich sowohl in Schizophrenie-Patienten (Depatie et al., 2002; Larrison-Faucher et al., 2004), als auch in gesunden Probanden (Bowling & Donnelly, 2010; Dawkins et al., 2007; Depatie et al., 2002; Ettinger et al., 2009; Rycroft et al., 2006; Rycroft et al., 2007). Basierend auf dem Modell von Newhouse und Kollegen (2004) wurde die Hypothese aufgestellt, dass Probanden mit einem niedrigen Leistungsniveau in der Antisakkadenaufgabe von einer Nikotingabe profitieren würden, und dass Probanden mit einem hohen Leistungsniveau in der Antisakkadenaufgabe durch nikotinerge Stimulation beeinträchtigt würden.

Genetikstudie = Studie 1: CHRFAM7A Genkopien-/2-bp-Deletionspolymorphismen und Antisakkadenleistung.

Methoden. *Probandenrekrutierung*. Die Probanden wurden durch Aushänge an der Universität und in der örtlichen Gemeinde rekrutiert. Abgefragt wurden Alter, Geschlecht, Ethnizität, Rauchstatus (Raucher, Nichtraucher), Bildungsjahre, mütterlicher und väterlicher sozio-ökonomischer Status (gemessen auf einer 1-4 Skala, 1=elementary, 4=professional). Ausschlusskriterien waren DSM-IV Achse I Störungen, die Probanden wurden dazu mithilfe des SKID-I Interviews gescreent. Weitere Ausschlusskriterien waren erlittene Kopfverletzungen mit Bewusstlosigkeit >1 min, neurologische Erkrankungen, ein Angehöriger ersten Grades mit einer psychotischen Erkrankung, Drogenmissbrauch oder Drogenabhängigkeit und Sehbehinderungen. Die Probanden füllten Gesundheitsfragebögen bezüglich ihres allgemeinen Gesundheitszustandes aus. Außerdem füllten die Probanden

standardisierte Persönlichkeitsfragebögen aus, um mögliche Genotypeneffekte auf Persönlichkeitsmerkmale zu erfassen: Rust Inventory of Schizotypal Cognitions (RISC) (Rust, 1988; Fragebogen zur Schizotypie), den Adult ADHD Self-Report Scale (ASRS) (Kessler et al., 2005; Fragebogen der Weltgesundheitsorganisation zu Symptomen der Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung), das Obsessive-Compulsive Inventory (OCI) (Foa et al., 2002; Fragebogen zur Erfassung von Zwangsgedanken und Zwangshandlungen), Neurotizismus-Skala des Eysenck Personality Questionnaire – Revised (EPQ-R) (Eysenck & Eysenck, 1991; Fragebogen zur Erfassung des Persönlichkeitsmerkmals Neurotizismus). Die Zulassung der lokalen Ethikkommission wurde eingeholt und alle Probanden unterzeichneten eine schriftliche Einverständniserklärung.

Antisakkadenparadigma. Die Augenbewegungen wurden per Infrarotokulographie (IRIS Skalar 6500, Skalar Instruments GmbH, Deutschland) aufgezeichnet; die Abtastrate betrug 500 Hz. Die Teilnehmer saßen mit einem Abstand von 57 cm vor einem 17-Zoll Computermonitor. Kopfbewegungen wurden dadurch minimiert, dass die Teilnehmer ihr Kinn auf einer Kinnstütze ablegten. Der Zielreiz war ein weißer Punkt (0.3 Sehwinkel Durchmesser) auf schwarzem Hintergrund. Zunächst wurde eine 3-Punkt-Kalibrierung vorgenommen (0°, ±12°), anschließend folgten 60 Antisakkadendurchgänge. Ein Durchgang beinhaltete die Darbietung des Zielreizes in der Bildschirmmitte für eine randomisierte Dauer von 1000-2000 ms, anschließend wurde der Zielreiz an einer von vier möglichen Positionen (±6°, ±12°) für 1000 ms gezeigt. Die Teilnehmer wurden instruiert, auf den Zielreiz zu schauen, wenn er sich in der Bildschirmmitte befindet und genau zur spiegelbildlichen Stelle zu blicken wenn der Zielreiz zur Seite springt. Die Auswertung der Augenbewegungen erfolgte mittels Eyemap (AMTech GmbH, Deutschland) mit den folgenden automatischen Kriterien zur Detektion einer Sakkade: minimale Amplitude (1°), Geschwindigkeit (30°/s), minimale Latenz (100 ms), sowie Einstufung je nach Richtung der Sakkade als korrekte

Antisakkade oder Antisakkadenfehler. Als Antisakkadenvariablen wurden die Antisakkadenlatenz (in ms), die Antisakkadenfehlerrate (in %, d.h. reflexive Sakkaden zum Zielreiz hin geteilt durch die Gesamtanzahl der Durchgänge), der Antisakkaden-Gain (d.h. das Antisakkadenamplitudenverhältnis in % = Sakkadenamplitude geteilt durch Zielreizamplitude) und der räumliche Antisakkadenfehler berechnet. Der räumliche Antisakkadenfehler berechnete sich folgendermaßen: die Zielreizamplitude wurde von der Sakkadenamplitude abgezogen und das daraus resultierende Ergebnis durch die Zielreizamplitude geteilt. Der absolute Wert wurde anschließend durch alle Sakkaden geteilt und mit 100 multipliziert.

Genotypisierung. Genotypisiert wurden die Probanden hinsichtlich zweier *CHRFAM7A*-Polymorphismen: Copy Number Variation (CNV) Polymorphismus (=Anzahl der Kopien des *CHRFAM7A*-Gens) und 2bp-Deletionspolymorphismus. Daraus ergaben sich fünf Genotypen-Gruppen: „1C0D“: eine Kopie und keine Deletion (N=16), „1C1D“: eine Kopie und eine Deletion (N=12), „2C0D“: zwei Kopien und keine Deletion (N=15), „2C1D“: zwei Kopien und eine Deletion (N=42), sowie „2C2D“: zwei Kopien und zwei Deletionen (N=18). Der sehr seltene Genotyp, der keinerlei Kopie von *CHRFAM7A* aufweist, fand sich bei den Probanden nicht.

Statistische Auswertung. Die statistischen Analysen wurden mit SPSS 15.0 (SPSS Inc., USA) durchgeführt. Der Genotyp (1C0D, 1C1D, 2C0D, 2C1D, 2C2D) fungierte dabei als unabhängige Variable, soziodemographische Variablen (Alter, Bildung, mütterlicher und väterlicher sozio-ökonomischer Status) und Antisakkdenvariablen (Fehlerrate, Latenz, Gain, räumlicher Fehler) waren die abhängigen Variablen in separaten univariaten Varianzanalysen (ANOVA). Die Beziehung zwischen Genotyp und Geschlecht wurde mittels des χ^2-Tests untersucht. Rauchstatus (Raucher, Nichtraucher) wurde als zusätzliche unabhängige Variable zum ANOVA-Modell hinzugefügt. Außerdem wurde mittels χ^2-Test untersucht, ob

Rauchstatus mit dem Genotyp assoziiert war. (Mittelwerte (M) und Standardabweichungen (SD) sind im Folgenden als M±SD dargestellt.)

Resultate. Insgesamt wurden 111 gesunde Probanden untersucht. Bei acht Probanden schlug die Genotypisierung fehl, was zu einer endgültigen Stichprobe von N=103 führte. Die Stichprobe von N=103 Probanden enthielt 57 männliche Probanden, das Durchschnittsalter betrug 25,87±5,50 Jahre, die Probanden wiesen durchschnittlich 17,64±3,36 Bildungsjahre auf, der väterliche sozio-ökonomische Status betrug im Durchschnitt 3,12±0,84, der mütterliche sozio-ökonomische Status betrug im Durchschnitt 2,83±1,02; 27 der untersuchten Probanden waren Raucher. Alle Probanden waren kaukasisch.

Die Genotypen-Gruppen unterschieden sich statistisch signifikant in keiner der soziodemographischen Variablen (alle p-Werte>0,20). Die Genotypen-Verteilung unterschied sich ebenfalls nicht signifikant vom Hardy-Weinberg-Equilibrium (χ^2=4,51, d.f.=3, p=0,21). Die Analysen der Antisakkadenvariablen zeigten keine Assoziation der Genkopien-/Deletionsgenotypen mit den Antisakkadenvariablen (alle p-Werte>0,37). Die Gruppierung der Probanden in Probanden nach 2-bp Deletionspolymorphismus (N=31 ohne Deletion, N=72 mit mindestens einer Deletion) und in Probanden nach Anzahl Genkopien (N=28 mit einer Kopie, N=75 mit zwei Kopien) zeigte in separaten Analysen ebenfalls keine signifikanten Effekte (jeweils p>0,34 und p>0,59).

Auch bei Hinzufügung des Rauchstatus als unabhängige Variable blieben die Genotypeneffekte blieben unverändert, es gab keine signifikanten Haupt- oder Interaktionseffekte mit dem Faktor Rauchstatus (alle p-Werte>0,18). Des Weiteren gab es keine Assoziationen der kombinierten Genkopien-/Deletionsgenotypen mit den Daten aus den Persönlichkeitsfragebögen (alle p-Werte>0,48). Die Gruppierungen der Probanden nach Deletionspolymorphismus alleine sowie nach Anzahl Genkopien alleine zeigten ebenfalls

keine signifikanten Effekte (P-Werte jeweils p>0,25 und p>0,22). Ebenfalls gab es keine Haupt- oder Interaktionseffekte der Faktoren Rauchstatus und Genotyp auf die erzielten Werte in den Persönlichkeitsfragebögen.

Nikotinstudie = Studie 2: Differentielle Modulation der Antisakkadenleistung durch Nikotin in gesunden männlichen Nichtrauchern, die nach niedriger und hoher Leistung eingeteilt wurden.

Methoden. Probandenrekrutierung und Nikotinapplikation. Dreißig gesunde, kaukasische, nichtrauchende, männliche Versuchsteilnehmer wurden über Aushänge an der Universität und aus einer Zufallsstichprobe aus der Allgemeinbevölkerung durch das örtliche Melderegister rekrutiert. Als Nichtraucher wurden dabei Personen qualifiziert, die in ihrem Leben nicht mehr als 100 Zigaretten und im letzten Jahr überhaupt nicht geraucht haben. Die Versuchsteilnehmer mussten zwischen 18 und 55 Jahren alt sein und wurden mit dem SKID-Interview hinsichtlich möglicher DSM-IV Diagnosen untersucht. Ausschlusskriterien waren eine aktuelle oder eine lebenszeitliche Achse I Störung, ein Angehöriger ersten Grades mit einer psychotischen Erkrankung, eine neurologische Erkrankung, andere schwere körperliche Erkrankungen, erlittene Kopfverletzungen mit einer Bewusstlosigkeit >5 min, eine Lebenszeitdiagnose Alkoholmissbrauch oder Alkoholabhängigkeit, Sehbehinderungen, Adipositas (BMI>30), die Einnahme von zentralnervös-wirksamen Medikamenten. Weitere Ausschlusskriterien, um schwere nachteilige Wirkungen der Nikotingabe auszuschließen, waren: kardiovaskuläre Erkrankungen, Hypertonie, Ekzeme und atopische Dermatitis, schwere Nieren- und Leberfunktionsstörungen, Magen- und Zwölffingerdarmgeschwüre, Schilddrüsenüberfunktion, Phäochromozytom, insulinpflichtiger Diabetes, Überempfindlichkeit hinsichtlich Pflaster, Nikotin oder sonstiger Bestandteile von Pflastern. Um keine negativen Effekte durch Koffein-„Entzug“ zu erzeugen, durften die Probanden ihre gewohnte Menge an Kaffee, Tee oder anderen koffeinhaltigen Getränken trinken. Der

Koffeinkonsum der Teilnehmer wurde für beide Testsitzungen dokumentiert. Der bildungsabhängige Verbal-IQ wurde mit dem Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B, Lehrl, 1989) eingeschätzt. Die Zulassungen der lokalen Ethikkommission sowie des BfArMs wurden eingeholt und alle Probanden unterzeichneten eine schriftliche Einverständniserklärung. Die Studie wurde unter http://www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT01315002) registriert.

Bei allen Probanden wurde der Blutdruck gemessen, um eine Hypertonie auszuschließen (diastolischer Wert nicht größer als 90). An beiden Testtagen wurde ein Urin-Drogen-Test durchgeführt, um den akuten Konsum von Amphetaminen, Benzodiazepinen, Kokain, Cannabis und Opiaten auszuschließen. Das Nikotin wurde sodann in einem doppel-blinden, Placebo-kontrollierten, ausbalancierten Messwiederholungsdesign verabreicht, jeder Proband erhielt also einmal Nikotin und einmal Placebo. Die Nikotingabe erfolgte über ein Nikotinpflaster (NiQuitin Clear 7 mg, GlaxoSmithKline Deutschland), das Placebopflaster war ein sehr ähnlich aussehendes Pflaster (Fink and Walter GmbH Deutschland). Beide Pflaster wurden (für den Probanden nicht sichtbar) von einem Studienassistenten, der nicht Testleiter war, auf das rechte Schulterblatt geklebt. Drei Stunden nach Applikation der Pflaster begannen die Antisakkadentestungen. Am Ende einer Testsitzung wurden die Probanden aufgefordert, einzuschätzen, welches Pflaster sie bekommen hatten. Die Stimmung und körperliche Symptome wurden über visuelle Analogskalen erfasst. Den Probanden wurden die visuellen Analogskalen vor der Pflasterapplikation (=erster Messzeitpunkt) und drei Stunden nach der Pflasterapplikation (=zweiter Messzeitpunkt) vorgelegt. Folgende Items wurden mit den Analogskalen erfasst: „entspannt“, „munter“, „nervös“, „schläfrig“, „angenehm“, „unruhig“, „konzentriert“, „benommen“, „angeregt“, „aufmerksam“, „Mir gefällt die Wirkung der Substanz“, „Ich bin schlechter Stimmung“, „Mir ist übel“ und „Ich bin guter Stimmung“.

Antisakkadenparadigma. Die Augenbewegungen wurden per Elektrookulographie (EOG) aufgezeichnet. Alle Teilnehmer führten zunächst einen Block Prosakkaden, anschließend einen Block Antisakkaden aus. Die Teilnehmer saßen mit einem Abstand von 41 cm vor einem 17-Zoll Computermonitor. Zunächst erschien zufällig für 1000, 1500, 2000 oder 2500 ms ein weißes Fixationskreuz auf schwarzem Hintergrund. Anschließend wurde der Zielreiz, ein weißer Punkt, an einer von vier möglichen Positionen (±6°, ±12°) für 1000 ms gezeigt. Das Fixationskreuz erlosch beim Auftauchen des Zielreizes (Step-Paradigma). Es wurden 48 Prosakkaden- und 48 Antisakkadendurchgänge gezeigt. Die Reihenfolge der Zielreizpositionen war pseudorandomisiert. Vor dem Pro- und dem Antisakkadenblock erfolgten 5 Übungsdurchgänge. Die Instruktion für die Prosakkaden-Aufgabe erfolgte dahingehend, bei Erscheinen des Punktes so schnell und so genau wie möglich auf den Punkt zu schauen, die Instruktion für die Antisakkadenaufgabe lautete, bei Erscheinen des Punktes so schnell und so genau wie möglich auf die spiegelbildliche Stelle des Punktes zu schauen. Die Auswertung der Augenbewegungen erfolgte mittels Brain Vision Analyzer. Eine Sakkade wurde dabei angenommen, wenn eine Abweichung von mindestens 1.5 Standardabweichungen von der Baselineamplitude vorlag, anschließend erfolgte die Einstufung je nach Richtung der Sakkade als korrekte Prosakkade, Prosakkadenfehler, korrekte Antisakkade oder Antisakkadenfehler. Diese Einstufung wurde anschließend manuell überprüft und gegebenenfalls korrigiert, wobei dies verblindet, also ohne Kenntnis der experimentellen Bedingung (Placebo/Nikotin), erfolgte.

Als Outcome-Variablen wurden Prosakkadenfehler, Prosakkadenlatenz, ICV der Prosakkadenlatenz, Antisakkadenfehler, korrigierte Antisakkadenfehler, Antisakkadenlatenz und ICV der Antisakkadenlatenz berechnet. Mit ICV wird ein Maß für die Reaktionszeitvariabilität dargestellt (englisch: ICV= intra-individual coefficient of variation, deutsch: intraindividueller Variabilitätskoeffizient der Reaktionszeit). Der ICV berechnet sich

aus der Standardabweichung der Reaktionszeit geteilt durch die mittlere Reaktionszeit (Nandam et al., 2011). Somit stellt der ICV ein Maß für die Konsistenz von Reaktionen innerhalb eines Individuums dar. Ein Vorteil des ICVs als Maß gegenüber der reinen Standardabweichung ist, dass der ICV adjustiert für den Einfluss der Reaktionsgeschwindigkeit der jeweiligen Person ist und somit ein vorteilhafteres Variablitätsmaß darstellt.

Statistische Auswertung. Die statistischen Analysen wurden mit SPSS 18.0 (SPSS Inc., USA) durchgeführt. Um zu testen, ob Nikotin differentielle Effekte auf Probanden mit einer hohen Genauigkeit (d.h. einer niedrigen Antisakkadenfehlerrate) versus einer niedrigen Genauigkeit (d.h. mit einer hohen Antisakkadenfehlerrate) im Antisakkadenparadigma hat, wurden die Probanden mittels eines Mediansplits in Probanden mit niedriger bzw. hoher Leistung eingeteilt. Die Nikotineffekte auf die Sakkadenvariablen wurden mit einer 2×2×2×2 Kovarianzanalyse mit Messwiederholung analysiert mit Pflaster (Placebo, Nikotin) und Exzentrizität (6° Exzentrizität, 12° Exzentrizität) als Innersubjektfaktoren, sowie Gruppe (Probanden mit niedriger Leistung, Probanden mit hoher Leistung) und Reihenfolge (Nikotin zuerst, Placebo zuerst) als Zwischensubjektfaktoren. Verbal-IQ wurde als Kovariate in alle Analysen eingefügt, weil sich die Probandengruppen hinsichtlich dieser Variable unterschieden. Die Blindheit der Probanden für die Pflaster wurde mittels Chi-Quadrat-Test überprüft. Die Daten der visuellen Analogskalen wurden mittels einer 2×2×2 Varianzanalyse mit Messwiederholung mit Pflaster (Placebo, Nikotin) als Innersubjektfaktor, sowie Messzeitpunkt (erster Messzeitpunkt, zweiter Messzeitpunkt) und Gruppe (Probanden mit niedriger Leistung, Probanden mit hoher Leistung) als Zwischensubjektfaktoren analysiert. (Mittelwerte (M) und Standardabweichungen (SD) sind im Folgenden als M±SD dargestellt.)

Resultate. Dreißig Probanden nahmen an der Studie teil. Die explorative Analyse der Daten ergab, dass dabei ein Proband ein Ausreißer war; er lag hinsichtlich Antisakkadenfehlerrate

mehr als drei Standardabweichungen außerhalb des Interquartilsabstand des Boxplots und wurde deshalb bei den weiteren Analysen nicht berücksichtigt. Der Median der Antisakkadenfehlerrate betrug 25.91%. Der Ausschluss des Median-Probanden führte zu zwei gleich großen Probandengruppen mit jeweils N=14 Probanden. Die zwei Probandengruppen unterschieden sich nicht signifikant hinsichtlich Alter (Probanden mit niedriger Leistung: 30,50±11,71 Jahre, Probanden mit hoher Leistung: 25,71±5,21 Jahre), Bildungsjahren (Probanden mit niedriger Leistung: 16,21±2,75 Jahre, Probanden mit hoher Leistung: 16,29±0,73 Jahre), täglichem Koffeinkonsum (Probanden mit niedriger Leistung: 31,07±46,93 mg, Probanden mit hoher Leistung: 39,00±68,20 mg) und BMI (Probanden mit niedriger Leistung: 24,04±3,68, Probanden mit hoher Leistung: 23,97±1,53) (alle p-Werte>0,17). Allerdings unterschieden sie sich hinsichtlich des Verbal-IQs (Probanden mit niedriger Leistung: 126,07±18,21, Probanden mit hoher Leistung: 111,57±15,41) (p=0,03). Daher wurde der Verbal-IQ kovarianzanalytisch in den weiteren statistischen Analysen berücksichtigt.

Die Probanden konnten die Art der verabreichten Pflaster signifikant korrekt erraten: bei der jeweiligen ersten Sitzung rieten 69,2% der Probanden korrekt – dies war signifikant besser als Zufallsniveau ($\chi^2(1)=4,14$; p=0,042), bei der zweiten Sitzung rieten 92,3% der Probanden korrekt ($\chi^2(1)=16,45$; p=0,0001). Die Ergebnisse der visuellen Analogskalen zeigten, dass die Probanden sich unter Nikotin beim zweiten Messzeitpunkt unruhiger fühlten (p=0,017), ebenso war den Probanden unter Nikotin beim zweiten Messzeitpunkt übler (p=0,034). Tendenziell fühlten sich die Probanden unter Nikotin beim zweiten Messzeitpunkt auch weniger angenehm (p=0,050).

Die Korrekturrate der Antisakkadenfehler war generell hoch – dies zeigt, dass die Probanden die Antisakkadenaufgabe verstanden hatten und auch gewillt waren, sie korrekt auszuführen. Die Probandengruppen unterschieden sich nicht hinsichtlich dieser Variable (F(1,23)=0,80,

p=0,78): Probanden mit niedriger Leistung: 92,97±9,66% in der Placebobedingung, in der Nikotinbedingung 89,90±17,92%; Probanden mit hoher Leistung: 98,30±3,46% in der Placebobedingung, 91,74±17,72% in der Nikotinbedingung. Es gab keine weiteren Haupt- oder Interaktionseffekte hinsichtlich Antisakkadenfehler-Korrekturrate (alle p-Werte>0,35).

Die explorative Datenanalyse ergab, dass es so gut wie keine Varianz innerhalb der Prosakkadenfehlerrate gab, die Probanden machten so gut wie gar keine Prosakkadenfehler. Daher wurde diese Variable von weiteren Analysen ausgeschlossen. Es gab weder einen Haupteffekt des Faktors Reihenfolge (Nikotin zuerst, Placebo zuerst) noch Interaktionseffekte mit dem Faktor Reihenfolge (alle p-Werte>0,14). Es gab keine Effekte der Nikotingabe auf die Prosakkadenlatenz (alle p-Werte>0,18). Für den ICV der Prosakkadenlatenz fand sich ein Trend für einen Haupteffekt des Pflasters: unter Nikotin war die Variabilität der Prosakkadenlatenzen geringer ($F(1,23)=3,78$, $p=0,064$, $\eta_p^2=0,14$). Es gab keine weiteren Haupt- oder Interaktionseffekte für diese Variable (alle p-Werte>0,15). Aufgrund der Mediansplit-Prozedur gab es einen signifikanten Haupteffekt von Gruppe für die Antisakkadenfehlerrate: die Probanden mit der niedrigen Leistung machten mehr Antisakkadenfehler als die Probanden mit der hohen Leistung ($F(1,23)=34,93$, $p=5\times10^{-7}$, $\eta_p^2=0,60$). Für die Antisakkadenfehlerrate gab es keinen Haupteffekt des Pflasters ($F(1,23)=1,06$, $p=0,31$). Die aufgestellte Hypothese bestätigte sich teilweise: es gab eine signifikante Pflaster×Gruppe Interaktion ($F(1,23)=6,45$, $p=0,018$, $\eta_p^2=0,14$): die Probanden mit schlechter Leistung machten weniger Fehler unter Nikotin als unter Placebo (post hoc Vergleich: $F(1,12)=6,83$, $p=0,023$, $\eta_p^2=0,36$), hingegen gab es bei den Probanden mit guter Leistung keinen Unterschied zwischen Placebo- und Nikotinsitzung (post hoc Vergleich: $F(1,12)=0,30$, $p=0,596$, $\eta_p^2=0,02$). Es gab keine weiteren Haupt- oder Interaktionseffekte für diese Variable (alle p-Werte>0,08). Für die Antisakkadenlatenz fanden sich keine signifikanten Haupt- oder Interaktionseffekte bezüglich Exzentrizität und Nikotingabe (alle p-

Werte>0,09). Der ICV der Antisakkadenlatenz zeigte keinen signifikanten Haupteffekt des Pflasters ($F(1,23)=0,04$, $p=0,84$), aber es fand sich ein Trend für eine Pflaster×Exzentrizität Interaktion ($F(1,23)=4,07$, $p=0,056$, $\eta_p^2=0,15$). Post-hoc Vergleiche zeigten, dass die Variabilität unter Nikotin für die 12° Exzentrizitätsbedingung vermindert wurde ($F(1,27)=4,52$, $p=0,043$, $\eta_p^2=0,14$), diese Reduktion der Reaktionszeitvariabilität fand sich nicht für die 6° Exzentrizitätsbedingung (($F(1,27)=0,03$, $p=0,87$, $\eta_p^2=0,001$). Des Weiteren gab es eine signifikante Interaktion der Faktoren Pflaster×Exzentrizität×Gruppe ($F(1,23)=5,39$, $p=0,029$, $\eta_p^2=0,19$). Post hoc Vergleiche zeigten, dass die Interaktion von Pflaster×Exzentrizität nur in den Probanden mit niedriger Leistung signifikant war ($F(1,11)=4,97$, $p=0,048$, $\eta_p^2=0,31$), nicht aber in den Probanden mit hoher Leistung ($F(1,11)=0,10$, $p=0,76$, $\eta_p^2=0,009$). Es gab keine weiteren Haupt- oder Interaktionseffekte (alle p-Werte>0,23).

Diskussion. In Studie 1 fanden sich keine signifikanten Assoziationen zwischen den *CHRFAM7A* Genkopien-/2-bp-Deletionspolymorphismen und der Antisakkadenleistung. Die Stichprobengröße war für molekulargenetische Standards relativ klein, dies schränkt die Aussagekraft der Studie ein. Kleine Geneffekte der *CHRFAM7A* Genkopien-/2-bp-Deletionspolymorphismen auf kognitive Leistungen können daher nicht ausgeschlossen werden. Um diese mögliche Effekte sichtbar zu machen, müssten allerdings sehr große Stichproben getestet werden, d.h. bei einem angenommenen kleine Effekt mit einer Effektstärke von d=0,2 und einer Teststärke (Power) von über 80% müssten über 600 Probanden gemessen werden. Die Durchführung einer Multi-Center-Studie wäre hierfür eine realistische Möglichkeit, um diese große Probandenanzahl zu erreichen.

Es ist auch denkbar, dass die *CHRFAM7A*-Polymorphismen spezifische Effekte auf durch den Hippocampus vermittelte kognitive Funktionen haben, da die bisherigen Effekte von *CHRFAM7A* auf episodisches Gedächtnis (Dempster et al., 2006) und auf die P50-

Suppression (Raux et al., 2002) beide durch den Hippocampus vermittelt werden. Nichtsdestotrotz stellt die Antisakkadenaufgabe einen Schizophrenie-Endophänotyp mit hoher Erblichkeit dar, daher kann das Antisakkadenparadigma zur weiteren Aufklärung genetischer Unterschiede dienen, die interindividuelle Unterschiede in der Leistung erklären.

Zukünftige Studien sollten neben Varianten in den *CHRNA7*- und *CHRFAM7A*-Genen auch andere cholinerge Polymorphismen im Zusammenhang mit Antisakkaden untersuchen, wie z.B. Polymorphismen in den α4β2 nikotinergen Acetylcholinrezeptoren. Neben den α7 nikotinergen Acetylcholinrezeptoren, stellt der α4β2 nikotinerge Acetylcholinrezeptor-Subtypus den Rezeptorsubtypus dar, der am häufigsten im Gehirn vorkommt (Boess et al., 2007). Daher ist es möglich, dass auch genetische Varianten im α4β2 nAChR interindividuelle Unterschiede in der Antisakkadenleistung erklären könnten. Diese Erweiterung auf andere Polymorphismen legt auch eine kürzlich publizierte Studie an 534 Probanden nahe, wonach die Antisakkadenleistung nicht mit dem *CHRNA7*-Gen assoziiert ist (Greenwood et al., 2011). Insoweit kämen Polymorphismen in *CHRNA4* und *CHRNB2* in Betracht, um sie hinsichtlich ihres möglichen Einflusses auf die Antisakkadenleistung zu untersuchen.

Studie 2 zeigte, dass Nikotin die Antisakkadenfehlerrate bei Probanden mit einem niedrigen Leistungsniveau verringert, hingegen hatte Nikotin bei Probanden mit einem hohen Leistungsniveau keinen Effekt auf die Antisakkadenfehler. Außerdem verringerte Nikotin tendenziell bei allen Probanden die Variabilität der Prosakkadenlatenzen. Schließlich gab es noch einen weiteren differentiellen Effekt der Nikotingabe: Nikotin verringerte die Reaktionszeitvariabilität der Antisakkaden in der 12° Exzentrizitätsbedingung nur bei Probanden mit niedrigem Leistungsniveau, nicht aber bei Probanden mit hohem Leistungsniveau. Die Ergebnisse von Studie 2 bestätigen das erwähnte Modell von Newhouse und Kollegen (2004), das differentielle Wirkungen von Nikotin abhängig vom anfänglichen

Leistungsniveau annimmt. Allerdings wurden in Studie 2 – anders als das Modell hätte erwarten lassen – keine nachteiligen Wirkungen des Nikotins bei Probanden mit hohem Leistungsniveau gefunden. Möglicherweise war die Nikotindosis nicht hoch genug um eine beeinträchtigende Wirkung bei Probanden mit hohem Leistungsniveau zu entfalten. Eine höhere Nikotindosis könnte diesen Effekt herbeiführen und würde außerdem möglicherweise zu einer noch stärkeren Verbesserung bei Probanden mit niedrigem Leistungsniveau führen.

Die Wirkung von Nikotin auf Variablen, die Reaktionszeitvariabilität abbilden, zeigt, dass diese Variablen sensitiv auf eine cholinerge Stimulation reagieren und Nikotin zu konsistenteren Reaktionen führt. In zukünftigen Studien mit cholinergen Agonisten sollten daher Variablen der Reaktionszeitvariabilität berücksichtigt werden. Studie 2 zeigt auch, dass klinische Prüfstudien, die die Wirkung von neuen cholinergen Agonisten zur Behandlung von kognitiven Defiziten testen, das anfängliche Leistungsniveau der Testpersonen berücksichtigen sollten. Die bisherige Strategie, möglichst ein Medikament für die Gesamtgruppe von Patienten eines Störungsbildes zu entwickeln, muss möglicherweise verfeinert werden. Es ist denkbar, dass lediglich eine Subgruppe von Patienten von einem bestimmten Medikament profitiert. Eine Idee wäre daher, Patienten bei einer Medikamentenprüfstudie in eine Gruppe von Patienten mit keinen oder nur leichten kognitiven Einschränkungen und in eine Gruppe mit persistierenden schwereren Einschränkungen einzuteilen. Hinweise darauf, dass diese Strategie erfolgreich sein könnte, gibt auch eine Studie von Larrison-Faucher et al. (2004), die zeigte, dass Nikotin nur in denjenigen Schizophrenie-Patienten zu Verbesserungen in der Antisakkadenaufgabe führte, die bei dieser Aufgabe beeinträchtigt waren.

Die Aussagekraft von Studie 2 ist insoweit eingeschränkt, als dass die Probanden die Art des Pflasters (Placebo/Nikotin) erkennen konnten. Daher sollten zukünftige Studien versuchen, die Gewährleistung der Doppelblindheit zu verbessern. Dies könnte durch die zusätzliche

Verwendung einer Capsaicin-Salbe zum Placebopflaster geschehen, um hierdurch das Jucken des Nikotinpflasters nachzuahmen und die Art des Pflasters besser zu verdecken. Dies wurde in einer kürzlich publizierten Nikotinstudie von Wignall und de Wit (2011) praktiziert. Allerdings haben Wignall und de Wit ihre Probanden leider nicht zur vermuteten Art des Pflasters befragt, sodass unklar bleibt, ob die Capsaicin-Salbe tatsächlich zur besseren Verblindung beitragen konnte. Nichtraucher verspüren nämlich oftmals Nebenwirkungen durch die Nikotingabe. Studie 2 konnte insoweit zeigen, dass diejenigen Probanden, die zuverlässig unter Nikotin, nicht aber unter Placebo Nebenwirkungen verspürten, das Nikotinpflaster richtig identifizieren konnten. Alle Nebenwirkungen durch eine Nikotingabe wird man in zukünftigen Studien wahrscheinlich nicht vermeiden können. Man könnte allerdings versuchen, Nebenwirkungen wie z.B. Übelkeit zu minimieren, indem man zusätzlich zur Nikotingabe ein Medikament verabreicht, das gegen die Übelkeit wirkt (z.B. eine Kapsel) und dementsprechend zusätzlich zur Placebogabe eine Placebokapsel verabreicht.

Zusammenfassend lässt sich sagen, dass in der vorliegenden Arbeit kein Einfluss von Polymorphismen im *CHRFAM7A*-Gen auf die Antisakkadenleistung gefunden wurde. Jedoch konnte gezeigt werden, dass interindividuelle Unterschiede im anfänglichen Antisakkadenleistungsniveau das Ansprechen auf eine cholinerge Stimulation beeinflussen. Zukünftige Studie sollten auch die Kombination aus genetischem Forschungsansatz und pharmakopsychologischem Experiment in pharmakogenetischen Untersuchungen verfolgen, um so zum besseren Verständnis beizutragen, welche genetischen Faktoren prädiktiv für eine cholinerge Wirkung sind.

Abbreviations

5-HT	serotonin
5-HTT	serotonin transporter
5-HTTLPR	serotonin transporter-linked polymorphic region
ACh	acetylcholine
AChEI	acetylcholinesterase inhibitor
AChR	acetylcholine receptor
AD	Alzheimer's disease
ADHD	attention deficit/hyperactivity disorder
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ANT	attention network test
APOE	apolipoprotein E gene
ASRS	Adult ADHD Self-Report Scale
BfArM	German Federal Institute for Drugs and Medical Devices
BMI	body mass index
BOLD	blood oxygen level-dependent response
Ca^{2+}	calcium ions
CGI	Clinical Global Impressions scale
CHRFAM7A	hybrid gene, fusion of *CHRNA7* (exons 5-10) and *FAM7A* (exons A-E)
CHRNA1	α1 nicotinic acetylcholine receptor sub-unit gene
CHRNA2	α2 nicotinic acetylcholine receptor sub-unit gene
CHRNA3	α3 nicotinic acetylcholine receptor sub-unit gene
CHRNA4	α4 nicotinic acetylcholine receptor sub-unit gene

CHRNA5	α5 nicotinic acetylcholine receptor sub-unit gene
CHRNA7	α7 nicotinic acetylcholine receptor sub-unit gene
CHRNB2	β2 nicotinic acetylcholine receptor sub-unit gene
CHRNB4	β4 nicotinic acetylcholine receptor sub-unit gene
CNS	central nervous system
CNV	copy number variation
COGS	Consortium on the Genetics of Schizophrenia
COMT	Catechol-O-methyltransferase gene
CPT	Continuous Performance Test
CRHR1	corticotropin releasing hormone receptor 1 gene
DA	dopamine
DAT	dopamine transporter
DAT1	dopamine active transporter 1 gene (also known as SLC6A3)
DISC1	disrupted in schizophrenia 1 gene
DLPFC	dorsolateral prefrontal cortex
DMXB-A	3-(2,4-dimethoxybenzylidene) anabaseine (also known as GTS-21)
DNA	deoxyribonucleic acid
DRD2	dopamine receptor D2 gene
DRD3	dopamine receptor D3 gene
DRD4	dopamine receptor D4 gene
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
EEG	electroencephalography
EOG	electroocculography
EPQ-R	Eysenck Personality Questionnaire – Revised
ERBB4	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian) gene, receptor tyrosine-protein kinase erbB-4 gene

ERP	event-related potential
FAM7A	family with sequence similarity 7A gene
FEF	frontal eye fields
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GLU	glutamate
GRIK4	glutamate receptor, ionotropic, kainate 4 gene
HEOG	horizontal electroocculogram
HPA axis	hypothalamic-pituitary-adrenal axis
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A gene
ICC	intraclass correlations
ICV	intra-individual coefficient of variation
IQ	intelligence quotient
LTD	long-term depression
LTP	long-term potentiation
mAChR	muscarinic acetylcholine receptor
MFFT	Matching Familiar Figures Test
MMN	mismatch negativity
mRNA	messenger ribonucleic acid
MWT-B	Mehrfachwahl-Wortschatz-Intelligenztest, a standardized German vocabulary test
NA	noradrenalin
Na^+	sodium ions
nAChR	nicotinic acetylcholine receptor
NRG1	neuregulin 1 gene
OCI	Obsessive-Compulsive Inventory

PD	Parkinson's disease
PET	positron emission tomography
PFC	prefrontal cortex
PPI	prepulse inhibition
PRODH	proline dehydrogenase (oxidase) 1 gene
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
RELN	reelin gene
RISC	Rust Inventory of Schizotypal Cognitions
RT	reaction time, response time
SC	superior colliculus
SCID	Structured Clinical Interview for DSM-IV Disorders
SES	socio-economic status
SLC18A1	solute carrier family 18 (vesicular monoamine), member 1 gene
SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4, serotonin transporter gene
SNP	single nucleotide polymorphism
SPEM	smooth pursuit eye movement
SSRT	stop-signal reaction time
STDP	spike-timing-dependent plasticity
SWM	spatial working memory
THC	tetrahydrocannabinol
TOL	Tower of London test
TPH1	tryptophan hydroxylase 1 gene
TPH2	tryptophan hydroxylase 2 gene
VAS	visual analogue scale
VEOG	vertical electroocculogram

VTA	ventral tegmental area
WCST	Wisconsin card sorting test
WDYWS	“Why do young women smoke?” sample
WHO	World Health Organization

Glossary

Term	Definition and reference
allele	An alternative form of a gene at a locus. (Plomin et al., 2001)
agonist	Drugs that increase the effectiveness of neurotransmission at a particular receptor are called agonists for that receptor. (Kolb & Wishaw, 2003)
antagonist	Drugs that decrease the effectiveness of neurotransmission at a particular receptor are called antagonists for that receptor. (Kolb & Wishaw, 2003)
base pair (bp)	One step in the spiral staircase of the double helix of DNA, consisting of adenine (A) bonded to thymine (T) or Cytosine (C) bonded to guanine (G). (Plomin et al., 2001)
copy number variation (CNV)	A copy number variation (CNV) is when the number of copies of a particular gene varies from one individual to the next. (National Institutes of Health, National Human Genome Research Institute, USA, 2011)
deletion	A deletion is a type of mutation involving the loss of genetic material. It can be small, involving a single missing DNA base pair, or large, involving a piece of a chromosome. (National Institutes of Health, National Human Genome Research Institute, USA, 2011)
DNA (deoxyribonucleic acid)	The double-stranded molecule that encodes genetic information. The two strands are held together by hydrogen bonds between two of the four bases, with adenine bonded to thymine and cytosine bonded to guanine. (Plomin et al., 2001)
electroencephalography (EEG)	A technique used to noninvasively measure electrical brain activity via sensors placed on the scalp. EEG measures volume conduction currents from apical dendrites of post synaptic cortical pyramidal cells. The electrical signals recorded by the scalp sensors result from the summation of the coordinated electrical activity of thousands of neurons in a given region. (Ettinger (Ed.) & Klein (Ed.) 2008, Glossary, *Brain Cogn 68*)

electrooculography (EOG)	Method for measuring eye movements based on changes in the electrostatic field with changes in the concurrent changes in eye position as the eyes rotate in the orbit. The resulting electrical potential differences are measured using skin electrodes placed around the eye. (Ettinger (Ed.) & Klein (Ed.) 2008, Glossary, *Brain Cogn 68*)
endophenotype	A biobehavioral characteristic that appears to reflect the action of genes predisposing an individual to a specific disorder even in the absence of diagnosable pathology. As a measurable, reliable manifestation of genetic risk for a disorder, an endophenotype: (1) is associated with an illness, (2) is heritable, (3) shows trait-like properties, (4) co-segregates with illness in the family, and (5) identifies individuals at increased genetic risk for the disorder. (Ettinger (Ed.) & Klein (Ed.) 2008, Glossary, *Brain Cogn 68*)
epistasis	Nonadditive interaction between genes at different loci. The effect of one gene depends on that of another. (Plomin et al., 2001)
exon	DNA sequence transcribed into messenger RNA and translated into protein. (Compare with intron.) (Plomin et al., 2001)
functional magnetic resonance imaging (fMRI)	fMRI is a neuroimaging method that allows in-vivo measurements of blood oxygen level dependent (BOLD) changes in the vasculature that supports neuronal activity. While a subject lies inside a magnetic bore performing a task, magnetic resonance pulse sequences are used that allow the relative changes in oxygenated and deoxygenated hemoglobin during neuronal activity to be measured localizing brain regions that support task performance. (Ettinger (Ed.) & Klein (Ed.) 2008, Glossary, *Brain Cogn 68*)
gene	The basic unit of inheritance. A sequence of DNA bases that codes for a particular product. Includes DNA sequences that regulate transcription. (Plomin et al., 2001)
genetic association study	A class of genetic research designs whose goal is to test whether a genetic variant (a particular allele, genotype, or haplotype of a polymorphism) is associated with a particular disease or trait. If association is present, the variant will be seen in an affected individual more often than expected by chance. Genetic association strategies include case-control, family-based, quantitative trait loci (QTL), and genome wide association studies. (Ettinger (Ed.) & Klein (Ed.) 2008, Glossary, *Brain Cogn 68*)

genetic polymorphism	A locus with two or more alleles. Greek for "multiple forms". (Plomin et al., 2001)
haplotype	A haplotype is a set of DNA variations, or polymorphisms, that tend to be inherited together. A haplotype can refer to a combination of alleles or to a set of single nucleotide polymorphisms (SNPs) found on the same chromosome. (National Institutes of Health, National Human Genome Research Institute, USA, 2011)
Hardy-Weinberg-equilibrium	Allelic and genotypic frequencies remain the same generation after generation in the absence of forces, such as natural selection, that change frequencies. If a two-allele locus is in Hardy-Weinberg-equilibrium, the frequency of genotypes is $p^2 + 2pq + q^2$, where p and q are the frequencies of the two alleles. (Plomin et al., 2001)
homologous recombination	Homologous recombination is a type of genetic recombination that occurs during meiosis (the formation of egg and sperm cells). Paired chromosomes from the male and female parent align so that similar DNA sequences from the paired chromosomes cross over each other. Crossing over results in a shuffling of genetic material and is an important cause of the genetic variation seen among offspring. (National Institutes of Health, National Human Genome Research Institute, USA, 2011)
infrared oculography	Method for measuring eye movements based on the reflection of infrared light illumination by the border of the sclera and the pupil (called the "limbus") or by the depth of the pupil. The reflection is measured using infrared photodetectors placed with the source of infrared light into goggles. (Ettinger (Ed.) & Klein (Ed.) 2008, Glossary, *Brain Cogn 68*)
intron	DNA sequence within a gene that is transcribed into messenger RNA but spliced out before translation into protein. (Compare with exon.) (Plomin et al., 2001)
inversion	An inversion polymorphism describes a genetic polymorphism which consists of a duplicated segment of a gene which is in opposite orientation to the DNA strand. (Makoff & Flomen, 2009)
linkage	Close proximity of loci on a chromosome. Linkage is an exception to Mendel's second law of independent assortment because closely linked loci are inherited together instead of being inherited independently. (Plomin et al., 2001)

linkage disequilibrium (LD)	The nonrandom association between two or more alleles such that certain combinations of alleles are more likely to occur together on a chromosome than other combinations of alleles. (The American Heritage Medical Dictionary, 2007)
locus (plural, loci)	The site of a specific gene on a chromosome. Latin for "place". (Plomin et al., 2001)
microsatellite polymorphism	Also known as simple sequence repeat marker. Two, three, or four base pairs are repeated at a particular locus. The number of repeats at each locus differs among individuals. For example, a microsatellite polymorphism might have three alleles, in which the two-base sequence C-G repeats 14, 15, or 16 times. (Plomin et al., 2001)
mRNA (messenger RNA)	Processed RNA that leaves the nucleus of the cell and serves as a template for protein synthesis in the cell body. (Plomin et al., 2001)
mutation	A heritable change in DNA base pair sequences. (Plomin et al., 2001)
nucleobase (also: base)	Basic building blocks of DNA and RNA. There are four DNA bases: adenine (A), thymine (T), guanine (G), and cytosine (C). As a result of the structural properties of these bases, A always pairs with T, and G always pairs with C. (Plomin et al., 2001)
positron emission tomography (PET)	PET is a neuroimaging method. Either a small amount of water, containing radioactive molecules to label it, is injected into the bloodstream of the subject, or a gas containing the radioactive molecule is inhaled. Positrons from the radioactivity are released, they collide with electrons in the brain, and photons are produced (i.e gamma ray), this gamma ray exits the head and is recorded by pairs of radiation detectors. Active Areas of the brain will use more blood and thus will emit more photons. On PET scan images, differences are usually portrayed by a color gradient with active areas being depicted in yellows and reds, and less active areas depicted in greens and blues. It is also possible to record a receptor PET with a radioactive tracer binding to specific brain receptors in order to measure receptor distribution and activity. (Kolb & Wishaw, 2003)
promoter	A promoter is a sequence of DNA needed to turn a gene on or off. The process of transcription is initiated at the promoter. Usually found near the beginning of a gene, the promoter has a binding site for the enzyme used to make a messenger RNA (mRNA) molecule. (National Institutes of Health, National Human Genome Research Institute, USA, 2011)

single nucleotide polymorphism (SNP)	A single-base mutation. This single nucleotide change may or may not alter the function of the relevant protein depending on whether the affected mRNA triplet (codon) results in a different amino acid. (Plomin et al., 2001)
splicing	Splicing is a modification of the RNA after transcription, in which introns are removed (i.e. spliced out) and exons are joined (i.e. spliced back together). (Plomin et al., 2001)
transcription(al) factor	A transcription factor is a protein that binds to sequences of DNA adjacent to genes, thereby influencing transcription either positively or negatively. Thus, transcription factors influence the rate at which genes produce proteins. In some cases introns regulate gene transcription. (Latchman, 1997; Plomin et al., 2001)

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