A REPEATED AMPHETAMINE MODEL OF IMPAIRED ATTENTION IN SCHIZOPHRENIA.

by

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Abstract

Cognitive impairments, including deficits in attention processing, represent major and central elements of schizophrenic symptoms. First- and second-generation antipsychotic drugs can effectively mitigate the florid symptoms of psychosis. However, treating schizophrenia's cognitive deficits remains problematic and has met with limited success. Evidence indicates that the basal forebrain cholinergic system (BFCS) is an essential component of the neuronal circuitry involved in mediating attention processing attention processing- an important aspect of cognition. The present thesis is based on the core hypothesis that cholinergic dysregulation contributes to the cognitive impairments associated with schizophrenic symptoms. Using a repeated-amphetamine (AMPH) rat model of schizophrenia, the following main hypotheses are tested:

1) Repeated, escalating AMPH administration, followed by 'AMPH-challenges' at previously innocuous doses, results in performance impairments on a task that measures sustained attention.
2) The consequences of repeated-AMPH administration and subsequent challenge dosing in task-performing animals include dysregulated cortical cholinergic transmission.
3) In AMPH-pretreated animals performing a sustained attention task; sub-chronic, low-dose administration of antipsychotic drugs will attenuate performance impairments.

The present findings provide evidence for aberrant regulation of the basal forebrain cholinergic system and impaired sustained attention processing in a repeated-AMPH model of schizophrenia. Specifically, pretreatment with AMPH resulted in markedly attenuated performance associated cortical cholinergic

transmission. This effect was evident only under the condition of task performance, and was not apparent in passive, non-performing animals. Cholinergic abnormalities were found to actually precede task onset, indicating that they contributed to rather than resulted from impaired performance. In addition, low doses of commonly prescribed first- and second-generation antipsychotic drugs were found to attenuate these attentional impairments effectively, although the effects on cortical cholinergic transmission in task-performing animals remain speculative. The present data are consistent with the hypothesis that the basal forebrain cholinergic system represents a principle component in the neuronal dysregulation mediating schizophrenia's cognitive impairments. Expanding upon this hypothesis, the present data elucidate the dynamic nature of this dysregulation in response to different stimulus environments. Collectively, these experiments demonstrate the potential usefulness of this procedure for modeling aspects of impaired cognition in schizophrenia and may serve as a potential starting point for pre-clinical efforts aimed at discovering and developing novel, pro-cognitive drugs to improve the cognitive deficits of schizophrenia.

CHAPTER 1

INTRODUCTION

1.1 Why study impaired cognition in schizophrenia?

Schizophrenia is a severely debilitating neuropsychiatric disorder whose essential characteristics include hallucinations, delusions, paranoia, inappropriate affect and cognitive deficits. The term 'cognitive deficits' describes functional deficiencies or reduced capacities for cognitive operations that span multiple domains, including attention, mnemonic processing, and verbal memory. Cognitive impairments are persistent, are present in the majority of schizophrenics (between 55-85%), and predict functional outcomes more accurately that the severity of psychosis (Green 1996; Keefe, Eesley *et al*. 2005). Cognitive deficits are considered to be elemental 'state-characteristics' and are conceptualized as predisposing factors for disease onset rather than the secondary consequence of florid symptoms (Green and Braff 2001; Heaton, Gladsjo *et al*. 2001). Cognitive impairments can be detected in children at risk for psychosis; they become more pronounced during the disease prodrome, and worsen during first-break psychosis. Thereafter, the severity of impaired cognition either remains constant or further deteriorates- even in the face of illness phases marked by substantial improvements in the severity of psychosis (Heaton, Gladsjo *et al*. 2001). Presently, the American Psychiatric Association defines the primary treatment goals for persons with schizophrenia as the reduction of psychotic symptoms and the prevention of injurious behavior (APA

2006). In parallel, the majority of preclinical research efforts geared towards antipsychotic drug discovery to date, have focused on the determination antipsychotic mechanisms for the alleviation of florid psychotic symptoms. Despite Kraepelin's early emphasis on the cognitive components of schizophrenia, efforts directed at determining cognition-enhancing treatment strategies for the disease have been initiated only recently (Green and Braff 2001; Green, Nuechterlein *et al*. 2004). The pervasiveness of cognitive impairments and their impact on multiple functional domains necessitates the development and application of novel, pro-cognitive drugs targeted explicitly towards improving cognition in schizophrenia.

Impaired attention processing is evident in schizophrenia and represents a prime target of therapeutic intervention for several reasons discussed below. The following paragraphs will define attention processing, review the role of impaired attention in schizophrenia, and describe the application basic neuroscientific techniques, including animal models, towards the problem of schizophrenia. Subsequently, the relevant neurobiological components of attention and schizophrenia will be addressed. I will then review the currently available treatments for schizophrenia and discuss their mechanisms of action. Finally, the discussion will describe how this body of knowledge can be applied towards an animal model of the cognitive deficits in schizophrenia.

1.2 What is attention?

Attention is defined as the ability to detect, select and process relevant stimuli while filtering out irrelevant stimuli. Attention is a non-unitary construct that

involves multiple processes and capacities comprised of several interrelated components, including sustained attention, divided attention, and selective attention. Experiments assessing sustained attention in humans typically measure a subject's ability to detect and report the occurrence of rarely and unpredictably occurring stimuli over an extended period of time. A subject's capacity to sustain attention is thought to be limited by multiple factors including finite attentional resources and limited processing capacities. During testing, attentional resources can be taxed or over extended through the manipulation of task parameters to increase cognitive demands and produce performance decrements. Such manipulations include the successive rather than simultaneous presentations of signal or non-signal events to necessitate cognitive switching, the variation of stimulus features (i.e. intensity or duration) to prevent the establishment of detection criteria, the randomization of signal or non-signal event types, the increased rate of stimulus presentations, and the variable timing of trial occurrences (Parasuraman 1986; Parasuraman 1987). Collectively, these manipulations are thought to extend the cognitive demands of task performance beyond those of simple stimulus detection by limiting the implementation of routine-based strategies, necessitating additional processing (i.e. symbolic or conditioned significance of signals), and imposing the guided allocation of cognitive resources (Sarter, Givens *et al*. 2001).

1.3 The role of impaired attention in schizophrenia

Attentional deficits in schizophrenia can be ascertained through clinical observation and have been characterized using a variety of attention tasks.

Impairments in attention have been reported consistently in patients with schizophrenia since the earliest accounts of the illness by Kraeplin and Bleurler (Hoenig 1983). Generally, schizophrenics perform approximately ~1-2 standard deviations worse than control groups on commonly used tests assessing attention, working memory and executive functioning (Heinrichs 2005). Attentional impairments can be accentuated during stressful or cognitively demanding situations and are indicative of heterogeneous impairments, including deficits in stimulus detection, selection and filtering (Cattapan-Ludewig, Hilti *et al*. 2005).

The clinical presentation of attentional deficits can be illustrated with the following examples: a 21 year old male diagnosed with schizophrenia noted apparent difficulties in stimulus detection and the allocation of attentional resources that contributed to academic difficulties: "...*it's like I'm in class and trying to pay attention to the prof…my mind goes elsewhere…and then I miss the point."* Alternatively a 28 year old female dual-diagnosed with schizophrenia and substance abuse disorder described apparent gating deficits that contributed to vocational difficulties "*...I was working behind the counter and then it was like all of a sudden, everything (got real loud) and it felt like it was all coming at me at once…I couldn't handle it and took off…"* (unpublished). Preclinical experiments have substantiated such anecdotal observations with data describing impairments in various aspects attention processing. For example, deficits in attention and related cognitive processes have been detected in nearly all varieties of the Continuous Performance Task (i.e. CPTX, CPT degraded, and

CPT continuous pairs) (van den Bosch, Rombouts *et al*. 1996; Elvevag, Weinberger *et al*. 2000; Cattapan-Ludewig, Hilti *et al*. 2005; Braff 1993;Javitt, Shelley *et al*. 2000). Continuous performance tasks require the subject to detect and respond to a rarely and unpredictably occurring target stimulus (or set of stimuli) while inhibiting responses to distracting, non-target stimuli. CPT performance is commonly used to assess sustained attention and its dependent measures (i.e. commissions, omissions and reaction time) are used to gauge a subject's ability to rapidly detect and select target stimuli and discriminate non-target stimuli (Rosvold, *et al*. 1956). Depending on the task-version, schizophrenics demonstrate impaired stimulus detection, increased incorrect responses to non-target stimuli, and delayed reaction times (references above). Importantly, and as will be discussed later, valid measures of sustained attention can be ascertained in animals through the use of tasks with demands that bear some analogy to those of the CPT (McGaughy and Sarter 1995; Robbins 2002; Bushnell 1998).

1.4 Challenges in the development pro-cognitive drugs for schizophrenia and alternative research approaches: *what to model*

Recent insights into the cognitive deficits of schizophrenia and current advancements in basic, preclinical neuroscience have not been paralleled by advancements in drug development. Hymen and Fenton have described a "translational bottleneck" which exists between clinical- and preclinical-science (Hyman and Fenton 2003). This bottleneck refers to the difficulties in shifting the recent advancements in the fundamental pharmacology, neural circuitry, and

psychology of schizophrenia into the discovery of novel pro-cognitive drugs for schizophrenia (Hyman and Fenton 2003). This is due, at least in part, to a lack of well-defined targets as a focus for pre-clinical research, the use of animal models incapable of detecting cognitive impairments or improvements, and attempts to model disease components that bear little consequence to improved functional outcomes in patients. Further contributing to this failure are unsuccessful experimental approaches based on 'holistic animal models' that attempt to reproduce the entire clinical syndrome. Such models target non-specific clinical endpoints (i.e. positive symptoms) (Nielsen, Lyon *et al*. 1983; Castner and Goldman-Rakic 2003), that are rooted in traditional psychiatric diagnostic tools (i.e. the DSM-IV; Kilts 2001). Alternatively, other models have attempted to replicate poorly understood aspects of the disease such as its etiology (Weiss and Feldon 2001; Meyer, Feldon *et al*. 2005). The primary goal of such experimentation has been the discovery of comprehensive 'monotherapies' for what are in fact, very complex and heterogeneous clinical entities. As an alternative, more suitable experimental objectives (such as specific cognitive components of the disease) can be derived from the domains of cognitive impairment set forth by the National Institute of Mental Health Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS). MATRICS has determined seven separable domains of cognitive impairment that are evident in schizophrenia: attention, speed of processing, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving (Nuechterlein, Barch *et al*. 2004). This system is based on the

fractionation of schizophrenia's symptoms into discrete elements that can be addressed with a high degree of precision and specificity. The determination of these cognitive factors represents a step towards the identification of appropriate targets that can be explored to facilitate the development of new drugs to enhance cognition in schizophrenia (Green, Nuechterlein *et al*. 2004).

The following experiments will focus on sustained attentional processing in an animal model of schizophrenia. Sustained attention processing in schizophrenia represents an optimal target of experimentation for several reasons. First, sustained attention processing is a well-defined cognitive construct that is essential for a variety of other cognitive operations and has been well characterized in schizophrenia. Second, sustained attentional processing is amenable to study in animals and thus, in animal models of the disease; attentional impairments can be reproduced and assessed within animals with high fidelity through the application of tasks that bear remarkable analogy to those used in humans. Third, sustained attention processing possesses a relatively well-defined neurobiology that serves to facilitate experimentation and to operate as a discernable target for the development of novel drugs. Lastly, measures of attention processing in humans predict 'real-life' vocational and interpersonal outcomes in patient populations (references above).

1.5 Measuring attention in rats

The present experiments utilize an operant sustained attention task that requires the animal to report rarely and unpredictably occurring signals of varied durations over an extended period of time and to discriminate signal- from non-

signal events. Although alternative measures of attention in rats are available (i.e. divided attention; Turchi and Sarter, 1997), the sustained attention task is utilized as a matter of experimental convenience as it requires less time for acquisition (~3 months). The focus of this thesis on sustained attetention does not imply that *only* sustained attention is disrupted in schizophrenia, but rather, represents an efficient way to study one important aspect of this illness. Validation of this task as a measure of sustained attention performance was based on experiments conducted by McGaughy and Sarter (1995) and Bushnell *et al*. (1997). Manipulations of task parameters described above (i.e. signal duration, inter-trial interval, background 'noise', and randomization of signal and non-signal events) are known to affect task performance in a manner analogous to human tasks. Complete task methodology, including animal shaping, is described in the methods section of each subsequent chapter. Briefly, utilizing an operant chamber equipped with two retractable levers, a houselight, a signal light and reward port animals are required to indicate the presence or absence of a signal by pressing the left lever for signal events and the right lever for non-signal events. Correct responses to signal trials (hits) and non-signal trials (correct rejections) are followed by the presentation of a water reward. Incorrect responses signal trials (misses) and non-signal trials (false alarms) are not rewarded. Half of all animals are trained using reverse response rules (e.g. the right lever scores hits, the left lever scores misses). Importantly, this task provides a direct measure of false alarms, or discrete claims by the subject for the presence of a signal, when in fact, no signal was presented. Each session

consists of 162 trials divided equally between signal and non-signal events to promote trial type unpredictability. Signal and non-signal trials occurred randomly with 9±3 s inter-trial intervals to limit the prediction of trial type or trial onset. Signals consist of single light flashes of variable duration (500 ms, 50 ms, 25 ms); durations are varied for the purpose of preventing the establishment of fixed detection criteria. As expected, the number of detected trials declines in concert with diminishing signal duration reflecting a vigilance decrement. It is important to note that chance-level performance of this task is characterized by uniform detection rates of 50% for all signal durations accompanied by 50% correct rejections. Performance criteria consists of at least 70% correct responses to all 500 ms signal-trials and to non-signal trials, with fewer than 25 omitted trials per session. Overall levels of attention performance are calculated using the Vigilance Index ($VI = [(h-f)/2*(h+f)-(h+f)^2]$). VI is derived from the Sensitivity Index described by Frey and Colliver (1973), but calculated based on the relative number of hits and false alarms rather than the probability of such occurrences. Additionally VI is used expressly for describing data from tasks that include discrete non-signal events whereas the SI is generally not. VI values range from-1 to 1, with a score of 1 indicating correct responses to 100% of attempted trials. A VI value of 0 indicates a complete inability to dissociate signal- from non-signal events, and reflects chance-level task performance.

1.6 The neurobiology of sustained attention processing in wellness and pathology

The goal of the following section is to describe the anatomical basis of the neurobiological systems involved in attention processing and to discuss hypotheses regarding the functions of these systems during normal functioning and under conditions of pathology, specifically in the context of schizophrenia. Hypotheses regarding the neurobiology of attention processing have been informed by data from multiple levels of analysis. Posner and Peterson described the 'anterior attention system' as a macro anatomical network consisting of frontal-parietal cortical regions that influence attention processing (Posner and Petersen 1990). This conceptualization has been supported by human data demonstrating that damage to frontal or parietal regions of the cortex results in impaired performance (i.e. decreased hits, augmented vigilance decrement and slowed reaction times) on tasks that measure sustained attention (Rueckert and Grafman 1998, Rueckert and Grafman 1996). Corroborating data are provided by neuroimaging studies using intact subjects performing sustained attention tasks (Hager, Volz *et al*. 1998). These data provide evidence for a distributed cortical attention network that is comprised of frontal-parietal regions and lateralize primarily in the right hemisphere.

Additional neuroimaging data, as well as neuro-pathological data from Alzheimer's patients, implicates subcortical structures in attention processing. These structures include those located in the ventral pallidum (Paus 1997). The basal forebrain cholinergic system (BFCS) is thought to be of particular importance in this regard (Sarter and Bruno 1997; Sarter, Hasselmo *et al*. 2005). The cholinergic basal forebrain is a crucial component of the neuronal circuitry

mediating attentional processing (Chiba, Bucci *et al*. 1995; Voytko 1996; Everitt and Robbins 1997; Sarter and Bruno 1997). The basal forebrain cholinergic system is the most anterior of the major ascending neuromodulatory systems and it projects to all areas and all layers of the cortex (Woolf 1991, see Figure 1.1). The neuroanatomical organization of the BFCS reflects its capacity to regulate information processing in all cortical areas, including frontal-parietal attentional networks, via the release of acetylcholine. In humans cortically projecting cholinergic neurons are situated along the medial wall of the globus pallidus within the horizontal and vertical limbs of the diagonal band. The BFCS and is comprised of cholinergic neurons in the nucleus basalis of Meynert, the substantia inominata and the magnocellular preoptic nucleus. Analogously in rodents, cortical cholinergic projections originate in the substantia inominata and nucleus basalis magnocellularis. These neurons are approximately 20-30μm in size and display a multi-polar morphology (Saper 1984).

Cortical cholinergic projections comprise two major pathways, one medial and one lateral (Saper 1984). The medial pathway arises in the medial septal nucleus, substantia innominata and the medial diagonal band. It courses dorsally along the genu of the corpus callosum and terminates primarily in the prefrontal and cingulated cortices and extends to the hippocampal formation via the fornix. The lateral pathway consists of those axons arising from neurons in the medial septum, diagonal band, and magnocellular preoptic nucleus and terminating primarily in the entorhinal and pyriform cortices. Cortically projecting neurons terminating in the prefrontal cortex (PFC) demonstrate a rough topographic

organization, with the most rostal neurons of the nMB projecting to the medial prefrontal regions, and neurons arising from medial and posterior areas terminating in lateral prefrontal regions. Collectively, these nuclei comprise the main source of cholinergic innervation of the cortex, with particularly dense innervation layers 1, 3, and 5. The earliest hypotheses of cholinergic functions based on these neuroanatomical data, and those describing the non-uniform distribution of cholinergic soma, described the basal forebrain cholinergic neurons as the rostral extension of a unitary 'reticular activating system' comprised also of brainstem nuclei and mediating general states of arousal. Notably, current evidence has determined that cholinergic neurons possess a modular organization and are arranged in longitudinally oriented, structurally distinct bands that have been hypothesized to possess the capacity to influence discrete cortical regions independent of one another (Zaborszky 2002).

1.7 Functions of the basal forebrain cholinergic system

Evidence indicates that the integrity of the basal forebrain cholinergic system is required for normal attentional operations and capacities. Experiments have demonstrated that selective depletion of cortical cholinergic inputs (produced by infusions of the selective immuno-toxin 192-IgG saporin) results in cortical cholinergic deaffrentation and impaired performance in animals performing a sustained attention task (McGaughy, Kaiser *et al*. 1996). These deficits manifest specifically with respect to decreased hits on signal trials. Intact performance of non-signal trials indicates that such impairments can not readily be described in terms of indiscriminant 'fundamental executive problems'

because the ability to process basic response contingencies remains intact. Instead the detection-specific characteristics of these impairments are indicative of impaired attentional capacities. Further evidence implicating cortical cholinergic transmission in attention processing has been derived from behavioral-dialysis experiments demonstrating augmented increases in cortical ACh during performance of tasks with explicit attentional demands, but not tasks that control for motor and reward retrieval aspects of performance (Arnold, Burk *et al*. 2002).

Neurophysiological data indicate that cortical cholinergic transmission biases cortical information processing towards sensory, rather than associational inputs (Hsieh, Cruikshank *et al*. 2000) and at the same time selectively inhibits responses to weak inputs while enhancing, or at least suppressing to a lesser degree, responses to strong inputs (Metherate and Ashe 1995). These actions are thought to enhance the 'signal to noise' ratio during stimulus processing, thus facilitating stimulus detection and discrimination.

1.8 Schizophrenia and cortical acetylcholine: the mesolimbic link

Cholinergic neurons in the basal forebrain are thought to be influenced by multiple input systems and are known to receive glutamatergic, GABAergic, noradrenergic, serotonergic, dopaminergic, and cholinergic innervations. These contacts are thought to arise from a variety of structures including the PFC, the amygdala (Zaborszky, Leranth *et al*. 1984), the NAC (Zaborszky and Cullinan 1992), the locus coeruleus (Zaborszky, Cullinan *et al*. 1993), the VTA (Gaykema and Zaborszky 1996), the dorsal raphe nucleus (Gasbarri, Sulli *et al*. 1999), and

the pedunculo pontine gigantocellular nucleus (Jones and Cuello 1989). To explain the individual and interacting contributions of each system in modulating the excitability of basal forebrain cholinergic neurons is beyond the scope this discussion and beyond the author's comprehension. Instead, the following paragraphs will focus primarily on the role of mesolimbic networks in modulating cortical cholinergic transmission. These systems have been selected as the focus of this discussion due to their putative role in the pathogenesis of schizophrenia, their neuroanatomical relationships to the basal forebrain cholinergic system, and their relevance to psychostimulant-based models of schizophrenia.

Dysregulated (i.e. hyperactive) mesolimbic dopamine transmission is considered a hallmark of schizophrenia and has been substantiated by neuroimaging studies in schizophrenic patients (including non-medicated, first-episode populations (Breier, Su *et al*. 1997; Laruelle, Abi-Dargham *et al*. 1999), and by data demonstrating the efficacy of dopamine antagonists for attenuating psychotic symptoms (described below). The precise contributions of abnormal mesolimbic dopamine transmission to the attentional impairments in schizophrenia remain unsettled. However, abnormal dopamine transmission in the nucleus accumbens has been hypothesized to mediate aberrant cortical cholinergic transmission and thus, may influence attentional processes and capacities.

Importantly, the mesolimbic dopamine system can influence cortical cholinergic transmission via multiple pathways. This modulation is thought to

occur either mono-synaptically via direct, presumably dopaminergic contacts on cholinergic neurons (Gaykema and Zaborszky 1996), or trans-synaptically via dopaminergic regulation of multiple output structures that synapse in the basal forebrain (i.e. the prefrontal cortex and nucleus accumbens). Of particular interest are the medium-spiny neurons arising in the accumbens shell and terminating in the basal forebrain. These projection neurons represent the majority of inputs into the basal forebrain (Zaborszky and Cullinan 1992) and are met with a high density of basal forebrain GABA receptors (Gao, Hornung *et al*. 1995). Medium-spiny projection neurons are likely GABAergic and are thought to influence the excitability of cholinergic neurons both directly via mono-synaptic connections (Zaborszky and Cullinan 1992), and trans-synaptically via the regulation of basal forebrain interneurons (Zaborszky, Cullinan *et al*. 1991).

Hypotheses regarding the contributions of NAC GABAergic transmission in regulating basal forebrain excitability have been formulated based on the capacity of systemic or intra-basalis administration of benzodiazepine receptor agonists or inverse agonists to modulate cortical cholinergic transmission in a bi-directional manner (Moore, Sarter *et al*. 1993; Moore, Sarter *et al*. 1995). Furthermore, additional experiments have demonstrated that the ability of benzodiazepine receptor partial inverse agonists to augment cortical cholinergic transmission is moderated by the administration of dopamine antagonists (Moore, Fadel *et al*. 1999). GABAergic inputs from the NAC are thought to converge with glutamatergic inputs from multiple sources, including reciprocal inputs from the prefrontal cortex, to regulate the excitability of basal forebrain

cholinergic neurons. Likewise, the effects of glutamatergic transmission in the basal forebrain have been explored. Intra-basalis infusions of glutamate antagonists or agonists have been shown to attenuate or augment cortical cholinergic transmission in response to food stimulus (Fadel, Sarter *et al*. 2001). Furthermore, experiments demonstrating that intra-basalis infusions of NMDA or APV (an NMDA antagonists) affected sustained attention performance in rats by increasing the number of false alarms, and decreased the number of hits, respectively (Turchi and Sarter 2001).

Despite anatomical evidence demonstrating direct dopaminergic projections from the VTA to cholinergic basal forebrain neurons, the influence of these projections on cortical cholinergic transmission remains poorly understood. Acute administration of AMPH increased dopamine transmission in the NAC by 700%, and augmented cortical cholinergic transmission by 150%. Curiously, these effects were unaltered by administration of D1 or D2 antagonists into the basal forebrain. However, intrabasalis infusions of glutamatergic antagonists or GABA agonists successfully attenuated AMPH-induced augmentations in cortical cholinergic transmission (Arnold, Fadel *et al*. 2001). Additional evidence suggests that activation of D2 receptors in the nucleus accumbens attenuates cortical cholinergic transmission following intra-accumbens administration of NMDA (Brooks and Bruno 2007). Thus dopamine is thought to exert its influence on cortical cholinergic transmission primarily via regulation of NAC output neurons- particularly on D2 receptors in the high-affinity state. Collectively, these data indicate that GABAergic projections from the NAC to the basal forebrain

may represent a neural substrate by which abnormal mesolimbic dopamine transmission can result in aberrant cortical cholinergic transmission, thus contributing to schizophrenic pathology. The ability of repeated-AMPH administration to produce psychotic like symptoms (discussed below) is thought to be mediated, at least in part, by this neural circuitry.

1.9 Evidence for abnormal cortical cholinergic transmission in schizophrenics

Converging lines of evidence have implicated abnormal cortical cholinergic transmission in the neurobiology of schizophrenia. Alterations in the distribution of nicotinic and muscarinic receptors in cortical regions of schizophrenic brains have been reliably demonstrated (Breese, Lee *et al*. 2000; Crook, Tomaskovic-Crook *et al*. 2001; Adams and Stevens 2007). The involvement of cholinergic transmission in the pathogenesis of schizophrenia has been further substantiated by genetic linkage studies showing abnormalities in the region of chromosome 15 coding for the α-7 nicotinic receptor in schizophrenics (Freedman, Coon *et al*. 1997). Abnormal cholinergic transmission in schizophrenia is thought to result from dysregulation rather than overt pathology. Neuropathological studies comparing post-mortem tissue of schizophrenics to that of control or Alzheimer's groups failed to demonstrate any gross pathological abnormalities of the basal forebrain cholinergic neurons of schizophrenic patients (el-Mallakh, Kirch *et al*. 1991). Furthermore, there does not appear to be a decreased enzymatic capacity for schizophrenics to synthesize acetylcholine (Powchik, Davidson *et al*. 1998), although data

examining the high-affinity choline transporter, known to be the rate limiting step in acetylcholine synthesis, is currently unavailable.

Pharmacological evidence also implicates dysregulated acetylcholine transmission in schizophrenia. Second-generation antipsychotic medications, including clozapine, have high-affinity antagonistic properties at muscarinic receptors and are metabolized into active compounds that affect cortical cholinergic transmission. It is thought that the ability of these drugs to modulate cortical cholinergic transmission may contribute to their therapeutic actions, particularly with regard to their moderate pro-cognitive effects (Li, Huang *et al*. 2005; Davies, Compton-Toth *et al*. 2005; Crook, Tomaskovic-Crook *et al*. 2001). Despite these findings, hard evidence of cortical cholinergic dysregulation in schizophrenics remains elusive due to technological limitations prohibiting the direct assessment of cholinergic transmission in humans. Multiple groups have put forth hypotheses regarding the precise nature of cortical cholinergic dysregulation in regards to specific disease states; the following discussion serves to describe two separate and opposing theories that address this matter. One theory purported by Tandon *et al.* (1991) suggested that cholinergic hyperactivity contributed to the affective and social symptoms in schizophrenia, whereas decreased cholinergic transmission resulted in the manifestation of florid psychosis. Support for these hypotheses was provided by data demonstrating the ability of acute administration of muscarinic antagonist to improve scores on a negative symptoms scale. Sarter *et al.* (2005) separately interpreted muscarinic down-regulation in schizophrenics as evidence of

cholinergic hyperactivity, but hypothesized that cortical cholinergic hyperactivity resulted in a breakdown of sensorimotor gating mechanisms that escalated into psychotic symptoms. This 'hyper-cholinergic' hypothesis of schizophrenic psychosis was corroborated by data indicating that chronic exposure to cholinomimetic agents (i.e. cholinesterase inhibitors) can potentially result in psychotic symptoms in humans and that these effects can be treated successfully with antipsychotic medications (Sarter, Nelson *et al*. 2005). Despite their differences, both of these hypotheses predict that drugs that 'normalize' cholinergic transmission will improve schizophrenic symptoms. Several studies have determined the effects of adjunct treatment with cholinesterase inhibitors (which results in increased extra-cellular concentrations of acetylcholine) on cognitive and psychotic symptoms in schizophrenics. By and large, the results of these studies have been negative, with only a few groups reporting non-significant trends for cognitive improvement and generally no improvement on the severity of psychosis (Bora, Veznedarolu *et al*. 2005; Buchanan, Summerfelt *et al*. 2003; Freudenreich, Herz *et al*. 2005; Mazeh, Zemishlani *et al*. 2006; Sharma, Reed *et al*. 2006). Importantly, many of these studies were conducted as open-label designs, with only a few being double-blind and placebo controlled. Moreover, these studies have frequently included heterogeneous subject populations including geriatric patients or patients with heterogeneous disease durations. Further complicating the interpretation of these data are those studies allowing the co-administration of anti-cholinergic agents (i.e. biperdine) or the primary administration of antipsychotics with cholinergic antagonist properties

(i.e. clozapine or chlorpromazine). Oddly, those studies that did not allow for anti-cholinergic drugs to be co-administered had primarily negative findings and those that permitted the use of anti-cholinergic agents reported the most trends for positive improvements. The outcomes of these studies should not be interpreted to indicate that cholinergic dysregulation is not involved in the pathogenesis of schizophrenia. Rather, it should be concluded that these data do not support the hypothesis that the cognitive impairments of schizophrenia can be treated with cholinesterase inhibitors.

1.10 Current treatments for schizophrenia

Presently, the available medications for schizophrenia are divided into two categories, first-generation drugs (FGAs, sometimes called typical antipsychotics) and second-generation drugs (SGAs, also referred to as atypical antipsychotics).The primary characteristics distinguishing these two classes of drugs are their relative propensities for inducing extrapyramidal side effects (i.e. parkinsonism and tardive dyskinesia) and their relative affinities for D2 and serotonin 5-HT_{2A} receptors (Seeman 2002). FGAs possess a higher affinity for D2, and SGAs possess a higher affinity for 5-HT_{2A} (Pretorius, Phillips *et al*. 2001). Both classes of drugs are lipophylic, readily cross the blood brain barrier, and are metabolized by cytochrome P450 enzymes (Linnet and Olesen 1997; Fang, McKay *et al*. 2001). The individual mechanisms of action for haloperidol and clozapine are discussed in more detail below.

1.11 First-generation drugs: focus on haloperidol

Haloperidol is a first-generation antipsychotic drug that is effective for the treatment of psychotic symptoms. The antipsychotic effects of haloperidol (and all first-generation drugs) are generally attributed to its antagonism of D2 receptors. Haloperidol is a potent D2 receptor antagonist with a D2 binding affinity 50 times greater than that of chlorpromazine (Lieberman 2006). D2 receptor occupancy produced by haloperidol is known to predict the clinical efficacy of first-generation drugs. Utilizing PET imaging in first-episode schizophrenic patients, Kapur and colleagues demonstrated that 65-70% of D2 occupancy is required for an optimal clinical response. Occupancy of ≥72% was found to abnormally augment prolactin levels and D2 receptor occupancy beyond 78% was found to induce extrapyramidal symptoms (Kapur, Zipursky *et al*. 2000). High affinity D2 receptors (D2 High) have garnered a good deal of attention recently, and are thought to be the primary target for first-generation drugs including haloperidol (Seeman, Schwarz *et al*. 2006). Haloperidol is known to affect other neurotransmitter systems and has been shown to be a low-affinity antagonist for all muscarinic receptor subtypes as well as to act as an antagonist for several classes of histamine receptors (Bymaster, Calligaro *et al*. 1996). Haloperidol is not known to affect cortical cholinergic transmission in passive, non-performing rats (Ichikawa, Dai *et al*. 2002), and has not been shown to alter the distribution of muscarinic or nicotinic receptors in the cortex (Terry, Gearhart *et al*. 2006).

The effect of first-generation antipsychotic drugs on impaired cognition in schizophrenia remains controversial. Only a few studies have shown that first-

generation drugs exert cognitive benefits schizophrenic patients, with the majority of experiments demonstrating no change in cognition or even an exacerbation of cognitive impairments (Saeedi, Remington *et al*. 2006). However, accurate interpretation of the available literature is impeded by multiple factors. First, much of the available data are confounded by the inclusion of dose-ranges that would, by modern standards, be categorized as high (e.g. >15 mg per day). The range of dosing represents critical design variable since administration of FGAs is thought to produce inverted U-shaped dose response patterns, with low-to-moderate doses resulting in cognitive improvements and higher doses producing impairments (Strauss, Lew *et al*. 1985). Furthermore, high-dose administration of first-generation drugs is known to increase the incidence and severity of extrapyramidal symptoms. Extrapyramidal symptoms may contribute to poor cognitive performance and are commonly treated with adjunct administration of anti-cholinergic agents (i.e. benztropines) that could further impair cognition (McGurk, Green *et al*. 2004). Additional concerns arising from basic design flaws (i.e. unequal group sizes, non-randomized drug assignments, lack of placebo control groups, lack of drug naïve groups, unequal proportion of male to female inclusion, inconsistent dose ranges, and variable illness durations) have severely hindered the interpretation of these data and have made accurately assessing the pro-cognitive effects of FGAs extremely difficult.

To address these issues and make a quantitative assessment of the available literature, Mishara and Goldberg (2004) conducted a meta-analysis of studies spanning almost 50 years to examine clinical studies comparing the

effects of FGAs with placebos or non-treatment conditions on various domains of cognition (e.g. attention, executive functions, memory, and others). The group determined effect sizes for experiments comparing first-generation drugs treatments with placebo, or non-medicated conditions. Based on calculations of 224 effect sizes from 36 studies, first-generation drugs were shown to improve cognition above levels seen in placebo groups. The collective Cohen's '*d*-score' of 0.22 was sufficient to surpass the criteria for a small effect size (Mishara and Goldberg 2004). The largest positive effects of treatment in these studies were observed on measures of attention, automatic processing, memory and perceptual processing. Not surprisingly, treatment with neuroleptics impaired motor functions on a variety of tasks (i.e. Perdue Pegboard, tapping task). Collectively, these results have provided evidence in support of the hypothesis that FGAs produce modest improvements across multiple domains of cognition. Importantly, these findings are not the result of experimental artifacts and are not limited to meta-analytic studies. Recently, several well controlled studies have extended these findings. Harvey, *et al.* (2005). performed experiments controlling for dosing and adjunct treatments found first-generation drugs improved multiple domains of cognitive impairment (Harvey, Rabinowitz *et al*. 2005) . Similar effects were demonstrated by Rollnik, *et al.* (2002) who found that over the course of 3 months, neurocognitive functioning in schizophrenics is moderately responsive both first- and second-generation drugs (Rollnik, Borsutzky *et al*. 2002). These data demonstrate the moderate pro-cognitive

effects of low doses of first-generation antipsychotics and indicate that the effects of FGAs on cognition warrant further investigation.

1.12 Second-generation drugs: Focus on clozapine

Clozapine is considered to be the prototypical second-generation antipsychotic drug based on its weaker affinity for D2 receptors and reduced risk for causing extrapyramidal symptoms. Clozapine's mechanisms of action are complex and not fully understood. Clozapine is generally considered to be a potent antagonist for certain receptor types including those for acetylcholine (all 5 muscarinic types and α-7nicotinic) and serotonin (5-$HT_{2a,}$ 5-$HT_{2c,}$ 5-$HT_{6,}$), and a weaker antagonist for others (D2 and α2). Its low propensity for inducing extrapyramidal side effect has been attributed to its lower affinity for D2 receptors and the high un-binding rate (K_{off}) of clozapine from dopamine receptors (Seeman 2002; Kapur and Seeman 2001). Studies examining clozapine's actions at muscarinic receptors have demonstrated both antagonistic and agonistic properties, including antagonism of M2, M3, and M5 receptors, and partial agonist actions at M1, M2, and M4 receptors. The degree to which clozapine functions as an muscarinic agonist or antagonist is thought to depend on a variety of factors such as high- or low- receptor density, the relative number of reserved receptors, and the type of preparation utilized *ex vivo* (clonal cell lines transfected with muscarinic receptors, or rat brain tissue preparations) (Kenakin, Bond *et al*. 1992). Additional factors affecting clozapine's possible effects include its metabolism into active moieties including N-desmethylclozapine (Li, Huang *et al*. 2005). Multiple *in vivo* microdialysis studies have characterized the effects of

clozapine on ACh in cortex, nucleus accumbens and striatum. Using triple-micro dialysis in the presence of a cholinesterase inhibitor, Parada, *et al.* (1997) demonstrated that clozapine augmented extra cellular ACh in a dose-response fashion in the all three areas of interest. These effects were largest in the PFC with the smallest increases shown in the striatum (Parada, Hernandez *et al*. 1997). Using cholinesterase free preparations, Ichikawa *et al.* (2002) demonstrated that clozapine increased in cortical ACh without affecting levels in the nucleus accumbens or striatum. The precise mechanisms underlying clozapine's ability to preferentially increase cortical ACh remain speculative; generally, these effects have been attributed to antagonism of M2 and M4 auto-receptors in the nucleus basalis magnocellularis, which projects to the cortex but not to the striatum or nucleus accumbens. Alternative explanations attribute these effects to increased cholinesterase density in accumbens and striatal regions relative to the cortex. The heightened density of striatal cholinesterase could result in the rapid hydrolysis of ACh, preventing any measurable increases from being detected. Previous hypotheses attributing these effects specifically to antagonism of muscarinic autoreceptors have not been supported by experiments demonstrating undiminished cholinergic transmission in the hippocampi of M2/M4 knock out mice following administration of second-generation drugs. Although notably, the ability of scopolamine to increase ACh via autoreceptor antagonism was markedly attenuated in these animals (Tzavara, Bymaster *et al*. 2006)). These results were taken to indicate that muscarinic auto-receptor antagonism is not necessary for second-generation

drugs to facilitate cholinergic transmission. Separate experiments utilized a variety of receptor antagonists (i.e. 5-HT2a, 5-HT2c, 5-HT6, and D2) to determine the contributions of antagonism of individual monoamine receptor types on ACh levels in the hippocampus (Shirazi-Southall, Rodriguez *et al*. 2002). Administration of several drugs produced only modest increases of ACh that did not parallel those of clozapine administration. These data indicate that clozapine's ability to increase ACh cannot be attributed to any singular mechanism, but may be the result of complex interactions of multiple receptors or some unknown mechanism. (Shirazi-Southall, Rodriguez *et al*. 2002). Although it remains difficult to conceptualize the specific interactions of clozapine on acetylcholine receptors, the ability of clozapine to preferentially increase cortical ACh while not affecting transmission in the striatum may account for a portion of clozapine's pro-cognitive effects (described below), as well as its low propensity to induce extra-pyramidal symptoms.

1.13 Comparing first- and second-generation drugs

Compared to the relatively small number of experiments that demonstrate improved cognition using first-generation treatments, evidence in support of the pro-cognitive effects of second-generation antipsychotics is more abundant. Multiple studies have determined that second-generation drugs (i.e. clozapine) produce measurable cognitive benefits in schizophrenia, including improved attention, verbal fluency and executive functions (for review see: Meltzer and McGurk 1999). However, the presumed superiority of second-generation drugs over first-generation drugs remains a topic of contention. Many of the studies

making direct comparisons between first- and second-generation drugs suffer from many of the same methodological weaknesses addressed above. The major limitation of these studies remains the use of inappropriately high doses of FGAs relative to SGA doses. In addition, such studies are seldom able to dissociate the benefits of symptomatic improvement, the reduced occurrence of extrapyramidal symptoms, or the avoidance of adjunct anti-Parkinsonian drugs, from cognitive improvements, *per se*. Other experimental confounds arise when considering the 'drug-switching' design that is commonly employed. Schizophrenics are seldom treatment free before entering into a study and as a result, no drug-free baseline data are available. Studies frequently change treatments from FGAs to SGAs often do so without counterbalancing for order effects and use an insufficient washout period. Likewise, these studies often fail to consider the effects of repeated practice on task performance.

A few experiments are notable exceptions that permit a more accurate assessment of first- versus second-generation drug effects. Purdon *et al* (2000) compared the effects of haloperidol and second-generation drugs (olanzapine and risperidone) on neurocognitive impairment in early-phase schizophrenics over the course of 12 months. The results demonstrated superior effects of olanzapine over risperidone and haloperidol, with risperidone and haloperidol producing the same magnitude of cognitive benefits (Purdon, Jones *et al*. 2000). Similarly, separate experiments have demonstrated that the cognitive improvements on measures of attention, verbal fluency and digit span, did not differ between groups treated with either first-generation drugs (haloperidol,

perazine, etc.) or second-generation drugs (i.e. clozapine, olanzapine, etc.) (Rollnik, Borsutzky *et al*. 2002), however this study was conducted with a small number of subjects. A large scale, long term study conducted by Keefe, *et al.* (2004) utilized a randomized, double-blind experimental design to compare low dose treatment with haloperidol (~5 mg per day) to treatment with the second-generation drug olanzapine over the course of two years. This study showed significant improvements in neurocognitive function in both haloperidol and olanzapine treatment groups on a variety of tasks (i.e. CPT, working memory task and verbal learning test). Although olanzapine demonstrated superiority on the weighted composite score of all measures, the composite scores assessing cognitive improvement indicate that the advantage of olanzapine or haloperidol is negligible. Furthermore, the improvements demonstrated by subjects treated with low-doses of haloperidol suggest that previous results showing exacerbation of cognitive impairments may be ascribed to the excessively high dose ranges used. Despite the methodological complications evident in much of the literature, the available evidence supports the hypothesis that both first- and second-generation antipsychotic drugs can improve multiple domains of impaired cognition in schizophrenia. As such, animal models attempting to assess impaired cognition in schizophrenia should also be sensitive to these improvements.

1.14 Modeling aspects of schizophrenia using repeated amphetamine in rats

Amphetamine administration can produce psychotic symptoms that closely resemble those observed in paranoid schizophrenia; this condition is known as amphetamine psychosis (Ellinwood 1969; Ellinwood 1970). Similar to schizophrenia, the psychotic symptoms associated with AMPH include hallucinations, delusions, paranoia, flattening of the affect, and avolition. AMPH-psychosis typically initiates during acute drug intoxication (not withdrawal), is generally brief, and spontaneously remits. Hallucinations in AMPH-users usually subside in less than two days and the symptoms of paranoia and delusions generally desist in approximately 1-2 weeks. Following the remission of AMPH-psychosis, psychotic symptoms can be readily reinstated through subsequent exposure to small doses of AMPH or following exposure to stressful stimuli (Sato 1992).

The first reports of psychosis in AMPH-users were initially described in by Young and Scoville in 1938. However, due to the rarity of the condition, AMPH-psychosis was generally dismissed as interesting clinical anomaly. In 1959, Connell's seminal Maudsley monograph reported that that AMPH-psychosis occurred with greater frequency than was commonly believed (Connell 1959). However, Connell's conclusion that AMPH-psychosis could not be discriminated from schizophrenia was controversial and was contested on the basis that the observed psychoses were potentially idiosyncratic (i.e. the result of preexisting schizophrenia) or had been produced by other substances that were abused concurrently with AMPH. Slater (1959) noted that patients with AMPH-psychosis displayed a greater prevalence of visual hallucinations, emotional reactivity, and

anxiety than was typically observed with schizophrenia (Slater 1959). Additionally, Bell (1965) postulated that the two conditions were phenomenologically distinct and that a differential diagnosis could be formulated upon the presence of symptoms related to thought disorder (Bell 1965).

To address these issues in controlled clinical settings, multiple prospective studies were undertaken to further characterize the symptoms of AMPH-psychosis and to determine if the condition could be produced in individuals who had been carefully prescreened for the exclusion of preexisting psychotic disorders (Angrist and Gershon 1970; Griffith 1968; Bell 1973). Using diagnostic methods set forth by the American Psychological Association and by Schneider (1957), these studies unequivocally demonstrated that the full spectrum of schizophrenic symptoms including paranoid delusions, hallucinations (auditory, visual, olfactory, and tactile), and thought disorder occurred in the majority of patients administered AMPH or methylated AMPH. In fact, the symptoms of AMPH-psychosis replicate those of schizophrenia with such fidelity that they meet the DSM diagnostic criteria for paranoid schizophrenia. In summary, the utilization of AMPH-psychosis as a model of schizophrenia was originally based upon the ability of amphetamine to produce paranoid schizophrenic-like symptoms in healthy individuals, to 'trigger' the manifestation of psychosis in at risk individuals, and to exacerbate psychotic symptoms in chronically ill patients (Yui, Ikemoto *et al*. 2000; Lieberman, Kane *et al*. 1987; Lieberman, Kane *et al*. 1987; but see Barch and Carter 2005). Data from multiple human studies were summarized in a review by Janowsky (1979), who concluded that the induction of

AMPH-psychosis in healthy individuals represents a “pharmacologic parallel” of schizophrenia and is therefore suitable for the study of schizophrenia.

Beyond the identical clinical presentations of AMPH-psychosis and schizophrenia, the central hypothesis providing the basis for experimentation with this model posits that these two conditions share common neural underpinnings. Sensitization of the mesolimbic dopamine system has been studied extensively in this regard. Repeated, intermittent, escalating exposure to AMPH results in potentiated neurobiological and behavioral responses during administration of subsequent smaller challenge doses (Robinson and Becker 1986; Paulson, Camp *et al*. 1991). Importantly, animals exposed to repeated, escalating AMPH display sensitized mesolimbic dopamine transmission in response to AMPH challenges (Paulson and Robinson 1995) these neuronal changes are thought to contribute to altered behavioral profiles (e.g. overt motor stereotypies, perseverative responding, impulsivity) seen in AMPH-pretreated animals (Segal and Kuczenski 1992; Paulson and Robinson 1995). The contributing role of sensitization for humans with AMPH-psychosis is less clear and remains controversial. However there is evidence to suggest that AMPH-psychosis is the end result of a developing process whereby the severity of anxiety worsens during successive drug binges until finally culminating in a full-blown episode of paranoid psychosis. In fact, some reports indicate that following the first-break episode of AMPH-psychosis, subsequent drug-induced episodes are of intensified severity (Gawin and Khalsa, 1996).

Historically, primary experimentation utilizing of AMPH-models of schizophrenia has focused chiefly on predictive validity. AMPH-models have been used extensively to estimate the potency of dopamine antagonists and their efficacy for ameliorating florid psychotic symptoms. Critics of psychostimulant-based models have noted that these models are unable to replicate the entire disease entity, specifically with regards to reproducing or even exploring the etiological or developmental components of schizophrenia. Additionally, Weinberger and Lipska have questioned the future heuristic value of AMPH-models as well as their utility for developing non-dopaminergic drugs based on the incorrect perception that such models rely principally upon 'dopamine-in, dopamine out' logic (Lipska and Weinberger 2000). Such criticisms are unfounded and based upon the assumption that the psychotogenic effects of AMPH stem purely from dopaminergic mechanisms. A simple examination of AMPH pharmacology reveals that amphetamine's mechanisms of action are diverse and involve multiple neurotransmitters beyond dopaminergic mechanisms. Furthermore, these criticisms do not pose limitations to paradigms targeting *specific* disease components while assessing well-defined constructs with behavioral measures that do not directly reflect, and are not critically dependent upon mesolimbic dopamine transmission (e.g. attention processing). In fact, a rudimentary understanding of the functional and neuroanatomical substrates of schizophrenia's cognitive deficits makes it apparent that the utility of psychostimulant models can be readily expanded and applied to target neurotransmitter systems that bear relevance for schizophrenia's cognitive

deficits, specifically those critical for attentional processing. In fact, repeated exposure to AMPH has been shown to augment cortical cholinergic transmission in response to AMPH challenges (Nelson, Sarter *et al*. 2000), and has been demonstrated to result in attentional impairments in rats (Fletcher, Tenn *et al*. 2007; Dalley, Theobald *et al*. 2005).

1.15 Summary of experiments

The above discussion forms the rationale for experiments exploring the role of cortical cholinergic transmission in the attentional deficits observed in a repeated-AMPH model of schizophrenia, and furthermore, justifies experiments designed to assess the ability of first- and second-generation antipsychotic drugs to attenuate these impairments. The main points of these experiments are summarized below.

Experiment 1:

The purpose of Experiment I was to characterize the effects of a repeated escalating dosing regimen of AMPH on sustained attention performance in rats. Rats were trained in a sustained attention task and then treated with saline or in accordance with an escalating dosing regimen of AMPH (1-10 mg/kg). Performance was assessed during the pretreatment and withdrawal periods and following the subsequent administration of AMPH "challenges" (0.5, 1.0 mg/kg). Compared with the acute effects of AMPH, AMPH "challenges," administered over 21 days after the pretreatment was initiated, resulted in significant impairments in attentional performance. Fos-like immunoreactivity (Fos-IR) in selected regions of these rats' brains was examined to test the hypothesis that

AMPH-sensitized attentional impairments are associated with increased recruitment of basal forebrain cholinergic neurons. These data provide limited evidence of dysregulation of the basal forebrain cholinergic system by showing that in AMPH-pretreated and -challenged animals, an increased number of Fos-IR neurons were observed in the basal forebrain. The majority of these neurons were cholinergic. The evidence supports the hypothesis that abnormally regulated cortical cholinergic inputs represent an integral component of neuronal models of the attentional dysfunctions of schizophrenia. The results from this experiment formed the basis for subsequent experiments directly examining the role of cortical cholinergic transmission in this animal model of schizophrenia.

Experiment 2:

Experiment II provided evidence in support of the hypothesis that pretreatment with AMPH results in cortical cholinergic dysregulation. This dysregulation contributes to, rather than results from, the impaired attentional performance observed in an animal model of the cognitive deficits of schizophrenia. Using *in vivo* microdialysis methods, cholinergic transmission during AMPH-challenges was assessed in the prefrontal cortices of attentional task-performing and non-performing rats pretreated with an escalating dosing regimen of AMPH or saline. In non-performing rats, pretreatment with AMPH did not affect the increases in ACh release produced by AMPH-challenges. In contrast, the increases in ACh release that are normally associated with attention task performance in rats were attenuated following AMPH-pretreatment and AMPH-challenges. This was already apparent before task-onset, suggesting that

the observed performance impairments were a result of cholinergic dysregulation. These findings indicate that the demonstration of repeated AMPH-induced dysregulation of the prefrontal cholinergic input system depends on interactions between the effects of repeated AMPH exposure and performance-associated recruitment of this neuronal system. Experiment II expands current paradigms used to investigate the neuronal mechanisms contributing to the cognitive impairments of schizophrenia by demonstrating that in order to show dysregulation in a neuronal system of interest, it is sometimes necessary to actively recruit that system using tasks known to be mediated by, or critically dependent on that system.

Experiment 3:

There are currently no bench-mark drugs available for the use in validating an animal model of schizophrenia's cognitive deficits. As a result, present efforts geared towards the development of pro-cognitive drugs for schizophrenia requires the use of paradigms sensitive to the moderate pro-cognitive effects of currently available antipsychotic drugs. Experiment III tests the hypothesis that moderate pro-cognitive effects of low dose sub-chronic treatment with haloperidol (HAL; 0.025 mg/kg 0.2% acetic acid, s.c.) or clozapine (CLOZ; 2.5 mg/kg in 0.2% acetic acid, s.c.) can be detected by a paradigm assessing sustained attention performance in a repeated-AMPH model of schizophrenia. Using the same paradigm as the first two studies, this experiment first replicated the findings shown in Experiment I indicating that in response to AMPH-challenges; the hit rate of AMPH-pretreated rats was robustly impaired. In contrast, in AMPH-

pretreated animals receiving haloperidol or clozapine, the attentional impairment produced by the challenges were attenuated. Collectively, these data lend predictive-validity to this model of impaired cognition in schizophrenia and demonstrate the potential usefulness of this paradigm for the development and screening of novel, pro-cognitive drugs for the treatment of schizophrenia's cognitive deficits.

1.16 Figures

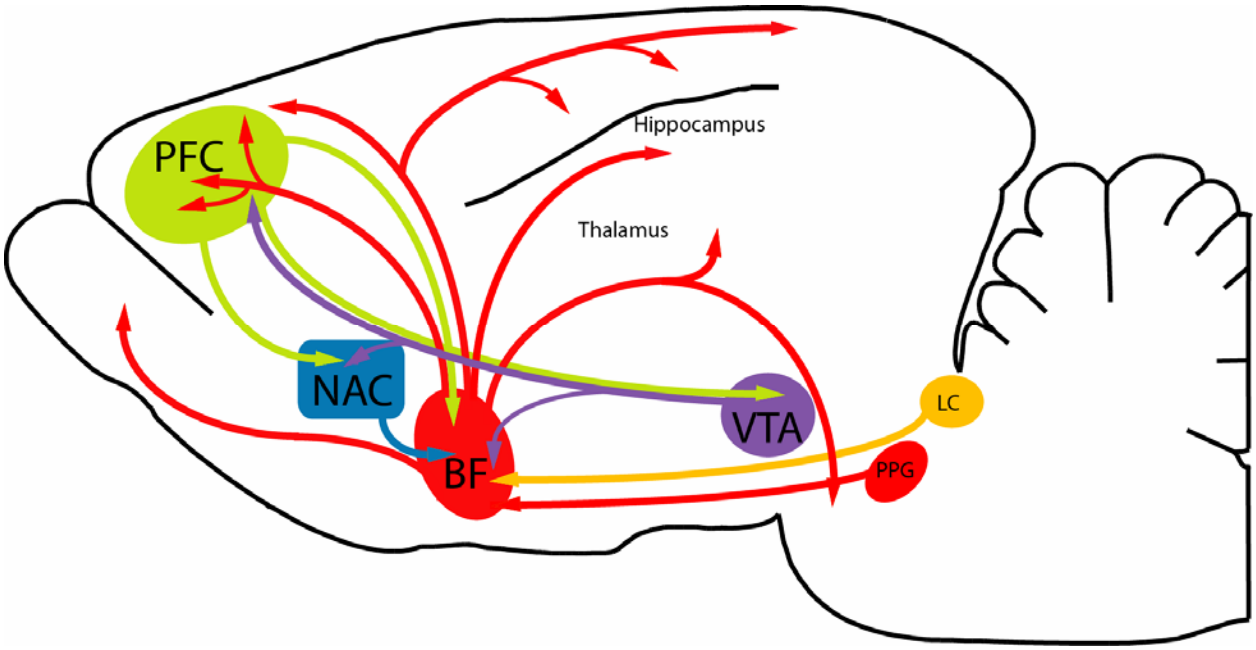

Figure 1.1: Diagrammatic representation of the basal forebrain cholinergic system. The cholinergic basal forebrain (BF) receives multiple sources of input including GABAergic (blue) inputs from the nucleus accumbens (NAC), dopaminergic input (purple) from the ventral tegmental area (VTA), noradrenergic (orange) input from the locus coeruleus (LC), cholinergic input (red) from the pedunculo pontine gigantocellular nucleus (PPG). The basal forebrain sends cholinergic projections to all areas and all layers of the cortex. The sources of input and targets of output depicted in this diagram are not exhaustive, but rather represent the major neurotransmitter systems system relevant to the present experiments. Figure modified from Woolf (1991).

CHAPTER 2

SENSITIZED ATTENTIONAL PERFORMANCE AND FOS-IMMUNOREACTIVE CHOLINERGIC NEURONS IN THE BASAL FOREBRAIN OF AMPHETAMINE-PRETREATED RATS

2.1 Summary

The consequences of repeated exposure to psychostimulants have been hypothesized to model aspects of schizophrenia. This experiment assessed the consequences of the administration of an escalating dosing regimen of amphetamine (AMPH) on attentional performance. Fos-like immunoreactivity (Fos-IR) in selected regions of these rats' brains was examined to test the hypothesis that AMPH-sensitized attentional impairments are associated with abnormal recruitment of basal forebrain cholinergic neurons. Rats were trained in a sustained attention task and then treated with saline or in accordance with an escalating dosing regimen of AMPH (1–10 mg/kg). Performance was assessed during the pretreatment and withdrawal periods and following the subsequent administration of AMPH "challenges" (0.5, 1.0 mg/kg). Brain sections were double-immunostained to visualize Fos-IR and cholinergic neurons. Compared with the acute effects of AMPH, AMPH "challenges", administered over 2 months after the pretreatment was initiated, resulted in significant impairments in attentional performance. In AMPH-pretreated and -challenged animals, an increased number of Fos-IR neurons was observed in the basal forebrain. The majority of these neurons were cholinergic. The evidence supports the hypothesis that abnormally regulated cortical cholinergic inputs represent an

integral component of neuronal models of the attentional dysfunctions of schizophrenia.

2.2 Introduction

Sensitization of mesolimbic dopamine systems is considered to be a neuropathologic hallmark of schizophrenia (Lieberman, Sheitman *et al*. 1997; Laruelle 2000) and thus of animal models of this disease (Robinson and Becker 1986). Schizophrenic patients exhibit increased AMPH-induced displacement of dopamine D2 receptor ligands during periods when positive symptoms manifest and intensify, but not during remission (Abi-Dargham, Gil *et al*. 1998; Laruelle, Abi-Dargham *et al*. 1996; Laruelle, Abi-Dargham *et al*. 1999). Moreover, the dopaminergic system of first-episode psychotic patients may already be maximally up-regulated (Strakowski, Sax *et al*. 1997). The effects of repeated AMPH exposure, particularly the sensitized effects of subsequent AMPH-challenges, have served as a model in research on the role of sensitized dopamine systems in the mediation of psychosis. Furthermore, AMPH-sensitized animals exhibit behavioral and cognitive abnormalities that model aspects of psychosis (Castner, al-Tikriti *et al*. 2000; Seeman, Tallerico *et al*. 2002; Tenn, Fletcher *et al*. 2003).

Attempts to reduce the complex symptoms of psychosis to dysfunctions in elementary cognitive operations have focused on the disruption of attentional processes and capacities and related executive functions (McGhie and Chapman 1961; Braff 1993; Andreasen, Paradiso *et al*. 1998; Gray 1998; Kapur 2003;

Venables 1964). Moreover, lasting cognitive, particularly attentional, impairments in schizophrenia patients have emerged as a new and distinct treatment target (Braff and Light 2004). Little is known, however, about the long-term consequences of AMPH-sensitization on attentional performance. Likewise, the neuronal systems underlying the cognitive consequences of AMPH-sensitization are poorly understood. Based on extensive evidence on the role of the cortical cholinergic input system in attentional functions (Sarter, Hasselmo *et al*. 2005),on the regulation of cortical cholinergic transmission by dopaminergic systems (Moore, Fadel *et al*. 1999), and on the available evidence suggesting abnormalities in the regulation of cortical cholinergic inputs in schizophrenia (Crook, Tomaskovic-Crook *et al*. 2001; Tandon 1999), the attentional impairments of schizophrenia have been hypothesized to be mediated specifically via abnormally reactive cortical cholinergic inputs (Sarter, Hasselmo *et al*. 2005). The finding that repeated AMPH administration sensitizes cortical acetylcholine (ACh) release corresponds with this hypothesis (Nelson, Sarter *et al*. 2000).

The experiment described here was designed to determine the consequences of repeated AMPH administration on attention performance. Performance in this task reflects the status of cortical cholinergic transmission (Arnold, Burk *et al*. 2002; McGaughy, Decker *et al*. 1999; McGaughy and Sarter 1995; McGaughy, Kaiser *et al*. 1996; Turchi and Sarter 2001). Once animals achieved criterion performance, they were treated with saline or AMPH in accordance with a 40-day escalating dosing treatment regimen that included

days without AMPH administration to model aspects of “runs” and “crashes” observed in AMPH abusers (Paulson, Camp *et al*. 1991). The administration of escalating doses of AMPH did not produce neurotoxic effects but was demonstrated by Paulson, *et al.*(1991) to yield lasting (> 1 year) behavioral sensitization. Such lasting, sensitized effects of AMPH pretreatment are thought to model the ability of low doses of AMPH to trigger psychotic periods following long withdrawal periods in AMPH abusers (Robinson and Becker 1986; Nuechterlein, Dawson *et al*. 1994; Moghaddam 2002; Muller 2004). Furthermore, selected brain regions, particularly the region of the cortically projecting cholinergic neurons in the nucleus basalis of Meynert and substantia innominata (nbM/SI) of the basal forebrain, were inspected for Fos-immunoreactivity (IR) to test the hypothesis that the effects of ‘AMPH ‘challenges’ on attentional performance are associated with increased activity of cortical cholinergic inputs.

2.3 Methods

Animals: Sixteen male Fischer/Brown Norway hybrid rats (aged 3 months and weighing 229±19 g (Mean±SEM) at the beginning of the experiment; Harlan, Indianapolis, Indiana) were housed individually in single-standard cages in a humidity and temperature-controlled environment accredited by the American Association of Laboratory Animal Care (Ohio State University, Townshend Hall). All animal care and experimental procedures were approved and supervised by the Ohio State University Institutional Laboratory Animal Care and Use Committee. Lighting followed a 12-hour light-dark cycle (lights on at 6 am).

Testing occurred between 8:00 am and 5:30 pm. Animals were handled extensively before testing and were water deprived by limiting access to water to an 8-min period that followed the completion of the daily behavioral test session. Food was provided ad libitum.

Apparatus: Behavioral training and testing were conducted using 12 operant chambers (MedAssociates, East Fairfield, Vermont). Each chamber was outfitted with two retractable levers, three panel lights (2.8 W), one house light (2.8 W), and a 2900-Hz sonalert tone generator with the water dispenser located on the same wall as the panel lights and levers. Operant chambers were housed within sound-attenuating compartments.

Behavioral Training: Training took place 7 days per week. After being trained to lever press for water in accordance with a fixed-ratio (FR) 1 schedule of reinforcement, subjects were placed in darkened (houselights off) operant chambers for 20 minutes before task onset. Rats were first trained to discriminate between signal (illumination of the central panel light for 1 sec) and non-signal (non-illumination) events. Two seconds following an event, both levers were extended and remained available for 4 sec or until a lever press occurred. If no lever press occurred after 4 sec, an omission was scored and the intertrial interval (ITI; 12±3 sec) was reinstated. On signal trials, a left lever press was scored as a hit, and 0.25-mL water was delivered as reward; depression of the right lever was considered an incorrect response and scored as a miss. During non-signal trials, a right lever press was scored as a correct rejection, and

animals again received water reward. During non-signal trials, a left lever press was considered an incorrect response and scored as a false alarm. Animals were not rewarded for incorrect responses. If a rat failed to respond correctly for three consecutive trials, up to three correction trials were presented during this stage of training. During a correction trial the ITI was reinstated and the trial was repeated up to three times. If a rat failed to respond correctly during these trials, a forced-choice trial was initiated that was designed to block the development of side biases. A forced-choice trial consisted of a signal or non-signal event, followed by the extension of only the correct response lever into the chamber. During a forced-choice trial the extended lever remained active for 90 sec or until a press occurred. In the event that the forced-choice trial was a signal event, the central panel light remained lit for as long as the lever was active. The presentation of signal and non-signal trials was pseudo-randomized (162 trials/session, plus correction and forced-choice trials). After 3 consecutive days of 70% correct responses to signal and non-signal trials and less than 25 omissions, animals progressed to the second phase of training. During this phase, the duration of signals was decreased to 25, 50, or 500 msec and correction and forced choice trials were eliminated. Sessions consisted of 27 trials of each of the three signal lengths and 81 trials of the non-signal events, yielding a total of 162 trials per session. Each session was divided into three blocks of 27 signal and 27 non-signal trials. Each signal type was presented nine times per block. After reaching a criterion of 59% correct responses to signal and non-signal trials and less than 25 omissions for three consecutive sessions, houselights were illuminated

throughout the session and the ITI was decreased to 9±3 sec (see Figure 2.1). Illumination of the houselights requires the animals to focus on the stimulus panel and thus represents a crucial step in the acquisition of this sustained attention task. The drug treatment regimen was initiated after animals reached the final criterion of greater than 70% correct responses for 500 msec signals, greater than 70% correct responses during non-signal trials, and fewer than 25 omissions per session, for at least 3 consecutive sessions.

Pretreatment Regimen and Challenges: After reaching performance criterion, animals were randomly divided into two groups. Animals were given either d-amphetamine sulfate (AMPH; 1–10 mg/kg intraperitoneally *i.p.*); drug concentrations include salt weight; dissolved in 1 mL/kg of 0.9% saline; Sigma, St. Louis, Missouri) or vehicle (0.9% saline; 1 mL/kg) twice daily, with approximately 8 hours separating the two injections. Animals received the first of the two daily injections at approximately 8:00 am in the testing room and were immediately placed in their operant chambers for 20 min before task onset. Following the completion of operant testing, animals were promptly returned to their home cages. The second dose of drug or vehicle was given in the home cage at approximately 4:00 pm. The AMPH doses were administered in elevating increments over the course of 40 days in accordance to the regimen described in (Paulson, Camp *et al*. 1991; Figure 2.2); AMPH was administered every day of the week excluding weekends to mimic purposely the pattern of abuse seen in AMPH-abusers (Paulson, Camp *et al*. 1991). Animals were given injections of saline on weekends. Animals treated with AMPH exhibited high levels of

omissions (> 90% of all trials) following doses ≥2.0 mg/kg, but they completed significantly more trials during the drug-free weekends (< 20% omissions). To control for the potentially confounding consequences of substantial differences in the amount of behavioral practice between the two groups of animals, saline-treated animals were placed into the chambers for 1 hour on weekdays without being allowed to perform; however, similar to animals undergoing AMPH pretreatment, saline-treated animals performed the task on weekends.

Following completion of the pretreatment regimen, animals underwent a 20-day drug-free period consisting of twice-daily saline injections and continued operant testing. Saline was administered to avoid potential performance changes due to termination of the injection procedure. The length of the withdrawal period was based on the observations by Paulson and Camp *et al*. (1991) that the symptoms of behavioral depression were most pronounced within the first couple of days of withdrawal, and that behavioral sensitization was fully developed 2 weeks following completion of the pretreatment regimen. As detailed later, the performance of AMPH-pretreated rats was almost completely recovered by the end of this period.

Subsequently, the effects of AMPH were assessed in AMPH and saline-pretreated animals. Animals received 0.5 mg/kg AMPH on days 61 and 81 (with day 1 being the first day of the pretreatment regimen) and 1 mg/kg AMPH on days 71 and 86 (see Figure 2.2). The selection of these “challenge” doses was based on the observation that an acute dose of 1.0 mg/kg (which was the first

dose given as part of the pretreatment regimen) did not significantly alter the animals' performance and on the hypothesis that AMPH-sensitized performance should be sensitive to the detrimental effects of even smaller doses. Subsequent to the administration of these AMPH doses, animals were placed in their operant chambers for a 20-min acclimation period followed by a standard behavioral test session.

Immunohistological Analysis: At the conclusion of the test session on day 86, animals were immediately anesthetized with pentobarbital (100 mg/kg IP) and transcardially perfused with 100 mL of ice-cold heparinised saline followed by 300 mL of 4% paraformaldehyde in 0.1 mmol/mL phosphate-buffered saline (PBS; pH 7.4). The brains were removed and postfixed in paraformaldehyde solution for 4 hours (with shaking) at 4°C and stored in 30% sucrose in PBS (pH 7.4) for 72 hours. The brains from six animals per group were processed for immunohistochemical analyses.

Coronal sections (50 μm) were cut using a cryostat microtome (Leica CM 3050 S; Leica Microsystems, Chantilly, Virginia) and stored in cryoprotectant solution (15% glucose, 30% ethylene glycol, and 0.04% sodium azide in 0.05 mmol/mL PBS, pH 7.4) at -20°C until further processing. Sections were thawed, washed twice in 0.1 mmol/mL PBS for 5 min, and incubated with 0.3% H_2O_2 for 10 min to block endogenous peroxidase. After washing with PBS, the sections were incubated with blocking solution (10% goat serum in 0.1 mmol/mL PBS) with constant shaking for 30 min followed by overnight incubation with rabbit anti-

Fos antibody (H-125:sc-7202; Santa Cruz Biotechnology, Santa Cruz, California) diluted 1:1000 in 0.1 mmol/mL PBS containing 1% goat serum and 0.3% triton X-100 at 4°C. The following day, sections were washed in PBS three times for 5 min and incubated with 1:2000 diluted biotinylated goat antirabbit immunoglobulin G (IgG; Vector Labs, Burlingame, California) for 2 hours at room temperature. After washing in PBS, the sections were incubated with avidin-horseradish peroxidase complex (Vectastain ABC Kit, Vector Labs, Burlingame, California) for 1 hour at room temperature. Sections were washed again in PBS and staining was developed with 3-3'-diaminobenzidine (DAB) and in the presence of 0.01% nickel chloride. Stained sections were mounted onto gelatin coated slides; after air drying, the slides were dehydrated and coverslipped with Permount (Biomedia, Foster City, California). Omission of the primary antibody from this procedure eliminated Fos-like immunoreactivity (Fos-IR).

To assess whether basal forebrain cholinergic neurons express Fos-IR, nBM sections were double-immunostained to reveal choline acetyltransferase (ChAT) and Fos-IR. The sequential immunostaining procedure revealed first ChAT-IR with DAB and second Fos-IR with DAB/Ni. Briefly, sections were blocked with 10% donkey serum (Chemicon International, Temecula, California) and sequentially incubated with goat-anti-ChAT antibody (1:2000; Chemicon International) for ChAT staining and rabbit anti-Fos antibody (1:1000) for c-Fos. Appropriate secondary antibodies (1:3000 diluted biotinylated donkey anti-goat IgG or 1:2000 diluted biotinylated donkey anti-rabbit IgG; Jackson Immunoreseach Laboratories, Westgrove, Pennsylvania) were used after the

respective primary antisera. Overnight incubations at 4°C were carried out for primary antibodies, and incubations were made for 2 hours at room temperature for secondary antibodies. Dilutions of all antibodies were made in 0.1 mmol/mL PBS containing 1% donkey serum and 0.3% triton X-10. The avidin-biotin peroxidase method using the Vectastain ABC kit was employed to stain and visualize the brown reaction product for ChAT with DAB and the grayish-black reaction product for Fos with DAB/Ni. No cross-reactivity was observed between the antibodies, and adequate controls were included by reversing the order of primary antibodies or omitting the primary or secondary antibodies.

Sections were analyzed using an Olympus AX 70 microscope (Olympus America, Melville, New York) equipped with an Olympus Magnafire digital camera; digital photographs were captured and processed using Olympus Magnafire software (Olympus America, Melville, New York). Microscopical examinations were performed by an experimenter (VP) who remained blind to the animals' pretreatment condition. Three sections per region of interest were selected for analysis: dorsal striatum (DS; between 1.60 and 1.30 mm anterior to bregma), nucleus basalis of Meynert and substantia innominata (nbM/SI, SI; 1.20 mm posterior to Bregma), frontoparietal cortex (PC, 1.40–1.70 mm posterior to bregma), and ventral tegmental area (VTA; 4.8–5.2 mm posterior to bregma; (Paxinos 1998). Fos-IR neurons in these areas were counted in the right hemispheres of three brain sections per brain at 10× magnification. Counts were made inside predefined areas per region; DS: 0.80 mm^2; nBM/SI: 0.36 mm^2; PC: 0.64 mm^2; VTA: 0.16 mm^2 (see Figure 2.7 for an illustration of sampling areas).

The average count from 3 sections per region per animal was used for statistical analysis.

Data Analysis: The total number of hits (h), correct rejections (cr), misses (m), false alarms (fa), and omissions were calculated for the entire behavioral session and for each block of 54 trials. Based on these values, the relative number of hits (h = h/(h + m) and correct rejections (cr = cr/(cr + fa) were calculated. In addition, the vigilance index (VI), an overall measure of sustained attention performance, was calculated based on the relative number of hits and false alarms using the formula: VI = (h-fa) / (2(h fa) - $(h+fa)^2$)). This index is similar to the sensitivity index (Frey 1973) except that omitted trials are excluded from the calculation. Values for VI can vary from +1.0 to -1.0, with +1.0 indicating that all responses were scored as hits or correct rejections, 0 indicating an inability to discriminate between signal and non-signal events, and -1 indicating that all responses to signals were misses and all responses to non-signals were false alarms. The index was calculated for each signal duration ($VI_{500,50,25}$). Hits and correct rejections were analyzed using angularly transformed values (Zar 1999). Performance data from the last 3 days before the start of the pretreatment regimen were used for baseline comparisons. Performance during the pretreatment period was not analyzed except for data from the 2-day off- drug sessions. Data from the first and second off-drug days were collapsed over 2 weeks, resulting in 3 blocks of data. The 20-day drug-free period following pretreatment was divided into two time periods (days 1–10 (W1) and days 11–20 (W2)) and analyzed to assess the time course of effects of AMPH withdrawal.

Because the subsequent administration of AMPH was expected to produce augmented performance effects when compared with the first exposure to AMPH, the effects of both doses of AMPH, collapsed over the two tests of the individual doses, were compared with the effects of the first administration of AMPH (1.0 mg/kg). Data depicting the number of Fos-IR neurons are expressed as Mean±SEM per group and the four anatomic regions of interest. Group-based comparisons of the average number of Fos-positive cells were conducted using planned, two-tailed, unpaired Student t tests. Mixed factor analysis of variance (ANOVAs) were used to assess the effects of pretreatment, block, signal duration and AMPH on hits, misses, false alarms, correct rejections, omissions and response latencies (time from lever extension to lever press). Post hoc comparisons were made using the Least Significant Difference (LSD) for within-subject comparisons. The reported statistical results reflect Huyn-Feldt-corrected degrees of freedom. Exact *p* values are reported as recommended by (Greenwald 1996). Statistical analyses were performed using the SPSS/PC+ 11.5 Version (SPSS, Chicago, Illinois).

2.4 Results

Baseline performance before the pretreatment phase: The baseline performance of the animals designated for pretreatment with AMPH or saline, respectively, did not differ during the last three sessions before the pretreatment phase (hits, correct rejections, omissions; all main effects and interactions involving the factor "Group"; $p > 0.05$). The animals' performance was signal duration-dependent

(F(2,56) = 176.81; $p < 0.001$; see Figure 2.3). Animals omitted a relatively small proportion of trials (6.56±0.72/session), and the number of omissions increased over blocks of trials (F(1.42,19.85) = 9.07; p =0.004).

Drug-free weekend performance during the pretreatment period: As described earlier, animals undergoing the AMPH-pretreatment regimen were given saline on weekends, and saline-treated animals were placed into the operant chambers on weekdays without being allowed to perform the task. During drug-free weekends, the omission rate in AMPH-treated animals was sufficiently low to permit a meaningful analysis of their performance on those days (averaged per animal over 2 weekends, yielding 3 blocks of data).

In terms of overall performance (VI), the animals undergoing AMPH-pretreatment were impaired compared with saline-treated control animals (F(1,14) = 18.84; p =0.001). This effect interacted with signal duration (F(2,28) = 19.94; $p < 0.001$; Figure 2.3). Multiple comparisons indicated that VI_{500} and VI_{50} values differed between the groups (VI_{500}: F(1,15) = 53.06; $p < .005$; VI_{50}: F(1,15) = 89.79; $p < 0.01$; VI_{25}: F(1,15) = .227; p =0.607). All animals performed better on the second drug-free day (F(1,14) = 18.52; p =0.001; day 1: 0.18±0.15; day 2: 0.22±0.13); however, the difference in performance between the two groups was consistent across the three blocks of weekend data (all interactions involving group and week, p >0.14). The significant interaction between group and signal duration on VI was based on similar effects revealed in the analysis of hits (F(1.92,26.93) = 32.06; $p < 0.001$). The animals' non-signal performance

remained unaffected by ongoing treatment (all effects and interactions involving group: $p > 0.12$).

Animals undergoing AMPH-pretreatment exhibited an increased number of omissions that interacted with day (first vs. second drug-off day) and week ($F(2,280) = 5.76$; $p = 0.008$). As illustrated in Figure 2.4, this interaction was due to a relatively high number of omissions in AMPH-treated animals on the first off-day during the last two weekends of the pretreatment period when compared with their omission rate on the first off-day during weeks 3–4 (LSD = 14.22; $p = 0.015$), and with the omissions generated by AMPH-treated animals on the 2nd off-day during weeks 5–6 (LSD = 16.09; $p < 0.001$) and by saline-treated animals on both days during this late pretreatment phase ($t(14) < 0.001$).

Attentional performance during the withdrawal period: The performance of the two groups during the 20-day withdrawal period differed significantly ($F(1,14) = 12.51$; $p = 0.003$; VI for saline animals: 0.26±0.02; AMPH: .15±.02). Furthermore, all animals improved their performance from W1 to W2 ($F(1,14) = 11.65$; $p = 0.004$), but post hoc analyses indicated that this improvement was primarily due to the recovery in AMPH-pretreated rats ($F(1,8) = 16.69$; $p = 0.004$; saline-pretreated: LSD = 0.01; $p = 0.44$; AMPH-pretreated: LSD = 0.06; $p = 0.04$; Figure 2.5). Although all animals maintained signal duration-dependent performance ($F(2,28) = 372.07$; $p < 0.001$), the effects of group and signal duration interacted ($F(2,28) = 24.57$; $p < 0.001$). Multiple comparisons failed to locate this interaction, however (Figure 2.5).

Although the overall rate of omissions remained small in both groups of animals ($F(1,14) = 1.01$; $p = 0.33$; saline-pretreated: 6.63±0.89 omissions/session; AMPH-pretreated: 5.43±0.79), significant interactions between group and W1/W2 and group and block and among group, block, and W1/W2 were found (all $p < 0.03$). Collectively, these complex interactions reflected an increase in omissions in AMPH-pretreated animals during W2 when compared with W1, a less pronounced increase in omissions across trial blocks in these animals when compared with saline-pretreated animals, and a normalization of the block-related increase in omissions in W2 when compared with W1 in AMPH-pretreated animals.

Effects of AMPH challenges on attentional performance: The administration of 1.0 mg/kg AMPH as a challenge produced impairments in performance in AMPH-pretreated animals when compared with the acute effects of this dose. The first administration of this dose (which was the first dose given during the pretreatment regimen) did not affect their performance when compared with their baseline performance before the pretreatment regimen (all $p > 0.41$; Figure 2.6). Likewise, in animals pretreated with saline, administration of AMPH as a "challenge" (which was their first AMPH-exposure) did not affect their performance when compared with the effects of the very first AMPH exposure in the other group of animals (all $p > 0.05$; Figure 2.6).

The main effect of the 1.0 mg/kg AMPH-challenge on the animals' overall performance (VI; $F(1,8) = 14.46$; $p = 0.005$) was due to a lower hit rate following

the AMPH-challenge ($F(1,8) = 26.31$; $p = 0.001$; first administration of 1.0 mg/kg (averaged over all signal durations): 57.56±4.86% hits; 1.0 mg/kg challenge: 36.97±5.47% hits). The decrease in hits following the AMPH-challenge occurred across all signal durations ($F(2,16) = 1.05$; $p = 0.37$; Figure 2.6) and blocks ($F(3.51, 28.10) = 0.18$; $p = 0.93$). The relative number of correct rejections (Figure 2.6 and the number of omissions did not differ between the first and the challenge administration of AMPH (all $p > 0.12$). Omissions remained generally low (7.98±1.73 omissions/session).

Moreover, the administration of a smaller dose of AMPH (0.5 mg/kg) as a challenge produced impairments in performance when compared with the innocuous effects of the very first exposure to 1.0 mg/kg AMPH. This effect interacted with the blocks of trials and reached significance in the analysis of the overall performance (VI: $F(1.71,13.69) = 7.33$; $p = 0.009$; Figure 2.7), but not hits ($F(2,16) = 2.84$; $p = 0.09$; not shown). This significant interaction was due to a significantly lower performance following AMPH-challenge during the middle block of trials (block 2: $t(8) = 3.44$; $p = 0.009$; $p > 0.25$ for blocks 1 and 3; Figure 2.7). No other performance measure was affected as a result of the challenges with 0.5 mg/kg AMPH.

The impairment in attentional performance produced by AMPH-challenges in AMPH-pretreated animals may have been confounded by a lower baseline performance at the end of the withdrawal period. The performance during the last three sessions before the administration of the challenges differed only in one

respect from the animals' baseline performance before the first AMPH exposure. There was a significant interaction between signal duration and the two baselines on hits ($F(2,16) = 3.68$; $p = 0.048$), but multiple comparisons failed to reveal significant differences for individual signal durations.

Finally, to substantiate hypotheses about the nature of the effects of AMPH-sensitization (see Discussion), response latencies (time period from lever extension to lever press) were analyzed. Compared with the latencies observed before the pretreatment period, neither the very first administration of AMPH (1.0 mg/kg) nor the administration of this dose as a challenge altered response latencies ($F(1.60,12.81) = .27$; $p = 0.72$). Latencies did not differ by trial type (signal vs. non-signal trials) or response type (hits, misses, false alarms, correct rejections), and there were no interactions between treatment and trial type/response type (all $p > 0.43$). Animals required 909.35±44.13 ms to press a lever.

Fos-immunoreactivity (IR): In saline-pretreated animals, considerable Fos-IR was found in the frontoparietal cortex, caudate/putamen, and VTA (Figure 2.8). In the nbM/SI region, Fos-IR cells were only found sporadically in saline-pretreated rats, and they were primarily situated in the SI portion of the basal forebrain (Figure 2.8 and Figure 2.9). In AMPH-pretreated animals, significant increases in the number of Fos-IR cells were found in the nbM/SI ($t(1,10) = 7.30$; $p < .0001$), frontoparietal cortex ($t(1,10) = 4.40$; $p = 0.002$), and VTA ($t(1,10) = 5.99$; $p < .001$) but not the caudate-putamen ($t(1,10) = 1.15$; $p = 0.27$; see Figure 2.8). Double-

immunolabeling indicated that approximately 80% of Fos-positive cells in the nbM/SI region were also ChAT-positive (Figure 2.9). Fos-IR cells that were not also ChAT-positive were intermingled with double-labeled cells and also primarily located in the SI region.

2.5 Discussion

The main findings of our study indicate that as a result of AMPH-pretreatment with an escalating dosing regimen, the administration of doses of AMPH that did not produce significant effects on attentional performance when given acutely resulted in substantial impairments in attentional performance when administered as challenges. We also recorded the performance of animals at drug-off days during the pretreatment period and during the withdrawal period; these data assist in interpreting the effects of AMPH challenges. Furthermore, in AMPH-pretreated and AMPH-challenged rats, the number of Fos-positive neurons was found to be increased in the basal forebrain, and the majority of these neurons were cholinergic. The discussion will focus on the nature of the behavioral and attentional effects of AMPH challenges, the significance of the Fos-data, and the relevance of these findings for models of the cognitive impairments of schizophrenia.

This experiment utilized a more extensive pretreatment regimen and a longer withdrawal period than previous studies addressing cognitive consequences of repeated AMPH exposure (Kondrad and Burk 2004). As discussed in the literature, short pretreatment periods, consisting of the

intermittent administration of relatively low doses of AMPH, have been fruitful in psychostimulant-sensitization research (Robinson and Becker 1986), but they do not adequately model the escalation of AMPH doses in AMPH abusers and the "runs and crashes" that are associated with the emergence of psychotic symptoms in humans and analogous symptoms in animal models (Angrist 1994; Kuczenski and Segal 1997; Castner and Goldman-Rakic 2003). Furthermore, the administration of escalating doses and longer withdrawal periods produces lasting neurochemical changes, without generating neurotoxic effects, that differ qualitatively from those achieved by shorter pretreatment regimens (Paulson, Camp *et al*. 1991; Paulson and Robinson 1995).

Our demonstration of AMPH-sensitized attentional impairments cannot be readily attributed to overt behavioral effects. Given the overwhelming evidence on AMPH-sensitized locomotor activity and stereotypies, the possibility that such effects confounded the present impairments in attentional performance needs to be carefully considered. AMPH-challenges did not affect the animals' performance in non-signal trials and, although suppressing the hit rate, AMPH-challenges did not abolish the effects of signal duration on this measure. Putative consequences of sensitized locomotion or stereotypies would be expected to manifest primarily as side- or lever biases, or randomized lever selection, and to result in a high number of omissions. As indicated by the relatively short response latencies, and as confirmed by observations, animals positioned themselves in front of the correct rejection lever (while withdrawn) and, upon signal detection, switched to the hit lever, awaiting its extension into the

chamber, therefore producing similar latencies for all responses. AMPH-challenges did not affect response latencies and thus may not have altered this behavior. Thus, sensitized locomotor activity or stereotypies, increased switching behavior (Evenden and Robbins 1983) or complex changes in lever-choice behavior (Evenden and Robbins 1983) appear highly unlikely to have occurred as a result of AMPH challenges. Likewise, it is noteworthy that such doses of AMPH do not affect visual discrimination performance *per se* (Andrews and Holtzman 1988). Collectively, it is difficult to envision how the selective impairments in performance observed following AMPH challenges could have been due to fundamental changes in the animals' behavior.

Our findings and the discussion presented here also suggest that sensitized locomotor activity or increased stereotypies, and possibly the associated increases in striatal dopamine release (Paulson and Robinson 1995), do not represent necessary consequences of AMPH-pretreatment but that their manifestation may depend on the absence of behavioral or operant constraints. As demonstrated by Wolgin and colleagues, repeated AMPH-induced stereotypies and appetitive behaviors, if interfering continuously with goal-directed behaviors or operant performance, are increasingly suppressed, in part as a result of the acquisition of counteracting operant contingencies (Hughes, Popi *et al*. 1998; Wolgin 2000; Wolgin 2002). Our data indicate that during the pretreatment period, omissions increased substantially in AMPH-treated animals, as seen during the first of the two drug-off days and during the final 2 weeks of pretreatment. These omissions may have reflected in part the transient

manifestation of overt behavioral effects of higher doses of AMPH, rather than the effects of acute withdrawal, because increased omissions were not observed during the withdrawal period.

The decrease and eventual disappearance of AMPH-induced overt behavioral effects in continuously performing subjects contrasts with the demonstration of persistent AMPH-sensitized impairments and may be of significance for the usefulness of AMPH-sensitization as a model for schizophrenia-associated cognitive dysfunction. The focus on the cognitive consequences of AMPH-sensitization not only enhances the face validity of the animal model, but the sensitized attentional effects may be mediated via abnormally regulated neuronal systems that overlap only partly with the sensitized striatal systems that have been traditionally in the focus of research on AMPH-sensitized locomotor activity.

The exact mechanisms that mediated the AMPH-challenge-induced decrease in the animals' ability to detect signals remain speculative. In terms of signal-detection theory, a parallel downward shift of the signal duration-hit curve may indicate a conservative shift in the animals' decision criterion. Such a shift could result from increased costs for false alarms, decreased costs for misses, or both, or from decreased benefits for hits (Swets 1982). The costs for misses and false alarms were identical (no reward), however, and the absence of changes on omissions do not support the speculation that the rewarding effect of hits was

diminished after AMPH-challenges. Likewise, the number of correct rejections, which resulted in identical reward, remained unchanged.

An alternative source of an increasingly conservative criterion is a decreasing expectation of signal occurrence (Swets 1970). Because of the absence of factual changes in signal probability in this experiment, multiple mechanisms—including impairments in monitoring perceptual (particularly visual channels) and, more generally, source monitoring, or general fragmentation of cognitive operations—may have contributed to the manifestation of a more conservative criterion. Impairments in the ability to monitor the frequency and distribution of signal events could result in the underestimation of signal probability, and thus in changes in performance indicative of a shift toward a more conservative criterion. It is intriguing that signal detection impairments observed in schizophrenic patients tested in sustained attention tasks have been attributed to similar impairments in cognitive operations (Braff 1993; Keefe, Arnold *et al*. 1999; Li 2002; Braff and Light 2004; van den Bosch, Rombouts *et al*. 1996) and that such impairments may also contribute to the attentional and executive symptoms observed in chronic amphetamine abusers (Rogers, Everitt *et al*. 1999; Ornstein, Iddon *et al*. 2000).

This experiment was not designed to assess comprehensively the increases in Fos-expression following repeated AMPH exposure (Ostrander, Badiani *et al*. 2003; Uslaner, Norton *et al*. 2003) but to determine Fos-IR primarily in the cholinergic basal forebrain of AMPH-pretreated, AMPH-challenged, and task-performing animals. In saline-pretreated rats, Fos-positive cells were rarely

seen in the nbM section of the cholinergic basal forebrain. In contrast, Fos-positive cells were distributed more evenly in the nbM/SI area in AMPH-pretreated animals. There is little evidence in support of discrete differences between the topographic organization of these two structures (Zaborszky 2002), although the projections from the nucleus accumbens (Mogenson, Swanson *et al*. 1983) and amygdala (Jolkkonen, Miettinen *et al*. 2002) appear to contact SI neurons preferentially. Thus, the increase in the number of Fos-positive cells in the ventral section of the cholinergic basal forebrain may reflect the transsynaptic consequences of a sensitized mesolimbic system on basal forebrain cholinergic neurons.

Our data, together with prior findings indicating mesolimbic influences over cortical cholinergic transmission (Moore, Fadel *et al*. 1999; Neigh-McCandless, Kravitz *et al*. 2002; Neigh, Arnold *et al*. 2004), AMPH-sensitized cortical ACh release (Nelson, Sarter *et al*. 2000), and the role of cortical cholinergic inputs in attention (see Introduction), correspond with the hypothesis that the cortical cholinergic input system is an integral branch of neuronal circuitry involved in the mediation of the attentional effects of psychostimulant sensitization. These data indicate that the attentional consequences of repeated AMPH exposure in rats may serve as a model to investigate further the role of the cortical cholinergic input system in the cognitive symptoms of schizophrenia and for the test of the potential therapeutic significance of cholinergic modulators (Tzavara, Bymaster *et al*. 2004; Tzavara, Bymaster *et al*. 2006).

2.6 Figures

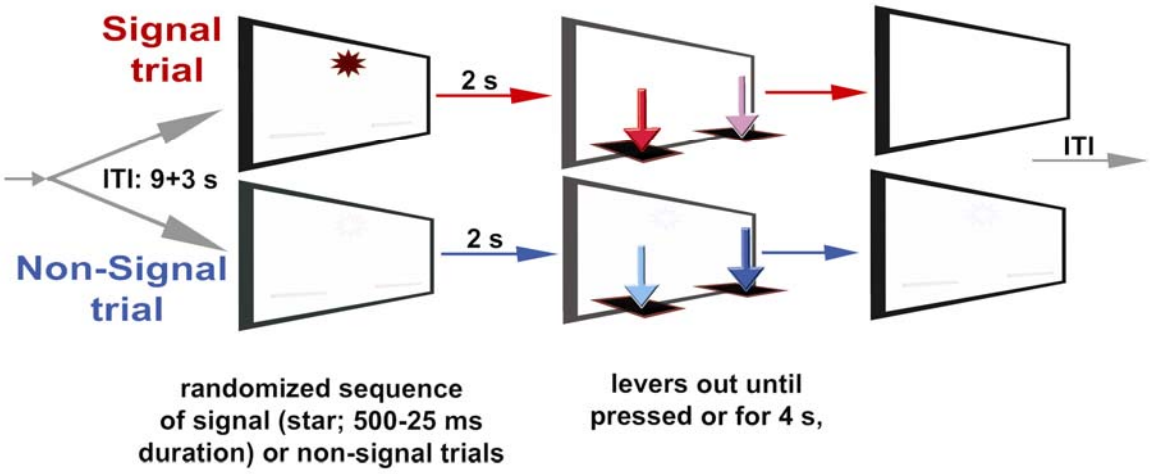

Figure 2.1 Schematic illustration of the sustained attention task. A session consisted of 162 signal or non-signal trials. Two seconds following a signal or non-signal event levers extended into the operant chamber. Correct responses in signal trials (left lever presses: hits) and non-signal trials (right lever presses: correct rejections) were rewarded, and incorrect responses (misses and false alarms, respectively) were not. The intertrial interval (ITI) was variable to limit the animals' ability to time an event.

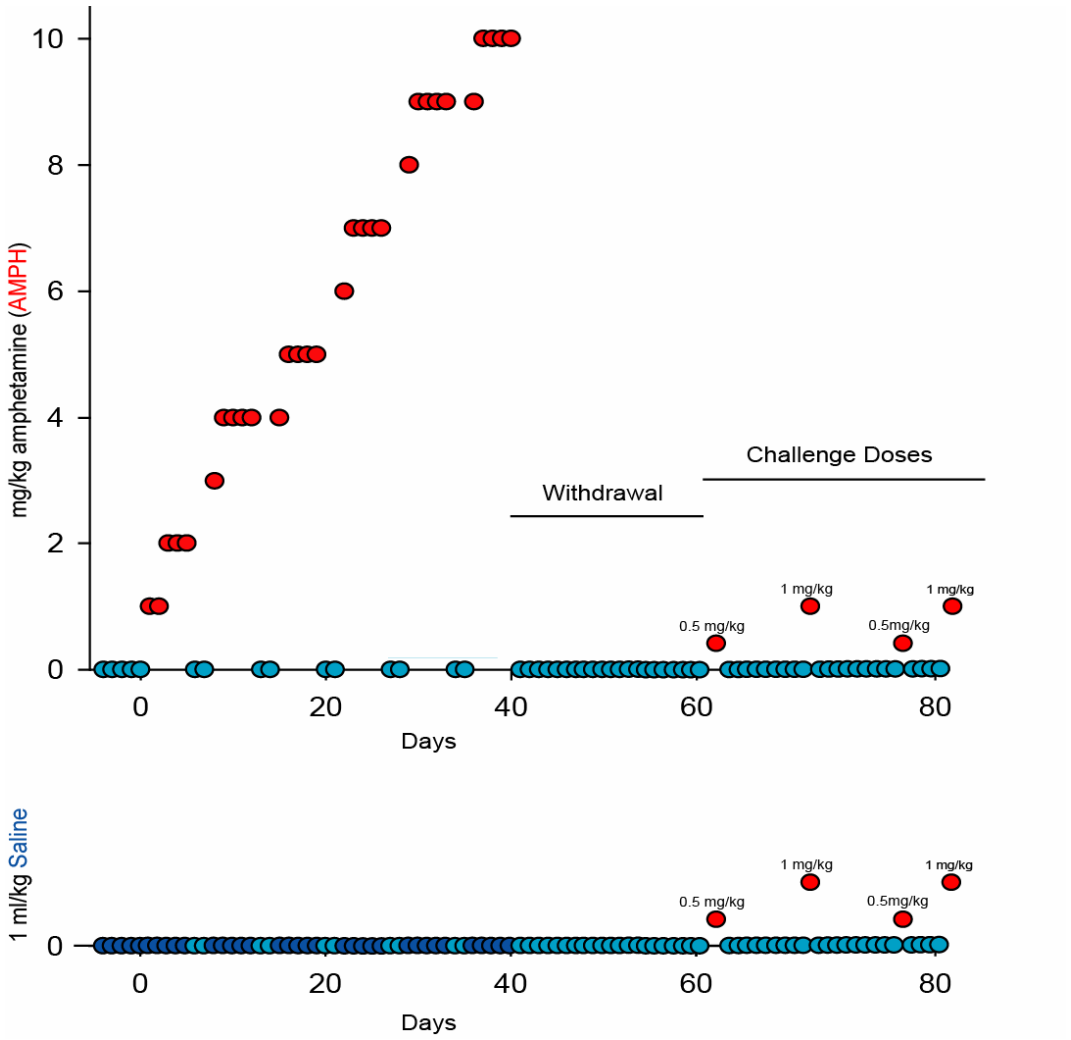

Figure 2.2 Experimental time line of escalated AMPH or saline pretreatment and challenge doses. Animals received AMPH or saline twice a day during the pretreatment phase, once before the daily training session and again 8 hours later (the ordinate depicts the dose that was given twice daily; each dot depicts one day and dose; see Methods and Materials for details). Control animals received vehicle throughout the pretreatment regimen and, similar to AMPH-pretreated rats, AMPH-"challenges" following the drug free period.

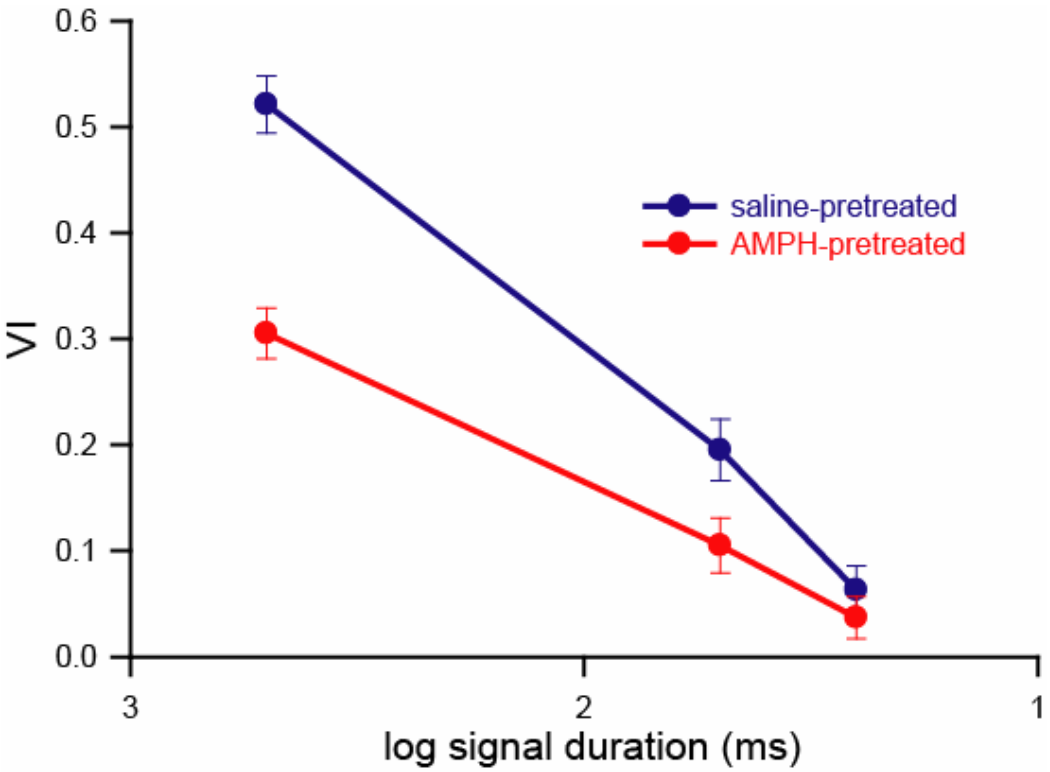

Figure 2.3: Drug-off day performance of rats during the pretreatment phase (M±SEM). amphetamine (AMPH)-treatment resulted in an impairment of the overall performance (indicated by the "vigilance index" (VI)) during drug-off days when compared with the performance of saline-treated animals. The effect on VI was largely due to a decrease in hits that was most pronounced in trials presenting the longest (500 msec) signals, possibly because of "floor"—effects with respect to the relative number of hits to shorter signals.

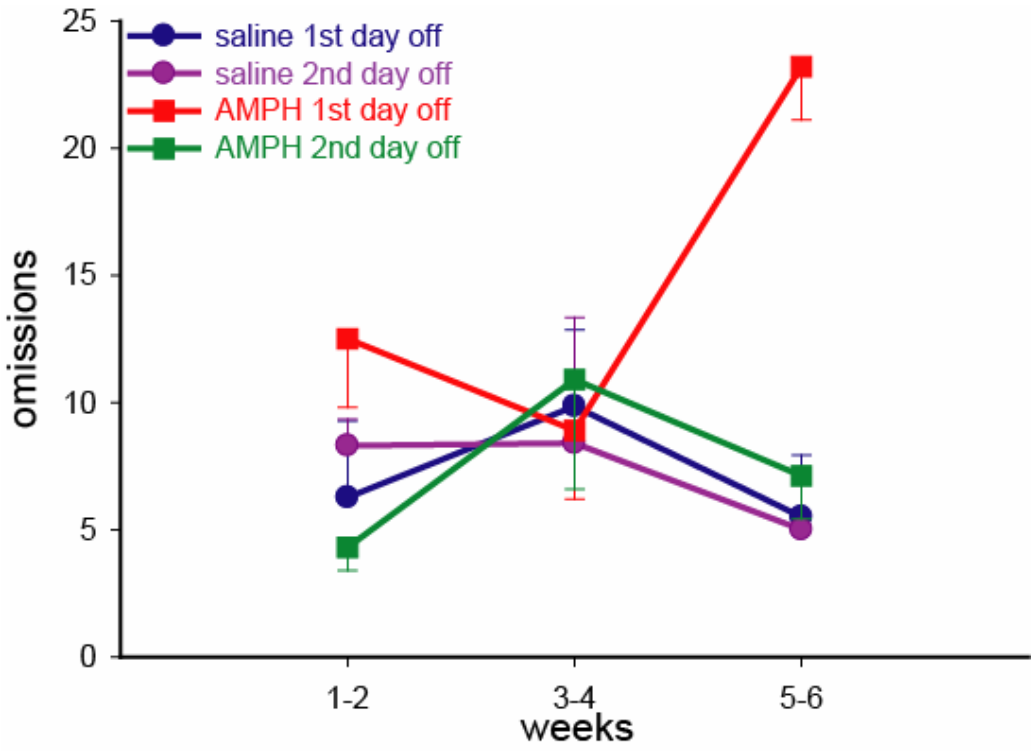

Figure 2.4 Omission data from the final two drug-free periods during pretreatment. During the last two, 2-day drug-off periods, the omissions in AMPH-treated animals were relatively high on the first when compared with the second drug-off day, possibly reflecting the acute effects of, or immediate withdrawal from, relatively high doses of AMPH administered during the later part of the pretreatment phase.

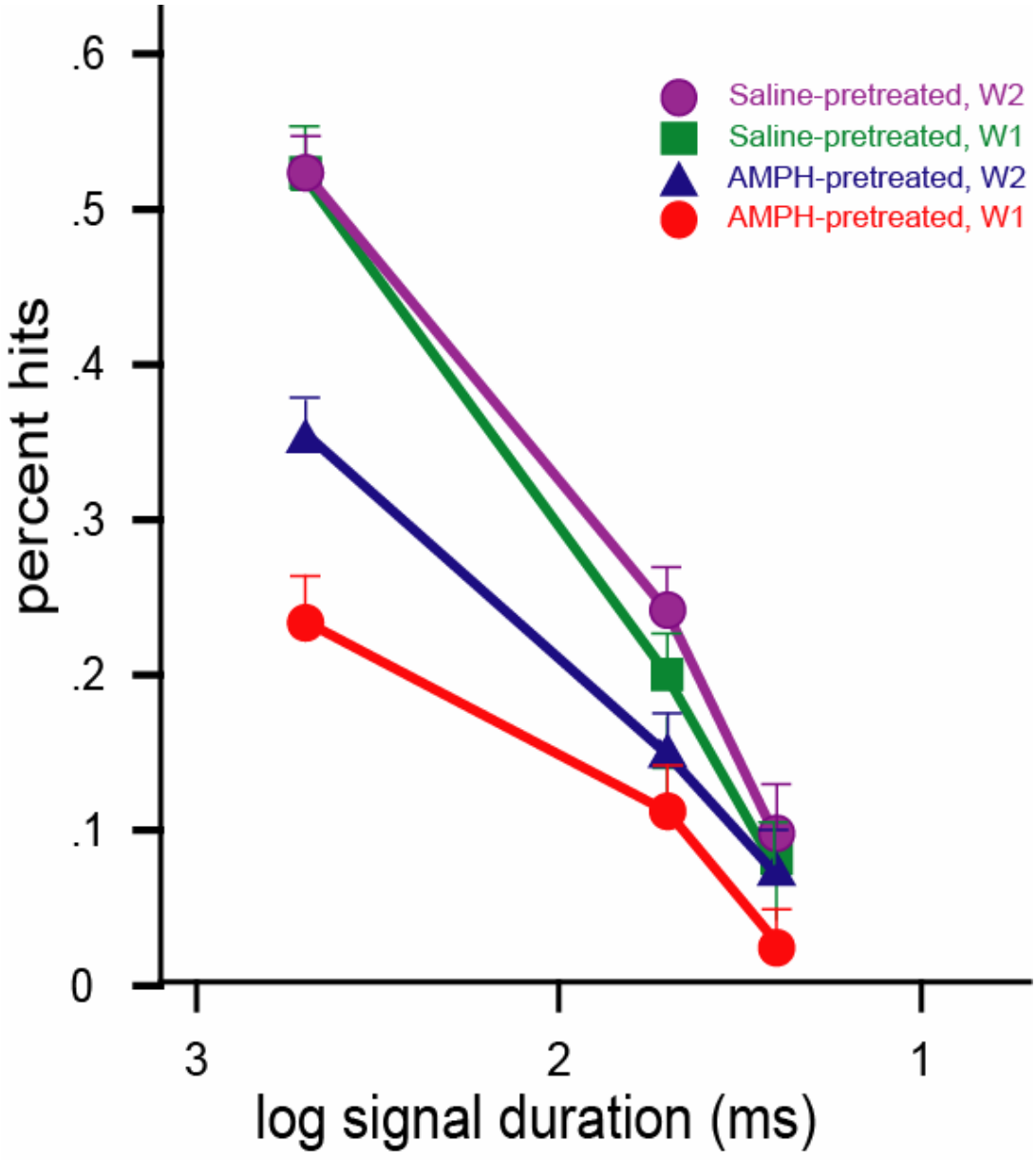

Figure 2.5: Average overall performance (vigilance index; (VI)) of amphetamine (AMPH)- and saline-pretreated animals during the the 20-day withdrawal period. Pretreatment with AMPH resulted in an impaired performance as indicated by VI. The performance of AMPH-pretreated rats improved from W1 to W2.

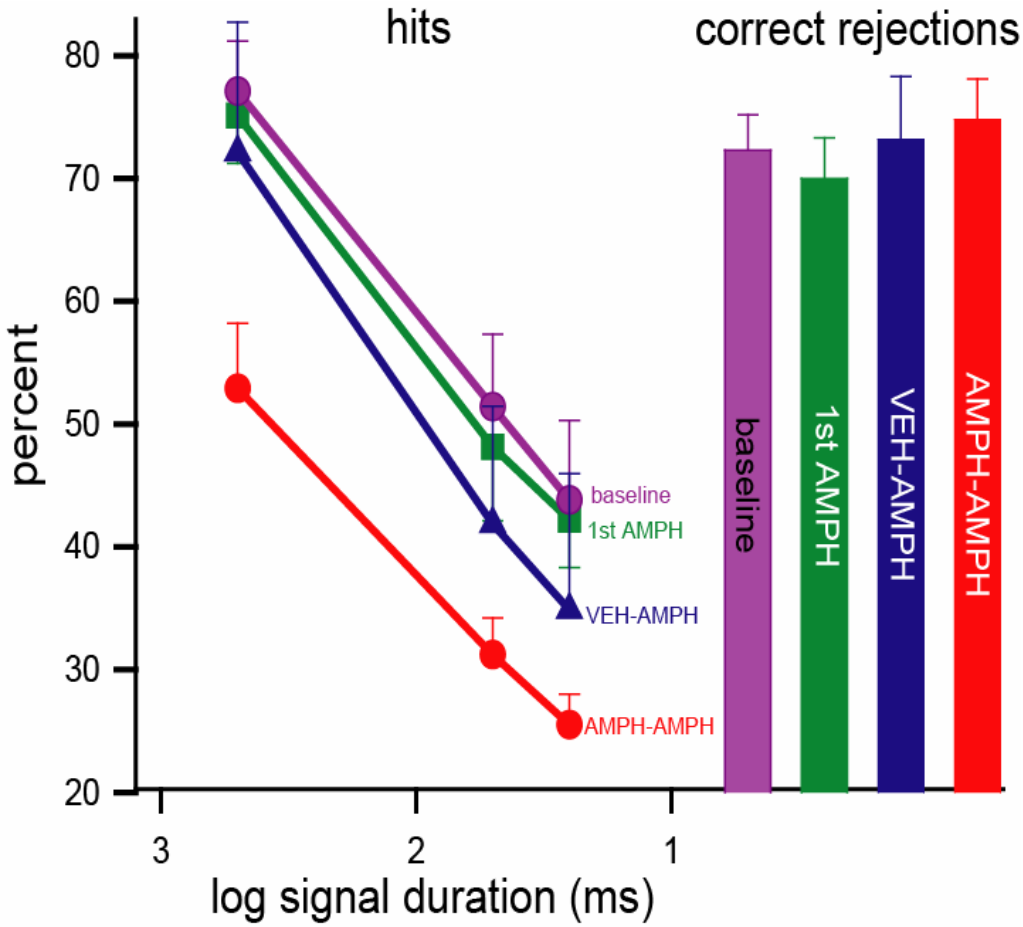

Figure 2.6. Effects of 1.0 mg/kg AMPH-challenges on the relative number of hits and correct rejections. The figure depicts the relative number of hits (at left) and correct rejections (at right) of animals at baseline (before any drug administration; "baseline"), following the first administration of amphetamine (AMPH; 1.0 mg/kg) at the beginning of the pretreatment regimen ("first AMPH"), and following the administration of this dose as a challenge ("AMPH-AMPH"). Furthermore, the effects of this dose given as a "challenge" to vehicle-pretreated rats are shown ("VEH-AMPH"). The effects of first AMPH and VEH-AMPH did not differ from baseline performance. In contrast, the administration of AMPH-challenges in AMPH-pretreated rats produced a significant decrease in hits but did not affect the animals' ability to respond correctly in non-signal trials.

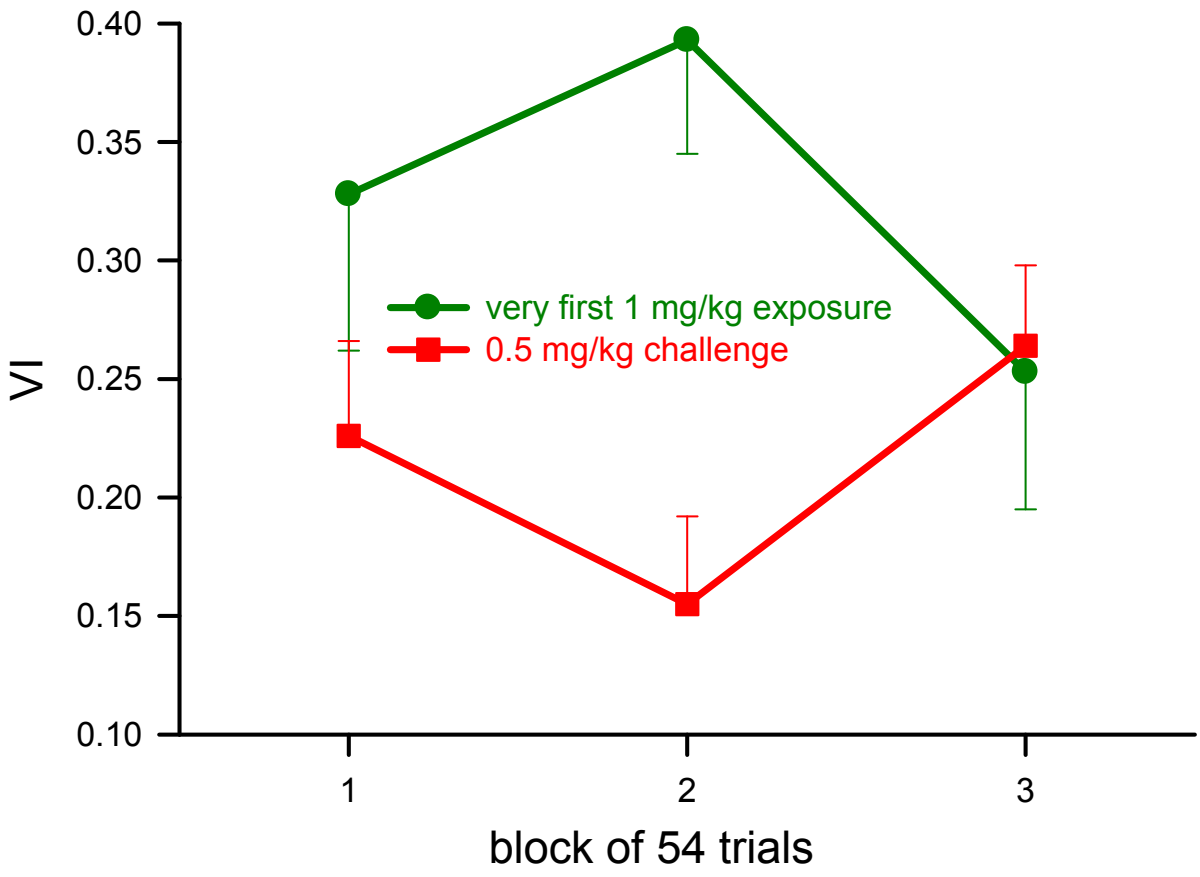

Figure 2.7 Comparison between the effects of the administration of 0.5 mg/kg following the drug-free period ("challenge") and the first administration of 1 mg/kg of AMPH. In the analysis of effects on overall performance (VI), the smaller dose of AMPH given as a challenge resulted in a significant impairment in performance when compared with the acute effects of twice the amount of drug. The reason for a significant difference specifically during the second block of trials is unclear.

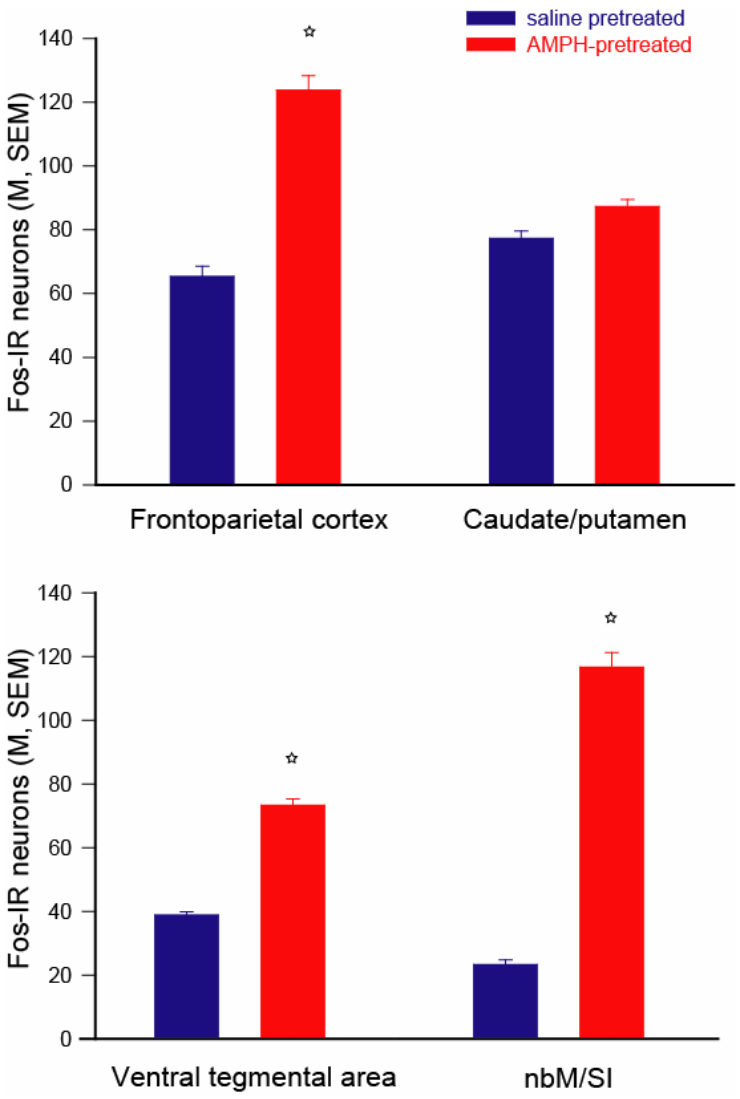

Figure 2.8. Number of Fos-immunoreactive (IR) neurons in the four regions of interest (see Methods for counting and averaging procedures). In animals pretreated and challenged with amphetamine (AMPH), compared with saline-pretreated and AMPH-'challenged' animals, significant increases in Fos-IR were found in all regions except the caudate/putamen. The increase in Fos-IR neurons in the nucleus basalis of Meynert/substantia innominata (NbM/SI) region was particularly robust. The majority of Fos-IR neurons in the latter region were also cholinergic (Figure 2.7).

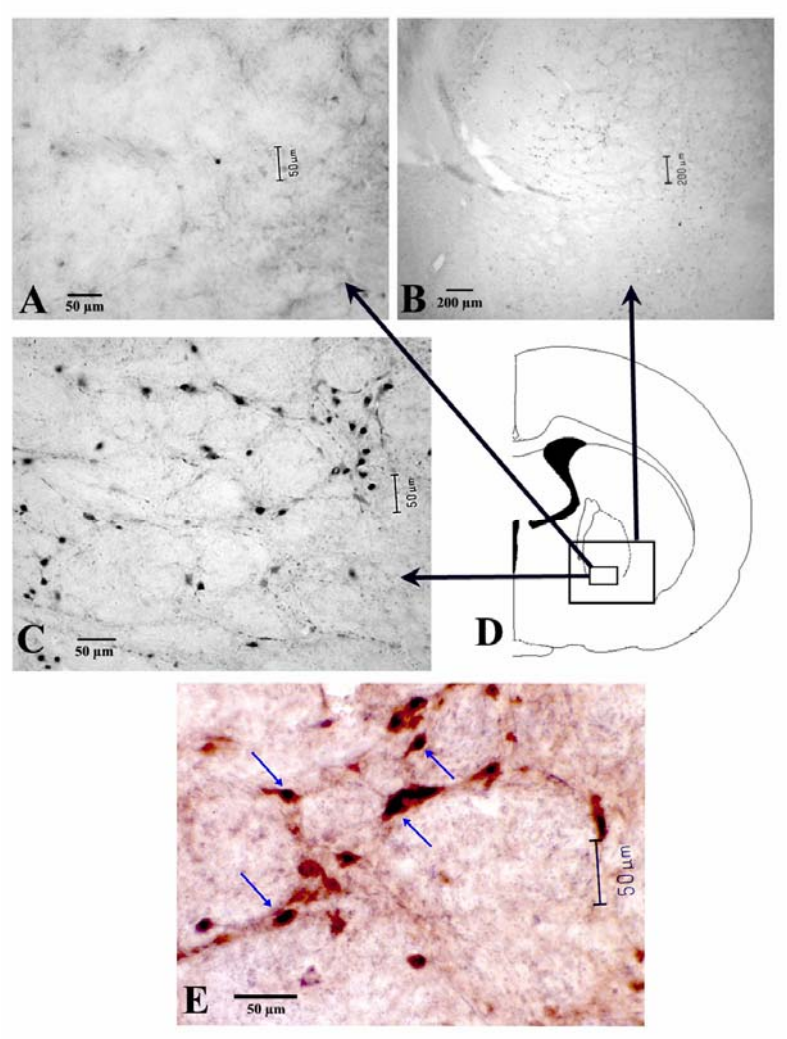

Figure 2.9 Schematic illustration of the sampling area and photomicrographs of Fos-immunoreactivity in the basal forebrain of a saline- **(A)** and an amphetamine (AMPH)-pretreated **(C)** animal. **(B)**The region in the basal forebrain shown in (**D)** which includes the dorsal substantia innominata and the medioventral part of the nucleus basalis of Meynert. A section, from an AMPH-pretreated rat, that was double-immunostained for Fos (see the blackish nuclei) and choline acetyltransferase (ChAT; see the reddish-brown cytoplasm) (**E)**. Approximately 80% of all Fos-positive cells in this area were also ChAT-positive (see arrows). Bars in A, C, E: 50 µm.

CHAPTER 3

TOWARD A NEURO-COGNITIVE ANIMAL MODEL OF THE COGNITIVE SYMPTOMS OF SCHIZOPHRENIA: DISRUPTION OF CORTICAL CHOLINERGIC NEUROTRANSMISSION FOLLOWING REPEATED AMPHETAMINE EXPOSURE IN ATTENTIONAL TASK-PERFORMING, BUT NOT NON-PERFORMING, RATS

3.1 Summary

Impairments in attentional functions and capacities represent core aspects of the cognitive symptoms of schizophrenia. Attentional performance has been demonstrated to depend on the integrity and activity of cortical cholinergic inputs. The neurobiological, behavioral, and cognitive effects of repeated exposure to psychostimulants model important aspects of schizophrenia. In the present experiment, prefrontal acetylcholine (ACh) release was measured in attentional task-performing and non-performing rats pretreated with an escalating dosing regimen of amphetamine (AMPH) and following challenges with AMPH. In non-performing rats, pretreatment with AMPH did not affect the increases in ACh release produced by AMPH-challenges. In contrast, attentional task performance-associated increases in ACh release were attenuated in AMPH-pretreated and AMPH-challenged rats. This effect of repeated AMPH exposure on ACh release was already present before task-onset, suggesting that the loss of cognitive control that characterized these animals' performance was a result of cholinergic dysregulation. The findings indicate that the demonstration of repeated AMPH-induced dysregulation of the prefrontal cholinergic input system

depends on interactions between the effects of repeated AMPH exposure and cognitive performance-associated recruitment of this neuronal system. Repeated AMPH-induced disruption of prefrontal cholinergic activity and attentional performance represents a useful model to investigate the cholinergic mechanisms contributing to the cognitive impairments of schizophrenia.

3.2 Introduction

As reflected by the NIMH Initiative 'Measurement and Treatment Research to Improve Cognition in Schizophrenia' (MATRICS), the determination of the neuronal abnormalities that underlie the cognitive impairments of schizophrenia and the development of novel pro-cognitive treatments are pressing research objectives. Based on the fundamental role of attentional processes for learning and general cognitive control, attentional dysfunctions have been considered a central and even essential characteristic of schizophrenia (McGhie and Chapman 1961; Braff 1993; Braver, Barch *et al*. 1999; Braff and Light 2004; Keefe, Bilder *et al*. 2006; Nuechterlein, Dawson *et al*. 1994; Venables 1964).

Attention describes the cognitive states and operations that govern the readiness for the detection of changes in the stimulus situation, the selection of such changes over irrelevant 'noise' for further processing, and the management of attentional resources for the detection and processing of competing stimuli. Substantial evidence supports a crucial role of the cortical cholinergic input system in the mediation of attentional functions and capacities (Everitt and Robbins 1997; Sarter, Givens *et al*. 2001; Sarter, Hasselmo *et al*. 2005).

Removal of cortical cholinergic inputs produces persistent impairments in attentional performance. Furthermore, attentional performance is associated with increases in cortical acetylcholine (ACh) release that are not observed in animals performing tasks controlling for the non-attentional aspects of performance (McGaughy, Kaiser *et al*. 1996; Himmelheber, Sarter *et al*. 1997; Turchi and Sarter 1997; Himmelheber, Sarter *et al*. 2000; McGaughy, Everitt *et al*. 2000; Passetti, Dalley *et al*. 2000; Turchi and Sarter 2000; Dalley, McGaughy *et al*. 2001; Kozak, Bruno *et al*. 2006).The available evidence indicates that the cortical cholinergic input system mediates not one particular aspect of attention but supports a range of attentional abilities, including sustained, selective, and divided attention.

Although reduced muscarinic receptor densities in the cortex of schizophrenics have been reported (Crook, Tomaskovic-Crook *et al*. 2000; Crook, Tomaskovic-Crook *et al*. 2001; Hyde and Crook 2001; Raedler, Knable *et al*. 2003; Newell, Zavitsanou *et al*. 2007), the status of cortical cholinergic neurotransmission in schizophrenia remains poorly understood. Owing in part to the lack of methods capable of assessing dynamic aspects of cholinergic dysregulation in humans, the potential contribution of cholinergic dysregulation to the cognitive symptoms of patients is unknown.

Repeated exposure to psychostimulants has long been known to produce psychotogenic effects in humans (Bell 1965; Bell 1973; Kokkinidis and Anisman 1981; Kokkinidis and Anisman 1981; Snyder, Aghajanian *et al*. 1972; Snyder

1973; Wallis, Mc *et al*. 1949; Caton 2000). Furthermore, the effects of repeated psychostimulant exposure in healthy humans and animals model important neurobiological, behavioral, and cognitive aspects of schizophrenia (Robinson and Becker 1986; Lieberman, Sheitman *et al*. 1997; Castner and Goldman-Rakic 1999; Laruelle 2000; Castner and Goldman-Rakic 2003; Kapur 2003; Segel 1978; Strakowski, Sax *et al*. 1997; Yui, Goto *et al*. 1999), including the deficits in sensorimotor gating and attentional processing (Crider, Solomon *et al*. 1982; Tenn, Fletcher *et al*. 2003; Martinez, Parikh *et al*. 2005; Sarter, Nelson *et al*. 2005). Furthermore, repeated psychostimulant exposure models the sensitization of the mesolimbic dopamine system that has been demonstrated in never-medicated patients and during psychotic periods (Abi-Dargham, Gil *et al*. 1998; Laruelle, Abi-Dargham *et al*. 1996; Strakowski, Sax *et al*. 1997; Laruelle, Abi-Dargham *et al*. 1999; Laruelle 2000). Based on evidence suggesting close links between the mesolimbic dopamine system and basal forebrain cholinergic neurons (Moore, Fadel *et al*. 1999; Neigh-McCandless, Kravitz *et al*. 2002; Neigh, Arnold *et al*. 2004; Zmarowski, Sarter *et al*. 2005), abnormal regulation of the cortical cholinergic input system has been hypothesized to represent an integral component of the dysregulated forebrain systems responsible for the cognitive symptoms of schizophrenia (Sarter, Hasselmo *et al*. 2005). Previous findings indicating psychostimulant exposure-induced alterations in the regulation of basal forebrain cholinergic neurons are consistent with this hypothesis (Nelson, Sarter *et al*. 2000; Martinez, Parikh *et al*. 2005). However, this evidence does not form the basis for hypotheses describing unidirectional, causal

relationships between dysregulated dopaminergic and cholinergic systems. Escalating bidirectional interactions between abnormally regulated mesolimbic projections to the basal forebrain and prefrontal cortex, and aberrant cholinergic recruitment of prefrontal neurons projecting to mesolimbic regions may ultimately be responsible for the disruption of prefrontal-mesolimbic information processing that is widely hypothesized to underlie the cognitive symptoms of schizophrenia. The present focus on the regulation of the prefrontal cholinergic input system is based on the extensive evidence linking this system to fundamental attentional processes.

The current experiment utilized an escalating dosing pretreatment regimen of amphetamine (AMPH) that is known to generate neurobiological and behavioral characteristics resembling psychostimulant psychosis (Paulson, Camp *et al*. 1991). This regimen produces lasting psychomotor sensitization without yielding neurotoxicity (Robinson and Camp 1987; Paulson, Camp *et al*. 1991; Paulson and Robinson 1995; Robinson, Jurson *et al*. 1988). Importantly, psychomotor sensitization, locomotor hyperactivity, or stereotypies are not observed in attentional task-performing rats following the repeated administration of AMPH, perhaps as a result of the constraining of the animals' behavior by the operant and attentional requirements of the task (Martinez, Parikh *et al*. 2005). Furthermore, it is important to note that the administration of AMPH-challenges is thought to model the role of stressors in eliciting psychotic episodes, and/or in revealing a sensitized mesolimbic system (Moghaddam 2002; Robinson and Becker 1986) that can trigger active disease periods (Ventura, Nuechterlein *et al*.

1989; Lieberman, Sheitman *et al*. 1997; Yui, Goto *et al*. 1999, Ujike and Sato 2004; Sato 1992; Sato, Numachi *et al.* 1992).

The present study was designed to determine the effects of pretreatment and challenge with AMPH on ACh release in the medial prefrontal cortex (mPFC) in animals performing a task taxing attentional capacities and in animals that did not perform a task. This experiment was guided by the general hypothesis that in order to demonstrate the abnormal regulation of a neurotransmitter system, recruitment of that system, by behavioral and cognitive operations relevant to that system, is required. The results support this hypothesis and indicate that repeated exposure to AMPH disrupts the regulation of cholinergic projections to the prefrontal cortex and thereby cognitive task control. Furthermore, these findings indicate the usefulness of experiments designed to measure the effects of repeated AMPH exposure on cognitive performance and, simultaneously, performance-associated increases in ACh release, as a model for research on the neuronal mechanisms underlying the cognitive symptoms of schizophrenia as well as on the development of novel treatments for the cognitive symptoms of this disorder.

3.3 Methods

Animals: Twenty-four male Fischer-344/Brown–Norway F1 hybrid rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing between 300 and 350 g at the beginning of behavioral training, were housed individually in a temperature (23°C)- and humidity (45%)-controlled environment with a 12:12 light/dark cycle

(lights on at 0700 hours). Animals were handled extensively before the beginning of training. Food (Rodent Chow, Harlan Teklad, Madison, WI) was available *ad libitum*, whereas water was available only during behavioral training as reward (below) as well as for 8 min in the home cage following daily operant training. Animal care and experimentation were performed in accordance with protocols approved by the University Committee On Use and Care of Animals of the University of Michigan (UCUCA).

Apparatus: Behavioral training was conducted using 12 operant chambers (Med-Associates, St Albans, VT), each enclosed within a sound-attenuating compartment and equipped with three panel lights (2.8 W), two retractable levers and a water dispenser delivering 30 µl water per reward into a cup located between the two levers. A house light (2.8 W) was located on the rear wall. Signal presentation, lever operation, water delivery, and data collection were controlled by a PC running Med-PC for Windows software (V 4.1.3; Med-Associates).

Behavioral Training: Training methods and evidence in support of the validity of performance measures in terms of reflecting sustained attention performance have been previously described (McGaughy and Sarter 1995; McGaughy and Sarter 1998). It should be noted that the use of this particular task in this experiment does not necessarily imply that performance of specifically this task would uniquely activate the cortical cholinergic input system; rather, this form of attention can be more readily trained and tested in rats when compared with

more complicated tasks designed to assess other aspects of attention, such as divided attention (Turchi and Sarter 1997; Turchi and Sarter 2000).

Training occurred between 08:00 and 18:30 hours 7 days a week. Animals were initially trained to lever press for water in accordance with a modified FR-1 schedule. Following at least three consecutive sessions of over 120 reinforced lever presses, animals advanced to the second stage of task acquisition. Animals were first trained to discriminate between signal (1 s illumination of the central panel light) and non-signal (no illumination) events. Two seconds following the occurrence of a signal or non-signal, both levers were extended into the operant chamber and remained active for 4 s or until a lever press occurred. During signal trials, a left lever press was scored as a hit, whereas a right lever press was scored as miss. Conversely, during non-signal trials, a right lever press was scored as a correct rejection and a left lever press was scored as a false alarm. Half of all animals were trained to acquire the task using reversed response rules. Hits and correct rejections were rewarded, whereas false alarms and misses were not. During this stage of training, incorrect responses resulted in the initiation of correction trials. During correction trials, the previous trial was repeated up to three times. If an animal continued to respond incorrectly, a forced-choice trial was initiated by presenting the correct lever only following a signal or non-signal event. Correction and forced-choice trials served to facilitate the acquisition of response rules and prevent the development of a side bias.

Once animals achieved at least three consecutive days of stable performance defined as ≥59% correct responses to both signal- and non-signal

events, they advanced to the third stage of task acquisition. Signal duration was shortened and signals were presented for 25, 50, or 500 ms. The sequence of signal duration and the occurrence of signal and non-signal trials were pseudo-randomized to yield 27 trials per signal duration and 81 non-signal trials. Correction trials and forced trials were discontinued, and the intertrial interval (ITI) was shortened from 12±3 to 9±3 s. As will be further detailed below, measures of performance included the relative number of hits (hits/hits+misses), calculated for each signal length, and the relative number of correct rejections (correct rejections/correct rejections+false alarms). Once animals achieved at least 3 days of stable performance, defined as ±70% hits to 500 ms signals, ±70% correct rejections, and ≤50% omissions to 25 ms signal), they began training in the final task (see Figure 3.1a). House lights were illuminated throughout the session. This important final modification requires that animals constrain their behavior to maintain persistent orientation toward the intelligence panel. Each session lasted approximately 40 min. The pretreatment regimen was initiated after animals maintained criterion performance (≥70% hits to 500 ms signals, ≥70% correct rejections, and ≤20% omissions) for 3 consecutive days.

Measures of performance included hits, misses, correct rejections, false alarms, and omissions. The relative number of hits and correct rejections was calculated as described above. To obtain an overall index of performance that reflects the performance in trials involving signals as well as non-signal events, the vigilance index (VI) was calculated based on the relative number of hits (h) and false alarms (fa) using the formula: $VI=(h-fa)/[2(h+fa)-(h+fa)^2]$). This index is

comparable to the sensitivity index (SI) (Frey 1973)except that VI is based on the relative number of hits and false alarms, whereas SI is calculated using the probabilities for hits and false alarms. Thus, VI values are not confounded by errors of omission. Values for VI can vary from +1.0 to -1.0, with +1.0 indicating that all responses were scored as hits or correct rejections, 0 indicating an inability to discriminate between signal and non-signal events, and -1 indicating that all responses to signals were misses and all responses to non-signals were false alarms. The index was calculated for each signal duration (VI: 500, 50, 25 ms). Finally, errors of omission were recorded. Performance measures were calculated collectively for the entire session as well as separately for each of four task blocks (10 min each; see Figure 3.2).

Pretreatment Regimen and Challenges: After reaching performance criterion, animals were randomly divided into two groups (n=7 each) designated to be pretreated with AMPH or vehicle (saline; SAL). Animals were administered either *d*-AMPH sulfate (1–10 mg/kg; i.p.; concentrations included salt weight; dissolved in 1.0 ml/kg of 0.9% saline; Sigma, St Louis, MO) or saline (1.0 ml/kg i.p.) twice per day, with approximately 8 h separating the two injections (Figure 3.1b). Animals received the first injection at approximately 08:00 hours and were placed immediately into the test chambers. Task onset was 20 min post-injection. Following the completion of the test session, animals were promptly returned to their home cages. The second dose of drug or vehicle was given in the home cage at approximately 16:00 hours. AMPH doses were administered in elevating increments over the course of 40 days (Paulson, Camp *et al*. 1991). All animals

were given injections of saline on weekends to mimic the 'runs and crashes' known to foster psychostimulant-induced psychoses (see Figure 3.1b; for references see Introduction). Following AMPH doses of 2 mg/kg or higher, task performance was disrupted and animals omitted all trials. In order to control for the potentially confounding effects of substantially lower amounts of task practice in AMPH-treated animals, animals treated with vehicle were placed in the testing chambers but not allowed to perform for an equivalent number of sessions during weekdays, whereas AMPH-treated animals received doses >2 mg/kg (Martinez, Parikh *et al*. 2005). Both saline- and AMPH-treated animals performed the task on weekends.

In our previous experiment employing this AMPH regimen in task-performing animals (Martinez, Parikh *et al*. 2005), we found that following termination of the pretreatment regimen, AMPH-treated animals' performance required about 2 weeks of continued training to return to baseline, reflecting the general behavioral depression observed during this period of withdrawal (Paulson, Camp *et al*. 1991). As this experiment was not designed to study aspects of withdrawal, and in order to implant guide cannula (for the later insertion of microdialysis probes) relatively close to the actual microdialysis test sessions, surgery was conducted 7 days into this withdrawal period. The daily testing continued until surgery (below) and resumed following 5 days of post-surgery recovery during which food and water were available *ad libitum*. All subsequent testing was conducted in operant chambers modified to accommodate the procedures for microdialysis (see below).

The effects of AMPH-challenges were determined following the administration of 1.0 mg/kg of AMPH. This dose is the first dose given during the pretreatment regimen and was observed earlier not to produce acute effects on attentional performance (Martinez, Parikh *et al*. 2005). Likewise, this dose did not affect the performance of saline-pretreated animals when given as a 'challenge' (Martinez, Parikh *et al*. 2005). Therefore, significant differences between the effects of 1.0 mg/kg when given as a challenge to animals pretreated with AMPH or vehicle can be attributed to the differential pretreatment history. Thus, final comparisons were based on data from animals pretreated with SAL or AMPH and challenged with either SAL or AMPH, resulting in four treatment conditions and groups (SAL/SAL; AMPH/SAL; SAL/AMPH; AMPH/AMPH).

Non-Performing Rats: Non-performing animals (n=10) were handled extensively using procedures identical to task-performing rats, including the daily transport between home cages and operant chambers and the number and the timing of injections of AMPH (n=6) or SAL (n=4). However, the task was never activated for these animals and, as water reward was not delivered, they were not water deprived. Similar to task-performing animals, non-performing animals underwent stereotaxic surgery 7 days following the cessation of pretreatment. Animals were allowed to recover for 5 days and then resumed the handling procedures for the remainder of the withdrawal period. The effects of AMPH- or saline-challenges on ACh release were determined 33.6±6.4 days (M±SEM) after completion of the pretreatment period (in order to match the interval that was required for performing rats; see below).

Surgical Methods: Surgery was performed under aseptic conditions. Initial anesthesia was induced with 4–5% isoflurane by placing the animal in an anesthetic chamber (Anesco/Surgivet, Waukesha, WI). Gas was carried via oxygen at a flow rate of 0.6 ml/min. Animals were also given a preoperative injection of an antibiotic (Amikacin, 100 mg/kg; s.c.). Heads were shaved using electric clippers and cleaned with 70% ethanol and iodine tincture. Ophthalmic ointment was applied to lubricate the eyes. Animals were then mounted into a stereotaxic instrument (David Kopf, Tujunga, CA). Isoflurane was administered via a face mask at 1.5–2% for the duration of surgery. Microdialysis guide cannula (0.38 mm o.d.; Sci Pro, Sanborn, NY, USA) were implanted dorsal to the prelimbic region of the right hemisphere at the following coordinates: AP (from Bregma: 2.9 mm, ML: 0.6 mm, DV: 0.6 mm (from dura). After surgery, rats were returned to their home cages and allowed to recover for 5 days with free access to food and water. Thereafter, the water deprivation schedule resumed and animals were returned to behavioral training until they regained performance criterion (if applicable). Before daily test sessions, the dummy stylets were removed and polyethylene tubing was attached in order to habituate the animals to performing while being dialyzed.

Microdialysis Methods: Following recovery, animals resumed operant training in chambers modified to accommodate microdialysis procedures. The modified operant chambers used to measure ACh release in task-performing rats featured a taller recessed water delivery area (9.0 X 5.0 cm, height X width) to allow access for animals with a probe inserted and inlets and outlets attached, and to

accommodate the liquid swivels, syringes and pumps outside the chambers. This was performed in order to allow collection of dialysates outside of the chambers without interfering with the animals' ongoing performance.

Furthermore, the length of the test sessions was set to 40 min to correspond exactly with the timing of four dialysate collections (10 min each). Procedures designed to foster habituation to microdialysis procedures, particularly the tethering during task performance, were initiated at this point. Because of the subsequent requirement for extended pretask microdialysis discard periods (3 h), the collection of four baseline dialysates, and an additional two dialysates following drug treatment and before task onset (see Figure 3.2), rats were placed in the operant chambers 240 min before task onset. The houselight was illuminated for the entire time the animals were in the operant chambers.

After being transferred to the modified operant chambers, animals were retrained to a performance criterion (≥60% hits to 500 ms signals, ≥65% correct rejections, and ≤20% omissions for three consecutive sessions). This criterion was more lenient than for the original acquisition (above), because the performance of tethered animals was more variable and slightly impaired relative to the performance of non-tethered animals. As these animals required a relatively large number of sessions to meet performance criterion, on average 34.7±3.7 days elapsed between completion of the pretreatment period and the first microdialysis session. Animals were dialyzed at least twice, first following the

administration of saline and 6±1 days later following the administration of AMPH. Considering evidence suggesting that even a single dose of AMPH produces sensitizing effects (Vanderschuren, Schmidt *et al*. 1999), the effects of saline-'challenges' were always tested first. In the event that dialysis sessions preceded by saline administration did not result in detectable levels of ACh as a result of probe failure or severed tubing, a maximum of two additional sessions were conducted in order to generate a complete data set for each animal. Repeated insertion of probes over a period of weeks, up to four insertions, was repeatedly demonstrated to generate similar basal ACh release levels (see Results) sensitive to the blocking of voltage-regulated sodium channels with tetrodotoxin (Moore, Sarter *et al*. 1993; Moore, Sarter *et al*. 1995; Moore, Stuckman *et al*. 1995; Moore, Stuckman *et al*. 1996; Moore, Fadel *et al*. 1999).

Before insertion of a concentric microdialysis probe into the brain (Model MAB4; membrane o.d.: 0.24 mm; membrane length: 3.0 mm; Sci Pro), probe recovery was determined *in vitro* by placing the probe into a 1.0 pmol ACh solution and collecting for 10 min. Probes that were used exhibited recoveries of ≥9%. Probes were perfused at a rate of 2.0 μl/min with artificial cerebrospinal fluid, pH 6.9±0.1, containing the following (in mM): 126.5 NaCl, 27.5 $NaHCO_3$, 2.4 KCL, 0.5 NA_2SO_4, 0.5 KH_2PO_4, 1.2 $CaCl_2$, 0.8 $MgCl_2$, and 5.0 glucose. Note that the perfusion medium did not contain an acetylcholinesterase inhibitor.

Dialysate collections were frozen at -80°C until ACh contents were determined using high performance liquid chromatography coupled with

electrochemical detection (ESA, Chelmsford, MA), using a mobile phase containing 50 mM sodium phosphate. ACh was separated from choline on UniJet microbore analytical column (Bioanalytical Systems Inc. (BASi), West Lafayette, IN) and catalyzed on a post-column solid-phase reactor containing acetylcholinesterase and choline oxidase. ACh was hydrolyzed to acetate and choline, and choline oxidized to hydrogen peroxide and betaine. The amount of hydrogen peroxide corresponding to ACh was then detected using a 'peroxidase-wired' glassy carbon electrode with an applied potential of -200 mV (Huang, Yang *et al*. 1995). The concentration of ACh was calculated by integrating the area under the peak and fitting this value to a regression line containing values of ACh that were in the expected range of the *in vivo* dialysates. The detection limit of this system averaged 2 fmol/15 μl.

Histological Verification of Probe Placements: Within 1 week following the last microdialysis session, animals were given an overdose of sodium pentobarbital and underwent cardiac perfusion with 0.1 M of phosphate buffer followed by 4% buffered formalin. The brains were post-fixed in formalin overnight, and transferred to a 30% sucrose phosphate buffer solution. Sections (40 μm thick) surrounding the probe and cannula sites were mounted, stained with cresyl violet, and examined for probe placements.

Statistical Methods: Statistical analyses for performance and dialysis data were conducted using mixed model analysis of variance (ANOVAs). As basal ACh release data did not differ between groups (see Results), the effects of the

challenges on ACh release in performing and non-performing rats were conducted based on values expressed as the percent change values from mean basal ACh release (average of the last three collections before the administration of AMPH or saline). ACh release values were not corrected for probe recovery. To determine drug-induced changes in ACh release in performing rats, a mixed ANOVA on the effects of task (e.g. two post-drug/pre-task collections vs. four task-associated collections), pretreatment (AMPH or saline), and session (AMPH- or saline-challenge) was conducted and followed, where appropriate by two- and one-way ANOVAs and multiple comparisons. Task performance was analyzed on the basis of overall performance as indicated by VI (see above for calculation). In addition, the numbers of errors of omission were analyzed. The ANOVAs determined the effects of pretreatment (AMPH vs. saline), session (AMPH- or saline-challenge), and signal duration (500–25 ms) on VI and omissions. Significant main effects and interactions were followed by two- and one-way ANOVAs and Fisher's least significant difference test (LSD) for multiple comparisons. Exact p-values are reported for significant results, as was recommended earlier (Greenwald 1996). Statistical analyses were performed using SPSS Version 14.0 for Windows (SPSS Inc., Chicago, IL).

3.4 Results

Histological Findings: As illustrated in the inset in Figure 3.2, dialysis probes were placed into the middle layers of the pre-limbic region. In most cases, the active membrane extended either dorsally into the anterior cingulate cortex or

ventrally into the infralimbic region. Neither baseline release values nor performance- and/or AMPH-induced changes in ACh release systematically differed between these minor variations in placement, and thus evidence obtained from all these placements was used for final analysis.

Effects of Repeated AMPH on ACh Release in Non-performing Rats: Basal ACh release did not differ between non-performing animals pretreated with AMPH and vehicle ($F(1,8)=0.085$; $p>0.05$; Figure 3.3), nor did basal values differ between session (before vehicle-'challenge' or AMPH-challenge; $F(1,8)=0.006$; $p>0.05$), and the two variables did not interact significantly ($F(1,8)=0.09$; $p>0.05$). Basal ACh release was 7.26±1.09 fmol/15 μl. Because of the absence of pretreatment effects on basal ACh release, the effects of vehicle- or AMPH-challenge on ACh release were expressed as percent change from baseline.

Compared with the administration of vehicle, the AMPH-challenge resulted in a significant increase in ACh release in both saline and AMPH-pretreated rats (main effect of session: $F(1,8)=28.28$; $p=0.001$). The increase in ACh release that resulted from AMPH-challenge did not differ between animals pretreated with vehicle or AMPH (pretreatment: $F(1,8)=0.07$; $p>0.05$; 'pretreatment' X 'session': $F(1,8)=0.11$; $p>0.05$). Furthermore, ACh release did not vary over the four collections (T1–T4 in Figure 3.3), and this variable did not interact with pretreatment or session (all $p>0.05$). As illustrated in Figure 3.3, following saline-'challenges', ACh release was 18.06±15.22% over baseline (averaged over T1–T4); these values did not differ significantly from baseline values ($F(1,8)=0.42$;

p>0.05). The AMPH-challenges resulted in an increase of 210.66±35.44% over baseline ($F(1,8)=35.13$; $p<0.001$). *Post hoc* analyses indicated that AMPH significantly increased ACh release in both groups of animals when compared to the effects of saline-'challenges' (both $p<0.02$), and that neither the effects of saline- nor AMPH-challenges differed between animals pretreated with saline vs. AMPH (both $p>0.05$). Thus, the pretreatment history of non-performing animals did not influence the AMPH-challenge-induced increases in ACh release.

Effects of Repeated AMPH on ACh Release in Performing Rats: Basal ACh release did not differ between animals pretreated with vehicle or AMPH ($F(1,12)=2.23$; $p>0.05$; Figure 3.4). Likewise, basal release did not differ before the administration of an AMPH-challenge or vehicle ($F(1,12)=1.63$; $p>0.05$) and the two factors (group, session) did not interact significantly ($F(1,12)=1.62$; $p>0.05$). Basal ACh release was 7.53±1.58 fmol/15 μl. Basal release did not differ between non-performing (above) and attentional task-performing rats ($F(1,22)=0.00$; $p>0.05$).

Effects of AMPH-Challenges on Performance-associated ACh Release: As illustrated in Figure 3.2, following the administration of drug or vehicle as challenges, two dialysates were collected before task onset. An overall ANOVA on the effects of task-stage (pre-task vs. task), pretreatment, and challenge revealed a significant interaction between the effects of pretreatment and challenge ($F(1,12)=31.63$; $p<0.001$). However, there was no effect of task-stage and no interactions between task-stage, pretreatment or challenge (all $p>0.05$).

Thus, the effects of repeated AMPH exposure and AMPH-challenge on ACh release did not differ between the two collections taken before task onset and the four collections taken during task performance (see Figure 3.4).

Similar to the results from previous experiments (Arnold, Nelson *et al*. 2003) (Himmelheber, Sarter *et al*. 2000) (Kozak, Bruno *et al*. 2006), performance of the attention task increased ACh release in the mPFC over baseline. In SAL/SAL animals, ACh release increased by 158.28±18.49% over basal ACh levels during the performance of the task ($F(1,6)=66.98$; $p<0.001$; see Figure 3.4). The performance-associated increase in mPFC ACh release is comparable to the increase observed previously in animals performing this task and using similar microdialysis conditions, including the absence of an acetylcholinesterase inhibitor (Kozak, Bruno *et al*. 2006).

The effects of the AMPH-challenges differed significantly between groups of rats pretreated with SAL vs. AMPH (pretreatment ×challenge: $F(1,12)=30.74$; $p<0.0001$; main effect of challenge: $F(1,12)=6.14$; $p=0.03$; main effect of pretreatment: $F(1,12)=3.00$; $p>0.05$; the factor time (T1–T4) did not produce a main effect and did not interact with group and session; all $p>0.05$). Figure 3.4 illustrates that AMPH-challenges in animals pretreated with AMPH (AMPH/AMPH) resulted in the attenuation of performance-associated increases in ACh release. Several *post hoc* comparisons further substantiated this result.

The acute administration of AMPH in SAL-pretreated rats did not affect the elevated levels of ACh release observed in animals performing this task

(SAL/SAL vs. SAL/AMPH; $F(1,6)=3.88$; $p>0.05$). In contrast, the acute administration of, or the challenge with, AMPH in animals that were pretreated also with AMPH resulted in a significant attenuation of ACh release levels when compared with animals pretreated with SAL (SAL/AMPH vs. AMPH/AMPH ($F(1,12)=29.62$; $p<0.0001$). Multiple comparisons indicated that all data points (T1–T4) differed significantly by pretreatment (all $p<0.004$; Figure 3.4, lower graph). Averaged over all time points, performance-associated ACh release in SAL/AMPH animals was 235.93±27.47% over pretask baseline and 47.89±24.42% in AMPH/AMPH rats. The attenuation of ACh release levels in AMPH/AMPH animals was also revealed by the within-subject comparison (AMPH/SAL vs. AMPH/AMPH; $F(1,6)=40.83$; $p=0.001$).

The attenuated levels of ACh release observed in performing AMPH/AMPH rats did not differ significantly from release levels measured at baseline (before task onset and before drug treatment ($F(1,6)=2.65$; $p>0.05$). Moreover, a *post hoc* comparison between ACh release levels in AMPH/AMPH animals over all three phases (baseline, post-drug/pre-task, during performance) indicated that ACh release levels in these animals never changed from baseline ($F(2,12)=0.79$; $p>0.05$).

Finally, in animals pretreated with AMPH and dialyzed following vehicle-'challenge' (AMPH/SAL; Figure 3.4), performance-associated ACh release was significantly higher than in animals pretreated and 'challenged' with vehicle (SAL/SAL; $F(1,12)=5.34$; $p=0.04$; averaged over all time points: saline-

pretreated: 158.28±18.49% over baseline; AMPH-pretreated: 250.92±18.44%). Multiple comparisons indicated that release during T3 was significantly higher in animals pretreated with AMPH ($F(1,12)=5.20$; $p=0.042$; the effect neared significance during T1; $F(1,12)=4.10$, $p=0.06$; Figure 3.4, top graph).

Baseline performance before the administration of challenges: During the pretreatment period, administration of escalating doses of AMPH (Figure 3.1b) increasingly disrupted the animals' performance. During drug-free weekends, performance partially recovered. The pattern of the performance during the 40-day AMPH-pretreatment period and during the subsequent 2-week withdrawal period corresponded with the evidence described previously (Martinez, Parikh *et al*. 2005). Before the challenge with AMPH or vehicle, the performance of all animals as measured by VI remained impaired relative to the pretreatment baseline ($F(1,12)=5.05$; $p=0.04$; pretreatment baseline, VI averaged over all signal durations: 0.24±0.03; pre-challenge baseline: 0.12±0.03; see Figure 3.1 for timeline and Methods for additional details). Importantly, the performance of AMPH-pretreated rats did not differ from saline-pretreated rats before the administration of the challenges ($F(1,12)=0.01$; $p>0.05$), confirming that the relatively low level of performance at this point was not a result of AMPH-pretreatment but of the testing conditions, particularly the tethering procedures required to conduct microdialysis in task-performing animals. The number of trials omitted remained low and did not differ from pretreatment baseline levels ($F(1,12)=0.02$; $p>0.05$; pretreatment baseline: 12.1±6.0% trials omitted/session; pre-challenge baseline: 9.0±1.8%).

Performance during challenge doses: The analysis of the effects of AMPH-challenges on performance (VI) indicated a significant interaction between the effects of pretreatment, session and signal duration ($F(2,24)=5.27$; $p=0.01$). Figure 3.5 depicts VI scores individually for the four treatment conditions and each signal duration. *Post hoc* analyses were conducted to identify the nature of this interaction. First, as was expected, the administration of AMPH as a challenge in SAL-pretreated rats (SAL/AMPH) did not affect VI (SAL/SAL vs. SAL/AMPH; $F(1,6)=0.39$; $p>0.05$). Likewise, pretreatment with AMPH alone did not affect performance (SAL/SAL vs. AMPH/SAL; $F(1,12)=0.28$; $p>0.05$). In contrast, the AMPH-challenge in AMPH-pretreated rats resulted in a significant decrease in performance compared with the administration of SAL in AMPH-pretreated rats (AMPH/SAL vs. AMPH/AMPH; $F(1,6)=7.50$; $p=0.03$). Thus, interactions between the effects of pretreatment and challenge with AMPH were responsible for the disruption of performance.

Figure 3.5 also illustrates the role of signal duration as a factor in the significant overall interaction. One-way ANOVAs indicated significant effects of signal duration on performance in SAL/SAL ($F(2,12)=16.51$; $p=0.003$) and AMPH/SAL rats ($F(2,12)=9.33$; $p=0.004$; see Figure 3.5 for multiple comparisons). As indicated in Figure 3.5, in SAL/AMPH animals, data variability prevent the demonstration of a significant effect of signal duration on VI ($F(2,12)=3.48$; $p=0.07$), whereas the performance of AMPH/AMPH rats was depressed and varied between +0.1 and -0.1 for all signal durations ($F(2,12)=2.92$; $p>0.05$). Thus, pretreatment and challenge with AMPH abolished

signal duration-dependent performance; this effect is reflected in the overall significant interaction between the effects of pretreatment, challenge, and signal duration reported above.

A similar interaction between these three factors was found in the analysis of hits ($F(2,24)=4.41$; $p=0.02$), but not correction rejections ($F(1,12)=1.11$; $p=0.31$), indicating that the effects on VI were largely due to effects of the animals' ability to detect signals. This finding is consistent with the selective impairment in signal trial performance observed following removal of the cortical cholinergic input system (McGaughy, Kaiser *et al*. 1996).Finally, AMPH-challenges did not affect the animals' errors of omission ($F(1,12)=1.57$; $p=0.24$). Omission rates remained generally low (5.54±0.76 omissions/session, averaged over all four groups and both test sessions).

3.5 Discussion

The present results indicate that in animals habituated to testing and microdialysis conditions but that did not perform the attentional task, pretreatment with an escalating dosing regimen of AMPH did not alter the effects of AMPH-challenges on prefrontal ACh release. In contrast, in rats performing the attentional task, such a challenge profoundly attenuated the increases in ACh release normally observed in animals performing this task- but only in animals that were pretreated with AMPH. Furthermore, task performance was disrupted in AMPH/AMPH animals. Based on post-drug and pretask ACh release values, the disruption of performance in AMPH/AMPH animals is concluded to represent

a result of the attenuation of prefrontal cholinergic activity. These results suggest that the effects of repeated AMPH exposure on the regulation of cholinergic neurotransmission in the mPFC depend on the level of recruitment of the cholinergic system. Below, empirical limitations and interpretational complexities will be discussed, followed by an evaluation of the significance of these findings for research on the neurobiology of sensitized cognitive impairments and animal models of schizophrenia.

The present results have implications for the understanding of the relationships between ACh release and levels of attentional performance. Previous experiments indicated that increases in the demands on attentional performance, resulting from long-task periods or pharmacological challenges on performance, as opposed to increases in performance levels, correlate with increases in cortical ACh release in task-performing animals (Kozak, Bruno *et al*. 2006; Passetti, Dalley *et al*. 2000). This evidence corresponds with the hypothesis that increases in mPFC ACh release above normal performance-associated levels mediate the recruitment of the 'anterior attention system' and the resulting implementation of top-down mechanisms that counteract the performance decrements triggered by challenging conditions (Sarter, Gehring *et al*. 2006). Based on this hypothesis, the augmented increases in ACh release observed in AMPH/SAL rats, when compared with SAL/SAL animals, may reflect the greater demands on attentional effort required to maintain normal attentional performance. Thus, AMPH/SAL animals were able to perform at control levels

but required abnormally high levels of mPFC cholinergic activity to maintain performance.

The present evidence suggests that AMPH-pretreatment and AMPH-challenge disrupts attentional performance-associated increases in mPFC ACh release and therefore impaired the animals' ability to employ information concerning the presence or absence of a signal to guide the selection and execution of a response. The finding that following saline or AMPH-challenges, pretask ACh release levels did not differ significantly from ACh release levels during task performance suggests that continuous task performance, the expectation of performance and task onset, and/or being placed in the performance context, are sufficient to reveal the consequences of AMPH-pretreatment. Moreover, this finding supports the view that following repeated AMPH exposure in task-performing rats, AMPH-challenges disrupt the normal recruitment of cholinergic inputs to the PFC and therefore results in the loss of cognitive control. In contrast, the results do not support the alternative view that repeated AMPH, via unknown mechanisms, abolished cognitive task control and that the low levels of ACh release were merely secondary to the behavioral/cognitive effects of repeated AMPH exposure.

Previous studies demonstrated that the performance of operant schedules not involving explicit demands on attention do not produce significant increases in cortical ACh release, or produce increases that are substantially lower than those associated with attentional performance. For example, cortical ACh release

in rats performing a fixed interval 9 s schedule of reinforcement increased only by about 50% over baseline, despite a lever-pressing rate that was almost 10-fold the rate observed in sustained attention task-performing rats (Arnold, Burk *et al*. 2002). Likewise, operant procedures controlling for the effects of reward rate and the sensory effects of stimuli indicated that these variables do not account for the increases in ACh release observed in attentional task-performing rats (Dalley, McGaughy *et al*. 2001; Himmelheber, Sarter *et al*. 1997). As the performance in non-cognitive procedures does not yield the levels of cholinergic activity observed in attentional task-performing animals, the interactions between recruitment of the cholinergic system and repeated psychostimulant exposure demonstrated in the present experiment would not be expected in animals performing tasks that do not tax cognitive functions.

In AMPH/AMPH animals, VI scores varied around zero across all signal durations (Figure 3.5). A VI score of zero indicates a loss of the ability to discriminate between signal and non-signal trails. That is, responses in signal and non-signal trials reached chance level and the animals' performance was no longer controlled by the presence or absence of a signal. Therefore, the performance of these animals no longer involved attentional processes and the processing of stimulus-response rules. Levels of ACh release in AMPH/AMPH animals performing the attention task (present experiment) were similar to ACh release levels observed in rats performing simple operant procedures not involving cognitive operations (references above). This observation is consistent with the conclusion that in AMPH/AMPH rats, cognitive task control was

abolished. Indeed, ACh release in AMPH/AMPH rats did not increase significantly beyond pre-task baseline. Thus, both the behavioral data and the ACh release levels support the conclusion that as a result of the pretreatment and challenge with AMPH, the animals' ability to utilize the presence or absence of signals to guide the responses was drastically impaired or, in other words, cognitive task control was disrupted.

As the pretreatment history of the animals did not modulate the effects of AMPH-challenges in non-performing animals, the present evidence provides a rather stark illustration of the view that the effective demonstration of abnormal regulation of a neuronal system requires recruitment of this system by, for example, demands on relevant behavioral or cognitive functions. This view contrasts with the widespread practice of assessing drug effects on neurotransmitter release, or with the status of neurotransmitter systems in animal models, in animals that remain passive or even anesthetized, and in the absence of recruitment of the neuronal system of interest (Sarter, Bruno *et al*. 2007).

The mechanisms underlying such drastically different modulation of the cortical cholinergic input system in response to repeated AMPH exposure remain a subject of speculation. In non-performing but extensively habituated animals, repeated AMPH-induced increases in ACh release could reflect a purely pharmacological effect, due primarily to the release of norepinephrine (Rothman, Baumann *et al*. 2001; Vanderschuren, Schmidt *et al*. 1999) and dopamine (Robinson, Jurson *et al*. 1988), both of which are capable of stimulating

cholinergic neurons in the basal forebrain (Arnold, Fadel *et al*. 2001; Momiyama and Sim 1996; Berntson, Shafi *et al*. 2003; Napier, Simson *et al*. 1991).

In contrast, the recruitment of prefrontal cholinergic inputs in task-performing animals is thought to be mediated via direct prefrontal projections to the basal forebrain as well as via multi-synaptic circuits involving the nucleus accumbens and perhaps also the amygdala (Holland, Han *et al*. 2000; Neigh-McCandless, Kravitz *et al*. 2002; Sarter, Givens *et al*. 2001; Sarter, Hasselmo *et al*. 2005; Zaborszky, Gaykema *et al*. 1997; Zaborszky 2002; Zahm 2000). Together with evidence demonstrating the disruption of the prefrontal modulation of accumbens neurons following repeated psychostimulant exposure (Goto and Grace 2005), the present findings suggest that repeated AMPH-exposure disrupts such telencephalic innervation of basal forebrain cholinergic neurons and thus, prefrontal ACh release. A range of neuronal mechanisms could be responsible for such a disruption, including abnormalities in glutamatergic and dopaminergic neurotransmission in prefrontal and mesolimbic regions (Giorgetti, Hotsenpiller *et al*. 2001; Lu and Wolf 1999; Peterson, Wolf *et al*. 2006; Prasad, Sorg *et al*. 1995) as well as structural reorganization of prefrontal and mesolimbic neurons (Crombag, Gorny *et al*. 2005; Robinson and Kolb 2004).

As the administration of AMPH as a challenge was necessary to reveal the cholinergic and cognitive consequences of the pretreatment with AMPH, it can be speculated that these consequences were a result of interactions between increases in noradrenergic and dopaminergic neurotransmission and

the recruitment of the cholinergic system by cognitive task performance. Importantly, this conclusion does not imply that the dysregulatory consequences of repeated psychostimulant exposure remain restricted to monoaminergic systems; rather, the current results indicate that increases in noradrenergic/dopaminergic systems are necessary to reveal the cholinergic and cognitive consequences of prior psychostimulant exposure. In addition, the results indicate that AMPH-pretreatment alone (AMPH/SAL) affects performance-related regulation of ACh release, as higher levels of ACh release were required to maintain normal performance levels in these animals.

As pointed out in the Introduction, the effects of repeated AMPH exposure model essential neurobiological and behavioral/cognitive aspects of schizophrenia. The present results indicate that repeated AMPH exposure causes a fundamental loss of cognitive task control. Such failure of cognitive control has been proposed to form a general basis for the diverse cognitive symptoms of schizophrenia (Braver, Barch *et al*. 1999). Therefore, the effects of repeated AMPH exposure on attentional performance and performance-associated ACh release appear to form a useful model for further investigations on the cholinergic mechanisms underlying the cognitive impairments of schizophrenia. Furthermore, as ongoing experiments indicate the sensitivity of this animal model in terms of detecting the pro-cognitive effects of drug treatments (Martinez 2006), this model may serve as a tool for research on the role of cholinergic mechanisms mediating the beneficial cognitive effects of treatments, and also for the detection of such treatments.

3.6 Figures

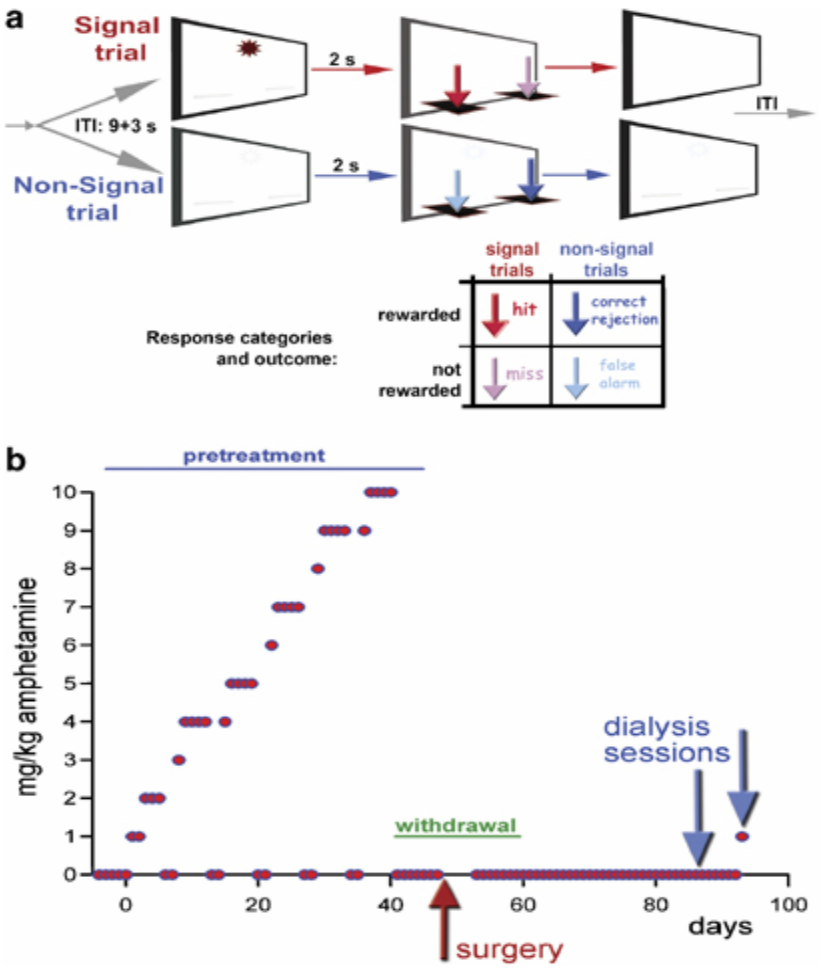

Figure 3.1(a): Schematic illustration of the sequences of events and the two trial types of the sustained attention task (a). A session consisted of 162 signal or non-signal trials. Correct responses in signal trials (hits) and non-signal trials (correct rejections) were rewarded (see arrows), whereas incorrect responses (misses and false alarms, respectively) were not. The ITI was variable to limit the animals' ability to time an event. **Figure 3.1(b):** Illustration of the AMPH-pretreatment regimen and the overall timeline of main events including pretreatment, withdrawal period, surgery and the assessment of AMPH-challenges on attentional performance and performance-associated ACh release in the prefrontal cortex. Animals were treated twice a day during the pretreatment phase, before the daily training session and 8 h later (the ordinate depicts the dose that was given twice daily; each dot depicts one day and dose; see Methods for details). Control animals received vehicle throughout the pretreatment regimen (not shown) and, similar to AMPH-pretreated rats, AMPH-'challenges' following the 'withdrawal' period. Non-performing animals were treated likewise, except that the task was n0ever activated.

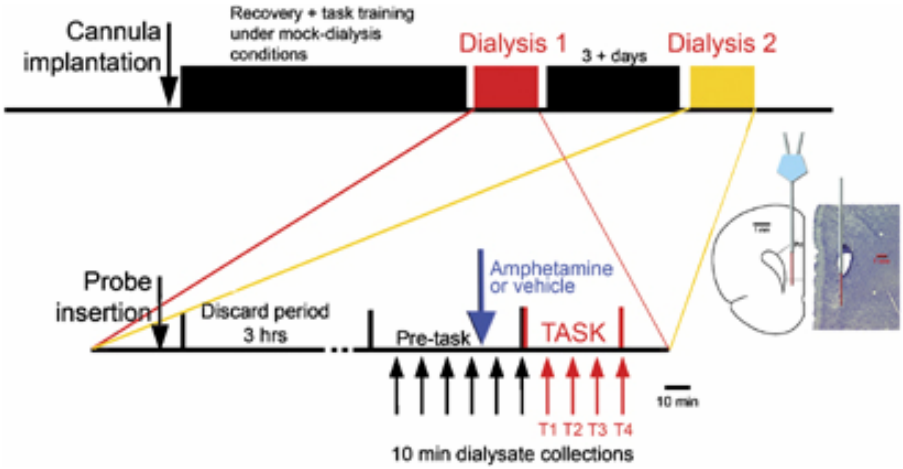

Figure 3.2: Main sequence of events following surgery (top) and detailed illustration of events during an individual dialysis session (lower line). As detailed in the Methods section, animals were implanted with a guide cannula for the later insertion of a microdialysis probe 7 days following the completion of the pretreatment regimen (see also Figure 3.1b). Following a period of subsequent recovery and daily behavioral training under mock-dialysis conditions, animals underwent two sessions during which probes were inserted into the prefrontal cortex and perfused. Vehicle or AMPH-challenges were administered during these sessions. During an individual dialysis session, the probe was inserted early in the morning, the animal was placed into the operant chamber, and the probe was connected to syringes and pumps. During the next 3 h, the probe was perfused and dialysates were discarded. The three collections before drug administration were used to determine the stability of ACh efflux and basal ACh efflux. Following two collections after the administration of drug or vehicle, the task was activated and four 10-min samples were collected. The inset shows a representative placement of a microdialysis probe in the prelimbic region (Prl), superimposed over a schematic section (left) and an actual coronal section (probe length reflects approximately the 1 mm scales inserted in both the schematic and the photomicrograph).

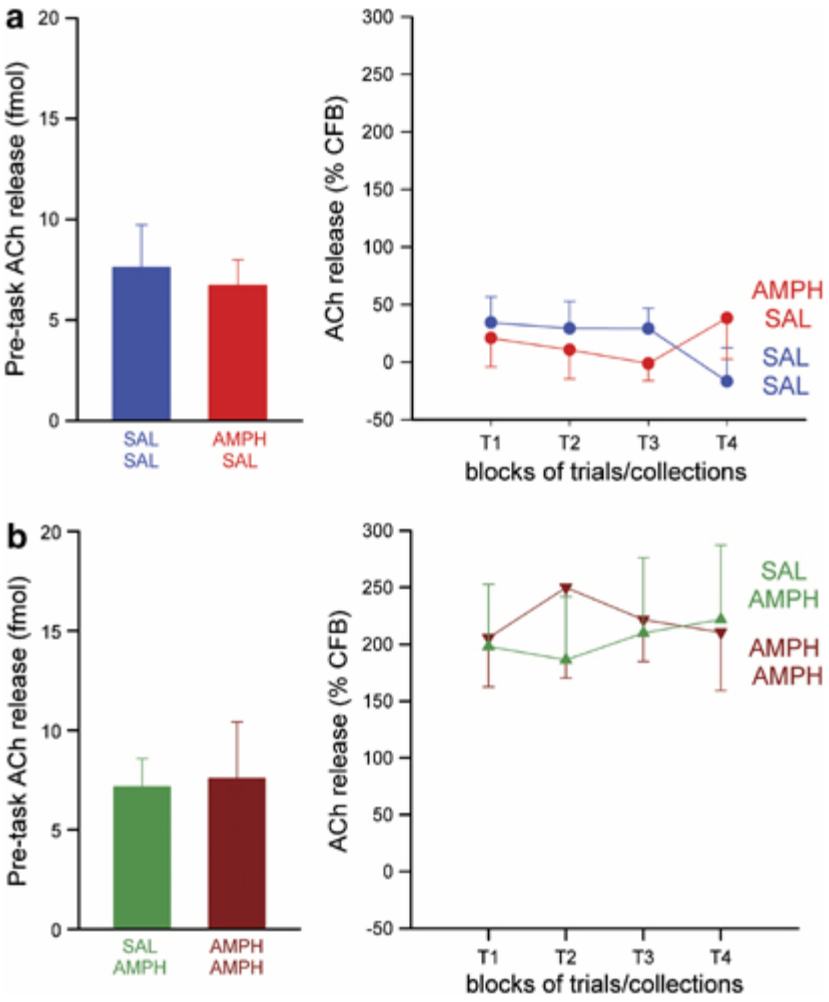

Figure 3.3: Effects of AMPH-pretreatment and AMPH-challenge on mPFC ACh release in non-performing rats (M±SEM). The bar graphs indicate absolute release levels (in fmol/15 μl) before the administration of saline (SAL) or AMPH. Following saline-'challenges', ACh release levels did not differ between saline- and AMPH-pretreated animals (**a**; see Results for statistical findings). AMPH-challenges resulted in comparable increases in ACh release in animals pretreated with SAL or AMPH (**b**). Thus, in non-performing animals, the type of pretreatment did not modify the effects of the acute challenges on ACh release.

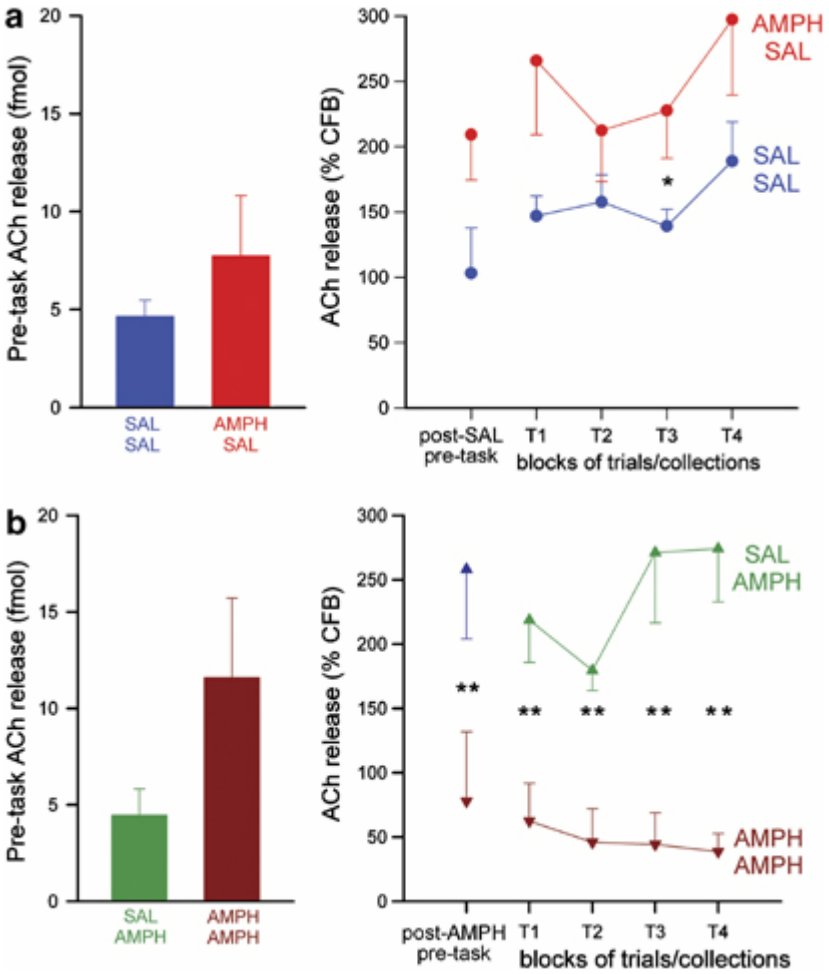

Figure 3.4: Effects of AMPH-pretreatment and AMPH-challenge on mPFC ACh release in task performing rats. In attentional task-performing rats, the type of pretreatment (saline vs. AMPH) determined the effects of the challenge (saline vs. AMPH) on ACh release (M±SEM). The bar graphs indicate absolute release levels (in fmol/15 μl) before the administration of saline (SAL) **(a)** or AMPH **(b)**. Absolute levels of ACh release did not differ significantly between the groups or session, and they did not differ from animals that did not perform the task (Figure 3.3; see Results for statistical findings). AMPH-pretreatment and AMPH-challenge (AMPH/AMPH) resulted in a highly robust attenuation of performance-associated increase in ACh release **(b)**. This effect was already present during the two collections taken before task onset, suggesting that continuous expectation of task onset and performance and/or the context of performance are sufficient to reveal the interactions between pretreatment and challenge. This observation also rejects the possibility that the attenuation of performance-associated increases in ACh release in AMPH-pretreated and -challenged animals represented merely secondary effects of the disruption of performance ($^{*}p<0.05$; $^{**}p<0.005$, significant differences between animals pretreated with saline vs. AMPH; see Results for ANOVAs).

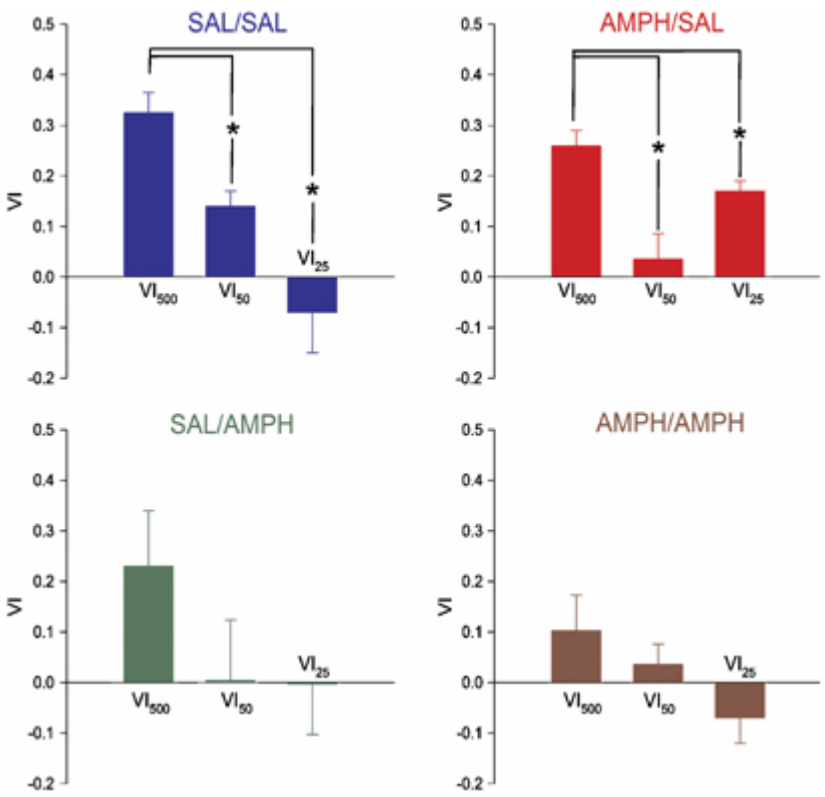

Figure 3.5: Effects of AMPH-pretreatment and AMPH-challenge on overall attentional performance as indicated by VI (M±SEM). As a result of performing under dialysis conditions, including the tethering of the animals, performance was variable and levels of performance were relatively low when compared with the performance before surgery. However, AMPH-challenges resulted in significantly lower levels of performance in AMPH-pretreated animals when compared to the effects of AMPH in saline-pretreated rats. Furthermore, in contrast to SAL/SAL and AMPH/SAL rats ($^{*}p<0.05$; multiple comparisons using LSD tests and conducted on the basis of significant ANOVAs), the depressed levels of performance of AMPH/AMPH animals did not depend on signal duration. The performance in SAL/AMPH animals appeared to remain dependent on signal duration; however, data variability prevented statistical significance (p=0.07).

CHAPTER 4

DETECTION OF THE MODERATE BENEFICIAL EFFECTS OF LOW-DOSE TREATMENT WITH HALOPERIDOL OR CLOZAPINE IN AN AMPHETAMINE MODEL OF THE ATTENTIONAL IMPAIRMENTS OF SCHIZOPHRENIA

4.1 Summary

Schizophrenic patients display attentional impairments. Low- to- moderate dose treatments of both first- and second-generation antipsychotic drugs have been demonstrated to produce moderate pro-cognitive effects in patients (Keefe, Seidman *et al*. 2004; Mishara and Goldberg 2004). The effects of repeated exposure to amphetamine (AMPH) model certain aspects of impaired attention processing in schizophrenia. Previous work has demonstrated that the consequences of repeated AMPH and subsequent challenge dosing include impaired attentional performance and attenuation of task-associated cortical cholinergic activity. The goal of the present experiment was to define an animal model capable of detecting the moderate beneficial effects of low dose treatment with haloperidol or clozapine on attention. To this end, the present experiment employed a repeated-AMPH model that has been shown to produce performance impairments in rats performing a sustained attention task. Specifically, AMPH-challenges produced robustly impaired hit rates in AMPH-pretreated rats, but not in rats pretreated with saline. Treatments with HAL or CLOZ attenuated these impairments. Collectively, these results indicate that the first-and second-generation drugs produce performance improvements sufficiently robust to be detected by this model. Therefore, the effects of this

particular AMPH pretreatment regimen on attentional performance may serve as a useful model for the preclinical detection and characterization of putative pro-cognitive treatments for schizophrenia.

4.2 Introduction

Cognitive impairments, including deficits in attention processing, represent central and enduring features of schizophrenia (Elvevag and Goldberg 2000). They are evident in the majority of schizophrenic patients (~65-85%) and are known to predict vocational and interpersonal outcomes in patients (Green 1996; Keefe, Eesley *et al*. 2005). Preclinical strategies for assessing drugs to improve attentional impairments in schizophrenics remain unavailable. Attention refers to a set of cognitive processes that facilitate the detection and processing of relevant stimuli, and filtering of irrelevant stimuli. Impairments in attentional functions are thought to represent a core aspect of the cognitive symptoms of schizophrenia (Sarter, Hasselmo *et al*. 2005) and represent a target of intervention to improve cognition in schizophrenia. This evidence highlights the need for the development and application of novel treatments targeted explicitly towards minimizing the attentional impairments associated with the schizophrenia.

The traditional views that first-generation drugs do not produce cognitive benefits, or that second-generation drugs produce superior cognitive benefits are not entirely substantiated. Meta-analyses and several well controlled experiments have determined that treatment with both first- and second-generation antipsychotics produce limited improvements on multiple domains of impaired

cognition in schizophrenia, particularly when administered at low doses (Mishara and Goldberg 2004; Harvey, Rabinowitz *et al*. 2005; Purdon, Jones *et al*. 2000; Rollnik, Borsutzky *et al*. 2002), and that the superior efficacy of second- over first-generation drugs may be negligible, or drug-specific in this regard (Keefe, Seidman *et al*. 2004; Keefe, Bilder *et al*. 2006). Because attentional impairments are central to impaired cognition in schizophrenia and represent a prime target of intervention, the present experiment employed

Exposure to psychostimulants including amphetamine (AMPH) produces paranoid schizophrenic-like symptoms in healthy individuals, to 'trigger' the manifestation of latent psychosis in at risk individuals, and to exacerbate psychotic symptoms in chronically ill patients (Yui, Ikemoto *et al*. 2000; Lieberman, Kane *et al*. 1987; Lieberman, Kane *et al*. 1987; but see Barch and Carter 2005). These effects are thought to be based on the capacity of psychostimulant drugs to induce sensitization of the mesolimbic dopamine system (Robinson and Becker 1986). Abnormal regulation the mesolimbic dopamine system is considered to be a central characteristic of the neurobiology of schizophrenia, and has been evidenced by neuro-imaging data from schizophrenic patients (Laruelle, Abi-Dargham *et al*. 1996; Breier, Su *et al*. 1997; Laruelle, Abi-Dargham *et al*. 1999).

Because attention processing represents a preclinical target for the development of pro-cognitive drugs, the present experiment assesses attention performance in rats using an AMPH-model of schizophrenia. Repeated, escalating administration of AMPH has been used to model various aspects of

schizophrenia in rats including mesolimbic dopamine dysregulation and attentional impairments (Paulson, Camp *et al*. 1991; Paulson and Robinson 1995) (Martinez, Parikh *et al*. 2005) (Kozak 2007). This dosing regimen is meant to simulate the 'runs and crashes' pattern of abuse frequently associated with AMPH-induced psychosis, and is considered a putative animal model of schizophrenia (Robinson and Becker 1986; Segal and Kuczenski 1997). Furthermore, this dosing regimen has been shown to produce robust and persistent neurochemical and behavioral sensitization in rats without the result of neurotoxicity (Paulson, Camp *et al*. 1991; Paulson and Robinson 1995). Repeated, escalating AMPH-treatment followed by a drug-free period and subsequent administration of AMPH-challenges results in cortical deficits in sustained attention performance as well as dysregulation of the cortical cholinergic input system that is necessary for attention processing (Martinez, Parikh *et al*. 2005; Kozak 2007). It should be noted that the subsequent administration of AMPH-challenges used in this paradigm are thought to model the precipitous events (i.e. a psychological, social, or chemical stressor) known to elicit psychotic relapse in humans (Robinson and Becker 1986; Nuechterlein, Dawson *et al*. 1994; Moghaddam 2002; Muller 2004).

The present experiment tests the hypothesis that moderate, sub-chronic administration of first- and second-generation drugs (haloperidol and clozapine) can attenuate the attentional impairments demonstrated in a repeated-AMPH model of schizophrenia. Specifically, that antipsychotic administration will mitigate the attenuated hit rates observed in animals pretreated and challenged

with AMPH. Rats were trained to perform a sustained attention task and then pretreated with either escalating AMPH or saline. Following a drug-free period, animals were administered a 10-day regimen of haloperidol, clozapine, or vehicle. All animals then received AMPH-challenge doses on days 1, 5, and 10 of antipsychotic treatment. Sustained attention performance was assessed 7 days per week during all points of the experiment. The present findings support the hypothesis that the present paradigm is sensitive to the beneficial effects of low doses of antipsychotic drugs. The present paradigm may prove useful for the development of novel drugs to treat attentional impairments in schizophrenia.

4.3 Methods

Animals: Forty-two male Sprague-Dawley rats (Harlan, Indianapolis, Indiana; aged 3 months and weighing 422±17 g (M±SEM) at the beginning of the experiment) were housed in single-standard cages with corn cob bedding in a humidity-(~45%) and temperature-(23°C) controlled environment. Testing occurred between the hours of 8:00 am and 5:30 pm. Animals were handling extensively prior to the initiation of behavioral training. All rats were water deprived to approximately 95% of free-access weight. Access to water was limited to a 30-minute period in the home cage following daily behavioral testing and approximately five additional milliliters of water reward could be earned during each daily session of operant testing. Food (Rodent Chow, Harlan Teklad, Madison, WI) was provided *ad libitum* in the home cage. All animal care, facilities, and experimental procedures were approved and supervised by the University Committee On Use and Care of Animals at the University of Michigan.

Lighting followed a 12-hour light-dark cycle (lights on at 6 am).

Apparatus: Behavioral training and testing took place in 12 operant chambers (Med-Associates, St Albans, VT). Each operant chamber was enclosed within a sound-attenuating compartment and equipped with two retractable levers, one house light (2.8 W), a central panel light, and a water dispenser located in between the levers. Ventilation and white noise were provided by a fan mounted on the wall of the sound-attenuating compartment.

Behavioral Training: Operant training took place 7 days per week. Rats were placed in unlit chambers for 20 minutes prior to task onset to acclimate. Animals were first trained to press a lever for a water reward in accordance with a modified fixed-ratio 1 schedule of reinforcement. During phase two of shaping, animals were trained to detect signals and discriminate between the presentation of signal events (illumination of the central panel light for 1 s) and non-signal events (non-illumination of light). Presentation of signal or non-signal events was randomized. Two seconds following the occurrence of a signal or non-signal event, both levers extended into the operant chamber and remained active for four seconds or until a response occurred. If the animal failed to respond within 4 s the levers were retracted and an omission was scored. Immediately following a response (either correct or incorrect), both levers were retracted and the variable ITI (12±3 s) was reset (Figure 4.1). During signal trials, depression of the left lever indicated a correct response and was scored as a hit whereas depression of the right lever indicated an incorrect response was scored as a miss.

Conversely, during non-signal trials depression of the left lever indicated an incorrect response and was scored as a false-alarm and depression of the right lever indicated a correct response and was scored as a correct rejection. Animals received water rewards only for correct responses (30 μl for each hit and correct rejection); incorrect responses (misses and false alarms) were not rewarded. During this phase of shaping incorrect responses resulted in the trial being repeated up to three times in the form of correction trials. If the animals continued to respond incorrectly following three correction trials, a forced-choice trial was initiated. A forced-choice trial consisted of a signal or non-signal event followed by extension of only the correct lever into the operant chamber for 90 s or until a lever press occurs. In the event that the forced-choice trial was a signal trial, the signal light remained illuminated for as long as the lever was extended. The house light was off during this shaping phase. Behavioral sessions consisted of 162 trials per session. After 3 consecutive days of responding correctly to ≥59% of both signal- and non-signal trials animals progressed to the subsequent step of shaping.

During the third phase of shaping, signal durations were shortened to 500, 50, or 25 ms (27 trials per duration) and the ITI was reduced to 9±3 s. Correction and forced-choice trials were also eliminated. Sessions were divided into three blocks of 54 trials each with all signal durations occurring randomly 9 times per block. Animals were advanced to the final stage of shaping when their performance met or exceeded a performance criterion of 70% hits to the 500 ms signal trials, 70% correct rejections and fewer than 20 omitted trials per session.

During the final stage of shaping the house-light was illuminated throughout the entire testing session. The addition of the illuminated house-light represents a crucial element of testing sustained attention as it requires the animal to constrain its behavior and focus on the central panel light during task performance. Upon reaching the final criterion of ≥70% correct responses to the 500 ms signal trials, ≥70% correct responses to non-signal trials and fewer than that 20 omissions per sessions for a minimum of 3 consecutive sessions drug treatment was initiated (described below).

Pretreatment Regimen and Challenges: Animals were separated into two groups designated to receive pretreatment with either escalating AMPH (1-10 mg/kg, n=21) or saline (1 ml/kg, n=21). Intraperitoneal injections were administered twice daily, once at 9 a.m. in the operant chamber (20 minutes prior to task onset) and again eight hours later in the home cage environment. AMPH pretreatment spanned 40 days, with doses ranging from 1-10 mg/kg bodyweight (Figure 4.2, represents salt weight, dissolved in 0.9 % saline). AMPH was administered 5 days per week and with saline (0.9%, 1 ml/kg) administered on the weekends. The intermittency and escalation of and this dosing regimen purposefully mimics the “runs and crashes” pattern of abuse that is typically displayed by amphetamine addicts and has been shown to result in psychosis (Segal and Kuczenski 1997). Following the cessation of AMPH or saline pretreatment, all animals received saline injections for a period of 10 days. This 10 day period of withdrawal was designed precede the administration of a 10 day sub-chronic antipsychotic dosing schedule. The timing of these events was

arranged such that the final challenge dose approximated the timing of challenges shown to disrupt attentional performance in Martinez *et al.* 2005.

Treatment with clozapine or haloperidol: Following the ten-day drug free period, pretreatment groups were further subdivide into cohorts designated to receive haloperidol (HAL: 0.025 mg/kg), clozapine (CLOZ: 2.5 mg/kg), or vehicle (described below: 1 ml/kg). In patient populations D2 receptor occupancies between ~60-75% are required to produce antipsychotic effects (Kapur, Zipursky *et al*. 2000). The low doses used in this experiment were designated as such based on their capacities to produce <50% D2 occupancy (Kapur, VanderSpek *et al*. 2003). To better replicate clinical conditions requiring the repeated administration of antipsychotic treatment, dosing lasted 10 days. Administration of haloperidol, clozapine or vehicle to AMPH or saline-pretreated rats resulted in the following six cohorts of animals: (see Figure 4.3, N=7 per cohort): 1) AMPH-pretreated: clozapine-treated (AMPH/CLOZ), 2) AMPH-pretreated: haloperidol-treated (AMPH/HAL), 3) AMPH-pretreated: vehicle-treated (AMPH/VEH), 4) saline-pretreated: clozapine-treated (SAL/CLOZ), 5) saline-pretreated: haloperidol-treated (SAL/HAL), and 6) saline-pretreated: vehicle-treated (SAL/VEH). The timing of antipsychotic dosing relative to task onset was delayed to permit escalation in drug/plasma levels prior to the time of testing; clozapine, haloperidol, and vehicle were administered 40, 30, and 30 minutes prior to task onset (see Figure 4.4), respectively. All animals underwent AMPH challenge doses (1 mg/kg) on days 1, 5, and 10 of the antipsychotic treatment schedule (days 11, 16 and 21 following cessation of treatment). The timing of these

challenges with regard to antipsychotic treatment was originally designed with consideration for the commonly held belief that the benefits of acute antipsychotic treatment are minimal and that protracted dosing is required to produce an optimum therapeutic response (Gelder 2000; Sadock 2000).

Data Analysis: Sustained attention performance yields measures of hits, misses, false alarms and correct rejections. These values are used to calculate the relative number of hits for each signal duration for signal trials (hits/hits+misses), and the relative number of correct rejections for non-signal trials (correct rejections/correct rejections+false alarms). Overall levels of performance are calculated using the Vigilance Index ($VI = [(h-f)/2*(h+f)-(h+f)^2]$). VI is derived from the Sensitivity Index described by Frey and Colliver, but calculated based on the relative number of hits and false alarms rather than the probability of such occurrences (Frey 1973). Additionally VI is used expressly for describing data from tasks that include discrete non-signal events whereas the SI is generally not. VI values range from -1 to 1, with a score of 1 indicating correct responses to 100% of attempted trials. A VI value of 0 indicates a complete inability to dissociate signal- from non-signal events, and reflects chance-level task performance. The number of omitted trials is also recorded.

Antipsychotic administration spanned 10 days. Challenge doses took place on days 1, 5 and 10. The days intermittent to challenge doses were examined by collapsing days 2,3,4 (T1) and days 6,7,8, and 9 (T2) into two time points. Analyses of clozapine and haloperidol treated animals were carried out using two

separate ANOVAs that included the factors group, time (T1 and T2) and signal duration.

Analyses were carried out for each performance measure; all omnibus analyses were conducted using mixed designs. Generally the main effects and interactions of the factors pretreatment- group (i.e. AMPH- vs. saline-pretreated) and treatment-group (CLOZ, HAL, or VEH treatment) were examine with respect to the factors signal duration (500, 50, 25 ms, where applicable) and the factor 'time' (described below). Baseline data were determined by averaging the final three days of performance prior to the start of pretreatment and conducting a mixed ANOVA using the factors group (AMPH- or saline-pretreated) and the factor signal duration. Next, the effects of acute administration of AMPH were determined by contrasting task performance of groups at baseline and following the acute administration of 1 mg/kg AMPH. Performance during the entire course of the 40 day escalating AMPH regimen could not be fully assessed due to high rates of omissions in AMPH-pretreated rats during higher doses of AMPH. Consequently, only data from the drug-free weekend periods were suitable for analyses. Saline-pretreated animals were only permitted to perform the task twice per week to control for potential practice effects. Data from weekends were averaged across the days to yield a total 5 time points. The subsequent analysis consisted of a mixed ANOVA that included the factors group (AMPH- vs. saline-pretreated), time (weekend) and signal duration. Following the cessation of pretreatment, both groups of animals continued to perform the task for 10 days. Data from these 10 days were analyzed by first collapsing data into three blocks

consisting of days 1-3 (P1), 4-7 (P2), and 8-10 (P3). Mixed ANOVAs for the factors group, time (P1, P2, and P3) and signal duration were then carried out. Using a similar analysis, data from P3 were contrasted with baseline data to determine the recovery of performance in AMPH and saline pretreated animals prior to challenge doses.

The effects of challenge doses on AMPH- and saline-pretreated rats were determined by running multiple analyses to contrast the performance of the six smaller treatment cohorts across all three challenges. Each analysis was conducted as a mixed design comprised of the factors group, time (challenge 1, challenge 2, and challenge 3), and signal duration. The primary analysis contrasted the effects of AMPH/VEH and SAL/VEH animals. Subsequent analyses were conducted separately on AMPH- and saline-pretreated groups undergoing treatment with clozapine or haloperidol. These analyses made the following comparisons: 1) AMPH/HAL versus AMPH/VEH versus SAL/VEH; and 2) AMPH/CLOZ versus AMPH/VEH versus SAL/VEH. SAL/VEH animals were used as a control group for two reasons (Martinez and Parikh, *et al*. 2005), first acute AMPH administration does not affect performance in drug naïve animals; and second, the effects of antipsychotics impair performance in saline-pretreated animals (below). A final analysis was then conducted to directly contrast the effects of haloperidol and clozapine in AMPH-pretreated animals. Where applicable, *post hoc* analyses were carried out using one-way ANOVAs and multiple comparisons (i.e. the Least Significant Difference Test). *Post hoc* tests generally consisted of direct comparisons between treatment groups for each

time point of interest, or within subject comparisons across time points. Statistical analyses were carried out on SPSS version 14. Prior to statistical analysis, percentage data underwent arcsine transformation (2*arcsine(√X)) (Zar 1999). P-values below 0.05 indicated statistical significance; exact p-values were reported where recommended by (Greenwald 1996).

4.4 Results

Performance at baseline: Baseline performance values for animals designated to receive AMPH- or saline-pretreatment were calculated by averaging the three days prior to the initiation of pretreatment. Groups did not differ on any measure of performance (VI: ($F(1,40)=2.53$, $p=0.11$); percent hits: ($F(1,40)=1.475$, $p=0.23$); correct rejections ($F(1,40)=1.15$, $p=0.29$); omissions ($F(1,40)=2.42$, $p=0.12$). The performance of signal trials was duration dependent ($F(2,80)=261,.97$, $p<0.001$, Figure 4.4), as were VI scores ($F(2,80)=247.24$, $p<0.001$). Animals omitted an average of 4.00±6.02% of trials per session.

Effects of acute-AMPH administration: AMPH-pretreated animals received 1 mg/kg AMPH acutely on the first day of pretreatment. A within subjects analysis using AMPH-treated animals revealed that a single exposure of drug at this dose did not affect any measure of performance relative to baseline (VI: ($F(1,40)=0.49$, $p=0.48$, hits: ($F(1,40)=0.56$, $p=0.49$), correct rejections: ($F(1,40)=2.71$, $p=0.06$), omissions: ($F(1,40)=2.71$, $p=0.10$). Saline pretreated rats received an acute 1 mg/kg dose of AMPH at the time of the first ‘challenge dose’ and their performance remained similarly unaffected (all p’s >0.06). Between group

comparisons indicated that performance following acute-AMPH exposure did not differ between groups (all p's>0.48).

Effects of escalating AMPH treatment and performance during drug-free weekends. Over the course of AMPH-pretreatment, doses of ≥2 mg/kg produced omission rates of ~100%. As described in the methods section, on weekends animals were administered saline and were capable of performing the task. Data from these drug free periods were analyzed by averaging values over two days, to yield 5 time points (1 per weekend, variable 'week'). Compared to saline-pretreated animals, the overall performance of animals receiving AMPH remained impaired during these drug-free days (VI: $F(1,40)=8.84$, $p=0.005$). This effect did not vary as a function of week or pretreatment (group x week: $F(4,160)=2.07$, $p=0.09$). The lower VI scores in AMPH-treated animals appeared to result from impaired performance on signal trials (main effect of group on hits: $F(1,40)=6.29$, $p=0.02$), however performance remained duration-dependent ($F(2,51)=233.99$, $p<0.01$). Further analysis of hits revealed a group X week interaction ($F(4,160)=0.047$). *Post hoc* comparisons between groups for each time point revealed significant group differences on weekend 3: ($F(1,40)=6.73$, $p=0.01$), weekend 4: ($F(1,40)=8.98$, $p=0.005$ and weekend 5: ($F(1,40)=5.94$, $p=0.02$) but not weekends 1 or 2 (both p's >0.52; Figure 4.5).

The performance of non-signal trials in AMPH-pretreated rats did not differ from that observed in saline-pretreated rats during the drug-free weekends (main effect of group: ($F(1,40)=0.34$, $p=0.56$). The number of trials omitted during drug-

free weekends did not differ between groups (F(1,40)=0.516, p=0.47) and did not change as a function of week (F(4,160)=2.33, p=0.058; week x group: F(4,160)=1.59, p=0.18; M±SEM: 9.80±2.00% omissions/session).

Performance during the withdrawal period: Following the stoppage of AMPH or saline pretreatment, all animals performed the task for a period of ten days (henceforth referred to as the 'withdrawal' period). To reiterate, here the term withdrawal is used specifically to describe the stoppage of treatment and does not refer to the psychological or physiological state of the animal. Analyses of these data were carried out by collapsing the first three, middle four, and last three days of the drug free period into a total of three time points (factor 'time': P1=days 1-3, P2=days 4-7, P3=days 8-10). Vigilance index scores differed between groups as a function of time over the course of withdrawal (group X time interaction: F(10,72)=2.06, p=0.04). Between groups *post hoc* analyses at each individual time point determined that the overall performance of AMPH-pretreated rats was significantly worse than rats pretreated with saline at P1: (F(1,40)=1.88, p=0.017), but that the groups did not differ at P2 or P3 (both p>0.15, Figure 4.7). The lower VI scores in AMPH-pretreated animals at W1 appeared to result from to impaired performance on non-signal trials (main effect of group: F(1,40)=5.18, p=0.03). Multiple comparisons performed over separate time points indicated that AMPH-pretreated rats performed worse on non-signal trials at P1 and P2, but not P3: (P1: (F(1,40)=7.02, p=0.01) ; P2:(F(1,40)=4.37, p=0.04). P3: (F(1,40)=3.29, p=0.07)). The performance on signal trials did not differ on the basis of group

(percent hits: main effect of group: F(1,40)=0.87, p=0.35), but produced a group X block interaction (F(2,80)=03.93, p=0.02). *Post hoc* comparisons examining each individual time point between groups were unable to reveal the locus of this interaction (P1: (F(1,40)=1.88, p=0.17), P2: (F(1,40)=0.01, p=0.97), P3: (F(1,40)=0.75, p=0.391).

Importantly, animals' overall performance (VI) had recovered to the baseline levels observed prior to the initiation of pretreatment (time: F(1,40)=0.37, p=0.54). Similarly, this analysis did not reveal a main effect of group (F(1,40)=0.02, p=0.96) but did indicate an interaction of time and group (F(2,80)=10.42, p=0.002). *Post hoc* analysis indicated that this interaction was non-orthogonal, and appeared to result from the performance of saline-pretreated animals being slightly better at W3 than at baseline (baseline: 0.34±0.03, W3: 0.40±0.04), whereas AMPH-pretreated animals showed a trend in the opposite direction (baseline: 0.41±0.04, W3: 0.32±0.03). By and large, all animals regained baseline performance prior to the initiation of antipsychotic treatment and the administration of AMPH-challenges. However, all animals (i.e. both pretreatment groups) omitted more trials at W3 than at baseline (main effect of time: (F(1,40)=7.4, =0.01), M±SEM: baseline: 4.00±0.92%, W3: 11.73±2.72%). Importantly, the number of omitted trials did not differ between groups, and was still well below the originally established performance criteria of ≤20% omissions.

The above analyses reflect comparisons made between the two larger pretreatment groups; all subsequent analyses compare data from the 6 smaller treatment cohorts (see Methods). To ensure that no group differences existed

between the smaller cohorts, secondary between-groups analyses were performed for all performance measures. These tests indicated that no between group differences existed between the smaller cohorts prior to challenge dosing (all main effects of group: p's>0.25).

Effects of clozapine or haloperidol in animals pretreated with AMPH or saline. Following withdrawal, all groups of animals were administered antipsychotics or vehicle for 10 days, with challenge doses occurring on days 1, 5, and 10 of treatment. Data from the days in between the challenge doses (henceforth termed 'drug-only days') were examined to determine the differential effects of antipsychotic treatment in AMPH- and saline-pretreated animals. To conduct this analysis, drug-only days were collapsed into two time points: T1 was comprised of days 2,3 and 4, and T2 was comprised of days 6,7,8, and 9. Groups were compared on all performance measure across the two time points (factor 'time'). Two separate ANOVAs were carried out, the first compared clozapine-treated groups (AMPH/CLOZ, SAL/CLOZ and SAL/VEH) and the second compared haloperidol-treated groups (AMPH/HAL, SAL/HAL, SAL/VEH). Analysis of clozapine-treated groups did not reveal any group effects for overall performance (VI: $F(2,18)=2.72$, $p=0.09$) and the factor group did not interact with time or signal duration (both p's>0.32). Subsequent analysis revealed a main effect of group on signal trials (hits: $F(2,18)=15.55$, $p<0.001$; Figure 4.8), with no interactions of time or signal duration (both p's>0.10). *Post hoc* analyses determined that SAL/CLOZ animals performed worse than both AMPH/CLOZ and SAL/VEH animals across both time points (AMPH/CLOZ vs. SAL/CLOZ: (LSD=0.59,

p<0.001); SAL/VEH vs. SAL/CLOZ: (LSD=0.73, p<0.001)), whereas the performance of AMPH/CLOZ and SAL/VEH groups did not differ (LSD=0.14, p=0.321). Furthermore, no group effects were observed in AMPH/CLOZ, SAL/CLOZ and SAL/VEH on the performance of non-signal trials (correct rejections: F(2,18)=1.30, p=0.29; p's for all interactions >0.35). However, a main effect of group arose from the analysis of omission rates (group: (F(2,18)=3.65, p=0.009). *Post hoc* analyses determined that SAL/CLOZ rats omitted significantly more trials than both AMPH/CLOZ animals (LSD=0.23, p=0.03) and SAL/VEH animals (LSD=0.33, p=0.003), while AMPH/CLOZ and SAL/VEH groups did not differ.

An identical analysis was carried out for animals treated with haloperidol (AMPH/HAL, SAL/HAL, SAL/VEH). These analyses did not reveal any main group effects or interactions for any performance measure: (VI: (F(2,18)=1.39, p=0.27); (hits: (F(2,18)=1.15, P=0.33; Correct rejections: (F(2,18)=2.03, p=0.16); omissions: (F(2,18)=2.79, p=0.08). None of these effects varied as a function of time and all interactions with the factor group were not significant (all p's >0.07).

<u>The effects of AMPH challenges in animals previously exposed to AMPH or saline.</u> As described above, acute administration of 1 mg/kg AMPH did not affect task performance on the first day of pretreatment; however an identical dose produced marked performance impairments in animals exposed to escalating AMPH. The following analyses compare two groups of animals AMPH/VEH and SAL/VEH (factor group) across all three challenges doses (factor 'challenge': Challenge 1, Challenge 2, and Challenge 3). Following the administration of

challenge doses, the overall performance of AMPH-pretreated rats was impaired relative to the performance of animals pretreated with saline (VI: $F(1,12)=5.55$, $p=0.04$). These effects did not fluctuate across challenge doses ($F(2,24)=0.078$, $p=0.92$) or interact with the factor challenge ($F(2,24)=0.652$, $p=0.51$). The group differences observed on VI resulted primarily from impaired performance of signal trials in AMPH-pretreated animals (hits: $F(1,12)=5.44$, $p=1.05$, $p=0.04$; Figure 4.6). Despite robust impairments in AMPH-pretreated animals, performance of signal trials remained signal duration dependent ($F(2,26)=62.17$, $p<0.005$), and did not interact with the factor group ($F(2,24)=1.04$, $p=0.36$). Performance of non-signal trials did not differ between groups (correct rejections: $F(1,12)=3.70$, $p=0.08$) or across challenge doses ($F(2,24)=2.06$, $p=0.14$). The number of omitted trials between groups did not differ ($F(1,12)=1.19$, $p=0.66$; 4.85±1.77 omissions per session).

Effects on haloperidol and clozapine treatment during AMPH-challenges. As demonstrated above, the administration of AMPH-challenges results in robust performance impairments in AMPH-pretreated animals. The following analysis examines the ability of sub-chronic administration of haloperidol or clozapine to attenuate these impairments. Antipsychotic administration impaired task performance in saline-pretreated animals. As a result, direct comparisons between AMPH or saline animals receiving identical antipsychotic treatments would be confounded by the performance deficits observed in the saline groups. To avoid these confounds, the SAL/VEH animals were used as a control group for analyses examining performance in AMPH/CLOZ and AMPH/HAL animals.

As described above, acute administration of a 1 mg/kg AMPH-challenge does not disrupt performance in drug-naïve animals. This is also the case for SAL/VEH animals receiving 1 mg/kg AMPH during challenge doses (all analyses relative to baseline: VI: ($F(3,18)=0.64$, $p=0.53$), hits: ($F(3,18)=0.45$, $p=0.53$), correct rejections: ($F(3,18)=2.58$, $p=0.12$), omissions ($F(3,18)=1.29$, $p=0.31$). These effects did not vary across challenge doses, and did not interact with any other factor (all p's>0.14).

Effects of challenge doses in AMPH/HAL, AMPH/VEH and SAL/VEH rats. The consequences of haloperidol administration in AMPH-pretreated rats during challenge doses were assessed using three separate analyses. The first compares the performance of AMPH/HAL with that of the impaired AMPH/VEH rats. The second examines AMPH/HAL animals with respect to SAL/VEH animals, and the third examines performance of AMPH/HAL animals during challenge doses with respect to their pretreatment baseline. The performance of AMPH/HAL rats, although slightly improved, was not significantly better than that of AMPH/VEH rats during AMPH-challenges (VI:($F(1,12)=4.39$, $p=0.056$). This effect did not vary across the three challenge doses ($F(2,24)=1.23$, $p=0.15$) or as a function of signal duration ($F(2,24)=0.94$, $p=0.40$), and these factors did not interact ($F(2,24)=0.77$, $p=0.47$). The minor elevation in overall performance demonstrated by AMPH/HAL rats could not be attributed to improved performance on signal trials (group: $F(1,12)=3.70$, $p=0.078$) and no effects were seen on the performance of non-signal trials (group: $F(1,12)=1.23$, $p=0.29$). These effects were not influenced by the factors challenge or signal duration (all

p's>0.09).

A second analysis was conducted to contrast the performance of AMPH/HAL and SAL/VEH groups. Results from this analysis indicated that that the performance of AMPH/HAL animals was restored to the levels observed in SAL/VEH animals on all measure of performance during AMPH-challenges (VI: ($F(1,12)=0.82$, $p=0.38$), hits: ($F(1,12)=0.25$, $p=0.624$), correct rejections: ($F(1,12)=0.39$, $p=0.54$), or omissions: ($F(1,12)=2.43$, $p=0.51$). These effects did not vary across the three challenge doses, or where applicable, on the basis of signal duration (all p's>0.21).

A third analysis was conducted within the AMPH/HAL group to determine if their performance during challenge doses was restored to baseline levels. Analysis of overall performance (VI) indicated that AMPH/HAL animals were significantly impaired during challenge doses relative to their performance at baseline (main effect of time: ($F(3,18)=3.42$, $p=0.04$). *Post hoc* analysis determined that AMPH/HAL animals were impaired relative to baseline during Challenge 1: (LSD=0.19, p=0.01) and Challenge 3: (LSD=0.189, p=0.040, but not Challenge 2: (LSD=0.155, p=0.12). However, separate analysis of signal and non-signal trials were unable to reveal specific differences between performance at baseline or during challenge doses for signal and non-signal trials (hits: $F(3,18)=0.99$, $p=0.39$; correct rejections: $F(3,18)=1.22$, $p=0.33$). The number of omitted trials did not differ between baseline and challenge doses ($F(3,18)=0.66$, $p=0.44$).

Effects of challenges in AMPH/CLOZ, AMPH/VEH, and SAL/VEH rats. A similar

progression of statistical analyses was applied to determine the effects of clozapine in AMPH-pretreated animals. The following analyses first compare performance of AMPH/CLOZ with AMPH/VEH rats and then contrast AMPH/CLOZ with SAL/VEH animals. In terms of overall performance (VI), AMPH/CLOZ animals performed significantly better than AMPH/VEH rats (group: $F(1,12)=4.79$, $p=0.049$). This effect did not interact with signal duration ($F(2,24)=1.56$, $p=0.23$) and these improvements did not vary over the course of the three challenge doses ($F(2,24)=2.89$, $p=0.08$), indicating that acute administration of CLOZ was sufficient to counter act the detrimental effects of AMPH-challenges in AMPH-pretreated rats. Separate analyses were conducted on signal and non-signal trials. Hit rates did not show significant effects on the basis of group $F(1,12)=3.11$, $p=0.10$) and did not differ across challenge doses ($F(2,24)=2.51$, $p=0.10$). However, a three way interaction was observed for the factors challenge, group, and signal duration ($F(4,48)=2.87$, $p=0.03$). *Post hoc* analyses conducted on each signal duration between groups were not able to determine the locus of this interaction (all p's>0.055). The performance of non-signal trials did not differ on the basis of group for AMPH/CLOZ and AMPH/VEH animals (group: $F(1,12)=3.77$, $p=0.08$) and this effect did not vary as a function of time ($F(2,24)=3.75$, $p=0.06$). Likewise the number of omitted trials did not differ by group ($F(1,12)=0.01$, $p=0.98$), and the factor group did not interact with time ($F(2,24)=1.51$, $p=0.24$).

A subsequent analysis was conducted to compare the performance of AMPH/CLOZ and SAL/VEH rats during challenge-doses. Administration of

clozapine in AMPH-pretreated rats restored performance to levels observed in saline-pretreated rats for all measures of performance. There were no significant group differences or interactions on any performance measure (all p's>0.21).

A final component of this analysis compared the baseline performance of AMPH/CLOZ animals to their performance during challenge doses. Treatment with clozapine during challenge doses counter-acted the detrimental effects of AMPH and allowed AMPH-pretreated animals to perform at levels analogous to baseline (VI: $F(3,18)=1.63$, $p=0.21$). Similar effects were demonstrated on the performance of signal and non-signal trials, as no difference were observed between baseline and challenge doses for hits ($F(3,18)=1.93$, $p=0.21$) or correct rejections ($F(1,6)=1.41$, $p=0.27$).

AMPH/HAL versus AMPH/CLOZ. To directly contrast the effects of haloperidol and clozapine treatment in AMPH-pretreated rats during challenge doses an additional analysis was carried out. This was done to directly test the hypotheses concerning the superior efficacy of clozapine over haloperidol. This analysis indicates that groups treated with haloperidol and clozapine did not differ for any performance measure, and that these effects were consistent over all signal durations and challenge doses (all p's>0.18).

4.5 Discussion

Similar to previous reports, escalating exposure to AMPH resulted in impaired performance during AMPH-challenge doses (Figure 4.6), but not during acute administration of the same dose (Martinez, Parikh *et al*. 2005; Kozak 2007). Importantly, these impairments cannot be attributed to overt stereotypies

or the development of side-biases, since animals do not display these behaviors when engaged in sustained attention performance (Martinez, Parikh *et al*. 2005). The principle findings of this study demonstrate that low-dose treatments of haloperidol and clozapine effectively attenuate the attentional impairments observed in this repeated-AMPH model of schizophrenia. The beneficial effects of haloperidol were not as statistically robust as those observed with clozapine, however the efficacy of both drugs appeared to be largely equivalent. The benefits of AMPH and CLOZ treatments on performance were most robust when assessed using the comprehensive measure VI. However, these effects appeared to result primarily from improved performance on signal trials.

The effects of clozapine treatment on attention performance were beneficial in AMPH-pretreated rats and detrimental in saline-pretreated rats. Specifically, in saline-pretreated rats clozapine administration impaired performance on signal trials and increased omission rates. Conversely, in AMPH-pretreated animals the same doses of clozapine attenuated the performance impairments observed during AMPH-challenges and did not affect performance on non-challenge days. Similar impairments have been observed in rats performing a five choice serial reaction time task (Amitai 2007) and in humans performing a task measuring sustained attention. Treatment with haloperidol at doses producing <50% D2 occupancy did not impair performance in saline-pretreated animals. Experiments demonstrating performance deficits following haloperidol administration generally use incomparably high doses, and may be of limited relevance to the effects observed here.

The present experiment applied a sub-chronic (10-day) schedule of dosing that was designed in part, to contrast the effects of acute and prolonged treatment with antipsychotics. This experimental design was based partially on the hypothesis that the beneficial effects of antipsychotic drugs have a delayed onset or lag in therapeutic efficacy. The present data demonstrate that both clozapine and haloperidol exert beneficial effects when administered acutely and that these effects did not change over the course of treatment. Although these results were initially surprising, a more thorough review of the literature indicated that the benefits of antipsychotic treatment in humans can appear on a much shorter timescale than conventionally expected, and in as little as 24 hours (Agid, Kapur *et al*. 2003; Kapur, Arenovich *et al*. 2005).

The exact mechanisms mediating the effects of clozapine and haloperidol in this model remain speculative. The hypothesis that the ability of these drugs to attenuate challenge-associated performance impairments results from D2 receptor blockade cannot be entirely excluded. That is to say, the performance effects observed in AMPH-pretreated animals following antipsychotic treatment may be a secondary consequence of D2 antagonism rather than pro-cognitive effects, *per se.* However, if the present results were a simple function of D2 antagonism, then the statistically more robust benefits of clozapine, with its weaker affinity for D2 receptors, would not be expected.

Alternative speculations regarding the neurobiological underpinnings of these effects focus on the basal forebrain cholinergic system (BFCS). Evidence suggests that the functional integrity of the BFCS is crucial for normal attentional

processing (McGaughy, Kaiser *et al*. 1996; Everitt and Robbins 1997). Previous work has determined that the challenge-associated performance impairments demonstrated by AMPH-pretreated animals coincide with robust attenuations in cortical cholinergic transmission (Kozak 2007). Hypotheses regarding the mechanisms driving this dysregulation have implicated multiple neuroanatomical circuitries associated with schizophrenia and involved in regulating BFCS activity; including the prefrontal cortex (Zaborszky, Leranth *et al*. 1984), the nucleus accumbens (Zaborszky and Cullinan 1992), and the ventral tegmental area (Gaykema and Zaborszky 1996).

Haloperidol and clozapine may normalize the aberrant mesolimbic activity associated with this model and allow the BFCS to be appropriately recruited to produce improved task performance. Despite these speculations, little is known regarding the effects of antipsychotic drugs on cortical cholinergic transmission in an operant context. Although previous work has demonstrated that clozapine, but not haloperidol, preferentially increases cortical cholinergic transmission, these experiments were carried out in passive rats and in environments that did not actively 'recruit' the basal forebrain cholinergic system (Ichikawa, Dai *et al*. 2002). As demonstrated in Kozak, *et al.* such recruitment would be necessary for an accurate determination of drug effects, since data taken from passive rats may be drastically different from those observed in task-performing animals (Kozak 2007). Nonetheless, these findings may provide a basis for, or at least provide justification to test the hypothesis that the pro-cognitive effects of

antipsychotic drugs are mediated, at least in part, via the normalization of cortical cholinergic transmission.

Despite the limitations of this model, its heuristic value is supported by its ability to detect the moderate cognitive benefits of low-dose treatment with both first- and second-generation antipsychotic drugs. Accordingly, model may have practical value as a screening and drug development tool for novel therapeutic interventions to improve cognition in schizophrenia.

4.6 Figures

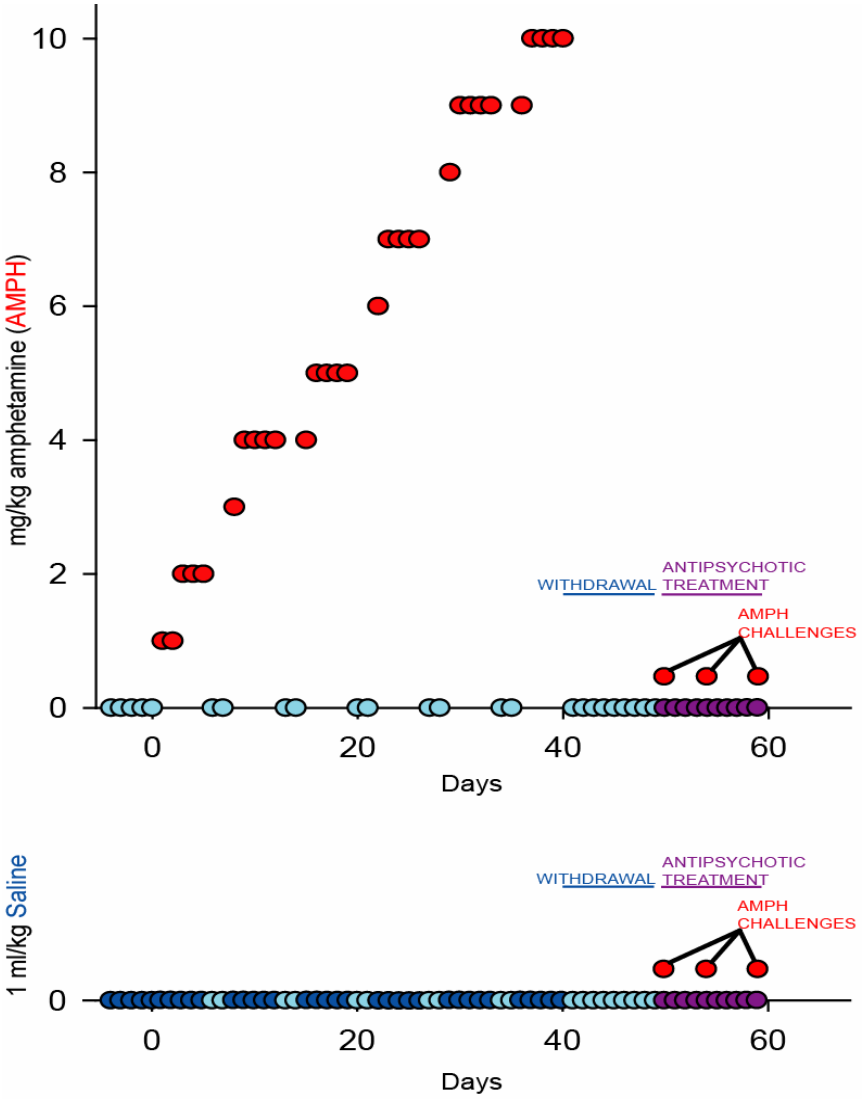

Figure 4.1: Diagrammatic representation of the drug treatment timeline. Animals were pretreated with either AMPH (1-10 mg/kg) or saline (1 ml/kg). Injections took place 5 days per week over a period of 40 days. Each dot represents the level of dosing that was administered twice daily. Injections took place in the morning prior to operant testing and again 8 hours later in the home cage. Note that this regimen was intermittent, with all animals receiving saline on the weekends and undergoing operant testing. Following the completion of pretreatment, all animals received saline injections for ten days and continued operant testing. Pretreatment groups were then divided into smaller cohorts (Figure 4.3) designated to receive antipsychotic or vehicle treatment for a period of 10 days. AMPH challenges were administered on days 1, 5, and 10 of this 10-day period

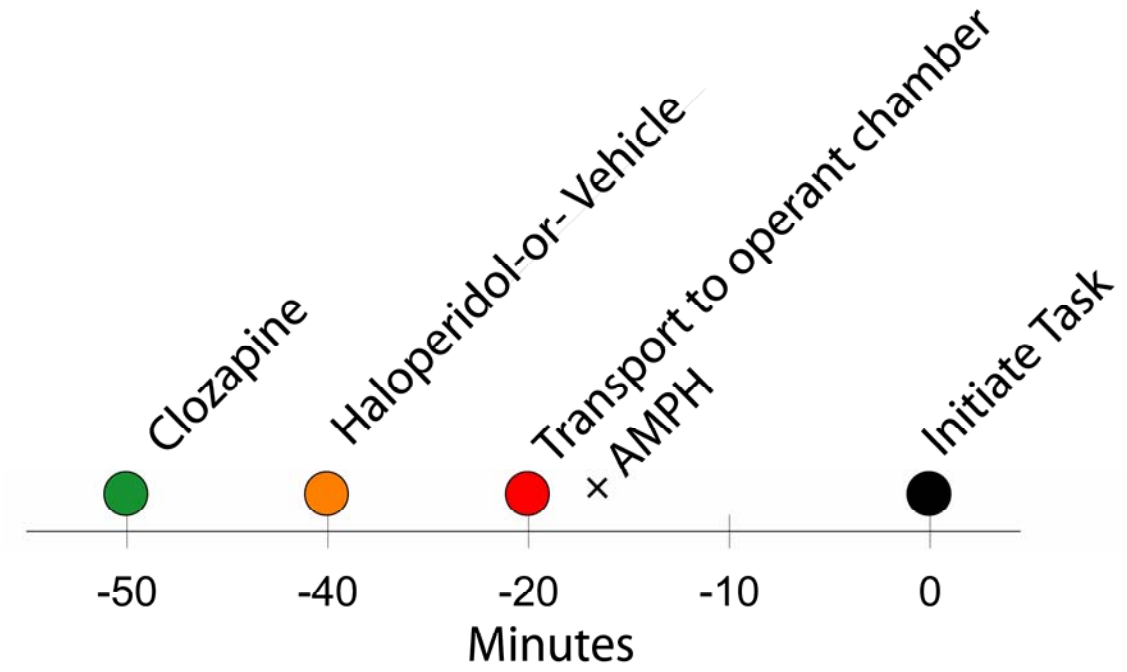

Figure 4.2: Timeline of daily events during antipsychotic treatment. During the 10-day period of antipsychotic treatment, animals were administered antipsychotics in the home cage environment. Timing of doses was staggered prior to testing to allow drug plasma levels to rise prior to testing. Animals were then transferred to the operant chambers, and then on the days of challenge dosing, received AMPH 20 minutes prior to task initiation.

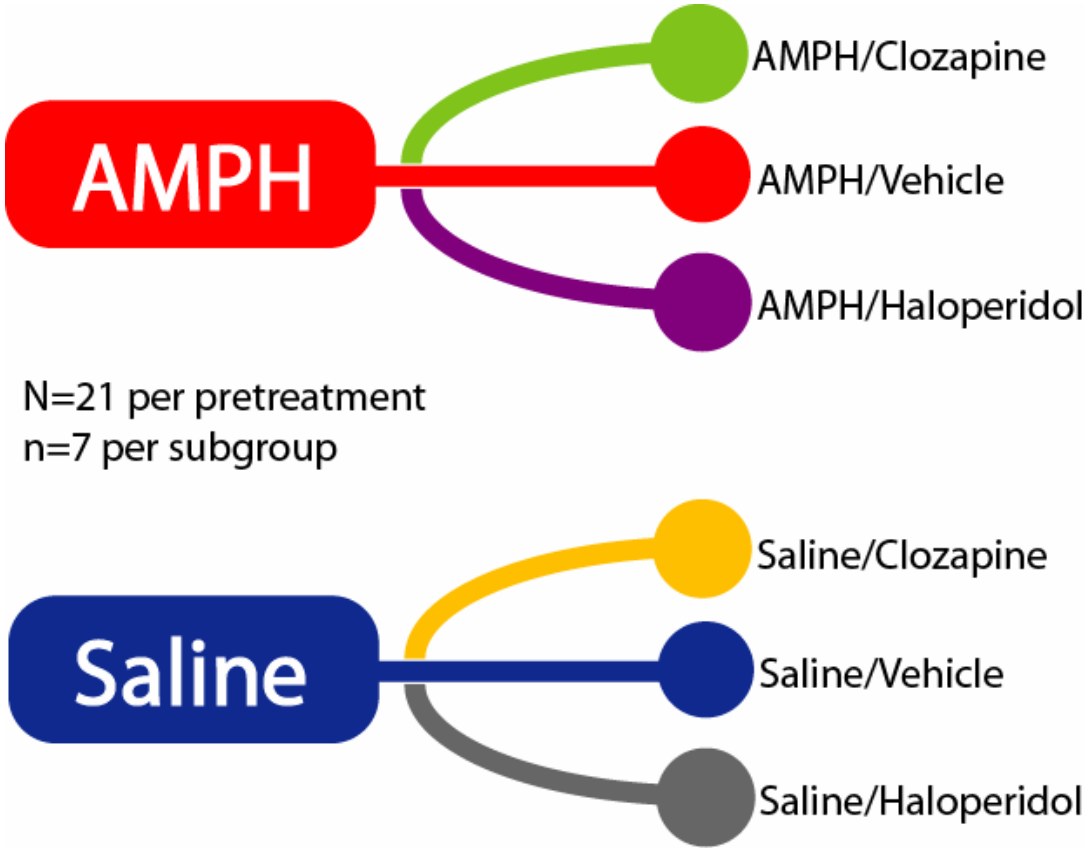

Figure 4.3: Schematic of treatment groups. Forty-two animals were first divided into groups receiving pretreatment with either saline or escalating AMPH (n=21 per group). Following pretreatment and withdrawal, pretreatment groups were further subdivided into cohorts designated to receive treatment with clozapine (2.5 mg/kg), haloperidol (0.025 mg/kg) or vehicle (1 ml/kg). This experimental design yielded the following 6 groups of animals (n=7 per group): AMPH-pretreated/vehicle post-treated (AMPH/VEH), AMPH-pretreated/haloperidol post-treated (AMPH/HAL), AMPH-pretreated/clozapine post-treated (AMPH/CLOZ), SAL-pretreated/vehicle post-treated (SAL/VEH), SAL-pretreated/haloperidol post-treated (SAL/HAL), SAL -pretreated/clozapine post-treated (SAL/CLOZ).

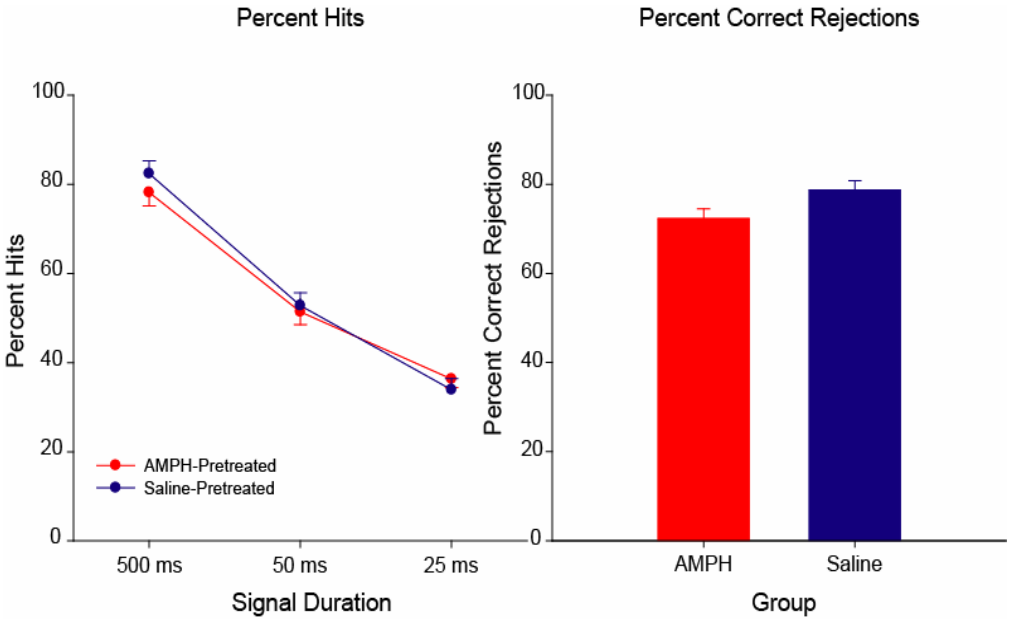

Figure 4.4: Performance of AMPH-and saline-pretreated groups at baseline. These data represent performance in AMPH- and saline pretreated groups averaged over a period of three days prior to AMPH- or saline-pretreatment. Groups did not differ on the performance of signal trials, and hit rates were signal duration dependent (percent hits; left panel). Similarly, animals' performance on non-signal trials did not differ between groups (percent correct rejections; right panel).

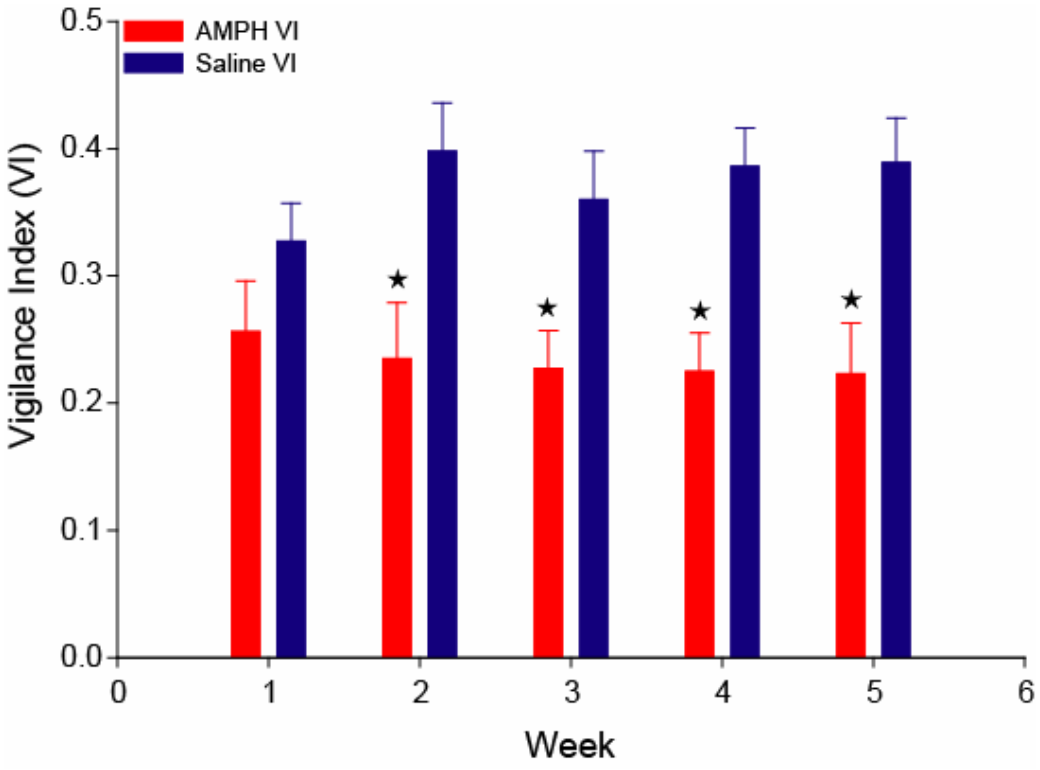

Figure 4.5: Overall performance (VI) during each of the five weekly-drug-free periods. Animals performed the task twice per week. Starting with the second drug free period, the overall performance of animals treated with AMPH was markedly impaired relative to performance in saline-pretreated animals.

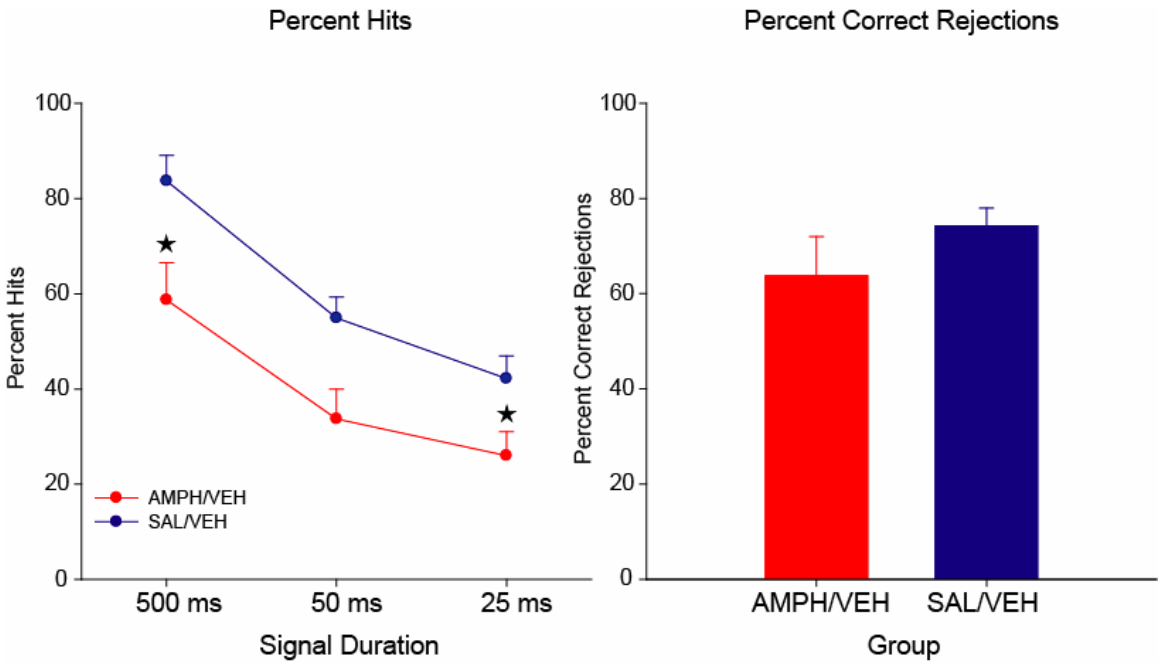

Figure 4.6: Performance of AMPH/VEH and SAL/VEH animals averaged over all three challenge doses. Compared to SAL/VEH animals, AMPH/VEH animals were robustly impaired on the performance of signal trials (left diagram). Notably, despite impairments hit-rates remained duration dependent in all animals. The performance of non-signal trials did not differ between groups. Acute administration of AMPH did not affect any measure of performance in SAL/VEH animals.

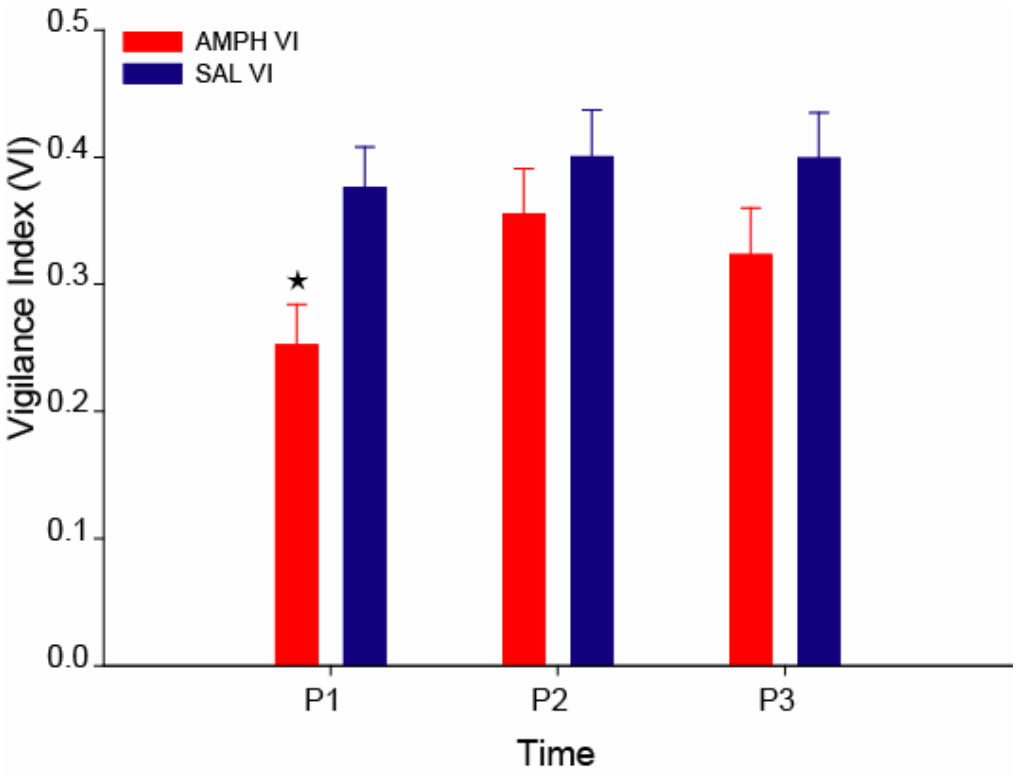

Figure 4.7: Performance following the completion of pretreatment. All animals received saline injections and continued task performance for the 10 days following the termination of pretreatment. Data was analyzed by dividing the 10 days into three blocks (P1=days 1,2,3; P2=days 4,5,6,7; P3=days8,9,10). Compared to saline-pretreated rats, AMPH-pretreated animals showed impairments in overall performance (VI) during P1. Performance then recovered markedly throughout the remainder of the drug--free period. Importantly, groups did not differ prior to the initiation of antipsychotic treatment and challenge dosing (P3).

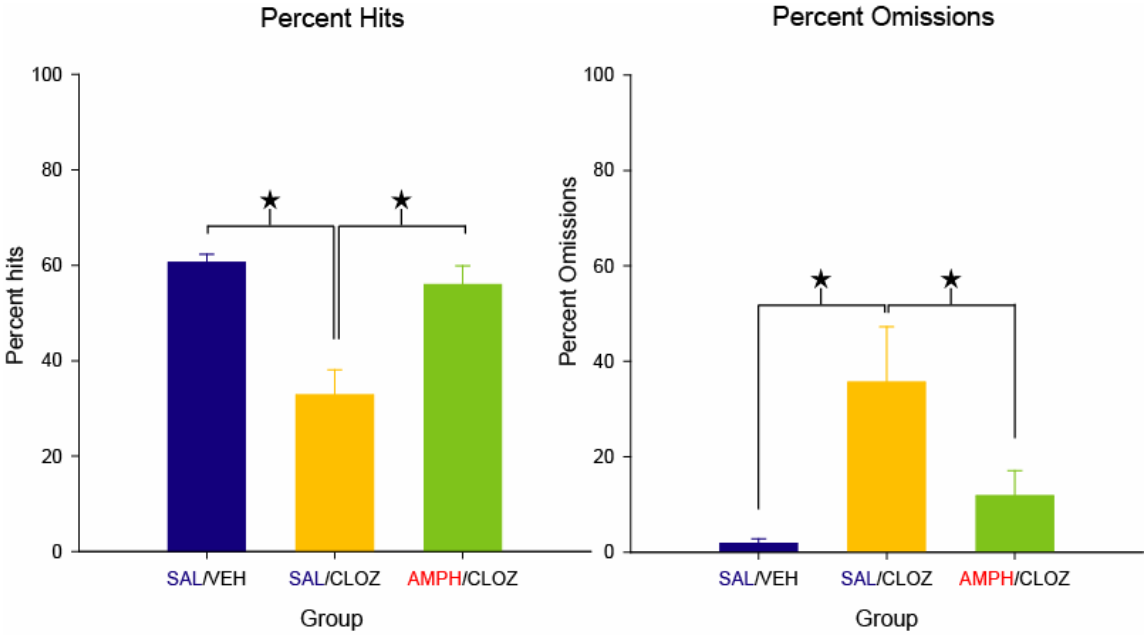

Figure 4.8: Effects of clozapine on performance in the absence of challenge doses (i.e. no challenge dose). Clozapine treatment lasted 10 days with challenge doses occurring on days 1, 5, and 10.These data reflect performance on the days in between challenge doses. Compared to the effects of vehicle, clozapine produced robust deficits on the hit rates of saline-pretreated animals. Pretreatment with AMPH appeared to protect animals from these deleterious effects (left graph). Similarly, clozapine treatment resulted in significantly increased rates of omitted trials in saline-pretreated rats while such impairments were not evident in animals pretreated with AMPH.

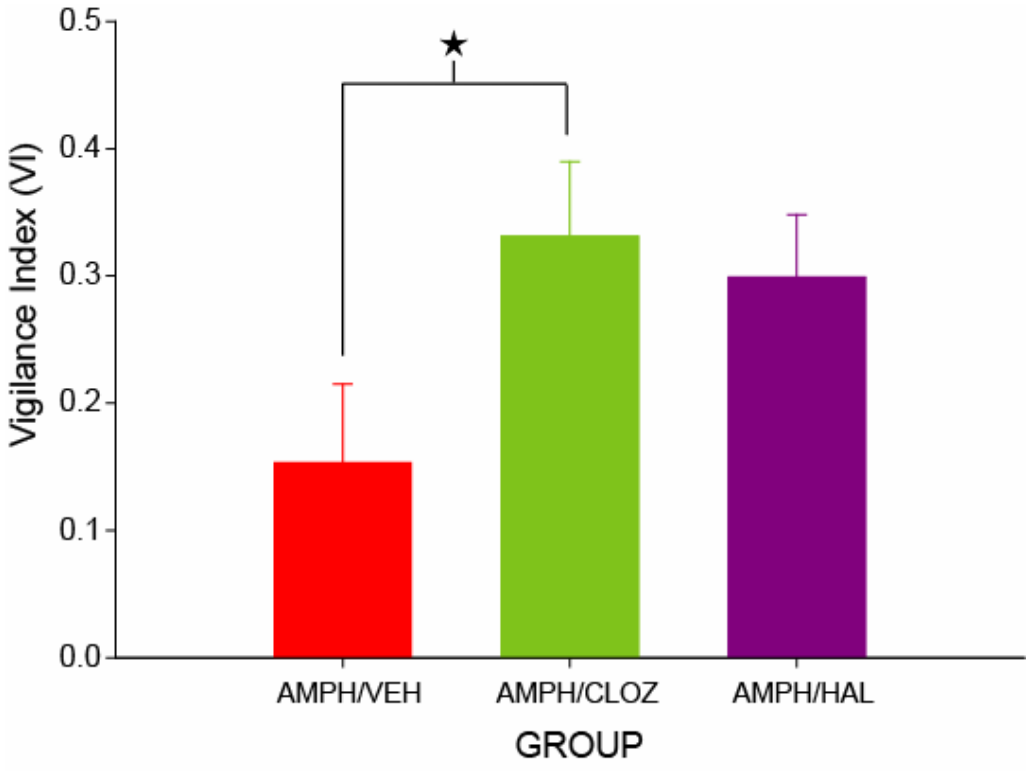

Figure 4.9: Performance effects of antipsychotic in AMPH-pretreated animals averaged over all challenge doses. Treatment with both clozapine and haloperidol attenuated performance impairments in AMPH-pretreated rats, however only the effects of clozapine were statistically robust. The performance of AMPH/CLOZ and AMPH/HAL groups did not differ.

CHAPTER 5

General Discussion

5.1 Synopsis

The experiments included in this dissertation were designed for three primary purposes: 1) To characterize nature of attentional impairments in an animal model of psychosis, 2) To test hypotheses regarding the nature of cortical cholinergic transmission in this model, and 3) to determine if this model of impaired attention in schizophrenia was sensitive to the moderate, pro-cognitive effects of commonly prescribed first- and second-generation drugs. The following section will attempt to summarize the main experimental results and describe some interpretational issues faced by the present data. Subsequent paragraphs will then delineate the significance of these findings and their relationships to hypotheses regarding cortical cholinergic dysregulation and impaired cognition in schizophrenia. Future directions will be explored at several points in this discussion and several testable hypotheses regarding the effects of antipsychotic treatment on cortical cholinergic transmission and attentional impairments are outlined. Finally the discussion will conclude with speculation regarding possible points of therapeutic relevance for improved cognition in schizophrenia.

5.2 Summary of findings and theoretical implications

The consequences of repeated, escalating AMPH exposure and subsequent challenge doses were explored in Experiment I. The results from Experiment 1 indicate that in AMPH-pretreated animals, administration of AMPH-

challenges results in marked performance impairments, particularly with regards to signal trials. These impairments are not evident following acute AMPH exposure, or following administration of AMPH-'challenges' in saline-pretreated animals. It is worth reiterating that these performance deficits cannot be attributed to overt motor stereotypy or side-bias. AMPH-associated impairments were restricted to decreased hit rates (i.e. responses to signal trials) and did not affect correct rejection rates or the number of omitted trials. Initially, the selectivity of these impairments for signal trials was surprising, as the observed impairment bore characteristics reminiscent of those seen following the selective depletion of cortical cholinergic transmission (McGaughy, Kaiser *et al*. 1996). These findings contrasted with original predictions regarding the nature of AMPH-associated performance impairments.

Using a pretreatment regimen and behavioral paradigm modified for operant-dialysis procedures, Experiment 2 aimed first to reproduce the behavioral deficits seen in Experiment 1, and second to characterize the effects of escalating AMPH on cortical cholinergic transmission during operant testing. Although the AMPH-associated attention deficits in Experiment II were somewhat obfuscated by micro-dialysis procedures, a similar constellation of impairments was evident. Microdialysis data indicated that in task-performing animals, repeated treatment with AMPH and subsequent administration of AMPH-challenges resulted in marked attenuation in cortical cholinergic transmission. Similar results were not evident in performing animals pretreated with saline or in untrained, non-performing, animals pretreated with AMPH. Reductions in the

magnitude of performance associated cortical ACh were shown to actually precede task initiation, indicating that the context or expectancy of task performance, rather than performance *per se*, may have been sufficient to produce these effects. These results seem to suggest that attenuated cortical cholinergic transmission contributed to, rather than resulted from, impaired task performance. The possible limitations of these data will be discussed.

The results of Experiment 3 demonstrate that low doses of haloperidol and clozapine can attenuate the AMPH-induced attentional impairments observed in a putative animal model of schizophrenia. By and large, both antipsychotic treatments displayed equal efficacy in AMPH-pretreated animals. In saline pretreated rats, clozapine produced detrimental effects (i.e. decreased hits and increased omissions), even in the absence of AMPH-challenge doses; similar impairments were not evident in AMPH-pretreated animals. These data lend predictive validity to this model as a screening tool for drugs to improve cognition in schizophrenia.

5.3 Experimental limitations and alternative interpretations of data

The present experiments provide data to support the core hypothesis that cortical cholinergic dysregulation represents an integral component of the neurobiology underlying schizophrenic symptoms, and that cortical cholinergic dysregulation may contribute to cognitive aspects of the disease. However, several methodological limitations prohibit the direct testing of hypotheses regarding the relationship of cholinergic dysregulation with respect to cognitive variables in this model. The following section will outline various limitations of the

present experiments and discuss their implications for the main hypotheses of these studies.

In Experiment 2, cortical cholinergic dysregulation is evident in AMPH-pretreated task performing rats. It is worth noting that the abnormalities in cholinergic transmission that are observed prior to task initiation cannot be singularly attributed to a specific factor such as general task expectancy, operational context, or explicit expectancy for cognitive task demands. The original hypothesis that "in order to demonstrate the abnormal regulation of a neurotransmitter system, recruitment of that system, by behavioral and cognitive operations relevant to that system, is required" has not been fully substantiated by these data and interpretations to this end should perhaps, be tempered. The main point of contention arises when determining the factors contributing to this dysregulation. That is, any claims that these effects result explicitly from expected 'cognitive demands' are not supported, as the data do not exclude the possibility that expectancies for reward or increased locomotion may have similar consequences. To substantiate these claims would require the addition of control groups to account for operational context as well as the non-attentional elements of task performance such locomotive behaviors, reward retrieval and consumption, and exposure to task-related stimuli (e.g. flashing lights and retracting levers). It has been demonstrated that bar pressing behavior and reward consumption are each sufficient to produce increases in cortical cholinergic transmission, albeit not to the same degree as sustained attention performance (Arnold, Burk *et al*. 2002). If, in fact, the context of sustained

attention performance is the key variable resulting in abnormal cortical cholinergic transmission, then trained AMPH-pretreated animals would exhibit cholinergic dysregulation only when placed into operant chambers associated with task performance, but not when placed in familiar environments with no attentional contexts. Similarly, claims that the expectancy for attentional demands (consequent to training history) drives the abnormal recruitment of cholinergic neurons could be substantiated using AMPH-pretreated animals trained to perform a simple reaction time task (as described in (Apparsundaram, Martinez *et al*. 2005). This task is thought to possess only minimal attentional demands and is designed to control for the motoric, reward, and stimulus associated components of task performance. In the absence of these controls, 'cognition oriented' interpretations of these data should be limited to imply that the challenge-associated cortical cholinergic dysregulation evident in task-performing, AMPH-pretreated animals is the result of abnormal recruitment of the BFCS by operational contexts and /or expectancy to perform a task with implicit attentional, motoric, stimulus, and reward associated components.

Experiment 3 applied a sub-chronic regimen of relatively low doses of clozapine and haloperidol. Although the effects of clozapine were more statistically robust, both classes of drugs were equally efficacious in attenuating the challenge-associated performance impairments observed in AMPH-pretreated animals. It remains to be determined if the performance improvements observed following antipsychotic treatment were the result of mechanisms other than direct D2 antagonism, or if these effects were simply the result of

antipsychotic drugs correcting the dopamine perturbations produced by an AMPH-challenge. If D2 antagonism represents the critical variable mediating these effects, then task performance should have improved as a function of a drug's affinity for D2 receptors. Although experimental design does not permit a formal test of this hypothesis (i.e. a correlation analysis), the more robust effects of clozapine compared to haloperidol, in conjunction with clozapine's lower affinity for D2 receptors, does not appear to support the interpretation that these effects were mediated solely by D2 antagonism.

The effects of clozapine in saline-pretreated animals were distinct from the effects of haloperidol. The beneficial effects of clozapine could only be demonstrated in AMPH-pretreated rats and its effects on saline-pretreated rats were actually detrimental. These mechanisms underlying these deficits are unknown, but speculatively, could be the result of clozapine's actions as a muscarinic antagonist. Interestingly, animals treated with haloperidol did not show performance deficits. Similar to the effects of acute treatment with AMPH, these findings may indicate that sustained attention performance is robust to minor manipulations of the dopamine system. Understanding the precise neural mechanisms mediating the differential effects of clozapine in saline- and AMPH-pretreated animals could inform hypotheses regarding beneficial actions of this drug on attention performance.

The 10-day antipsychotic dosing schedule was designed in part, to contrast the effects of acute and prolonged antipsychotic treatments, and was based partially on the hypothesis that the beneficial effects of antipsychotic drugs

have a delayed onset or lag in therapeutic efficacy. The present data demonstrate that both clozapine and haloperidol exert beneficial effects when administered acutely. Although these results were initially surprising, a more thorough review of the literature indicated that the benefits of antipsychotic treatment can appear on a much shorter timescale than conventionally expected (Kapur, Arenovich *et al*. 2005; Agid, Kapur *et al*. 2003).

5.4 Alternative animal models of schizophrenia

Alternative models attempting to reproduce aspects of schizophrenia have been based on etiological and developmental disease hypotheses; specifically, that pre- or peri-natal insults can produce developmental disturbances that confer susceptibility for the later development of schizophrenia. The common goal of these models has been to reproduce behavioral and neurobiological characteristics relevant to schizophrenia through the manipulation of putative 'causal' factors associated with the disease. For example, at least some epidemiological evidence suggests that obstetrical complications (Takagai, Kawai *et al*. 2006), maternal infection during gestation (Limosin, Rouillon *et al*. 2003), and maternal malnutrition (Brown, Susser *et al*. 1996) predispose the development of schizophrenia. These data have guided the development of animal models examining the behavioral and neurobiological consequences of early environmental and immunological insults, such as maternal deprivation (Ellenbroek, van den Kroonenberg *et al*. 1998), prenatal exposure to MAM (methylazoxymethanol-acetate; Featherstone, Rizos *et al*. 2007), or prenatal immunological challenges (Meyer, Feldon *et al*. 2005). Proponents of such

models note their ability to accurately reproduce hypothetical risk factors for schizophrenia while replicating many schizophrenia-like phenomena including sensorimotor gating deficits, neuroanatomical dysmorphogenesis, and irregularities in the dopaminergic and glutamatergic systems.

In addition to the etiological models described above, the cortical and hippocampal dysmorphogenesis observed in some schizophrenic brains has lead to the development of the neonatal ventral-hippocampal lesion (NNHL) model of schizophrenia (Lipska, Jaskiw *et al*. 1993). Ventral-hippocampal lesions produced on postnatal day 7 result in a variety of behavioral, neuro-chemical, and electrophysiological abnormalities that are manifested in adulthood, but are not apparent in pre-pubescent animals or in animals receiving hippocampal lesions as adults. Similar to schizophrenia, the behavioral consequences of NNHLs include hyper-responsiveness to stress, novelty, and psychostimulants; as well as deficits in latent inhibition and sensorimotor gating (Lipska, Jaskiw *et al*. 1993; Lipska, Swerdlow *et al*. 1995; O'Donnell, Lewis *et al*. 2002; Laplante, Stevenson *et al*. 2004).

Furthermore, additional pharmacologic models of schizophrenia have been based on the psychotogenic actions of NMDA-receptor antagonists, such as phencyclidine (PCP). These models are thought to reproduce the glutamatergic dysfunction associated with schizophrenia (Tenn, Kapur *et al*. 2005; Coyle 1996). Similar to the effects of AMPH, PCP administration in humans can exacerbate psychotic symptoms in patients and induce psychosis in healthy individuals. PCP treatments in rats and non-human primates have

likewise been shown to produce cognitive impairments, and alternations in neuronal systems associated with the disease (Flores 2007; Jentsch, Redmond *et al*. 1997).

As pointed out by critics of psychostimulant-based models, the repeated-AMPH paradigm used here fails to replicate the inducing factors of schizophrenia and is not capable of addressing hypotheses related to the time course of the illness. In certain cases any validity inherent to psychostimulant-based models can not be attributed to mechanisms beyond the scope of the induced dopamine dysregulation that is intrinsic to the model. In the simplest terms: following a direct manipulation to the dopamine system (i.e. administration of a dopamine agonist) an animal may display an abnormality in some dopamine-related behavior (i.e. stereotypy); that abnormality is then rectified by another direct manipulation of the dopamine system (i.e. a dopamine antagonist). Consequently, psychostimulant models have been criticized as being incapable of exploring disease mechanisms beyond those directly related to dopamine transmission. As a consequence, critics have indicated that such models could be of limited use for discovering novel, non-dopamine based drugs for schizophrenia (Geyer 2006; Lipska and Weinberger 2000).

Alternatively, developmental paradigms, such as the NNHL model, are thought to circumvent these logical constraints by engendering abnormalities in systems that are distinct from those they directly manipulate (Lipska and Weinberger 2000). Assertions that NMDA-based models share similar advantages have been based on data indicating that NMDA-antagonists (i.e.

PCP, ketamine), produce schizophrenic like characteristics that can are attenuated following treatment with clozapine, but not haloperidol (Geyer 2006; Linn, Negi *et al*. 2003). Because the consequences of PCP administration were responsive to second-, but not first-generation drugs, this evidence was taken to indicate that the application of NMDA-antagonist models could be applied toward the discovery of non- dopamine based drugs for schizophrenia (Geyer 2006). However, an alternative interpretation of these results reveals the same tautological reasoning that the author points out in psychostimulant-based models. Current data indicates that PCP has a high affinity for D2 and 5-HT2A receptors, along with affinities for dopamine and serotonin transporters (DAT and SRT, respectively). The fact that clozapine also possesses a high affinity for the same receptor sub-types once again makes it impossible to distinguish if the observed effects are mediated by systems distinct from those being directly manipulated or if they simply reflect the competition of drugs for common receptors.

In AMPH-pretreated animals, robust impairments in performance impairments are only apparent in the presence of an AMPH-challenge. This effect is problematic because, as discussed above, it occludes the mechanisms underlying the performance improvements observed following antipsychotic treatment, and furthermore is not representative of the persistent impairments observed throughout the entire disease course in patient populations. Perhaps future experiments will improve upon this model by determining ways to produce

persistent performance deficits in AMPH-pretreated animals in the absence of a direct dopamine manipulation (i.e. exposure to a stressful stimulus).

5.5 Future directions

Recent advances in technologies used to assess cortical ACh have made it possible to measure changes cortical ACh on a sub-second time scale. Such techniques have facilitated the examination of cortical cholinergic transmission in relation to discrete stimuli and specific behaviors (Parikh 2007). Furthermore, use of these techniques has revealed that changes in cortical cholinergic transmission can occur on multiple time scales. Slow rising 'tonic' changes are seen in response to general task performance, whereas faster, transient changes are seen in response to discrete stimuli. The microdialysis procedures employed in Experiment 2 are incapable of making such assessments and are generally thought to reflect a combination of both of these components (Parikh 2007). Future studies utilizing this model and applying newer technologies to determine the precise nature of cortical cholinergic dysregulation could provide data to guide attempts to normalize cholinergic transmission in schizophrenia. These techniques, in conjunction with the present paradigm could be employed to test hypotheses concerning the mechanisms by which haloperidol and clozapine achieved the performance-improving effects described in experiment 3; specifically that these drugs normalize both phasic and tonic components of cortical cholinergic transmission.

5.6 Normalizing cortical cholinergic transmission and attentional impairments in schizophrenia

It is not known whether the beneficial effects of clozapine and haloperidol in the present paradigm result from their antipsychotic actions or actual cognitive improvements. However, the more robust effects of clozapine compared to haloperidol, regardless of haloperidol's higher affinity for D2 receptors suggest that simple D2 antagonism may not be the single variable mediating these benefits. Speculatively, these benefits could stem from these drugs capacity to normalize afferent systems regulating the excitability of the basal forebrain, their direct actions on various receptor subtypes (i.e. D2, M2), or any combination of these effects. Attempts to alleviate the attentional impairments observed in schizophrenia via the direct manipulation of cholinergic mechanisms have not been successful. This failure is thought to stem from the inability of currently available drugs to restore the dynamic regulation of the cholinergic transmission with respect to changing stimulus environments and cognitive demands. The success of future endeavors to improve cognition in schizophrenia may be contingent upon the re-regulation basal forebrain cholinergic system via tran-synaptic modulatory mechanisms, rather than direct cholinergic agonism or antagonism.

5.7 Concluding remarks

Despite the experimental and interpretational challenges described above, the experiments included in this thesis aid in the refinement of current hypotheses regarding the neural mechanisms underlying schizophrenia's cognitive deficits. The above experiments provide direct evidence that abnormal

cortical cholinergic transmission occurs in association with attentional impairments in an animal model of schizophrenia. Importantly, this dysregulation was only apparent in task performing animals. As a consequence, the contributions of the basal forebrain cholinergic system to schizophrenia should no longer be conceptualized as trait characteristics such as excesses or deficiencies of cortical acetylcholine, but rather, hypotheses should reflect the expectation that the dysregulation of the basal forebrain cholinergic system in schizophrenia will vary widely depending on cognitive and contextual factors. In order for a drug to successfully improve cognition in schizophrenia its must be able to restore the dynamic regulation of this system. Furthermore, because this present model is sensitive to the performance improvements produced by antipsychotic drugs it may serve as a practical means to screen for such medications.

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