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Attention, prediction and sequence learning: roles of the cholinergic basal forebrain and the retrosplenial cortex

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Attention, Prediction and Sequence Learning: Roles of the Cholinergic Basal Forebrain and the Retrosplenial Cortex

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Cognitive Science

by

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2005
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Chair
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2005
DEDICATION

This thesis is dedicated to Suzanne Dufour; for her love, patience and support.
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PUBLICATIONS


Our ability to foresee and shape biologically important events relies on a combination of visuospatial attention, memory capacities, and an ability to learn new sequences of goal-directed action. A novel set of behavioral studies were conducted to investigate neurobiological processes that underlie selective attention and visuospatial sequence learning. The first experiment assessed a theorized computational role of basal forebrain cholinergic neurons in modulating attention by increasing stimulus processing in proportion to an animal's uncertainty of impending events in the environment. A selective lesion of basal forebrain cholinergic neurons in rats produced a graded impairment in
behavior that corresponds to the uncertainty elicited by different probabilistic classes of stimuli in the task. The findings thus provide initial support for a cholinergic role the regulation of Bayesian-like inference by regulating the balance of expectations and sensory processing in the brain. In a second experiment, the effects of sleep deprivation were assessed in a selective attention task to test hypothesized effects of the circadian regulation of attention through its suppressive effects on acetylcholine and other neurochemical systems. In a third experiment, the retrosplenial cortex of rats was reversibly inactivated to assess a role of the structure in temporal order learning suggested by retrosplenial pathologies in humans such as early Alzheimer’s disease. The reversible inactivation of neural activity in the retrosplenial cortex produced impairments during an early, but not a late stage of visuospatial sequence learning, providing direct evidence for a memory role of the structure during the encoding of ordered events in the environment. In combination, the studies provide new insights into the biological processes that underlie the human capacities for attention, memory and sequence learning in response to new challenges in the environment.
CHAPTER I

Introduction

Memory for temporally structured events is central to our ability to foresee and control biologically important outcomes in the environment. It underlies the basic ability to continually act within the environment, and is a broad, yet mostly invisible basis for apprehending the environment. In a changing environment, unpredictable events come to the fore of attention and lead to the development of new learning and instrumental actions, which gradually make those events invisible again within an expanding horizon of skills and apprehension.

This thesis considers the structural and neurochemical processes of the brain that allow animals to flexibly allocate attention, memory and behavior towards the learning of new structured events in the environment. Through a series of behavioral experiments, the thesis will first investigate the principles and neurochemical processes that underlie an animal’s ability to flexibly allocate attention to relevant stimuli in the face of change and novelty in the environment. Two studies investigated the role of the neuromodulator, acetylcholine in the modulation of attention by investigating the breakdown of attention that results from pharmacological and naturally occurring alterations of cholinergic function. The thesis then investigated neurobiological processes that allow animals to learn the sequential structure of visuospatial events in the environment, a process that entails the adaptive learning of new information that gradually allows animals to predict and control previously unexpected outcomes in the environment. The neurobiological study of sequence learning focused on the retrosplenial cortex, a region of the posterior cingulate cortex that may play a coordinating role among attention and learning processes
in the limbic system that allow animals to associate relevant events over time. The role of retrosplenial cortex in the adaptive learning of new sequences was assessed by investigating effects of pharmacological disruptions of the structure, and comparing behavioral effects across different stages of visuospatial learning that reflect the diminishing contributions of the retrosplenial cortex on performance of learned visuospatial sequences. In combination, the studies constituted a novel investigation into the neurobiological processes that allow animals to attend to relevant events in their environment, to learn their associative structure, and to gradually encode memories in a form that no longer requires learning and increased attention.

The following sections will briefly outline the functional anatomy of the retrosplenial cortex and the cholinergic basal forebrain. An overview of cortical and subcortical learning processes will help elucidate the functional contributions of each structure during attention and associative learning. The overview will also serve to outline the interactions of subcortical and cortical systems that will be a central theme in each of the studies in the thesis.

1. Cholinergic roles in attention

The cholinergic basal forebrain has been the focus of intensive studies, particularly since its implication in Alzheimer’s disease (Everitt & Robbins, 1997; Bartus & Emerich, 1999; Francis et al, 1999), but the development of pharmacological techniques for selectively lesioning cholinergic neurons has only recently allowed the experimental study of isolated cholinergic function (Wiley et al, 1991). Behavioral studies using
selective lesion techniques have thus largely inferred the function of the cholinergic system through animal models using tasks that assess the effects of pharmacological lesions on attention, memory and learning capacities of animals. Thus far, findings using selective pharmacological lesions have indicated that the basal forebrain cholinergic system facilitates selective increases in attention and learning by regulating the amount of processing devoted to cues that predict biologically relevant events (Muir et al, 1993; Chiba et al, 1995; Bucci et al, 1998; McGaughy et al, 2002).

The modulatory roles of acetylcholine have also been shown to function in the context of circadian processes that regulate the large-scale activity of the cortex, and thereby establish the waking, sleeping and dreaming states of brain. Much like disease models, the homeostatic effects of the sleep-wake cycle also have a profound suppressive effect on acetylcholine, and may underlie circadian alterations in the ability of intact subjects to flexibly allocate attention to new and changing events in the visuospatial environment.

1.1 Anatomy of the basal forebrain

Cholinergic neurons in the basal forebrain (BF) provide a major route by which subcortical leaning, motivational, and regulatory systems can affect activity and processing in the outlying cortex. The large cortically projecting cholinergic neurons in the basal forebrain of rats reside in a posterior area of the BF that spans the Nucleus Basalis Magnocellularis/Substantia Innominata (NBM/SI) region in rodents (Fig. 1.1). The NBM/SI is considered a ventral pallidal region of the striatopallidal system (Alheid & Heimer, 1988; Swanson & Petrovich, 1998), a circuit that been described as an
‘affective’ loop of the basal ganglia. Distinct from the motor and cognitive circuits in the dorsal striatum, the ventral striatopallidal system receives inputs from the frontal cortex, the amygdala, and hippocampus. On the basis of these connections, the ventral striatopallidal system has been described as a transition area between motivation, cognition and action that helps to regulate activity in the cortex with regards to cues in the environment that have biological relevance for an animal (Mogenson et al, 1980; Gallagher & Holland, 1994; Robbins & Everitt, 1996; O'Donnell et al, 1999).

In addition, the basal forebrain is one target of subcortical regulatory systems that controls the overall state of the cortex through the coordinated actions of monoamine systems in the basal forebrain, brainstem, and thalamus (reviewed by Mignot et al, 2002). The lateral hypothalamus and local adenosine in the basal forebrain (a by-product of metabolic adenosine tri-phosphate), mediate circadian regulatory processes and shape the activity of cholinergic neurons in concert with homeostatic processes that increase sleep drive and regulate the transitions between waking and sleeping states of the brain.

1.2 Cholinergic roles in attention

One major influence of activity in the NBM/SI is the central nucleus of the amygdala (CeA) (Alheid & Heimer, 1988), a sub-region that integrates information about neutral cues (Baxter & Murray, 2002) and instrumental actions (Killcross et al, 1997) that can predict biologically important outcomes. The NBM/SI is also strongly affected by the ventral striatum, particularly from the shell of the nucleus accumbens (NAcc). Dopamine release within the NAcc shell profoundly influences the learning of reward cues (Caine et al, 1995; McGinty, 1999), and has been proposed to directly convey the discrepancy
between actual and expected rewards (reward prediction error), a measure that parametrically increases attention in computational (Dayan et al, 2000) and animal learning theories of associative learning (Rescorla & Wagner, 1972; Pearce & Hall, 1980).

In the face of prediction errors, one effective strategy for selecting relevant cues is to increase attention to stimuli whose outcomes are less known (Hall & Pearce, 1982; Yu & Dayan, 2002). In a series of experiments, it was found that the integrity of a circuit that spanned the central nucleus of the amygdala (CeA), the cholinergic neurons in the NBM/SI and efferent cholinergic fibers projecting to parietal cortex were each necessary for the increased attention to stimuli that had a less consistent stimulus history (Holland & Gallagher, 1993; Chiba et al, 1995; Bucci et al, 1998). This relatively simple strategy has been proposed to underlie the selective processing of new conditioned stimuli in animal learning theories (Dickinson & Mackintosh, 1978; Hall & Pearce, 1982). However, it is also analogous to a central strategy in Bayesian learning that regulates the development of statistically optimal predictions on the basis of a cumulative stimulus history (Maybeck, 1979). Thus the attentional role of the basal forebrain cholinergic system has been interpreted in terms of an iterative Bayesian model called a Kalman filter, wherein the variance of predictions from present cues is used as a value for re-weighing associations between stimuli and subsequent events in the environment (Yu & Dayan, 2002).

Although these computational models ascribe a specific computational role to the attention-related functions of acetylcholine, the models have not yet been assessed. The first chapter of the thesis thus investigates the possibility that cholinergic neurons mediate selective increases in attention in proportion to the probabilistic uncertainty of stimulus
predictions, a degree of modulation that would facilitate stimulus detection, and would theoretically mediate selective increases of attention to stimuli that can predict relevant outcomes in the environment.

1.3 Circadian regulation of cholinergic system

The cholinergic basal forebrain also plays a prominent role in establishing the waking and sleeping states of brain activity with respect to the circadian cycles of the brain. Recent evidence has suggested that the homeostatic regulation of sleep onset appears to be mediated by adenosine, a molecule whose concentration in the extracellular space in the basal forebrain increases linearly with the duration of wakefulness (Porkka-Heiskanen et al, 1997). Extracellular adenosine directly inhibits the firing of cholinergic neurons (Rainnie et al, 1994) and thus appears to play a central role in the circadian regulation of the cholinergic system. The actions of adenosine on cholinergic modulation has been proposed to contribute to sleep onset by that diminishing the waking activities of the cortex and increasing the brain’s drive for sleep with extended wakefulness; the ‘sleepiness’ of the brain. An interesting possibility that is suggested by this mechanism is that attentional alterations that result from extended wakefulness would include parallels to attentional alterations that result from disease and pharmacological disruptions of the cholinergic system, a possibility that was assessed in the third chapter of the thesis.

The major neurochemical factors of the sleep-wake cycle have been studied extensively in rodent and animal models, and depend largely on the circadian activities of norepinephrine, acetylcholine, and serotonin (reviewed by Steriade et al, 2005). The attention-related functions of these and other neurochemical systems have also been
systematically investigated in a behavioral test of selective attention in rodents (the 5-choice reaction time task (5CSRTT) reviewed by Robbins, 2002). In the first systematic study of attention-related effects of sleep deprivation in animals, chapter 3 assesses a hypothesized link between the homeostatic suppression of cholinergic systems and subsequent attentional alterations by testing the effects of sleep deprivation on the 5CSRTT and comparing those effects to behavioral alterations that are known to arise from the selective disruptions of different monoamine systems. In combination with chapter 2, the study allowed a further investigation of cholinergic functioning in attention with respect to major subcortical influences that shape the modulatory actions of the basal forebrain.

2. The limbic system and sequence learning

The adaptive capacity for the learning of sequences of actions and stimuli relies on a combination of memory processes, behavioral control, and visuospatial attention to impending events in the environment. The neurobiological processes that integrate and encode new sequence memories depend critically on the coordinated functioning of multiple cortical and subcortical structures of the mammalian brain. A large body of clinical and neurobiological data have particularly implicated a connected circuit of cortical and neurochemical systems that form the paralimbic lobe first described by Broca (Morecraft et al, 1992; Mesulam et al, 2001; Heimer & Van Hoesen, 2005), a system of cortical structures that are joined by anterior thalamic nuclei, and include the frontal cortex, the hippocampus, the temporal lobes and the cingulate cortex. The retrosplenial
cortex appears to occupy a pivotal anatomical and functional position in the integrated functioning of the limbic system. Despite its prominent position, the understanding of the retrosplenial cingulate cortex has been limited by a rarity of clinical reports of humans with isolated retrosplenial damage, and limited experimental studies of the structure have not yet accumulated a converging body of evidence that define its major functions.

3. Sequence memory systems in the limbic system and the basal ganglia

Considerable evidence suggests that learning of sequential events results from a combination of learning strategies that are supported by distinct memory systems of the brain (Mishkin & Petri, 1984; Packard et al, 1989; Knowlton et al, 1996). A major distinction that has been found is between episodic memory processes of cortical limbic structures and stimulus-response learning in the subcortical basal ganglia. Concurring evidence from human and animal studies have suggested that during learning, limbic and basal ganglia systems are activated simultaneously and play complementary, and sometimes competitive roles in learning. In addition, there is a shifting competition between cortical and subcortical structures during associative learning, such that processing and behavior are initially dominated by cortical limbic structures and gradually transferred to memory systems in the dorsal regions of the basal ganglia (Poldrack et al, 2001).

3.1 Encoding of sequence memories by limbic cortex and basal ganglia
The nature of sequence encoding by limbic and basal ganglia systems appears to be defined by the respective anatomy of the two structures, and the distinct mechanisms by which the successive representation of events induce synaptic change in each of the structures. The basal ganglia encodes sequential events through the convergence of cortical inputs onto neurons in the dorsal striatum and the serial activation of circuits that loop from the striatum to the pallidum and back to their originating sites in the cortex (reviewed by Houk et al, 1995). The formation of behavioral circuits through the basal ganglia begins with the successful activation of striatal neurons from simultaneous, converging inputs from different regions of the neocortex (Graybiel et al, 1994), a physiological process that is suited for the development of context-specific and stereotypic sequences of thoughts and actions. Limbic structures, by contrast, are thought to encode sequential events by the brief maintenance of spatial and cognitive representations by neurons in the limbic system (Fuster, 1990; O’Keefe & Recce, 1993; Mehta et al, 2002), and the successful activation of post-synaptic neurons in the hippocampus by overlapping inputs from neurons that are activated by successive events in the environment.

Interestingly, the different cortical and subcortical learning strategies appear to follow a distinction between a Tolmanian cognitive strategy and stimulus-response learning strategy described by Thorndike and Hull. Consistent with Tolman’s notion of a cognitive map, there is evidence that limbic structures encode environment-centered representations, which allows flexible development of navigational behavior to reach goal locations (Tolman, 1932; O’Keefe & Dostrovsky, 1971; McNaughton et al, 1983). The learning processes of the dorsal striatum, by contrast, are suited for the reinforcement of particular responses and the gradual development of an S-R behavioral strategy. For
example, the performance of rats that are overtrained is not affected by reinforcer devaluation, but rats perform more slowly if a reward is devalued at an early stage of training (Sage & Knowlton, 2000). These and other findings provide evidence for the gradual acquisition of an S-R behavioral strategy by dorsal basal ganglia circuits where the reinforcer is not itself represented in the associations (Knowlton et al, 1996), an effect consistent with a nonrepresentational learning strategy proposed by Thorndike and Hull (Thorndike, 1933; Hull, 1943).

Though it is part of the pallidum, the basal forebrain has been more closely associated with representational learning processes of the limbic cortex (Alheid & Heimer, 1988; Heimer & Van Hoesen, 2005). Unlike the dorsal striatum, lesions of the core region of the nucleus accumbens that projects to the basal forebrain interfere with reinforcer devaluation effects in rats (Corbit et al, 2001, 2003), thus implicating both the basal forebrain and cortical limbic structures in new learning processes that entail the representation of associated outcomes.

4. The Retrosplenial Cortex

4.1 Anatomy of retrosplenial cortex

The posterior cingulate cortex occupies a mediating position between cortical limbic systems that play central roles in the representation and association of new temporally structured information. The posterior cingulate cortex of rodents and primates interacts heavily with the hippocampus (van Groen & Wyss, 1992; Wyss & Van Groen, 1992), entorhinal cortex (Burwell & Amaral, 1998) and the dorsolateral prefrontal cortex
(DLPFC) (Morris et al, 1999), a region that has been strongly implicated in encoding and working memory functions (Fuster, 1990; Goldman-Rakic, 1990). The posterior cingulate cortex is the largest cortical target of the hippocampus outside of the entorhinal cortex and is the major route through which the dorsal hippocampus interacts with working memory processes in the frontal cortex (Goldman-Rakic et al, 1984). The retrosplenial cortex also functions in close association with spatial memory and attention processes of the posterior parietal cortex and the dorsolateral prefrontal cortex (Goldman-Rakic et al, 1984; Goldman-Rakic, 1990; Morecraft et al, 1992; Kastner & Ungerleider, 2000).

### 4.2 Memory roles of retrosplenial cortex

Consistent with its connective anatomy, findings from imaging studies of intact humans in a cued attention task indicate that the retrosplenial cortex facilitates the rapid allocation of visuospatial attention in anticipation of impending stimuli (Mesulam et al, 2001), and that it does so in concert with the prefrontal cortex (Small et al, 2003). Humans with posterior cingulate damage also have profound impairments in the ability to remember the temporal order of recent events, while sparing order memory of remote events (Valenstein et al, 1987; Bowers et al, 1988). In addition, metabolic reductions in the retrosplenial cortex that are a hallmark of early Alzheimer’s disease are directly correlated with the degree of temporal disorientation (Hirono et al, 1998). The pattern of cognitive breakdown that results from disturbances of the retrosplenial cortex thus strongly implicate the structure in the learning of temporally structured information and events. However, the effect had not been isolated to focal disruptions of activity within
the retrosplenial cortex. This possibility was thus assessed local disruptions of neural activity of the retrosplenial cortex as described in chapter 4. A comparison of effects across early and late stages of visuospatial sequence learning also tested the possibility that sequence processing is gradually processed outside of limbic cortical structures, as suggested by clinical evidence and the anatomy of separate memory systems of the brain.

5. Outline

The second chapter of the thesis assesses the effects of selective cholinergic lesions of the NBM/SI in rats trained to respond to brief probabilistic stimuli to test a hypothesis that acetylcholine increases stimulus processing in proportion to the probabilistic uncertainty of an animal’s predictions of impending events in the environment. The third chapter of the thesis assesses the attentional influences of the circadian regulation of brain activity by measuring the effects of sleep deprivation on a well-characterized serial reaction time task and assessing its effects with respect to known effects of neurochemical disruptions on the task, which include acetylcholine, serotonin, and norepinephrine. The fourth chapter of the thesis describes a behavioral study of reversible pharmacological inactivations of retrosplenial cortex to assess its effects in visuospatial sequence learning. A fifth chapter will then review the findings of the thesis and discuss new directions of research that are currently being pursued to elaborate the findings and implications of the current studies. In combination, the experiments allowed a novel investigation of the processes and principles by which subcortical and cortical
interactions may allow the brain to detect, remember and behaviorally adapt to new information about temporally structured events in the environment.
Figure 1.1. Anatomy of the cholinergic basal forebrain. Groups of corticopetal cholinergic neurons in the basal forebrain modulate processing in different target regions of the cortex. VDB: vertical diagonal band of Broca, MS: medial septum, NMB/SI: nucleus basalis magnocellularis/ substantia innominata, CN: central nucleus of the amygdala.


1. Abstract

The dissertation aims to elucidate neural processes underlying attention and learning that allow humans and animals to adapt to a changing environment. The first section will focus on attentional processes that are mediated by acetylcholine. By facilitating the detection of new and changing events in the environment, cholinergic modulation of attention may constitute a first step of adaptive learning and behavior.

Our survival depends on the appropriate allocation of attention to biologically significant events in the environment. A recent convergence of behavioral, neurobiological and computational data has provided new insights into the way that the cholinergic neurons in the basal forebrain may regulate the allocation of attention to important, but relatively unpredictable events in the environment. Cholinergic neurons have recently been proposed to modulate the cortical processing of stimuli in proportion to the probabilistic uncertainty of ongoing stimulus predictions. Such a measure may allow the brain to combine past learning and new sensory information in a manner that optimizes the detection relevant stimuli in noisy and changing environments. The current study assessed the hypothesized role of cholinergic modulation by testing the effects of a selective lesion of BF cholinergic neurons in a task that elicited different degrees of predictive uncertainty to facilitate the detection of brief visuospatial stimuli. Consistent with predictions, a selective lesion of BF cholinergic neurons produced a graded
impairment in behavior that corresponds to the uncertainty elicited by different probabilistic classes of stimuli in the task. The findings thus provide support for a principled role of cholinergic modulation in the regulation of Bayesian-like inferential processes in the brain. The findings also shed new light on the limited capacities of humans with cholinergic pathologies to appropriately allocate attention in the face of change and novelty in the environment.

2. Introduction

Behavioral and neurobiological data suggest that cholinergic neurons in the basal forebrain help to regulate attention to relevant stimuli from a myriad of competing events in the environment. Cortically projecting cholinergic neurons in the Nucleus Basalis/Substantia innominata (NBM/SI) region of the basal forebrain promote activity and plasticity in the cortex in response to stimuli that predict significant events in the environment (Fig. 1.1); (Wilson & Rolls, 1990; Whalen et al, 1994; Bakin & Weinberger, 1996). NBM/SI cholinergic neurons are also required for the increased processing of stimuli with less known consequences (Chiba et al, 1995; Bucci et al, 1998), a strategy that has been proposed to underlie the learning of effective predictive associations between stimuli (Dickinson & Pearse, 1978; Hall & Pierce, 1982; Holland & Gallagher, 1993). In addition, NBM/SI cholinergic neurons contribute to the fluid allocation of attention by reducing the behavioral influence of incorrect stimulus expectations from visuospatial cues (Parasuraman et al, 1992; Baxter & Chiba, 1999) and promoting shifts of attention to less predictable stimuli (Moore et al, 1999; McGaughy et al, 2002).
In light of these findings, computational theorists have proposed that cholinergic neurons in the basal forebrain carry out attributed roles in attention by modulating stimulus processing according to the probabilistic uncertainty of stimulus expectations. Cholinergic uncertainty is hypothesized to mediate a central strategy of Bayesian inference that specifies the way that new sensory information should be combined with learned stimulus expectations to best infer the discrete state of events in the environment. One computational model has described how the uncertainty of ongoing expectations could be used as a value for optimally allocating processing to stimuli that can predict significant events in the environment following errors of prediction (Dayan et al., 2000). Another model has illustrated how the same measure of uncertainty could facilitate the detection of stimuli in noisy environments by establishing the correct balance of bottom-up sensory processing and top-down stimulus expectations according to how well events can be predicted from prior cues (Dayan & Yu, 2002; Yu & Dayan, 2002).

While the theories accord with a wide body of behavioral data on attention and learning processes in animals (Rescorla & Wagner, 1972; Dickinson & Mackintosh, 1978; Hall & Pearce, 1982; Dayan et al., 2000), they formalize the notion of optimal inference in terms of well-developed statistical learning models. Thus they describe the way that uncertainty should be represented in the brain during inference tasks and make specific predictions about how inference would break down following the loss of an uncertainty measure (Yu & Dayan, 2005). The computational role of acetylcholine in mediating that uncertainty, however, has not yet been assessed.

The current study assessed the theorized role of acetylcholine of by testing the effects of a selective NBM/SI cholinergic lesion in an inference task that parametrically manipulated the idealized levels of uncertainty represented by an animal that would
facilitate the detection of probabilistic stimuli. Rats were trained to respond to a light in one of four stimulus locations such that each stimulus could be predicted from the last in a probabilistic fashion (Fig. 2.1). In each trial, a stimulus was presented in the same port as the previous trial with a .7 probability and in a port two positions away with a .2 probability (Fig. 2.1), thus greatly facilitating the use of learned expectations for detecting very brief stimuli. The spatial locations of the probable stimuli, however were determined by an unsignaled context state that changed the likely locations of the stimuli every 2-30 trials, thereby introducing considerable uncertainty about the pending location of a stimulus on the basis of top-down expectations. Thus while expectations about likely stimulus locations could facilitate the detection of stimuli, an animal’s uncertainty about the current stimulus context would also facilitate detection in a subset of trials by appropriately decreasing use of expectations when top-down information is no longer useful. By administering a lesion to corticopetal cholinergic neurons, we were thus able to test if animals exhibited impairments reflecting an inappropriate use of learned expectations when responding to stimuli with greater contextual uncertainty.

We hypothesized that the degree of cholinergic modulation would correspond to the probabilistic uncertainty of the most likely stimulus context (Fig. 2.2), a simplified estimate that permits Bayesian inference from only one prior distribution (the most informative), but is much less computationally expensive (Yu & Dayan, 2002). It was hypothesized that the loss of cholinergic neurons would reflect the facilitative role of acetylcholine by producing a graded impairment in response to the less predictable stimuli under each context, and the largest impairment after a context had changed, when uncertainty about the current context is highest. Rats were given control and immunotoxic 192 IgG-saporin lesions of cholinergic neurons in the NBM/SI after they
had demonstrated learning of the underlying stimulus probabilities and post-lesion performance was assessed across the four types of probabilistic stimuli presented in the task (70%, 20%, 5% and after a context transition). Consistent with predictions, animals with selective NBM/SI cholinergic lesions were impaired in a graded fashion corresponding to the probabilistic uncertainty of the different stimulus classes in the task, an effect consistent with hypothesized levels of lost cholinergic modulation across trial types, and resulting impairments that result from the inappropriate use of learned expectations during inference.

### 2.1 Inference in a noisy environment

The theorized role of acetylcholine in stimulus inference has been illustrated with a three-layer hidden Markov model (HMM), a simple probabilistic model that employs a measure of predictive uncertainty to optimally infer the state of external events from very noisy stimuli. Using a probabilistic chain, stimuli presented to an observer (a computer or an animal) are generated by two hidden layers ($z$ & $y$) and accessible only by a third output layer ($x$). At each time step, the likely position of a stimulus is determined by the slow-moving dynamics $T_z$ of a context layer $z$, which can be in one of four states (Fig. 2.2, top). The discrete state of the context, in turn is multiplied by Markov stochastic matrix $O_{zy}$ (whose rows and columns sum to 1) to determine the trial-by-trial dynamics of the stimulus in layer $y$ (Fig. 2.2, middle). The state of layer $y$ is then filtered through Gaussian noise and projected onto the observable output layer $x$. The variance of the Gaussian noise is large, thus distinct states of the $y$ layer are mapped over wide overlapping regions in layer $x$ (Fig. 2.2, bottom).
In the experiment, the Gaussian noise may be considered analogous to reduced detectability introduced by a very brief signal, and the changing position of the stimulus relative to the rat’s movement in the chamber.

The inference problem is to use the current stimulus presented in observable layer $x$, and the history of $x$ to infer the most likely discrete state of $y_t$ that has produced the current stimulus. In a Hidden Markov model, an exact inverse model of the stimulus model can be built up from past experience and used as a source of information for disambiguating the stimulus. By learning the prior likelihood of $z$ given a stimulus history, $P[z_t|D_{t-1}]$, past information $P[z_t,y_t|D_{t-1}]$ can be computed and combined with sensory information $p[x_t|y_t]$ about the likelihood of the current stimulus:

$$P[z_t,y_t|D_t] \propto P[z_t,y_t|D_{t-1}]p[x_t|y_t]$$

The probability distributions of each possible state of $z_t$ are used to calculate the distribution of $y_t$, which is the belief about the discrete state that generated the current observation $x_t$ in the context of past experiences. In exact inference, all the possible states of $z$ are figured into the calculation of $y$ in proportion to their relative probabilities. While an exact inference algorithm achieves good performance, it is probably not computationally feasible for the brain to perform exact inference in all its mathematical complexity. One critical problem is maintaining and simultaneously manipulating the information about all possible contexts $P[z_t|D_t]$. There are potentially as many contexts as known environments, which greatly compounds the problem.
2.2 Acetylcholine-mediated approximate inference

In an analogous, but far less computationally expensive strategy, contextual information can still be used to disambiguate stimuli with a simplifying assumption that the brain calculates only the most likely state of context layer $z$ at a particular time point $\arg\max_{z} \tilde{P}(z|D_t)$, and the estimated probability that it is not in that state, $\alpha_t = 1 - \tilde{P}(z|D_t)$. With this measure of uncertainty, information about the most likely context can still be combined with sensory-based information in a correct proportion. In the case of approximate inference, a simplified prior distribution of layer $z$ is calculated by assigning a probability to the most likely state, and dividing the probability of ‘another’ state among the other contexts. The net effect is to limit the influence of prior information about the estimated current context in proportion to its uncertainty. During times of change, prior information is confusing or potentially incorrect, and so contextual information should be abandoned while external states are inferred mainly from incoming sensory information. This switch in inferential strategy is just the hypothesized effect of basal forebrain cholinergic modulation. By the well-timed release of acetylcholine to the cortex, the cholinergic system may initiate periods of increased attention to ongoing events, thus facilitating the detection of less predictable, but potentially significant events. Conversely, animals with cholinergic lesions would be expected to rely too strongly on prior information in periods of high predictive uncertainty.

3. Materials and methods
22 Long-Evans hooded rats (Charles River, Raleigh NC) began behavioral testing. Water was available *ad libitum*, but they were food restricted to maintain body weight at 85% of their free-feeding weight for motivation by a food reward.

### 3.1 Apparatus

Rats were tested using two four-hole operant chambers (Cambridge Cognition, Cambridge, UK, Fig. 2.1). Each aluminum chamber was 25x25 x25 cm and was equipped with a Plexiglas flap door that allowed access to a magazine where 45mg Noyes sucrose pellets were mechanically dispensed (Research Diets Inc., New Jersey). Four evenly-spaced apertures were located on the opposite wall of the chamber that registered nose pokes via the interruption of infrared photocell beams. At the rear of each aperture was a 3w bulb that served as a stimulus. A 3W bulb at the top of the chamber provided illumination (the house light). Each behavioral chamber was contained in a larger sound-attenuating chamber with a small fan that provided ventilation and background white noise. All stimulus presentations and behavioral measures were made with Cambridge Cognition Control 1.17 software on a PC Pentium IV class computer.

### 3.2 Behavioral training

Rats first were trained to nose poke in an aperture following the brief presentation of a visual stimulus within that aperture (the rodent serial reaction time task, Carli et al. 1983, Fig. 2.1). Each stimulus was presented with an equal probability in one of the four apertures and was thus spatially unpredictable. The rats were initially allowed 60 seconds to respond to each stimulus and were rewarded with a sucrose pellet immediately...
following a response into the illuminated port. Upon attaining 70% correct responses on
two consecutive days, the stimulus duration was shortened until the rats re-attained 70% accuracy. The stimulus was shortened repeatedly in this fashion until rats were able to respond correctly within 3 seconds to a 1s stimulus for 70 of 100 trials.

Thereafter, the stimuli were generated probabilistically and the rats were presented with stimuli generated by a 2 layer HMM (Fig. 2.2, layer y) The stimulus duration shortened to .6 secs, to promote the learning of probable stimulus locations. 100 daily stimuli were presented for five consecutive days each week, with each stimulus presented in equal numbers (+/- 7) over each five day period. During probabilistic training, the rats reliably performed faster and more accurately to the likely stimulus probabilities (70%, then 20%) in the first 2-3 weeks and then gradually improved performance in response to the rare events (5% stimuli and context transitions), indicating a progressive learning of the stimulus probabilities under the changing contexts. 17 reached behavioral criteria of responding to 70 out of 100 trials with .6 sec. stimuli.

3.3 Surgical methods

After rats demonstrated learning of the underlying stimulus probabilities, a cholinergic lesion was administered bilaterally to the NBM/SI region of the basal forebrain. Animals were anesthetized with isoflurane and 0.375 mg/ml of 192 IgG-saporin (n=9) (Advanced Targeting Systems, San Diego, CA) or artificial cerebrospinal fluid (n=8) was infused bilaterally into NBM/SI region of the basal forebrain using a 1.0 µl Hamilton syringe. 0.1 µl volume was infused stereotaxically into four sites at the following coordinates: -1.44 mm R/C, +/- 2.5mm M/L, -7.00mm D/V, +/- 4.0mm M/L, -
8.00 mm D/V. Injections were made at .01 µl per minute and the needle remained in place for 4 minutes after each infusion to allow for diffusion of the injected liquid. All rats were permitted 14 days to recover from the surgery before starting behavioral testing.

3.4 Histological methods

At the completion of behavioral testing, rats were given a lethal injection of sodium pentobarbital in preparation for perfusion. The brains were removed from the skulls and stored in a 40% paraformaldehyde-20% sucrose solution for three days before sectioning. Brains were cut into 30 µm coronal sections on a freezing microtome to assess the accuracy and extent of the lesions. Adjacent sections were processed for either acetylcholinesterase (AchE) histochemistry, Chat immunohistochemistry or parvalbumin immunocytochemistry. AchE histochemistry was used to reveal the presence or absence of cholinergic fibers in the cortex and to provide a measure of the degree of cholinergic denervation in the lesioned rats. CHAT immunocytochemistry was used to visualize cholinergic cell loss in the basal forebrain. Parvalbumin immunocytochemistry and Nissl-staining was used to verify the integrity of non-cholinergic cells in the basal forebrain. A butyrylcholine esterase inhibitor, tetraisopropyl pyrophosphoramide (Sigma, St. Louis, MO) was added to the solution (.137%) to reduce nonspecific staining.

An adjacent series of sections (also 240 µm apart) were processed for the p75 receptor according to previously described methods (Conner et al, 1992).

Free floating sections were also processed for p75 using the 192-IgG monoclonal antibody (Taniuchi, 1985) and parvalbumin using MAB1572 (Chemicon, Temecula, CA). Sections were rinsed for 30 minutes in 0.1 M tris-buffered saline (TBS), blocked
for 60 minutes in TBS containing 5% normal horse serum and incubated for 40 hours (at 4°C) in primary antibody (2.5 µg/ml for the 192 IgG and 1:3000 dilution for MAB1572). Bound antibodies were detected by sequentially incubating sections for 3 hours in 1.5 µg/ml biotinylated horse anti-mouse IgG (Vector Labs, Burlingame, CA) and for 90 minutes in an avidin-biotin peroxidase reagent (1:250 dilution ABC Elite, Vector Labs). Sections were rinsed and treated with a solution containing 0.04% diaminobenzidine tetrahydrachloride, 0.06% nickel chloride and 0.06% hydrogen peroxide in 0.1 M tris-HCl buffer (pH = 7.4). AChE and p75 stained sections were mounted on to gel subbed slides, dehydrated in a graded series of alcohols, cleared and coverslipped.

4. Results

4.1 Histological results

A previous study examining rats who underwent the precise surgical technique described above, in the same laboratory, with the same Batch and Lot of 192 IgG-saporin, indicated that 192 IgG-saporin lesions of the SI/NBM resulted in >99% loss in basal forebrain cholinergic innervation to the sensorimotor cortex (Conner et al, 2003; Fig. 2.4). These results were obtained using unbiased stereological techniques to randomly sample sites within layers II-III and layers V-VI throughout the rostral-caudal extent of the sensorimotor cortex. Visual comparison of sections from the present study indicate equivalent levels of depletion.

4.2 Behavioral results
Results were analyzed using JMP 4.0.2 by the SAS institute. A Maunchy sphericity crieterion was used to assess a possible confound of within-subject correlations and multiple comparisons were conducted using modified Bonferroni correction.

Training data indicate that animals learned about the stimulus probabilities, a necessary basis for the development of stimulus predictions and an accompanying estimate of uncertainty of those predictions. A one-way repeated measures ANOVA, analyzing the average performance over a 10 day period beginning 20 days before the end of pre-surgical training, showed a difference in the latency of performance of the different trial types ($F(3) = 2.76$, $p<.05$). The analysis revealed that rats responded to the 70% stimuli significantly faster than the other stimulus types ($p < .05$), indicating a cost in performance for less predictable stimuli. However, in the last 10 days of pre-surgical training, there was no longer a difference in latency of performance across the stimulus types ($F(3) = .889$, $P >.1$). Thus, learning across the last 20 days of pre-surgical training reflected differential acquisition of the stimulus probabilities and subsequent fluid performance.

After surgical recovery, rats were allowed to re-attain at least 40% overall accuracy in task performance before initiating experimental testing. Separate two-way repeated measures ANOVAs were used to analyze accuracy and latency of performance of lesioned and control rats in the last 10 days before the lesion and in three 10-day periods after the lesion (post-lesion days 1-10, 11-20 & 21-30) across the four stimulus types (70%, 20%, 5% and in the trial following a context transition) DAYS $\times$ STIMULUS.

Whereas the control rats were unchanged in their performance across the different test periods, the lesioned rats exhibited an overall increase in latency ($F(3,67) = 5.66$ $P < .01$), and a decrease in accuracy ($F(3,67) = 2.79$ $P < .05$) indicating an impairment in
attentional performance (Fig. 2.5A, 2.6A). This result replicates findings on the same task with random stimuli (the 5CSRTT) following nonselective lesions and selective cholinergic lesions of the basal forebrain (Muir et al, 1993; McGaughy et al, 2002).

A comparison of performance was made across the different probability types by analyzing the percentage difference of accuracy and latency (in separate analyses) of responses to the 20%, 5% and context transition stimuli from the responses to the 70% stimulus. This allowed performance on the 70% trials to serve as a baseline measure of performance for each rat. These differences across probabilities relative to baseline were measured across the four 10-day periods of pre- and post- lesion behavioral testing in a separate within-subject comparison of lesion and control rats (PROBABILITY x DAYS). The lesioned rats exhibited a significant interaction of stimulus type and testing period (PROBABILITY x DAYS) across the periods of behavioral testing in the latency of responses (F(9,67) = 2.43 P < .05), indicating a differential effect of the lesion across the different stimulus types (20%, 5% and following a context transition). Contrasts were used as a planned, post-hoc comparison of latencies across stimulus types, indicating that there was not a difference in the performance of lesion and control groups in the last 10 days of behavioral testing (p>.1), but in the lesioned rats there was a significant increase in latencies in the first ten days following the lesions response to the 5% stimuli (P<.05) and following a context transition (P<.01) (Fig. 2.5B). These data indicate that lesioned rats were slower to respond to the less probable events in the task during the first 10 days after the initiation of post-surgical experimental testing.

Similarly, there was a significant interaction in the accuracy of different responses across the different 10-day periods of testing for the lesioned rats(F(9,67) = 1.98 P < .05), but only during the last ten days of testing were rats significantly less accurate when
responding to the 5% stimuli (P<.05) and following a context transition (P<.01) (Fig. 2.6B).

In summary, the rats with selective NBM/SI cholinergic lesions exhibited a greater impairment when responding to the less likely stimuli, and were most impaired when responding after a stimulus context had changed, when predictive uncertainty would be highest (Fig. 2.4B). Over 30 days of post-lesion testing, the rats were less accurate and slower over time in response to all stimulus types (Fig. 2.4A). However, the greatest effect of stimulus types was first reflected in the speed of responding in the first ten days of post-lesion testing and the latency difference for the different stimulus types gradually diminished over the following 20 days of testing (P>.05). Additionally, in the last ten days of testing, rats became significantly less accurate when responding to the 5% stimuli (P <.05) and following the context transitions (P<.01). Thus, the nature of the deficit changed over time, whereas the degree of ensuing impairments was nearly constant from a baseline measure of response, with the percentage of lost accuracy nearly mirroring the previous cost in latency. At each point, the effect was limited to either a loss of accuracy or a loss of speed indicating a changing deficit in performance not due to a speed-accuracy trade off.

The possibility of increased errors from inappropriate expectancy was assessed by a separate two-way repeated measures ANOVA (LESION x PROBABILITIES) analyzing the types of commission errors made before and after the lesion (average of 10 days pre vs. average of 10 days of post-lesion performance). Commission errors were analyzed in terms of the types of errors made during the presentation of a 70% stimulus (either a response into the 20% port or into one of the two 5% ports) or during the presentation of a 20% stimulus (either a response into the 70% stimulus location or into one of the two
There was a significant difference in commissions made before and after the surgery, with both lesion and control rats making less commission errors overall; Control F(1,30)= 12.56 P < .001, Lesion F(1, 30) = P < .001) (Fig. 2.6), indicating that the primary error type for the lesioned rats was that of omissions. However, there was also a significant interaction of lesion × probability types for the lesioned rats (F(1,34) = 9.30, P < .01), but not for the control rats (F(1,30) = .37 , P = .54), as the lesion rats made considerably fewer errors into 5% ports in the two types of trials. This is likely to be indicative of a strategy shift in responding only to the more expected port locations.

5. Discussion

Our considerable ability to discern stimuli in a changing and noisy environment relies on a changing balance of new sensory information and learned stimulus expectations that allow us to infer the likely state of external events (Helmholtz, 1879). The current study explores the possibility that cholinergic neurons help maintain that balance by reporting the uncertainty of ongoing expectations. That degree of uncertainty may thereby increase stimulus processing in the cortex in a corresponding amount, to infer the location of stimuli on the basis of current sensory information. A novel probabilistic four-choice serial reaction time task was designed that allowed animals to infer the probable location of impending stimuli during each trial in a graded manner, thereby establishing that different levels of predictive uncertainty within the brain would facilitate the detection of brief probabilistic stimuli.
The rats’ learning of underlying probabilities was assessed from pre-lesion performance. During training, rats initially exhibited a selective cost in latency while responding to the less likely stimulus probabilities under each of the four contexts. The difference gradually decreased over successive weeks of training, which suggests a gradual learning of the more likely stimulus locations and an increasing capacity for responding to the less likely stimulus locations. While rats have been previously trained with probabilistic stimuli in a similar visuospatial environment (the covert attention task, Bushnell et al, 1998, Phillips et al, 2000), the current task required the learning of four probabilistic contexts of likely stimulus locations over the same spatial cues. The changing ambiguity of the current context within a behavioral session provided an opportunity to then parametrically manipulate the uncertainty of predictions.

Following selective NBM/SI cholinergic lesions, animals were impaired in a graded fashion corresponding to the hypothesized levels of uncertainty elicited by the different probabilistic classes of stimuli. This effect is consistent with the hypothesized role of acetylcholine in mediating uncertainty that would facilitate the detection of less predictable stimuli. In addition, rats with cholinergic lesions were much less likely to respond into unlikely stimulus locations on the basis of learned cues. This effect suggests that acetylcholine regulates appropriate increases in attention in part by decreasing the use of learned expectations when they are no longer advantageous for detecting stimuli in new and changing contexts.

Initially, rats exhibited an increased latency of responding to less predictable stimuli (Fig. 2.5), suggesting a lost effect of acetylcholine in mediating flexible allocation of attention to stimuli that deviate from expectations. After four weeks of recovery, rats were less accurate when responding to less unexpected stimuli. In combination, the
effects reflect an over-reliance on top-down information due to lost cholinergic modulation in a brain that is decreasingly capable of behavioral inhibition (McGaughy et al, 2002).

The pattern of impaired performance is consistent with a proposed computational role of acetylcholine in probabilistic inference. By parametrically decreasing the use of prior information during inference in proportion to the certainty of ongoing stimulus expectations, such a level of modulation could establish the correct balance of learned and new information for optimally detecting probabilistic stimuli in changing environments.

The hypothesized role of cholinergic modulation is broadly consistent with diverse attention and learning processes that have been attributed to acetylcholine. The selective modulation of cortex by acetylcholine is thought to establish brief periods of increased sensory processing in multiple targeted regions of the cortex (Bucci et al, 1998; Merzenich, 1998). Such cortical increases in processing are consistent with the hypothesis that the basal forebrain cholinergic system may be specifically implicated in forms of learning requiring plasticity of cortical representations (Conner et al, 2003). An additional proposed role for cortical acetylcholine is to suppress the behavioral and mnemonic influence of existing memories encoded in cortical and subcortical memory systems (Linster et al, 2001). One mechanism by which acetylcholine has been proposed to regulate the flow of information within the cortex is by increasing the influence of sensory inputs and reducing proactive interference from existing memories in the cortex (Linster et al, 2001, De Rosa et al, 2004). The release of acetylcholine has also been proposed to regulate balance of processing between cortical systems and striatopallidal
systems that encode well-learned sequences of actions, thoughts and stimuli (Mishkin, 1976; Alexander et al, 1986; Chang & Gold, 2003).

The current findings are consistent with existing theories of cholinergic function, yet they further suggest that the overall regulatory effects of acetylcholine operate in a principled manner described by normative theories of statistical inference and learning. We hypothesize that cholinergic uncertainty provides a mechanism for Bayesian-like inference by both increasing the influence of sensory information and by decreasing the influence of stimulus expectations on behavioral and sensory processes of attention. When uncertainty is high, potentially inaccurate and distracting contextual information would thereby be abandoned, and stimulus inference would rely more on sensory information to estimate the most likely external state that generated the current observation.

The proposed role of acetylcholine in mediating the relative influence of bottom-up information is suggested from behavioral findings from humans and animals in cued visuospatial attention tasks. On the basis of covert attention studies, it has been suggested that basal forebrain cholinergic modulation supports the fluid allocation of attention by facilitating shifts of attention to unexpected stimuli (Chiba et al, 1995, Baxter et al, 1999). A facilitative role of acetylcholine has been reported for shifts of attention in a Posner task where a normally reliable cue incorrectly signals the location of an impending stimulus (Parasuraman et al, 1992, Witte & Marrocco 1997; Davidson et al, 1999). Whereas selective lesions of cholinergic neurons in the NBM/SI (Chiba et al, 1999) and the blockade of muscarinic receptors with systemic drugs both increase the reaction time to a stimulus in an unexpected location, the systemic activation of nicotinic receptors shortens the reaction time (Phillips et al, 2000). Humans and non-human
primates with parietal cortex damage also exhibit the same increased cost from invalid spatial cues in the Posner task (Posner et al, 1987, 1994).

Although the current study only considered the possible role of cholinergic uncertainty in attention and inference, the same signal of uncertainty has been proposed to facilitate the selective allocation of attention during associative learning. A role for selective attention in learning has been proposed in animal learning theories, whereby attention is paid to stimuli with less known outcomes, such as novel stimuli or stimuli with a history of inconsistent outcomes (Pearce & Hall, 1980). Such a form of attention has been described as automatic (Pearce, 1997) and functions in an associative learning framework as a mechanism for regulating the amount of processing that is devoted to a stimulus that is a potential predictor of a surprising event (Mackintosh, 1975).

While earlier learning theorists initially formalized the notion of uncertainty in the appropriate allocation of attention, recent computational models have helped to formalize the role of uncertainty in attention in Bayesian models of learning and attention (Dayan et al, 2000, Dayan & Balleine, 2002; Yu & Dayan 2002, 2005). The current study provides support for the further possibility that such a measure of uncertainty is functionally mediated by acetylcholine in the context of visuospatial attention. The findings of the study thus help to outline the computational role of acetylcholine within framework for autonomous learning processes that have been proposed for closely related neurochemical systems, such as dopamine (Schultz & Dickinson, 2003), serotonin (Montague et al, 1996) and norepinephrine (Yu & Dayan, 2005). The current data provide initial support for a distinct computational role of cholinergic neuromodulation in the brain, and it suggests promising new directions of research using statistical models as a framework for linking together a rich set of behavioral learning processes,
neurochemical functions, and our seemingly effortless ability to discern the causal fabric of the environment.
Figure 2.1. The 5CRTT operant chamber. The behavioral chamber contained 4 evenly-spaced ports containing a light stimulus and a sensor that registered nose entry by the interruption of an infrared beam. In each trial, a .6 sec light stimulus was presented in one of four ports. A nose poke into the illuminated port triggered the delivery of a sucrose pellet into a reward tray in the opposite wall of the chamber that was accessible through a flap door.
Figure 2.2. Stimulus model for inference task. Each stimulus was determined by a two-layer probabilistic model. A: In each trial, a brief visual stimulus is presented in one of four stimulus locations (layer y) with pre-set probabilities that were allocated according to the current context (layer z). Under each context, the stimulus was presented with in the same location with a 70% probability (e.g. 1), or was presented two positions away with a 20% probability (e.g. 3- figure needs amending). E.g. P[y_t = z_t | z_t] = 0.7, P[y_t =2 | z_t =1] =0.2, P[y_t =3 | z_t = 1] = 0.05 and P[y_t =4| z_t =1] =0.05. The slow-changing dynamics in layer z were governed by dynamics P[Zt1 =Zt-1]=.96, and thus the probable stimuli alternated over the four locations every 16 trials on average. B: Example of the hidden context (z) and presented stimuli (y) across 400 trials.
Figure 2.3: Hypothesized degree of cholinergic modulation during the inference task. Ongoing uncertainty is equal to 1 minus the probability of current estimate of the most likely hidden context ($Z$) on the basis of the stimulus history to that point ($D_t$). With the Markov-generated stimuli (top), hypothesized cholinergic levels (bottom) are greatest when rats respond to the less probable stimuli under the current context and when the context had recently changed.

Adapted from Yu & Dayan, 2002
Figure 2.4. A representative 192-IgG Saporin lesion of the SI/NBM region. This is a representative lesion (Conner et al, 2003), using the same procedures, batch of 192 IgG-Saporin, antibodies and protocols as the current study. Intraparenchymal injections of 192-IgG saporin (SAP) into the NBM selectively destroy cholinergic cell bodies within the NBM and eliminate cholinergic afferent innervation to the cortex. Cholinergic fibers labeled for AChE were found throughout the cortex, including the primary motor cortex (A). Cholinergic fibers were rarely seen in the cortex of animals with bilateral injections of SAP into the NBM (B). Cholinergic cell bodies, expressing the p75 receptor, are normally scattered throughout the basal forebrain (BF) (C). SAP within the NBM eliminated virtually all p75-expressing cells in the NBM and substantia innominata. The arrow in (D) indicates the most rostral injection site. Sections stained for parvalbumin (E and F) demonstrated that SAP lesions did not damage GABAergic cells within the BF.
Figure 2.5. Post-lesion latencies (+ SEM).  A: Lesioned rats were significantly slower in responding to all the stimuli.  B: A comparison of relative performance was made across the four trial types (70%, 20%, 5% and one trial after a context change) in the ten day period following the lesion.  In the first ten days after the lesion, rats were disproportionately impaired responding to stimuli with greater predictive uncertainty.  The rats exhibited a greater impairment when responding the less likely stimuli, and were most impaired when responding after a stimulus context had changed.  (* p < .05, ** p < .01)
Figure 2.6. Post-lesion accuracies (+ SEM). A: Lesioned rats were significantly less accurate in responding to all the stimuli. B: A comparison of relative performance was made across the four trial types (70%, 20%, 5% and one trial after a context change) in the ten day period following the lesion. Thirty days after the lesion, rats were less inaccurate when responding the less likely stimuli, and were most impaired when responding after a stimulus context had changed. (* p < .05, ** p < .01)
Figure 2.7. Percent change of post-lesion commission errors (+/- SEM). A: Commission errors made during the presentation of a 70% stimulus into either a 20% stimulus location or into the two 5% stimulus locations. B: Commission errors made during the presentation of a 20% stimulus into a 70% stimulus location or the 5% stimulus locations. In both trial types, lesioned rats and control rats made a similar number of commission errors into the port where the more probable stimulus would appear, but lesioned rats made significantly fewer commission errors into the less probable 5% stimulus locations, indicating decreased responding into non-expected stimulus locations.
6. References


CHAPTER III

Effects of sleep deprivation on attention

1. Abstract

The second experiment of the thesis addresses the homeostatic regulation of neural processes underlying attention. The study elaborated the functioning of attention in the context circadian regulation of neurochemical processes that establish the waking and sleeping states of the brain. By characterizing the disruptive effects of sleep drive in a well-characterized test of attentional function of rodents, the study allowed a comparison to behavioral effects that arise from manipulation of different neurochemical systems that contribute to sleep onset, including acetylcholine, norepinephrine, dopamine and serotonin.

Sleep deprivation in humans leads to impairments of vigilance and selective attention. Although the neurochemical basis of sleep drive has been well established from animal models, the effects of sleep deprivation on attention had not been characterized in a comparable animal model. The attentional performance of rats was thus assessed after 4, 7 & 10h of total sleep deprivation (SD) induced by gentle handling on a 5-choice serial reaction time task, where rats detect and respond to brief visual stimuli. SD produced a monotonic increase in response latencies across the 4h, 7h and 10h deprivations. SD also led to increased omission errors, but the overall number of perseverative and premature responses was unchanged. Sub-groups of rats were differentially affected in the number of omission errors and perseverative responses. The effects of sleep deprivation on rats
are compatible with a range of human findings on the effects of sleepiness on selective attention, psychomotor vigilance and behavioral control. Rats also exhibited differential susceptibility to the effects of sleep deprivation, consistent with phenotypic susceptibility that has been found in humans. These findings indicate the feasibility of using the 5CSRTT as an animal model for investigating the direct links between homeostatic sleep mechanisms and resulting attentional impairments within a single animal subject.

2. Introduction

The effects of sleep deprivation on behavior and cognition have generally been understood in terms of a homeostatic process that increases the drive for sleep with extended wakefulness, a process whose neurochemical origins have recently begun to be elucidated (Mignot et al, 2002; Basheer et al, 2004; Steriade & McCarley, 2005). As decrements in vigilance and attention are among the first signs of sleep loss and sleepiness, it has been hypothesized that common neural systems are involved in the processes of the homeostatic sleep drive and sustained attention (Doran et al, 2001). For example, the activity of the basal forebrain cholinergic system has been proposed to promote cortical arousal and wakefulness (Jones, 1993; Szymusiak, 1995) as well as attention (McGaughy et al, 2002; Muir et al, 1992; Chiba et al, 1995; Sarter & Bruno, 1997). Furthermore, we and others have hypothesized that an inhibition of this cortically projecting cholinergic system mediates the sleepiness associated with prolonged wakefulness. Although the majority of biological studies investigating sleep processes have used animal models, we only know of one study that has used an attentional
measure (false alarms) in a behavioral assessment of sleep deprived animals (Rai et al., 2004). Hence, the goal of the present study was to develop a rodent model of the attentional dysfunction caused by sleep loss that could be used to elucidate the effects of sleep drive on neurobiological processes of attention.

The effect of sleep deprivation on attention has been well documented in humans. Total sleep loss in a single night leads to impairments in sustained attention tasks that require selective attention to an auditory channel (Johnsen et al., 2002) and psychomotor vigilance to unpredictable visual stimuli (Jewett et al., 1999; Van Dongen et al., 2003; Dorrian et al., 2005). Sleep deprivation also reduces performance when stimulus demands are higher, such as tasks that divide attention between arithmetic and verbal information (Drummond et al., 2001). More recently, it has been suggested that the pattern of deficits resulting from sleep loss reflects a dysregulation of behavioral control processes that rely on the prefrontal cortex (Muzur et al., 2002; Dorrian et al., 2005; Durmer & Dinges, 2002), such as the attention to relevant cues (Norton, 1970), flexible thinking (Harrison & Horne, 1999) and cognitive perseveration (Horne, 1988; Wimmer et al., 1992). Total sleep deprivation, however, spares some aspects of executive attention such as task shifting, response inhibition (Jennings et al., 2003) and tests of cognitive flexibility (Binks et al., 1999). The heterogeneous effects of sleep deprivation on attentional performance indicate that SD has different effects on underlying mechanisms of attention. Thus an important feature of an animal model would entail the ability to measure the separate effects of SD on behavioral measures that assess vigilance, selective attention and behavioral control.

The current study assessed the consequences of 4, 7, or 10h of total sleep loss in a well-developed rodent model of selective attention, the 5 choice serial reaction time task (5CSRTT). The task was designed to assess major aspects of attentional control by
measuring the ability of rats to continually monitor the location of a brief visual stimulus presented in one of five spatial locations (Carli et al., 1983; Robbins, 2002). The task is based on a 5CSRTT for humans that has been used to assess the effects of stressors on attention (Wilkinson, 1963; Broadbent, 1971) and it bears important similarities to several human sustained attention tasks, including continuous performance test and the psychomotor vigilance test (PVT) (Dinges & Powell, 1985). The 5CSRTT uses operational measures to assess the breakdown of performance according to different demands of controlled attention, including response latencies, omission errors, commission errors, impulsive responding and perseverative responding.

A considerable benefit of the 5CSRTT is that it has been used extensively in studies that have investigated the neurobiological mechanisms of attention in rats (reviewed by Robbins, 2002). Thus there is an established literature that has systematically outlined the effects of neurochemical and anatomical alterations on the behavioral measures of the task. These investigations have included studies of monoamine systems that contribute to homeostatic sleep onset, and cortical structures that have been associated with impaired attentional processing in sleep deprived humans, such as the parietal and frontal cortex (Muir et al., 1996).

Importantly, the rodent 5CSRTT uses measures that are analogous to measures in vigilance tasks that are affected by even a few hours of total sleep loss in humans, such as slowed reaction times and omission errors (Van Dongen et al., 2003), which are called ‘lapses’ in the PVT literature (Dorrian et al., 2005). The separate measures of inhibitory control could also allow a nuanced analysis of executive dysfunction that has been hypothesized to result from sleep loss. Whereas premature responses have been attributed to an impulsive disturbance in preparatory mechanisms, perseveration have
been attributed to a compulsive inability to disengage from responding once it has been initiated. Much like humans, these distinct impairments of executive control have been shown to result from the disruption of separate anatomical regions of the rodent prefrontal cortex in the 5CSRTT and other behavioral tests (Dias et al, 1996; Dalley et al, 2004).

3. Materials and methods

All procedures and animal care adhered strictly to AAALAC, Society for Neuroscience, and institutional guidelines for experimental animal health, safety, and comfort. Ten male Long-Evans hooded rats (Charles River, Raleigh NC) began behavioral testing. They were maintained in a temperature controlled room with a 12 hr light/dark cycle (lights on 08:00 - 20:00). Water was available *ad libitum*, but rats were food restricted to maintain body weight at 85% of their free-feeding weight for motivation by a food reward.

3.1 Apparatus

Rats were tested using two five-hole operant chambers (Cambridge Cognition, Cambridge, UK). Each aluminum chamber was 25x25x25 cm and was equipped with a Plexiglas flap door that allowed access to a magazine where 45 mg Noyes sucrose pellets were mechanically dispensed (Research Diets Inc., New Jersey). Five evenly-spaced apertures were located on the opposite wall of the chamber that registered nose pokes via the interruption of infrared photocell beams. At the rear of each aperture was a 3W bulb
that served as a stimulus. A 3W bulb at the top of the chamber provided illumination (the house light). Each behavioral chamber was contained in a larger sound-attenuating chamber with a small fan that provided ventilation and background white noise. All stimulus presentations and behavioral measures were made with Cambridge Cognition Control 1.17 software on a PC Pentium IV class computer.

3.2 Behavioral training

The five choice serial reaction time task has been described previously (Carli et al, 1983; Robbins, 2002). Rats were trained to nose poke in an aperture following the brief presentation of a visual stimulus within that aperture (Fig. 3.1). Each stimulus was presented pseudorandomly in one of the five apertures and was thus spatially unpredictable. The rats were initially allowed 60 seconds to respond to each stimulus and were rewarded with a sucrose pellet immediately following a response into the illuminated port. Upon attaining 70% correct responses on two consecutive days, the stimulus duration was shortened until the rats re-attained 70% accuracy. The stimulus was shortened repeatedly in this fashion until rats were able to respond correctly within 3 seconds to a 500 ms stimulus for 70 of 100 trials (8 rats reached this criterion).

The measures obtained in the 5CSRTT include the following 5 measures (Fig. 3.2): 1. Correct responses - defined as a nose poke response into the aperture where the stimulus was just presented; 2. Incorrect response – a response into an aperture where the stimulus was not presented; 3. Omission error – when no response was made in the 3 sec period after the presentation of the stimulus; 4. Premature response – when a response was made
during the 5 sec intertrial interval prior to the presentation of a stimulus; 5. Perseverative response- when two or more responses were made into an aperture after a single stimulus.

To facilitate learning, the house light was turned off for five seconds immediately following an incorrect response, an omission, or a premature response (a time out, Fig. 3.2). After a correct response or a time out, the rat initiated the next trial by opening and releasing the reward tray door. Each stimulus was presented 5 sec after egress from the reward magazine for both the training and testing periods to prevent the accumulation of omission errors during testing from possible lapses of performance, a variation from other 5CSRTT protocols (Carli et al, 1983; McGaughy et al, 2002).

3.3 Experimental design

Following training, rats were divided in two groups and tested on the 5CSRTT at ~16:00 on six successive days of the week (Sun-Fri) for four weeks (n=8). On alternating weeks, one group of rats underwent sleep deprivation prior to the behavioral test while the other group was left undisturbed in the colony room before testing. On the weeks of deprivation, the rats were kept awake before testing on alternating days (Mon, Wed & Fri) for 4, 7 and 10 hours, respectively, allowing a day for recovery between deprivations (Table 3.1). The rats were kept awake by the gentle handling method (Franken et al, 1993), which entailed continuous visual observation and gentle handling, using sensory, auditory and tactile stimulation whenever they became prone or immobile. EEG confirmation has indicated that the technique keeps rats awake 98% of the time over a six hour deprivation (~2% of the time they transition to sleep) while undisturbed controls are awake 25% of the time (Ramesh et al, 2004).
As the anticipation of daily feeding in food restricted rats can reduce behavioral motivation in instrumental tasks and shift circadian rhythms, the rats were fed no less than two hours after the last rats finished behavioral testing (~8pm). It has been shown that the body temperature and behavioral activity of food restricted rats increases approximately one hour before the daily consumption of rat chow (Mendoza et al, 2005; Saper et al, 2005) and before regular feedings of midday sucrose solution (~20 Kcal) presented 8 hours before daily feeding (Pecoraro et al, 2002). Although the current behavioral task provided smaller quantities of sucrose (~11 Kcal) at less regular intervals (5 days weekly), it is possible that the rats’ waking schedule was similarly shifted before testing and that they were sleep deprived for as little as 3, 6 & 9 hours from their actual sleeping times. Novel stimuli and daytime physical activity produce small and transitory changes on sleeping schedules in rodents (Mrosovsky, 1988; Mistlberger & Skene, 2004), so the periodic deprivations were not expected to significantly affect the sleeping schedule of the rats on the days of testing.

A within-subject comparison of performance was then made between the three days of incremental sleep deprivation and the corresponding three days on the weeks that they were not deprived. Data from recovery days after sleep deprivation were not analyzed. Results were analyzed using JMP 4.0.2 (SAS institute, Cary, NC). Following satisfaction of the Mauchly sphericity criterion that assesses a possible confound of within-subject correlations (Anderson, 1958), a repeated measures F test was used to assess the effects of deprivation, hours and subject for each of the 5 behavioral measures (correct responses, incorrect responses, omission errors, premature response and perseverative responses) across four weeks of behavioral data (2 weeks deprivation, 2 weeks control). A mixed- model design was used to account for the within- and between- subject
variance that might arise from the interindividual differences between subjects (Van Dongen et al, 2004b). Post-hoc comparisons were made using Fisher’s HSD. To address PVT data on the consistency of omission errors performed by individuals on repeated testing following sleep deprivation (Fleiss, 1986), an interclass correlation coefficient (ICC) was also calculated between omission errors made during the two 10hr deprivations. The ICC computes the ratio of between-subject variance to the sum of between-subject and within-subject variance and thus directly assesses the proportion of inter-individual variability in the repeated measures of the two 10hr deprivation conditions (Fleiss, 1986). One subject was not tested on the first 7hr control day following a finger injury.

4. Results

Sleep deprivation caused significant behavioral impairments across a number of performance measures of the 5CSRTT. Sleep deprivation produced a significant 33.14 msec increase in the mean latency of correct responses ($F(1,62) = 6.89, p = 0.0109$). There was also a significant interaction between the duration of sleep deprivation and the latency of correct responses ($F(2,62) = 3.32, p = 0.0426$) (Fig. 3.3). Post-hoc comparisons showed that there were no significant differences between the three 0-hr conditions over both control weeks ($p > 0.05$), but that within deprivation conditions, the longest deprivation produced significantly slower responses than the shortest deprivation (10 hrs vs. 4 hrs, $p < 0.05$). These results indicate a dose-dependent effect between the increasing length of sleep deprivation and the increasing speed of accurate responding.
There was also a significant reduction in the number of correct responses following sleep deprivation \((F(1,62) = 5.20, p = 0.026)\), indicating the absence of the potential confound of a speed-accuracy trade-off.

Sleep deprivation produced a significant increase of omission errors \((F(1,62) = 14.47, p = 0.0003)\) (Fig. 3.4), where rats did not respond to a stimulus within three seconds. In addition to the group effects on omission errors, there was also a significant interaction between the duration of sleep deprivation and individual rats for omission errors \((F(7,62) = 2.24, p = 0.0422)\) (Fig. 3.5), indicating an inconsistency in the effect of deprivation on the performance of individual rats. The interclass correlation coefficient of omission errors performed by the rats in the two 10hr deprivation conditions was almost significant \((r = 0.703; p = 0.0518)\) (Fig. 3.6). These data suggest a differential effect of sleep deprivation on individual rats on their ability to reliably execute a response within a short window of time. The variance of individual data was somewhat larger than has been reported for human data (Van Dongen et al, 2004a), and may reflect differences in motor preparedness between the PVT and the 5CSRTT, where rats move freely within the behavioral chamber.

Sleep deprivation did not affect the overall measures of behavioral inhibitory control. The number of premature responses was not significantly changed by sleep deprivation \((F(1,62), p > 0.1)\). Although the majority of rats made more perseverative errors following deprivation, the average number of perseverative responses into the same port was not significantly different \((F(1,62), p > 0.1)\). However, there was a significant interaction of rats and deprivation \((F(7,62) = 2.94, p = 0.01)\), as sleep deprivation produced mixed effects in the different rats and dramatic increases of perseverative responding in one rat (that also made increased omission errors).
5. Discussion

Ten hours of total sleep deprivation in rats produced a pattern of behavioral impairments in the 5CSRTT that is broadly consistent with the effects of sleep deprivation on vigilant attention performance in humans. Sleep deprivation produced a significant increase in the latency of correct responses in a dose-dependent manner, consistent with a monotonic effect of sleep debt on attention. Sleep deprivation also led to an overall increase in the number of omission errors, where a rat did not respond to the stimulus within a brief period. The same measures are comparably affected in the PVT following similar deprivation lengths (Doran et al, 2001; Dorrian et al, 2005), thus the behavioral effects of sleep deprivation closely resemble the findings in human studies using the PVT to assess vigilance and attention deficits after sleep deprivation. In the current task, care was taken to limit possible lapses of performance from sleeping by requiring the rats to behaviorally initiate each trial. While the increases of omissions could be due to failures in the detection of brief stimuli (Robbins, 2002), it is nonetheless possible that the rats had “microsleeps” in the brief period before the presentation of the stimulus that prevented both detection and responding, an effect of sleep deprivation that has been proposed to contribute to lapses of psychomotor vigilance (Dorrian et al, 2005).

Sleep deprivation, however, had a minimal effect on aspects of the task that require inhibitory control. Consistent with human findings in a visuospatial reaction time task, there was little difference in the number of premature responses between deprivation and control conditions (Jennings et al, 2003). In addition, sleep deprivation did not produce a
significant group effect in the number of perseverative responses. This finding is consistent with human performance on cognitive tasks such as the Wisconsin card sorting task (WCSRT) following short-term total sleep deprivation (Binks et al, 1999), although some studies have reported a nonsignificant trend towards more perseverative errors on the task following chronic partial sleep deprivation (Herscovitch et al, 1980; Redline et al, 1997). Perseverative responding has been shown to arise directly from lesions of medial prefrontal cortex both in the 5CSRTT and in a rodent task that requires attentional set-shifts equivalent those of the WCSRT in humans (Birrell & Brown, 2000). The region also shares functional and anatomical features with the lateral prefrontal cortex in primates, whose damage has been identified as a focal source of perseverative responding in WCSRT (Dias et al, 1996). The sparing of perseverative behavior in the current task may thus reflect a comparable, limited effect of sleep deprivation on regional processing in the prefrontal cortex.

Interestingly, individual rats appeared to be differentially susceptible to the effects of sleep deprivation. Although there was an overall group effect on sleep of omission errors, there was also a reliable difference between individual rats in the effects of deprivation on number of omission errors. Some rats exhibited almost no change in the amount of omission errors in response to sleep deprivation, suggesting different compensatory mechanisms across individuals that allow them to maintain consistent performance on the task. Moreover, the degree of impairment for each rat was consistent across the two 10hr deprivations (Fig. 3.6), and there was a strong trend towards a significant inter-individual performance difference between the rats. These effects are consistent with ‘trait-like’ differences between individuals in their vulnerability to the effects of sleep deprivation on omission errors in the PVT (Van Dongen et al, 2004a).
A similar individual pattern arose for the performance of perseverative responses. Following sleep deprivation, most rats made a greater number of perseverative responses into the same port, indicating a compulsive impairment of inhibitory control. Some rats, however, showed a decrease in the number of perseverative responses. Although there was not a significant main effect of perseverative responses, the significant interaction of sleep deprivation and individual performance could also be indicative of a selective susceptibility in sub-populations to the effects of sleep deprivation.

These data provide a systematic demonstration of attentional impairments in animals resulting from sleep loss. The pattern of effects on selective attention, behavioral control and individual susceptibility is consistent with findings from human studies where similar behavioral demands are made following sleep deprivation. These findings suggest that there is a broad preservation of sleep effects on attention and behavioral control across species.

The correspondence of human and animal impairments is significant in light of the paucity of data regarding the ways that sleep deprivation affects attentional processes in the brain. The extensive use of the 5CSRTT for neurobiological investigations of attention in rats, however provides a framework for understanding specific behavioral alterations in terms of their possible neurochemical and structural sources.

Much like sleep processes, some mechanisms of attention have been understood in terms of large-scale processes of the cortex that are regulated by ascending neuromodulatory systems. Attentional studies based on the 5CSRTT include studies of cortical structures that exhibit activity changes in sleep deprived humans (Muir et al, 1996; Drummond & Brown, 2001) and studies of neurochemical systems that help establish the sleeping and waking states of the brain, such as norepinephrine dopamine,
serotonin and acetylcholine. It is noteworthy that each of the monoamine systems are also the sites of orexin modulation that contribute to the stable maintenance of wakefulness (Taheri et al, 2002). The basal forebrain cholinergic system receives inputs from the monoamines and orexin, and is the major site of action of adenosine, a major homeostatic sleep factor (Porkka-Heiskanen et al, 1997; Eggermann et al, 2001; Beuckmann & Yanagisawa, 2002; Mignot et al, 2002; Steriade & McCarley, 2005). The considerable overlap of attention and sleep processes across the same neural systems highlights the potential value of the 5CSRTT as a model for relating the mechanisms of homeostatic sleep drive with processing changes in known systems subserving attention in the mammalian brain.

Of the neurochemical manipulations that have been tested with 5CSRTT- including systemic noradrenergic manipulations and dorsal noradrenergic bundle lesions (Carli et al, 1983; Cole & Robbins, 1987; Sirvio et al, 1993, 1994; Ruotsalainen et al, 1997), cholinergic basal forebrain antagonists (Muir et al, 1992) and lesions (McGaughy et al, 2002), striatal dopamine lesions (Cole & Robbins, 1989; Baunez & Robbins, 1999) and global serotonin depletion in the forebrain (Harrison et al, 1997) - the effects of sleep deprivation are most consistent with effects that arise from disruptions of basal forebrain cholinergic function, and to some degree, from drug manipulations of adrenergic systems. Alpha-2 agonists reduce the number of premature responses while also increasing omission errors on the 5CSRTT (Sirvio et al, 1993, 1994). Both the local blocks of acetylcholine and selective lesions of basal forebrain cholinergic neurons lead to a reduction in choice accuracy and lengthening of the latency of correct responses. Cholinergic disruption also leads to an increased number of premature and preservative responses, an effect that corresponds directly to the reduced cholinergic modulation of
the medial frontal cortex (McGaughy et al, 2002). Direct lesions of the medial prefrontal cortex produce a similar pattern of effects on latency, accuracy and preservative responding (Dalley et al, 2004). Interestingly, two major targets of the cholinergic basal forebrain, the frontal and parietal cortex, show increased activity in a divided attention task in humans following 35h of total sleep deprivation, possibly indicating compensatory processing as a result of altered subcortical modulation (Drummond et al, 2001; Drummond & Brown, 2001).

The multiple effects of total sleep loss on the 5CSRTT are thus consistent with known effects of acetylcholine and norepinephrine on attentional performance, and could partially be due to the combined suppression of these systems during sleep onset (McCarley & Massaquoi, 1992). The parallels of cholinergic function are particularly interesting in light of known processes in the basal forebrain that underlie the promotion of sleep. Adenosine directly inhibits cholinergic neurons in the basal forebrain of cats and rats and its concentration increases in the extracellular space of the basal forebrain in a manner that increases linearly with the duration of total sleep loss (Porkka-Heiskanen et al, 1997; Basheer et al, 1999). It could thus play an underlying role in the decreases of attentional function that accompany sleep loss. Increasing sedation is also likely to result from noradrenergic suppression (Hobson et al, 1975; Aston-Jones & Bloom, 1981; Cape & Jones, 1998; Berridge & Waterhouse, 2003; Cirelli et al, 2005), altered thalamocortical activity, hormones and metabolic changes. Each of these possibilities, however, remains to be tested.

While the current findings suggest a broad preservation of sleep effects across species, they only reveal behavioral parallels and many more studies will be needed to establish the biological nature of attentional alterations that result from sleep loss. In
light of considerable, but remarkably separate, scientific knowledge of sleep and attention processes in the brain, the successful use of the 5CSRTT for assessing attentional dysfunction in sleep deprived animals provides new opportunities for directly investigating the links between neurochemical sleep processes and our cognitive and behavioral impairments that result from sleep loss.

6. Acknowledgements

This chapter, in full, has been submitted for publication in the journal Sleep with co-authors Bishoy Said, Dr. Mark Baxter, Dr. Robert McCarley, Dr. Andrea Chiba and Dr. Robert Strecker. The dissertation author was the primary investigator and the first author of this paper.
Figure 3.1. The 5CRTT operant chamber. The behavioral chamber contained 5 evenly-spaced ports containing a light stimulus and a sensor that registered nose entry by the interruption of an infrared beam. In each trial, a .5 sec light stimulus was presented in one of five ports. A nose poke into the illuminated port triggered the delivery of a sucrose pellet into a reward tray in the opposite wall of the chamber that was accessible through a flap door.
Figure 3.2. Contingency diagram of the 5CSRTT. Each daily session began with the delivery of one free sugar pellet into the reward tray (bottom-left) and continued for 100 experimental trials. The release of the flap door, following either a reward or an error, triggered the next stimulus after a 5 sec intertrial interval (ITI). A timely response into a briefly illuminated port was rewarded with a sucrose pellet (a correct response) and an incorrect response turned off the house lights for 5 sec (a time out). Incorrect responses include responding before the presentation of a stimulus (a premature response), not responding to the stimulus within 3 sec period (an omission error), responding into a port that was not illuminated (incorrect response) and responding repeatedly into a port (a perseverative error, not shown). Credit: Cambridge Cognition.
Figure 3.3. Mean latency (+ S.E.M.) of correct responses across the three durations of sleep deprivation (4 hours, 7 hours & 10 hours). Average latency of all matched non-deprivation conditions is shown for comparison (0 hours). Sleep deprivation led to a significant increase in the mean latency of responses following sleep deprivation and a significant interaction of deprivation condition and testing day (deprivation length). Post-hoc comparisons of show that 10h of sleep deprivation led to significantly increased latency compared to 4h of sleep deprivation (* P < 0.05).
Figure 3.4. A- Mean number of correct responses in deprivation and control conditions (+S.E.M.). Correct responses were significantly lower following sleep deprivation (*P < 0.01). B- Mean number of omission errors in deprivation and control conditions. Omission errors were significantly higher following sleep deprivation. (**P < 0.01).
Figure 3.5. Effects of sleep deprivation on performance of individual rats. Each point represents the average omission errors across control or deprivation conditions for each rat. A significant interaction of rats by deprivation indicates that the performance lapses of individual rats were differentially affected by sleep deprivation. In most rats, sleep deprivation led to an increase in the number of omission errors. However, some rats committed fewer omission errors following sleep deprivation.
Figure 3.6. Comparison of omission errors of individual subjects during the first 10h deprivation (Dep 1) versus the second 10h deprivation (Dep 2). The subjects are rank-ordered on the basis of average performance across the two sessions. An interclass correlation of the subject’s scores between the two weeks was nearly significant ($r = 0.703, p = 0.051$), indicating a consistency of deprivation effects on the number of omission errors committed by individual rats.
Table 3.1. **Sleep deprivation schedule.** Eight rats that reached behavioral criterion were divided into two groups (Group 1 and Group 2) and tested five days a week at 16:00. The two groups were sleep deprived on alternating weeks. On the week of deprivation, the rats were sleep deprived prior to testing on Monday for 4 hours, Wednesday for 7 hours and Friday for 10 hours, allowing one day of recovery between the shorter deprivations and two days of recovery after the longest deprivation. The deprivation schedule depicted in the table was repeated twice, for a total of four consecutive weeks of testing.

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7. References


CHAPTER IV

Retrosplenial inactivation disrupts new, but not learned visuospatial sequences

1. Abstract

The third experiment of the thesis extends the investigation of adaptive behavior to learning processes of the retrosplenial cortex that contribute to the learning of temporally structured events in the environment. By facilitating the development of new memories of visuospatial sequences, retrosplenial cortex may allow animals to learn associative relations among structured events in the environment. Concomitantly, animals may gradually reduce attention and mnemonic encoding as stimuli become increasingly predictable to an animal.

Damage to the retrosplenial cortex in human subjects has been associated with temporal disorientation. A study was conducted for assessing the role of the rodent retrosplenial cortex in learning the temporal order of spatial sequences. Rats were trained to perform alternating fixed and random sequences of four cued nose pokes in a 9-port behavioral chamber. Before behavioral testing, neural activity retrosplenial cortex was reversibly inactivated by bilateral infusion of one microliter of bupivicaine at an early phase of learning of the fixed sequence or during a late phase of fixed sequence learning. Whereas an infusion of bupivicaine during the early phase produced a selective deficit in the performance of fixed sequences, an infusion in the late phase of learning did not have an effect in the performance of fixed or random visuospatial sequences. These data suggest a role for retrosplenial cortex in the processing of sequential spatial and behavioral information, a finding that is consistent with the anatomy of the retrosplenial
cortex as an intermediary structure with access to memory processes of the hippocampus, frontal cortex, and visuospatial information in the parietal cortex. These findings provide new confirmation of the memory functions of the retrosplenial cortex, and help to elucidate the sources of memory impairment in humans with retrosplenial pathologies, such as patients in the early stages of Alzheimer’s disease.

2. Background and significance

The retrosplenial cortex is a nodal point for communication between neural structures that play important roles in the representation and association of temporally separate events, including the posterior parietal cortex, dorsal hippocampal structures and the dorsolateral prefrontal cortex. The retrosplenial cortex of primates shares large bidirectional connections with the dorsolateral prefrontal cortex (DLPF) through the cingulum bundle, and constitutes a major route through which the hippocampus interacts with the DLPFC, (Morris et al, 1999) an area that has been strongly implicated in working memory functions (Goldman-Rakic, 1995; Kobayashi and Amaral, 2000) and behavioral control of goal-directed actions. Damage to each of the posterior hippocampus (Petrides and Milner, 1982), retrosplenial cortex (deToledo-Morrell et al., 2001) and dorsolateral prefrontal cortex (Petrides, 2000) produces similar impairments in self-ordering tasks that require a memory of previous actions for the selection of successive items in a sequence. Thus it has been proposed that the retrosplenial cortex contributes to the integrated functioning of frontal and hippocampal cortex memory processes during the development of ordering tasks that entail the recall and manipulation
of sequential information in working memory (Petrides, 2000; Petrides and Milner, 1982).

Consistent with its connective anatomy, human clinical data indicate that the retrosplenial cortex may contribute to the learning of temporal relations between recent stimuli. Human patients with retrosplenial lesions are profoundly impaired in recalling the temporal order of recent information, but have little difficulty remembering individual events or judging the order of events in the remote past (Bowers et al., 1988; Valenstein et al., 1987). In addition, focal metabolic reductions in the retrosplenial cortex that arise during an early stage of Alzheimer’s disease (AD) (Nestor et al., 2003) are directly correlated with impairments in the judgment of temporal order of recent events (Hirono et al., 1998).

Although the clinical testing of cingulate function has thus far been hindered by the rarity of patients with isolated retrosplenial cortex lesions, human and animal studies of retrosplenial cortex have implicated the retrosplenial cortex in encoding processes that require its integrated functioning with connected hippocampal and prefrontal structures. The retrosplenial cortex of rodents and primates interacts heavily with posterior regions of the hippocampal formation, including the presubiculum, the entorhinal cortex and anterior thalamic nuclei, forming a pivotal link in the flow of information through the medial lobe memory systems that help encode new episodic memories of recent life events (Burwell and Amaral, 1998; van Groen and Wyss, 1992; Wyss and Van Groen, 1992). Reversible inactivation of retrosplenial cortex disrupts the development of reliable place-related firing of neurons in the hippocampus during navigation and produces a correlated impairment in spatial memory, suggesting an influence of retrosplenial cortex in the encoding processes of the hippocampal system (Cooper and Mizumori, 2001).
One difficulty in interpreting human lesion data is that most clinical reports of retrosplenic damage include damage to neighboring brain regions, including neighboring cortex and underlying white matter (reviewed in (Maguire, 2001)). This is particularly significant for isolating the memory functions of retrosplenic cortex because memory problems have been shown to result from isolated lesions to the cingulum bundle, a finger tract which is immediately adjacent to most of retrosplenic cortex. Lesions of cingulum bundle in rats produce similar spatial deficits to those produced by small and isolated lesions of retrosplenic cortex (Harker and Whishaw, 2004; Neave et al., 1996; van Groen et al., 2004; Vann et al., 2003; Warburton et al., 1998), and damage to both the cingulum bundle and the retrosplenic cortex produces much more severe spatial memory impairments (Whishaw et al., 2001). Thus an important question that arises in understanding the specific roles of retrosplenic cortex is if temporal order memory problems result from direct disruptions of retrosplenic cortex, or from the disruption of neighboring structures and the interruption of the underlying white matter, which may disrupt encoding processes in closely associated memory systems. Similarly, accumulating damage in temporal lobe structures (Van Hoesen and Hyman, 1990) and diminished neuromodulation of connected limbic structures (Braak and Braak, 1991) in early Alzheimer’s disease makes it difficult to interpret links between metabolic reductions in retrosplenic cortex and sequence memory problems that arise at a correspondingly early stage of the disease.

The current experiment set out to investigate the role of retrosplenic cortex in sequence learning by assessing the effects of local pharmacological inactivations of the retrosplenic cortex of rats on the learning of visuospatial sequences. The retrosplenic cortex was inactivated in dysgranular region 29b, an area that makes extensive
connections with the dorsal hippocampus, the parietal cortex, and the anterodorsal and anteroventral thalamic nuclei, which anatomically connect the cortical structures of the limbic system (Shibata, 1998; Van Groen et al., 1993; van Groen and Wyss, 1992; Wyss and Van Groen, 1992). We hypothesized that the retrosplenial cortex contributes to memory functions in concert with temporal order encoding processes of connected limbic cortical structures, including the dorsal hippocampal complex (Gilbert et al, 2001; Kesner et al, 2002; Agster et al, 2001; Fortin et al, 2002). Considerable evidence indicates that sequence memories are encoded in parallel in hippocampal structures and the basal ganglia, but that increased learning leads to a gradual shift in processing to stimulus-response strategies of the basal ganglia (Bontempi et al., 1999), reviewed by (Packard & Knowlton, 2002). Thus we predicted that the inactivation of retrosplenial cortex would disrupt the performance of ordered sequences at an early stage of sequence learning, but that the inactivation of retrosplenial cortex would have a minimal effect at a later stage of learning, when limbic structures play a less prominent role in the organization and production of memory-guided behavior.

3. Materials and methods

All procedures and animal care adhered strictly to AAALAC, Society for Neuroscience, and institutional guidelines for experimental animal health, safety, and comfort. Twenty Long-Evans hooded male rats began the study (Harlan, Portage MD). Four rats failed to reach behavioral criterion and an additional five failed to complete the study because of flaws to their infusion cannula or damage to implant that prevented a
sufficient number of successful bilateral infusions. Following acclimation to the laboratory, the rats were housed in individual cages and food-restricted to 85% of their ad libitum weights to provide motivation for instrumental conditioning. The colony was temperature controlled with a 12 hr light/dark cycle (lights on 8am-8pm) and the rats were tested at a regular time during the light period. The rats were approximately 4 months old when they began behavioral training and weighed between 280-350 grams.

3.1 Behavioral apparatus

Rats were trained and tested in 25x25 x25 cm aluminum behavioral chambers, each with five evenly-spaced apertures located along a concave wall. Each aperture was equipped with a 3W incandescent lamp that served as a stimulus and photodetector that registered the entrance of the rat’s nose into the aperture by the interruption of an infrared light beam. 45mg Noyes sucrose pellets (Research Diets Inc., New Jersey) were mechanically dispensed into a reward tray located on the opposite wall and were accessible to the rat through a flap door. A 3w bulb at the top of the chamber provided illumination (the house light). Each behavioral chamber was contained in a larger sound-attenuating chamber with a small fan that provided ventilation and background white noise. All stimulus presentations and behavioral measures were made with Cambridge Cognition Control 1.17 software on a PC Pentium IV class computer with behavioral programs written by CC.
3.2 Behavioral training

A rodent serial reaction time task was developed where rats were trained to respond to visuospatial stimuli presented in repeating and nonrepeating sequences. Sequence learning was assessed through the increased accuracy and faster reaction time for repeated sequences of cued locations than for random sequences. Rats were initially trained to nose poke in an aperture following the presentation of a visual stimulus within that aperture (Fig. 4.1). A response made into the illuminated hole turned off the stimulus light and immediately delivered a reward pellet (a correct response). A response into a hole which has not been illuminated (an incorrect response) or a failure respond within a five second period (omission) turned off the houselight for five seconds to communicate an error. The rat's egress from the food tray initiated the stimulus of the next trial after 3 secs. Once the rats were able to reliably respond to a single stimulus, they were trained to serially respond to two sequentially illuminated ports with a .25 sec ISI with for a reward delivered upon the correct entry into the second port of the sequence. The rats were then trained to respond to three and then four non-overlapping stimuli for a reward delivered upon the correct completion of the entire sequence.

When the rats could reliably respond to randomly generated four-hole sequences, the rats were presented 32 repeating (‘fixed’) and 32 nonrepeating sequences (‘random’) in an alternating pattern (e.g. sequences a-b-a-c-a-d...). Each fixed sequence was shown for five sequential days with a two day break between different sequences. The fixed sequences changed directions at least once (e.g. 3-4-2-1) and were successively counterbalanced for direction, beginning and ending ports.
3.3 Inactivation schedule

The schedule of inactivation was determined by quantifying the development of a stimulus-response strategy across five days of learning. ‘Early’ and ‘late’ stages of sequence learning were determined by the gradual development of an s-r behavioral strategy across the five days that the rats performed each fixed sequence. For each fixed sequence, the influence of a stimulus-response (S-R) strategy was assessed from commission errors made during the performance of the random sequence that were comprised of segments of the fixed sequence (an interference error). For example, during the performance of the fixed sequence 3-4-2-1, the intrusion of the first segment of the fixed sequence into the random sequence was assessed by number of incorrect responses into hole 4 following a correct response into hole 3. Each four-hole sequence was comprised of three trajectories (e.g. 3-4, 4-2 & 2-1), thus the ballistic errors were classified according to their first, middle & last position in the fixed sequence.

3.4 Surgical procedure for cannula implant

Following training, animals were deprived of food and water 24 hrs prior to surgery and anesthetized with isoflurane anesthesia. Four burr holes were drilled and self-tapping anchor screws were inserted into the holes. Small drill holes (SS White ttp-1 drill bit) were drilled at 7.0 mm posterior to bregma and .75 mm lateral to midline. Dura was deflected and the guide cannulae were implanted .75 mm ventral from the surface of the brain.

Two guide cannula were spaced 1.5 mm apart for bilateral placement at the border of granular and disgranular retrosplenial cortex, area 29 b & c (A/P -6.0 mm, M/L +0.75
mm & -0.75 mm, D/V -1.3 mm). The cannulae were inserted at 6 mm posterior to bregma, within granular and dysgranular retrosplenial cortex (area 29b&c). Dental cement was applied to the base of the assembly, which anchored the guide cannula to the surface of the skull and anchoring screws. Buprenorphine was injected intramuscularly to provide post-surgical pain relief, triple antibiotic ointment was applied to the edges of the wound and cephalaxin (15 mg/kg) was administered orally for five days following surgery. After seven days of recovery, animals resumed food restriction and were re-trained for two weeks with new sequences and mock infusions to habituate them to the infusion procedure.

Dummy cannulae were inserted that sealed the guide cannula and a cap covered the assembly. The dummy cannulae protruded .5 mm beyond the tips of the guide cannula, which were removed at the time of inactivation and replaced with injection needles of the same length for infusion at a depth of -1.8 mm. The tips of the needles used for inactivations were beveled at a 25 degree angle to direct the flow of solutions in a medial direction into the cortex and away from white matter and the neighboring parietal cortex.

**3.5 Behavioral testing**

On alternate weeks, each rat was given an infusion of 1 µl of artificial cerebrospinal fluid (vehicle) or Bupivacaine infused bilaterally over a 3 minute period. Bupivacaine is a relatively long-lasting blocker of sodium channels, which thus prevents neuronal spiking across the cell membrane of neurons and axons at the site of infusion. The rats began testing in the behavioral chambers immediately following infusions. Each rat was
tested for a total of eight sequences over an eight-week period (two early inactivations, two early vehicles, two late inactivations, two late vehicles).

3.6 Statistical analyses
Data analyses were performed using JMP 4.0.2 (SAS institute, Cary, NC). Upon completion of behavioral training, sequence learning was assessed over five days of performance of fixed and random sequences with separate ANOVA assessing 1) the percentage of correct responses and 2) the median latency of correct responses. The resulting 2-way analyses were SEQUENCE (3) × DAY (5).

During post-surgical testing, a within-subject repeated measures ANOVA was used to assess each of the behavioral effects of inactivation and vehicle infusions on the performance of fixed and random sequences. Performance was measured with respect to five main effects: 1) percent correct responses, 2) average length of successfully performed sequence, 3) omission errors, 4) commission errors and 5) average latency to perform a sequence. Performance was measured on day two infusions (early inactivation) and day five infusions (late inactivation), with the first day as a baseline. The analyses for each of the five measures thus were INACTIVATION (2) × SEQUENCE (2) × DAY (3). A Maunchy sphericity crieterion was used to assess a possible confound of within-subject correlations and multiple comparisons were conducted using modified Bonferroni correction.

4. Results
4.1 Pre-surgical performance

Overall measures of performance accuracy at the end of training indicated a selective learning of fixed sequences over five days. A significant main effect of day (F(4, 57) = 6.608; p = .0002)), sequence type (F(1, 57) = 41.38; p < .00001)) and a significant interaction of day sequence type (F(4, 57) = 4.464; p = .0033)) indicated a differential effect on learning on the performance of fixed and random sequences. Rats performed a significantly greater number of fixed sequences than random sequences on days 3, 4 & 5 (fig. 2). In addition, performance of fixed sequences on day 3, 4 & 5 was significantly from day 1 (p < .05). The accuracy of random sequences, however, was not different across any days of the week (p > .05).

A comparison of latency of mean responses indicated a main effect of days on the latency of correct responses, F(4, 57) = 6.508; p = .0002), but not a significant difference between fixed and random sequences, as performance of both types of sequences became faster over the five days (fig. 3). An analysis of interference errors indicated that the lowest proportion of S-R errors were committed on the second day of the sequence, and the number increased on subsequent days, reflecting the increasing development of the sequence into an S-R habit (fig. 4). We inferred that lowest number of interference errors would correspond to a greater mediation of behavior by cortical systems, (Packard & McGaugh, 1996; Packard & Knowlton, 2002; McDonald & White, 1994). Thus early inactivations were done on day 2 of sequence learning and late inactivations were done on day 5; the last day of sequence learning.

4.2 Inactivation effects
Infusions of bupivicaine prior to the testing sessions on the second day of sequence learning produced impairments on measures of sequence performance accuracy (F (1,33) = 9.40; P = .004). Post-hoc comparison showed that inactivation on the second day produced a significant reduction in accuracy in the percentage of correct responses during the performance of the fixed sequences (p = 0.01) (fig. 5A&B), but not during the performance of the random sequences (p = .23). Inactivations produced a related reduction in the average length of correctly performed sequences (F (1,33) = 17.037; P = .002), reducing the average length of fixed sequences on day two from 3.31 responses to 2.99 responses (p = 0.013) (Fig. 5B), while not having a significant effect on the length of random sequences (3.1 vs. 2.9) (p = 0.219). By approximately a 2 to 1 ratio fixed sequences were shortened due to increased commission errors, where a response was made into the incorrect port, rather than an omission error, where no response was made within a 5 sec period (12.6 commissions vs. 5.2 omissions). Inactivations, however, did produce a significant main effect in the overall number of omission errors or commission errors. There was also not a significant main effect from the inactivations on the latency for correctly executing a four-hole sequence.

Inactivations of bupivicaine on the fifth day of sequence learning did not have a significant effect on the performance of sequence accuracy (F (1,33) =.51; P = .48)) and did not affect the average length of successful responses in a sequence ( p > .1) (fig. 5C&D),

5. Discussion
5.1 Sequence learning

Before behavioral testing, the days of cortically-mediated behavior were inferred from the proportion of S-R errors performed during the random sequences that were comprised of ballistic completion of behavioral segments from the fixed sequence. The least number of ballistic errors was during day 2 of the learning of the fixed sequence, and the number dramatically increased on successive days (fig. 4). The number of interference errors was also greatest for the first and last segments of the sequence, an effect consistent with primacy and recency effects in list learning, which have been proposed to result from associative learning of successive elements of a sequence and reduced encoding in the middle elements of the sequence when associative memory demands are greatest (Estes, 1985). In addition, a considerable increase of fixed sequence accuracy on days 3, 4 & 5 indicated that day 2 was a critical day of sequence encoding. Early inactivations were thus done on day 2 of sequence learning and late inactivations were done on the last day of sequence learning.

5.2 Inactivation effects

Inactivation of retrosplenial cortex had a detrimental effect on the performance of fixed sequences during an early stage of learning. While early inactivations had a dramatic effect on the accuracy of fixed sequences, they did not affect the performance of the random sequences, indicating the preservation of attention, sensory and motor capacities for responding to sequential stimuli. The selective effects on the performance of fixed sequences indicate that the inactivation of retrosplenial cortex diminishes the
benefits of sequence learning, where improvement of performance results from memories of predictive relations between structured stimuli.

From the increasing incidence of stimulus-response behavior from the repeating sequence, we predicted that sequence memory processes would be increasingly mediated by structures outside the limbic system during learning and would no longer be affected by inactivation of retrosplenial cortex. Consistent with this prediction, inactivating at a late stage of learning did not have an effect on the performance of fixed sequences.

The task used in the experiment is closely analogous to a serial reaction time task developed in humans in which sequence learning is assessed by faster reaction times for repeating than for random sequences. Humans can improve in responding to cued stimuli presented in a repeating sequence without becoming conscious of the sequence, thus the task has been characterized as an implicit form of learning. Although there is some clinical evidence for a functional distinction between explicit sequence learning in hippocampus and implicit sequence learning in subcortical structures (Reber & Squire, 1994; Clark et al, 2002), recent studies of sequence learning in intact subjects have found that explicit and implicit visuospatial sequence learning task activate the same regions of the brain, including the hippocampus, striatum, cingulate cortex, temporal lobes and the DLPFC, including sequences that are executed with and without reports of conscious awareness. (Schendan et al, 2003). From these and other findings, the role of dorsal hippocampal structures in sequence learning has been described by a relational account, where limbic cortical structures play a general role in the association of temporally separate information and events (Cohen et al, 1997; Rawlins & Tsaltas, 1983; Fortin et al, 2002).
There is also evidence that new implicit motor sequence learning (as assessed in a finger-tapping variant of a serial reaction time task) that humans are able to acquire within a single day has dual representation. After learning, the sequence is represented as an ordered series of spatial locations as well as a sequence of movements. (Willingham et al., 2000). Human subjects exhibit a good transfer of learning when a sequence is switched to the other hand, but performance suffers greatly if the locations are changed, even if the same pattern of movements occurs that had occurred during training. Such a form of encoding is consistent with spatial and episodic strategies that have been attributed to the cortical limbic system, and is thus likely to engage those structures at an early stage of learning.

Behavioral data from order judgment tests have strongly suggested that frontal and hippocampal systems play a cooperative role in the encoding of sequential events by a circuit mediated by the retrosplenial cortex. In rats, lesions of the CA1 region of the hippocampus or medial prefrontal cortex (an area that makes analogous connections to DLPFC in primates) impair order judgments of spatial locations that were recently visited in a fixed sequence (Chiba et al., 1994; Gilbert et al., 2001). The behavioral impairments from damage to both structures are most severe when rats have to judge the order of proximal elements in the sequence. Thus the encoding process within the hippocampus has been described in terms of a mnemonic separation of temporal contexts, where the hippocampus helps to distinguish discrete behavioral cues in situations with high degree of intertask interference (Shapiro & Olton, 1994; Rolls, 1996; Tanila, 1999; Holland & Bouton, 1999; Agster et al., 2001). These selective impairments of order memory are isolated to posterior regions of dorsal hippocampus (CA1), the originating site of most hippocampal outputs to the retrosplenial cortex (Fortin et al., 2002; Agster et al., 2001) and
is not produced by lesions to the ventral hippocampus or the temporal lobes. In light of
the current findings, these data suggest a combined processing of temporally structured
data by a hippocampal-cingulate-prefrontal circuit that whose interactions are
functionally mediated by the retrosplenial cortex.

One contribution of retrosplenial cortex may be in the appropriate allocation of
attention to impending events in a sequence. Recording studies in rodents have shown
that neurons in the hippocampus, retrosplenial cortex and subiculum which exhibit place-
related firing are best correlated with the immediate future location of an animal (O'Keefe
& Recce, 1993; Skaggs et al. 1996; Mehta et al, 2002; Siapas et al, 2005; Jones &
Wilson, 2005). Interestingly, place-related activity in the retrosplenial cortex of rodents
anticipates that of the hippocampus (Cho & Sharp, 2001) and imaging studies have also
shown that the retrosplenial cortex mediates the anticipatory allocation of spatial
attention to events that can be predicted by recent memories (Small et al, 2003). The
anticipatory nature of place fields in rodents is not disrupted by strong electrical
disruptions of the CA1 region of the place field, leading to the suggestion that the
anticipatory nature of spatial information in the hippocampus may arise from outside of
that structure (Zugaro et al, 2005; Cho & Sharp, 2001). It may thus be the case that
retrosplenial cortex encodes prospective information with the frontal cortex, which
results in the appropriate allocation of anticipatory visuospatial attention.

In summary, the findings from the current study corroborate human clinical findings
that indicate the retrosplenial cortex may play an important contributing role in the
encoding of temporally separate, but relevant information and events into a distinct
memory. These findings suggest a shared contribution of retrosplenial cortex with
encoding processes of frontal and hippocampal systems to the representation of successive events in time, a defining character of behavioral and episodic memories.
Figure 4.1. Rodent operant chamber. Each chamber contained 5 evenly-spaced ports containing a light stimulus and a sensor that registered nose entry by the interruption of an infrared beam. In each trial, the rats’ egress form the reward tray initiated the first light of the sequence after 3 secs. A nose poke into the illuminated port turned off the light and triggered the next light in the sequence, a process that repeated for a total of four non-overlapping lights that constituted a sequence. A nose poke into the fourth port of the sequence triggered the delivery of a sucrose pellet into a reward tray in the opposite wall of the chamber that was accessible through a flap door.
Figure 4.2. Accuracy of responses during training (+SEM). Percent correct responses were assessed for fixed and random sequences across five days. Performance was significantly different across days (F (4, 57) = 6.608; p = .0002), and sequence types (F (1, 57) = 41.38; p < .00001). There was also a significant interaction of day × sequence type (F (4, 57) = 4.464; p = .0033). Performance of fixed sequences was significantly greater than random sequences on days 3, 4 & 5 (* p < .05).
Figure 4.3. Median latency of correct responses during training (+SEM). Latency of responses were assessed for fixed and random sequences across five days. Latencies were significantly different across days, $F(4, 57) = 6.508; p = .0002$), but there was not a significant overall difference between the latencies of fixed and random sequences.
Figure 4.4. Interference errors committed per day. The gradual development of an S-R strategy was assessed by the proportion of omission errors committed during the performance of random sequences that were comprised of segments of the fixed sequence. An intrusion of the first segment of the fixed sequence (segment 1) was an incorrect response into the second hole of the fixed sequence following a response into the same location as the first hole of the fixed sequence. An intrusion of the second segment (segment 2) of the fixed sequence is an incorrect response into the third hole of the fixed sequence following a response into the second location of the fixed sequence. An intrusion of the third segment (segment 3) is an incorrect response into the final hole of the fixed sequence following a response into the third hole of the fixed sequence. Day 2 had the least intrusion errors overall.
Figure 4.5. Inactivation effects on day 2 and day 5 of sequence learning (+SEM).

Inactivations of neural activity in the retrosplenial cortex on day 2 of sequence learning produced significant impairment in accuracy of fixed sequences (A) and in the average length of successful sequential response (B). Inactivations on day 5 of sequence learning did not produce significant effects in accuracy (C) and in the average length of successful response in a sequence (D). (* p < .05)
6. References


In this thesis, a series of novel experiments assess the neural processes of attention, memory and behavioral learning. Such processes are critical in allowing the brain to foresee and ultimately shape the course of biologically important events in the environment. In each experiment, the underlying processes of adaptive behavior were inferred through a study of behavioral breakdown in animals following the compromised function of neural systems. A central theme of the thesis was, thus, the development of animal models of human cognitive abilities, allowing an assessment of their neurobiological origins from clinical and anatomical parallels that exist between the species. Each of the studies investigated attention or memory processes of the brain by characterizing changes in behavior that reflect an animal’s limited ability to detect and predict important events in a visuospatial environment that was common to all experiments. In combination, the studies shed new light on neural processes that allow humans and animals to discern the predictive relationships of relevant events in the environment, and ultimately adapt their behavior towards shaping those outcomes in a manner that benefits the life of an animal.

The behavioral design of each of the experiments addressed separate aspects of stimulus prediction, attention and learning. In combination, the findings of the experiments contribute to the understanding of adaptive learning processes in animals and humans.
The following conclusion will consider the complementary roles of the cholinergic basal forebrain and the retrosplenial cortex in the processing of changing or novel predictive relations between stimuli. The conclusion will begin with the processes of the brain that increase processing of new stimuli. It will then consider the way that new learning is mediated by cortical and subcortical structures across learning. Finally, it will review the implications of the studies as a model of disease states that result from damage to the structures, and the accompanying loss of behavioral flexibility.

1. Maintenance of vigilance and selective attention

The conclusion will begin with the findings from chapter 3, which addressed the effects of homeostatic sleep drive in the maintenance of vigilance and sustained attention. The study sought to investigate the effects of neurochemical dysregulation of attention through determining the effects of sleep deprivation on an established test of attention abilities in rodents (the 5-choice serial reaction time task). The effects of sleep deprivation were consistent with the hypothesis that sleep deprivation would result in diminished attentional abilities through the suppression of cholinergic neurons by homeostatic mechanism of sleep drive. The attentional impairments were comparable to those resulting from lesions of basal forebrain cholinergic neurons that have been found in the same task, including a reduction of accuracy and a dose-dependent increase of latencies in response to brief, unpredictable stimuli (McGaughy et al, 2002). In combination, the behavioral alterations in the task have been understood as a diminished
capacity for sustained visuospatial attention to select, unexpected events in the environment (Sarter et al, 1999).

Though the study did not assess underlying alterations of cholinergic or other neurochemical systems, it inferred those effects indirectly on the basis of the known neurochemical processes that regulate sleep and attention in animals. There had not been a study to date that systematically assessed the effects of sleep deprivation on the attentional capacities of animals. Thus the experiment also helped to establish an animal model of attentional dysfunction and to corroborate well-known effects of sleep deprivation on the attentional abilities of humans. The effects of sleep deprivation were broadly consistent with the effects of sleep deprivation in comparable tasks in humans on a range of behavioral measures, which included an increase of omissions and an increase in response latencies. Individual rats also exhibited a consistent, phenotypic susceptibility to the effects of sleep deprivation, wherein certain rats either consistently succumbed or failed to succumb to the effects of deprivation, a hallmark of sleep deprivation effects in humans.

2. Cholinergic modulation of attention

The experiment in chapter 2 of the thesis addressed a similar capacity of animals to maintain attention to visuospatial stimuli. The novel use of probabilistic stimuli in a serial reaction time task also addressed a possible role of cholinergic neurons in allocating attention to relevant stimuli during inference processes that regulate selective
attention. This is carried out in concert with existing stimulus expectations which have already been learned by an animal.

Animal learning theories have outlined a role of prediction error and uncertainty in the learning of new predictive associations in the environment (Rescorla & Wagner, 1972; Macintosh, 1975; Pearce & Hall, 1980). One important basis for adaptive learning in an environment is thus the brain’s ongoing prediction of impending events from cues, and learning processes that result from the limitations of those predictions. Behavioral studies of animals with selective lesions of basal forebrain cholinergic neurons have thus far implicated a cholinergic modulation in attentional processes as a consequence of expectancy violations (Chiba et al, 1995). In addition, NBM/SI cholinergic neurons contribute to subsequent learning of predictive stimulus-stimulus associations in the environment.

The experiment in Chapter 2 of this thesis elaborated upon findings that suggest that forebrain cholinergic neurons contribute to attentional increases that result as a consequence of expectancy violations. The experiment assessed a new computational hypothesis that cholinergic neurons mediate a probabilistic uncertainty of ongoing predictions. In doing so, they regulate the allocation of attention to predictive stimuli following errors of prediction. Such a measure was interpreted in analogy to a necessary computation of uncertainty in Bayesian models of inference. Distinct from reward prediction error, predictive uncertainty is proposed to play a role in selective attention by directly increasing the processing of select stimuli whose outcomes are less known and decreasing the use of learned expectations in inference (Pearce & Hall, 1980; Yu & Dayan, 2005). According to Bayesian models, the optimal prediction of the actual state
of the environment is developed from a mixture of prior associative learning (top-down expectancy), and new information (bottom-up sensory processing), which estimates the most likely stimulus that generated the current observation. Uncertainty thus allows a further understanding of the role of acetylcholine in regulating the statistically appropriate use of new stimulus information to optimally detect less predictable stimuli in a changing and noisy environment.

The overall impairments of accuracy and speed on the experiment replicated behavioral findings from rats with NBM/SI cholinergic lesions performing the 5 choice-serial reaction time task, (McGaughy et al, 2002) a closely analogous behavioral test conducted in the same visuospatial environment with brief, random stimuli. Consistent with those findings, the behavioral impairments from the cholinergic NBM/SI lesions were disproportionately larger when responding to less predictable stimuli (where purely random stimuli are the least predictable). However, responding to more predictable stimuli was relatively unaffected, highlighting the selective nature of cholinergic modulation of attention.

The probabilistic nature of stimuli also allowed an assessment of graded levels of cholinergic modulation that is suggested by computational models of probabilistic inference. Consistent with predictions, the graded nature of the impairment matched the hypothesized levels of lost cholinergic modulation that would support the optimal detection of different stimulus types in the task. The experiment thus provides the first supporting evidence for a distinct computational role of basal forebrain cholinergic neuromodulation.
The distinction between sensory processing and expectation suggested by the experiment is broadly consistent with the idea that cholinergic modulation of neural systems increases attention-related processing of cortical systems (Baxter & Chiba, 1999). For example, acetylcholine in the hippocampus has been directly correlated with the development of a place strategy indicative of hippocampal processing. In contrast, decreased acetylcholine has been correlated with habit-like response strategies indicative of the basal ganglia (Chang & Gold, 2004). Through their broad influence on the cortex, NMB/SI cholinergic neurons may similarly regulate learning and memory of structured events by altering the overall balance of cortical processing in regulating an adaptive or habitual response to stimuli (Gold, 2003). The selective modulation of cortex may allow new processing of stimuli to overshadow well-learned associations and habits from subcortical systems such as the basal ganglia, and facilitate the increased processing and association of select events in the environment.

The experiment only addressed the role of uncertainty in optimizing the detection of probabilistic stimuli during inference. However, the same measure of uncertainty has been proposed to underlie the appropriate selection and association of stimuli that may be used to predict biologically important outcomes. The cholinergic modulation of cortex may, thus, ultimately facilitate the development of stimulus-stimulus associations that allow the brain to autonomously predict biologically important outcomes in a changing environment.
3. Associative learning in cortical and hippocampal systems

At an early stage of learning, cholinergic modulation of the cortex and hippocampus has been shown to promote the learning of new memories and goal-directed actions (Olton et al, 1991 Conner et al, 2003). Cholinergic modulation plays a central role in the learning and memory functions of the hippocampus, including episodic memory formation (Hasselmo, 1999) and the reduction of interference from existing memories (De Rosa et al, 1999). In addition, cholinergic modulation of cortical structures helps promote attention and learning in diverse cortical systems, including the frontal, parietal, auditory and piriform cortex (McGaughy et al, 2002; Bucci et al, 1998, Merzenich, 1998; Linster & Hasselmo, 2001). Finally, cholinergic modulation also facilitates communication between hippocampus and connected neural structures, including entorhinal and frontal cortex (Chrobak & Buzsaki, 1998 Siapas & Wilson, 2005). Thus it is possible that the uncertainty of predictions mediated by acetylcholine promotes the processing and learning of new associations across cortical systems that gradually reduce both prediction errors and predictive uncertainty.

Chapter 4 addressed neural processes that may contribute to the learning of new predictive relations between visuospatial stimuli. Like the experiment in chapter 2, it also highlighted the role of multiple memory systems in mediating new and old forms of associative learning. It thus addressed the contributions of cortical structures and distinct memory processes outside of the retrosplenial cortex that encode well-learned visuospatial sequences.
The findings of the chapter 4 indicate that the retrosplenial cortex may play a critical role in the learning of new visuospatial sequences. On the basis of clinical and anatomical features of the retrosplenial cortex, sequence learning is likely to depend on interactions of retrosplenial cortex with other structures, including the dorsal hippocampus, with which it has strong bidirectional connections.

One prominent characterization of hippocampal dependent memory systems in animals is the proposal that the hippocampus is dedicated to the creation and use of spatial or cognitive maps (O'Keefe, 1978). Much like the hippocampus, there is considerable support from neuropsychological studies on animals showing that damage to the retrosplenial cortex results in a profound deficit in spatial memory (Whishaw et al, 2001; Cooper et al, 2001; Vann et al, 2003). In addition, neurophysiological studies have shown spatially-related firing patterns in both hippocampus (O'Keefe et al, 1971; McNaughton et al, 1983) and retrosplenial cortex (Cho & Sharp, 2001).

In recent studies, the cognitive processes involved in spatial mapping has been used as a framework for understanding episodic memory, and therefore has advanced our understanding of memory phenomena in humans from animal models. The representational processes of the hippocampus have also been characterized as explicit and underlying an ability to flexibly code new spatial relationships between stimuli, a process akin to Tolmanian cognitive mapping processes that allow flexible navigation to spatial goal locations. The hallmark of hippocampal-dependent memories has been the ability to flexibly express such memories (Knowlton & Squire, 1996). From this perspective, spatial memory processing in rodents has been regarded as a model for relational representation and flexible memory expression (Eichenbaum, 1999).
Several studies indicate that sequence learning entails a dual representation of spatial events and motor actions (reviewed by Packard & Knowlton, 2002). In humans and animals, a dissociable role has been attributed to the caudate nucleus and putamen in the gradual, incremental learning of associations that is characteristic of habit learning (Reber et al, 1996, Knowlton et al, 1996). Thus, the representational abilities are time-limited, in that they only contribute to the new coding of events, which are eventually mediated independently of hippocampal systems.

In chapter 4, a similar role was confirmed for retrosplenial cortex in the learning of new visuospatial sequences. The memory contributions of retrosplenial cortex were proposed to arise from the role of this structure in encoding new relations of events across a hippocampal and frontal network. Thus, inactivations were expected to have a similar time-limited effect that has been suggested by lesion studies of the hippocampus. While inactivations of the structure affected the performance of new sequences, they did not have an effect on behavior when sequences had been well-learned. The combined effects of inactivation thus provided evidence for a time-limited role of the structure in new sequence learning.

A similar, finger-pointing version of the behavioral task used in the experiment has shown that a nonconscious form of sequence learning can arise without the hippocampus (Reber & Squire, 1994). However, in an intact subject, the use of both hippocampal and basal ganglia systems would be expected to mediate spatial and behavioral aspects of sequence learning. The use of reversible inactivations in the experiment described in chapter 4 allowed a preservation of the neural system until the time of testing. In contrast to lesion studies, the use of inactivations also raises the possibility that animals continue
to rely on this structure, and thus contribute importantly to memory processing of new sequential information by the limbic system. More generally, we propose that intact neural systems normally require the hippocampus, frontal cortex and retrosplenial cortex in the new encoding of sequential events. Such a form of encoding may proceed more rapidly, through the flexible development of spatial memories by multiple cortical memory systems.

With increased learning, animals begin to predict and rapidly respond to impending stimuli. In addition, associated stimuli may become predictable for an animal, and no longer require attention and memory processes of the basal forebrain cholinergic system, including the frontal cortex, parietal cortex, hippocampus, and the retrosplenial cortex.

4. Overlearning, neural pathology and the loss of behavioral flexibility

The normal development of well-learned memories in intact brains is an important component of adaptive learning. One benefit is the ability to rapidly execute goal-directed behavior. In addition, the gradual consolidation away from cortical memory systems may allow processing of new stimuli by those structures, so helping animals to continually learn about new contingencies and events in the environment. However, the decreased functioning of cortical memory systems, and the remaining influence of well-learned memories, is characterized by automatic behavior and a lack of flexibility in the face of new stimulus demands.

The behavioral effects of the experiments shed new light on the breakdown of adaptive behavior resulting from the compromised function of each neural system. In the
three experiments of the thesis, compromised neural functioning led to a lack of ability to flexibly allocate processing to new and unpredictable visuospatial events.

Following sleep deprivation, animals were comparably impaired in responding to brief visuospatial events. This demonstrates a loss of ability to maintain increased attention to stimuli. The study took a first, but necessary step in the development of animal models of the neural basis of impairments that result from sleep deprivation. Sleep deprivation can be viewed as a natural form of the diminished capacity for the adaptive allocation of attention by intact humans, an effect that may result from the increasing suppression of cholinergic neurons in the basal forebrain (Porkka-Heiskanen et al, 1997).

The impairments of behavior following cholinergic lesions of the basal forebrain shed light on the underlying neurochemical processes that support the normal capacities of humans and animals to appropriately increase attention and abandon learned, but less relevant information, in the face of change and novelty in the environment. Following a cholinergic lesion rats were disproportionally impaired in their ability to respond to stimuli that were less predictable, on the basis of learned expectations. In addition, response errors were comprised of responses into expected stimulus locations on the basis of previous learning. The effects of cholinergic lesions in inference tasks, thus, reflect an inappropriate use of expectations in inference that limit an animal’s ability to detect and respond to unexpected events, an effect similar to that of humans with cholinergic diseases of attention (Parasuraman et al, 1999).

Finally, there was a similar lack of flexible behavior in late stages of sequence learning. The results of inactivation suggest that animals without retrosplenial cortex
were less able to use new learning to respond to sequential visuospatial stimuli. In a late stage of learning, animals were efficient in the execution of behavior, and responses were considerably faster than during new sequence learning. However, responding was characterized by increased stimulus-response form of behavior and the intrusion of stereotyped behavior in response to new, unstructured sequences. Thus the behavioral dependence on basal ganglia structures and the diminished involvement of retrosplenial cortex and hippocampus may represent an alternate, less flexible form of learning that arises at a late stage of encoding in intact subjects.

The findings of the experiment corroborate clinical findings in humans with damage and metabolic alterations in the retrosplenial cortex. In the early stage of Alzheimer’s disease, the ability to recall temporal order of stimuli is compromised (Hirono et al, 1998). In a corresponding manner, early hypofunction of the retrosplenial cortex is the best predictor that a patient with early cognitive decline will develop Alzheimer’s disease (Johnson et al, 1998; Kantarci et al, 2000, Valla et al, 2001). Thus the findings help to elucidate the nature of memory impairments that result from the compromised function in that structure in this widely prevalent disease.

This body of work constituted a novel investigation of the neural processes underlying the ability of animals to appropriately attend and learn about new biologically important events in the environment. The studies also investigated the ability of animals to affect those outcomes through the flexible development of instrumental action. Importantly, the experiments also provide new information on the underlying neural bases of adaptive behavior in rodents that is likely to provide insight into the human condition. The functional processes that were addressed in each of the studies were
modeled after clinical impairments in humans that diminish their capacities for adaptive action. Each of the studies developed new animal models for investigating processes of attention and learning in humans. Thus, they shed new light on the processes of the brain that allow us to discern and affect the causal fabric of a changing environment.

5. Future Directions

An ongoing study has begun to assess the effects of cholinergic lesions of the NBM/SI on the interactions of the retrosplenial cortex with the dorsal hippocampus, an interaction that may play an important role in the memory functions of both structures. The anatomical connections between the retrosplenial cortex and the basal forebrain raise the interesting possibility that cholinergic modulation of limbic cortical structures facilitates attention and learning by regulating communication between the dorsal structures of the cortex and the hippocampus. Whereas cortically projecting neurons in the NBM/SI increase selective attention through their actions on frontal, parietal and sensory cortices, cholinergic neurons medial septum/vertical diagonal band (MS/VDB) that project to the hippocampus contribute to episodic learning and background processes of attention.

Notably, the retrosplenial cortex is also the only the structure among the dorsal cortical systems that receives from the NBM/SI and from the MS/VDB. The anatomy retrosplenial cortex plays a unique role in the coordinating the functional interactions between dorsal spatial memory and attention systems and the hippocampus.
With the help of Laleh Quinn, the research scientist in Dr. Chiba’s laboratory, we implanted electrodes in the hippocampus, the retrosplenial cortex and the NBM/SI in two rats with NBM/SI cholinergic lesions and two control rats. We recorded the EEG in the two structures and analyzed the relationship of EEG oscillations in the 5-15Hz, range; a frequency that is thought to develop in the cortex as a result of cholinergic modulation. Initial results indicate that in rats without cholinergic modulation, the oscillatory activity of retrosplenial cortex slightly lags that of the hippocampus, an effect that may arise from the remaining influence of cholinergic neurons in the MS/VDB that modulate the hippocampus. However in control rats, the relationship of large-scale oscillations between the two structures alternates, where oscillations in the retrosplenial cortex periodically lead and follow those of the hippocampus. Such an effect may reflect the nature of functional interactions between the two structures as a result of intact NBM/SI modulation of cortical structures adjacent to the hippocampus.

Finally, I have established a post-doctoral research position in the laboratory of Barbara Jones in McGill University, where we will directly investigate the firing activity of cytochemically identified cholinergic neurons in the basal forebrain of awake head-fixed rats. We have designed simple conditioning tasks that will directly investigate the hypothesized levels of cholinergic neuron activity that reflect the predictive uncertainty in rats while they learn to associate cues with the probabilistic delivery of rewarding and aversive liquids.
6. References


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