A cross-species examination of cholinergic influences on feature binding: Implications for attention and learning

by

Leigh Cortland Perry Botly

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Department of Psychology
University of Toronto

© Copyright by Leigh Cortland Perry Botly (2010)
A cross-species examination of cholinergic influences on feature binding: Implications for attention and learning

Leigh Cortland Perry Botly
Doctor of Philosophy
Department of Psychology
University of Toronto
2010

Abstract
Feature binding refers to the fundamental challenge of the brain to integrate sensory information registered by distinct brain regions to form a unified neural representation of a stimulus. While the human cognitive literature has established that attentional processes in a frontoparietal cortical network support feature binding, the neurochemical contributions to this attentional process remain unknown. Using systemic administration of the cholinergic muscarinic receptor antagonist scopolamine and a digging-based rat feature binding task that used both odor and texture stimuli, it was demonstrated that blockade of acetylcholine (ACh) at the muscarinic receptors impaired rats’ ability to feature bind at encoding, and it was proposed that ACh may support the attentional processes necessary for feature binding (Botly & De Rosa, 2007). This series of experiments further investigated a role for ACh and the cholinergic basal forebrain (BF) in feature binding. In Experiment 1, a cross-species experimental design was employed in which rats under the systemic influence of scopolamine and human participants under divided-attention performed comparable feature binding tasks using odor stimuli for rats and coloured-shape visual stimuli for humans. Given the comparable performance impairments demonstrated by both species, Experiment 1 suggested that ACh acting at muscarinic receptors supports the attentional
processes necessary for feature binding at encoding. Experiments 2-4 investigated the functional
neuroanatomy of feature binding using bilateral quisqualic acid excitotoxic (Experiment 2) and
192 IgG-saporin cholinergic immunotoxic (Experiments 3 and 4) brain lesions that were assessed
for completeness using histological and immunohistological analyses. Using the crossmodal
digging-based rat feature binding task, Experiment 2 revealed that the nucleus basalis
magnocellularis (NBM) of the BF is critically involved in feature binding, and Experiment 3
revealed that cholinergic neurons in the NBM are necessary for feature binding at encoding.
Lastly, in Experiment 4, rats performed visual search, the standard test of feature binding in
humans, with touchscreen-equipped operant chambers. Here it was also revealed that cholinergic
neurons in the NBM of the BF are critical for efficient visual search. Taken together, these
behavioural, pharmacological, and brain-lesion findings have provided insights into the
neurochemical contributions to the fundamental attentional process of feature binding.
Acknowledgments

First and foremost, I would like to thank my advisor, Dr. Eve De Rosa, for her tremendous support, encouragement, and guidance throughout my doctoral training. It has been with kindness, an endearing sense of optimism, and a strong passion for research that Dr. De Rosa has taught me the skills and given me the confidence needed for a career in research. I would also like to thank the members of my thesis committee, Drs. Sara Shettleworth, John Yeomans, and Paul Fletcher, for their support and constructive feedback on my research and dissertation. Thank you to Adam Anderson for his advice and support of the cross-species study and thank you to Matt Dixon for his help with programming the human behavioural task and collecting the human behavioural data. Thank you to Wojtek Grabski for his technical expertise and invaluable help with setting up and programming the touchscreens. Thank you to all of the undergraduate students, Sanam Bamshad, Jamie Benoit, Bessie Chan, Milson Chan, Isabel Chiu, Jacquie Chung, Jasna Deluce, Kirsten Donovan, Robb Fatt, Steve Greening, Alin Khodaverdian, Mavis Kusi, Paul Luu, Michael Maksimowski, Bratislav Msic, Peter Poon, Allison Ritchie, Farah El-Sadi, Maryam Saheb-Al-Zamani, Caroline Strang, Alyssa Tam, Natalie Trent, Asif Virani, Gennie Wang, I-Zen Wang, and Evelyn Wei, who helped collect behavioural data over these past five years; your hard work has been invaluable to the completion of this dissertation. I would like to acknowledge the Natural Sciences and Engineering Research Council of Canada as well as the Ontario Mental Health Foundation for funding my dissertation research. To my fellow graduate students, Maha Adamo, Hanah Chapman, Norman Farb, Daniel Lee, Taylor Schmitz, and Joshua Susskind, the past six years have truly been a journey: thank you for making it such a memorable one and for your support and encouragement. To my husband, thank you for always being there for me, for giving me strength, for listening, and for never failing to make me laugh. To my parents, thank you for your unconditional love and support and for keeping me grounded.
Table of Contents

List of Tables .................................................................................................................. vi

List of Figures ........................................................................................................... vii-viii

Introduction ................................................................................................................. 1-38

Chapter 1: Experiment 1 ............................................................................................ 39-55

Chapter 2: Experiment 2 ............................................................................................ 56-74

Chapter 3: Experiment 3 ............................................................................................ 75-98

Chapter 4: Experiment 4 ............................................................................................ 99-126

General Discussion .................................................................................................... 127-169

References .................................................................................................................. 170-187

Tables .......................................................................................................................... 188-191

Figure Captions ........................................................................................................ 192-199

Figures ....................................................................................................................... 200-224

Copyright Acknowledgements .................................................................................. 225
List of Tables

*Table 1.* Experiment 1: List of odorants, colours, and shapes from which the experimental stimuli were created.

*Table 2.* Experiment 2: List of odorants and textures from which the experimental stimuli were created.

*Table 3.* Experiment 3: List of odorants and textures from which the experimental stimuli were created.

*Table 4.* Experiment 3: Correlations between task performance and ChAT immunoreactivity and AChE reactivity.
List of Figures

*Figure 1.* Experiment 1: Illustration of the two different trial types of the cross-species forced-choice tasks.

*Figure 2.* Experiment 1: Illustration of the features defining the intramodal Feature-Conjunction and Feature-Singleton stimuli.

*Figure 3.* Experiment 1: Acquisition of the Feature-Conjunction and Feature-Singleton tasks by rats (Experiment 1A) and humans (Experiment 1B).

*Figure 4.* Experiment 1: Performance costs of the cholinergic and attentional challenges on the Feature-Conjunction and Feature-Singleton tasks.

*Figure 5.* Experiment 2: Illustration of the two different trial types and a typical session of the forced-choice rat digging tasks.

*Figure 6.* Experiment 2: Illustration of the features defining the crossmodal Feature-Conjunction and Feature-Singleton stimuli.

*Figure 7.* Experiment 2: Choline acetyltransferase immunohistochemistry of the Nucleus Basalis Magnocellularis and Medial Septum/Vertical Limb of the Diagonal Band of Broca.

*Figure 8.* Experiment 2: Acetylcholinesterase histochemistry.

*Figure 9.* Experiment 2: Post-surgical performance by rats on the Feature-Conjunction and Feature-Singleton tasks.

*Figure 10.* Experiment 3: Illustration of the two different trial types and a typical session of the forced-choice rat digging tasks.

*Figure 11.* Experiment 3: Illustration of the features defining the crossmodal Feature-Conjunction, Feature-Singleton (FS), and FS Enhanced-Difficulty stimuli.

*Figure 12.* Experiment 3: Choline acetyltransferase and parvalbumin immunohistochemistry of the Nucleus Basalis Magnocellularis.

*Figure 13.* Experiment 3: Choline acetyltransferase and parvalbumin immunohistochemistry of the Medial Septum/Vertical Limb of the Diagonal Band of Broca.

*Figure 14.* Experiment 3: Acetylcholinesterase histochemistry.

*Figure 15.* Experiment 3: Pre-surgical performance and post-surgical retrieval by rats of the same set of Feature-Conjunction stimuli.

*Figure 16.* Experiment 3: Post-surgical performance by rats on the Feature-Conjunction, Feature-Singleton (FS), and FS Enhanced-Difficulty tasks.
Figure 17. Experiment 3: Scatterplots illustrating the relationships between Feature-Conjunction acquisition performance and Nucleus Basalis Magnocellularis choline acetyltransferase immunoreactivity and neocortical acetylcholinesterase reactivity.

Figure 18. Experiment 4: Overhead view of touchscreen-equipped operant chamber.

Figure 19. Experiment 4: Illustration of the different trial-types and stimulus set-sizes used in the rat Visual Search task.

Figure 20. Experiment 4: Pre-surgical performance of rats on the Visual Search task as measured by (A) accuracy and (B) correct latency.

Figure 21. Experiment 4: Choline acetyltransferase and parvalbumin immunohistochemistry of the Nucleus Basalis Magnocellularis.

Figure 22. Experiment 4: Choline acetyltransferase and parvalbumin immunohistochemistry of the Medial Septum/Vertical Limb of the Diagonal Band of Broca.

Figure 23. Experiment 4: Acetylcholinesterase histochemistry.

Figure 24. Experiment 4: Trial-type breakdown of the post-surgical performance of rats on the Visual Search task as measured by (A) accuracy and (B) correct latency.

Figure 25. Comparison of the impact of divided attention, scopolamine, and cholinergic-selective lesions of the NBM on the acquisition and retrieval of comparable Feature-Conjunction (FC) and Feature-Singleton (FS) tasks used in Experiments 1 and 3.
Introduction

Our environment is characterized by a multitude of objects and we must selectively focus on the most relevant objects in order to interact with them to achieve our goals. The cognitive process of selectively allocating processing resources to relevant information in the environment is referred to as attention. Each object in the environment is defined by a variety of features that may be processed by different sensory modalities. An important question in psychology and neuroscience is how the brain forms unified neural representations of objects when distinct brain regions are primarily responsible for detecting and processing different object features. This unknown mechanism of feature integration is referred to as feature binding (Reynolds & Desimone, 1999; Treisman & Gelade, 1980), and it has garnered much consideration in the cognitive psychology and neuroscience literatures.

The functional importance of feature binding is clear as it is a necessary first step of object recognition: the features of a given object must be correctly combined in order for an accurate representation of the environment to be made. Such feature integration can occur within a single sensory modality or across multiple sensory modalities depending on the nature of the stimuli in the environment. Visual feature binding is an example of unimodal binding, and the features of a visual stimulus can include colour, shape, size or motion. There are neurons located in distinct regions of the ventral and dorsal regions of the visual cortex that respond maximally to such specific features. For instance, area V4 of the visual cortex responds most strongly to the colour of stimuli, area V2 to the form of stimuli, and area MT/V5 to the motion of stimuli. Damage to each of these regions results in the inability to perceive the corresponding visual feature (Kolb & Whishaw, 1996). Thus, in order for a unified neural representation of a visual stimulus to be made, the brain must integrate neural signals from all of these sub-specialized regions of the
extrastriate visual cortex. Crossmodal feature binding involves the integration of stimulus features from multiple sensory modalities to form a unified neural representation of a multimodal stimulus. For example, a stimulus such as an angry running skunk is characterized by visual, auditory, and olfactory information, and spatially distinct regions of the brain are responsible for encoding and processing these different features, namely the visual, auditory, and olfactory cortices, respectively. Thus, in order for a unified representation of a multimodal stimulus to be made, the brain must integrate the neural patterns from multiple spatially distinct brain regions.

**Current Theories of Feature Binding**

Feature binding is an attention-dependent cognitive process. Evidence establishing this has come from three main sources: neuropsychological studies demonstrating the impaired feature binding performance of patients with attentional deficits as well as behavioural and functional neuroimaging studies with neurologically intact human participants demonstrating the need for attentional resources from a frontoparietal cortical network during feature binding (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Luck & Ford, 1998; Reynolds & Desimone, 1999; Tales et al., 2002; Treisman, 1998). According to the Feature Integration Theory of attention proposed by Treisman and Gelade (1980), the different features of a given visual stimulus are detected and encoded in separate feature-detection areas of the brain. Attention is then directed to the location of the stimulus to integrate its features and form a conjunction. Illusory conjunctions are believed to be formed when attention to the locations of stimuli is not deployed, resulting in the perception of unbound features “floating” in space without adherence to any specific stimulus. The “floating” features are then incorrectly
combined to form illusory conjunctions. For example, neurologically intact human participants who were briefly presented with visual arrays of coloured letters [e.g., a blue O, red T, and yellow Y] were found to erroneously recombine the features and reported with great confidence that they had seen a stimulus made up of the colour from one stimulus in the array and the shape from another stimulus in the array [e.g., a yellow O, blue T, or red Y] (Treisman, Sykes, & Gelade, 1977). Importantly, a greater number of illusory conjunctions were reported by participants when their attention was divided by a concurrent task.

Deficits in attention caused by damage to the parietal cortex result in feature binding impairments (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Friedman-Hill, Robertson, & Treisman, 1995; Robertson, 2003). Patients with unilateral neglect most commonly have lesions to the right parietal lobe and have difficulty processing stimuli presented to the contralesional (left) side of their visual field. Their impairment is a direct result of their attention being diverted to the ipsilesional (right) side of space. Such patients produce high rates of illusory conjunctions, as their attentional system cannot adequately deploy attention to the location of the stimuli for feature binding. However, their ability to detect a particular feature (colour or shape) presented to the contralesional side is preserved, suggesting that deficits in attention impair feature binding, but leave feature detection intact (Cohen & Rafal, 1991). Similarly, when a patient with bilateral parietal-occipital lesions was tested on a visual feature binding task, he reported a large number of illusory conjunctions, but was able to detect single features in a visual array (Bernstein & Robertson, 1998; Friedman-Hill, Robertson, & Treisman, 1995). Striate and extrastriate regions of the visual cortex are thought to be involved in feature detection, both of which were intact in these patients with parietal cortex damage.
The majority of research investigating the attention-dependent nature and functional
neuroanatomy of feature binding has relied on the visual search task, first pioneered by Treisman
and Gelade (1980). In this paradigm, participants must find a visual target stimulus embedded in
an array of distractor stimuli. On feature search trials, feature binding is not required to find the
target because participants can look for a single feature (e.g., look for the colour green to find a
green apple in a basket of red apples). However, on conjunctive search trials, feature binding is
required because participants must look for a conjunction of two features to find the target (e.g.,
look for the colour green and the shape of an apple to find a green apple in a basket of red apples
and red and green pears). During feature search, the demand on attention is minimal as targets
appear to “pop out” regardless of the number of distractors present, while during conjunctive
search trials, an attentionally-demanding serial search for the target is necessary, as demonstrated
by a significant increase in target detection time with increasing numbers of distractors.

Human functional neuroimaging studies with neurologically intact participants have revealed
selective activation of frontoparietal cortical networks during conjunctive but not feature search
trials of visual search task tasks (Corbetta, Shulman, Miezin, & Petersen, 1995; Nobre, Coull,
Walsh, & Frith, 2003), suggesting that single-feature processing constitutes a low attentional
load, while conjunctive processing requires greater attentional resources. Furthermore, in a
matching-to-sample study in which two sample visual stimuli were presented simultaneously
followed by the presentation of a single test stimulus, participants had to determine whether the
test stimulus matched either of the two sample stimuli on a single feature (shape, colour, or
location) or on a conjunction (shape and colour). After controlling for task difficulty and eye
movements, the parietal cortex was found to be activated during conjunction matching, but not
during feature matching (Shafritz, Gore, & Marois, 2002). Consistent with such findings, in an
event-related-potential study, the N2-posterior-contralateral component of the ERP waveform
which is associated with the deployment of selective attention was only found when subjects performed a task requiring the detection of conjunctions, but not the detection of single features (Luck & Ford, 1998).

Attention is not only important for unimodal feature binding, but also necessary for the binding of features between sensory modalities. Human functional neuroimaging studies have demonstrated activation in multimodal brain regions including the posterior parietal cortex, orbitofrontal cortex, hippocampus, intraparietal sulcus, and superior temporal sulcus (STS) during crossmodal auditory-visual, tactile-visual, and olfactory-visual feature binding tasks (Bushara et al., 2003; Fuster, Bodner, & Kroger, 2000; Gottfried & Dolan, 2003; Macaluso & Driver, 2005; Saito et al., 2005; Tanabe, Honda, & Sadato, 2005). Such multimodal brain regions receive either direct or indirect afferent input from unimodal sensory cortices, such as the visual, olfactory, somatosensory and auditory cortices (Gottfried & Dolan, 2003), and back-projections from multimodal to unimodal cortices have been identified (Macaluso, Frith, & Driver, 2000; McDonald, Teder-Salejarvi, Di Russo, & Hillyard, 2003). A spatially non-predictive cue presented in one sensory modality at a particular location can improve the discrimination of a second sensory cue presented in a different modality at that same location (Macaluso, Frith, & Driver, 2000; McDonald, Teder-Salejarvi, Di Russo, & Hillyard, 2003), and research suggests that such crossmodal facilitation is mediated by enhanced attention in unimodal brain regions via back-projections from multimodal cortices. Thus, unimodal cortices can readily and quickly influence each other via intermediary multimodal regions, creating a situation very conducive to feature binding between sensory domains. Recent studies investigating crossmodal integration in humans have illustrated numerous similarities between unimodal and crossmodal feature binding, such as the occurrence of crossmodal illusory visual-tactile conjunctions (Bushara et al., 2003; Cinel, Humphreys, & Poli, 2002; Gottfried & Dolan, 2003).
A cellular-based theory of feature binding proposes that temporal synchronization of neuronal activity is responsible for feature binding (Engel, Fries, & Singer, 2001; Senkowski, Schneider, Foxe, & Engel, 2008). This theory posits that when a stimulus is presented, neurons responsible for its representation are activated, and their action potentials begin to fire in synchrony providing a temporal code that distinguishes such neurons from those activated by a different stimulus. According to this theory, when a stimulus containing a conjunction of features is perceived, the neural discharges of the activated neurons are synchronized and the represented features bound together.

Synchronization theory is supported by electrophysiological studies of non-human animals showing the precise synchronization of neural responses within cortical regions of the brain. Single- and multi-cell recordings have found stimulus-specific synchronization of neuronal discharges within and between spatially separate regions of the striate and extrastriate regions of the visual cortex of anesthetized and awake cats and macaque monkeys (Engel, Kreiter, Konig, & Singer, 1991; Friedman-Hill, Maldonada, & Gray, 2000; Gray & Singer, 1989). Synchronization of neural firing with oscillation frequencies in the gamma range (20-70 Hz) occurred in the visual cortex when visual stimuli containing the same features were presented (Friedman-Hill, Maldonada, & Gray, 2000; Gray & Singer, 1989). For example, while recording from two sites of the cat visual cortex, each of which represented a distinct receptive field, Gray and Singer (1989) found synchronized gamma frequency neural firing when a long continuous light bar moving across both receptive fields was presented. When two separate light bars moving in the same direction or opposite directions were presented, no such neural synchronization occurred, suggesting that the presence of common features within a single stimulus is a requirement of neural synchronization. Using electroencephalography (EEG) with human participants, Muller et al. (1996) replicated Gray and Singer’s (1989) study using the
same visual stimuli and found consistent results. Furthermore, neural synchronization with oscillations in the gamma frequency range has also been found in the auditory, somatosensory, olfactory, and motor cortices of non-human animals, as well as crossmodal synchronization between the visual and motor cortices (Engel & Singer, 2001; Roelfsema, Engel, Konig, & Singer, 1997).

In both human and nonhuman animals, the occurrence of such high-frequency gamma-range neural oscillations is limited to states of alertness (Engel & Singer, 2001) and is correlated with cognitive activities requiring attention (Muller, Gruber, & Keil, 2000). Importantly, attention has been shown to modulate gamma frequency synchronization in the human cortex (Muller, Gruber, & Keil, 2000; Tiitinen et al., 1993). Using EEG, when participants were instructed to attend to a particular visual or auditory stimulus, gamma-band activity was much greater than when subjects were instructed to ignore the same stimulus. Furthermore, when participants shifted their visual attention to the opposite visual hemifield, cortical gamma-band activity shifted to the contralateral hemisphere (Muller, Gruber, & Keil, 2000). Such findings linking attention to a state of neural synchrony proposed to be necessary for feature binding are congruent with findings from the human cognitive literature demonstrating the attention-dependent nature of feature binding.

While the human cognitive neuroscience literature has established that attentional processing in frontoparietal cortical networks supports feature binding, and electrophysiological work has suggested that synchronization of cortical neural firing may be necessary for feature binding, the neurochemical contributions to feature binding remain unknown. We hypothesized that acetylcholine (ACh) may be critical to feature binding given this neurotransmitter’s established role in modulating attention. The following pages provide a discussion of the neuroanatomy and
functional importance of the cholinergic system to cognition to establish how this neurotransmitter system may support feature binding.

**Neuroanatomy of the Cholinergic Basal Forebrain**

*The Cholinergic Hypothesis* has become the basis for a vast amount of research investigating the role of ACh in cognition. It proposes that the deterioration of cognitive functioning characterized by neurodegenerative disorders, such as Alzheimer’s disease and age-related dementia, was in part due to the effects of neurodegeneration of cholinergic neurons in a region of the mammalian brain known as the basal forebrain [BF] (Coyle, Price, & DeLong, 1983). The BF is a collection of four deeply-set magnocellular cholinergic nuclei (Ch 1-4) within the inferior frontal portion of the brain, the axons of which project to brain regions important for cognition, such as the neocortex and hippocampus. Mesulam, Mufson, Wainer, and Levey (1983) have delineated the central cholinergic nuclei pathways of the BF in the rat brain, which are highly similar to those found in the human brain.

The BF projection system is topographically organized. The septohippocampal pathway projects to the hippocampus and related structures from the medial septum (Ch 1) and vertical limb nucleus of the diagonal band of Broca [MS/VDB] (Ch 2). The basalocortical pathway projects to the entire neocortex and amygdala from the nucleus basalis magnocellularis and substantia innominata [NBM/SI] (Ch 4). The final BF projection pathway originates in the horizontal limb nucleus of the diagonal band of Broca [HDB] (Ch 3) and projects to the olfactory bulb and cortex (Mesulam, Mufson, Wainer, & Levey, 1983).
While the roles of both the basalocortical and septohippocampal pathways in cognition have been investigated, the basalocortical pathway has been studied most extensively in this regard due to its widespread projections to the neocortex and presumed importance to a broad range of cognitive functions. The septohippocampal BF pathway has been primarily implicated in hippocampal-dependent forms of learning and memory that involve the encoding and retrieval of environmental and spatial cues (Chang & Gold, 2004; Fritingsdorf, Thal, & Prizo, 2006; Janisiewicz, Jackson, Fiez, & Baxter, 2004; Pang & Nocera, 1999; Xu, Datta, Wu, & Alreja, 2004), while the basalocortical BF pathway has been shown to be important to numerous cognitive functions, including information encoding, attention, response strategy selection, cognitive flexibility, and socially transmitted food preference (Bailey, Rudisill, Hoof, & Loving, 2003; Berger-Sweeney, Stearns, Frick, Beard, & Baxter, 2000; Butt & Bowman, 2002; Butt, Noble, Rogers, & Rea, 2002; Gibbs & Johnson, 2007; Harati, Barbelivien, Cosquer, Majchrzak, & Casell, 2008; Lehmkuhl, Grottick, Casella, & Higgins, 2003; McGaughy, Dalley, Morrison, & Casell, 2008; Lehmkuhl, Grottick, Casella, & Higgins, 2003). Importantly, recent research suggests that disruption of the basalocortical BF pathway primarily results in attentional deficits that then translate into impairments on a variety of types of cognitive tasks (Dashniani, Burjanadze, Beselidze, & Tschakalidze, 2006; Sarter, Bruno, & Turchi, 2006; Sarter, Gehring, & Kozak, 2006; Sarter & Bruno, 2002; Zaborszky, & Duque, 2000). GABA is the most abundant inhibitory neurotransmitter in the central nervous system and GABAergic projections arising from the ventral striatum, nucleus accumbens, and the amygdala innervate the nuclei of both the basalocortical and septohippocampal pathways in the BF. The cholinergic neurons in the BF are co-localized with a substantial population of non-cholinergic neurons, γ-aminobutyric acid (GABAergic) neurons represent the main population of non-cholinergic neurons in the BF (Gritti, Mainville, & Jones, 1993; Gritti, Mainville, Manca, & Jones, 1997; Sarter & Bruno, 2002; Zaborszky & Duque, 2000).
septohippocampal and basolocortical BF pathways, the MS/VDB and NBM/SI, respectively (Zaborszky, Heimer, Eckenstein, & Leranth, 1986). While the influence of GABAergic neurotransmission in the septohippocampal pathway has been moderately investigated, revealing a critical role for GABAergic neurons in the MS/VDB in maintaining the hippocampal theta rhythm necessary for efficient sensory processing in the hippocampus (Dwyer, Servatius, & Pang, 2007; Hangya, Borhegyi, Szilagyi, Freund, & Varga, 2009), much less is known about the role of GABAergic neurotransmission in the basolocortical BF pathway.

GABAergic neurotransmission may critically influence cholinergic neurotransmission in the basolocortical BF pathway given that the number of GABAergic neurons outnumber the number of cholinergic neurons in the NBM by 2:1 (Gritti, Mainville, & Jones, 1993). While many of the GABAergic neurons in the NBM are locally-projecting interneurons that inhibit nearby cortically-projecting cholinergic neurons, there is a subpopulation of GABAergic neurons in the NBM that are cortically projecting and parallel the cortical innervation of the cholinergic basolocortical BF projection pathway (Freund & Meskenaite, 1992; Gritti, Mainville, Mancia, & Jones, 1997). These cortically-projecting GABAergic neurons from the NBM make widespread connections to the neocortex, mostly onto inhibitory interneurons that function to suppress excitatory neurotransmission in cortical pyramidal neurons, including cholinergic neurotransmission. This suggests that cortical GABAergic neurotransmission may play an important role in cognition given its potential to modulate cortical cholinergic activity.

Experiments in which GABA receptor agonists were injected into the NBM resulted in significant reductions of cortical cholinergic activity (Casamenti, Deffenu, Abbamondi, & Pepeu, 1986; Lamour, Dutar, Rascol, & Jobert, 1986; Wenk, 1984; Wood & Richard, 1982), confirming that cortical cholinergic projection neurons in the NBM are inhibited by GABA. Brain lesioning work in rodents (see introduction section entitled “Cholinergic Contributions to Learning and
“Memory” below) suggests that GABAergic neurons in the NBM may play an important role in memory given that indiscriminate destruction of NBM neurons impairs retrieval performance on numerous memory tasks (Berman, Crosland, Jenden, & Altman, 1988; Connor, Langlais, & Thal, 1991). Cholinergic activity can modulate GABAergic activity: muscarinic receptor agonists induce GABAergic post-synaptic currents in cortical pyramidal neurons, suggesting a reciprocal relationship between GABAergic and cholinergic activity in the neocortex (Kawaguchi, 1997).

Both types of GABAergic receptors, ionotropic GABA-A and metabotropic GABA-B, are distributed throughout the NBM (Chu, Albin, Young, & Penney, 1990). Research suggests that it may be via binding at GABA-B receptors that GABAergic input to the NBM influences cognitive functioning (Ogasawara et al., 1999). Most recently, an important role for cortically-projecting GABAergic neurons from the BF in disinhibiting cortical activity and creating a synchronous neuronal firing pattern that encodes the motivational salience of a stimulus has been proposed (Lin, Gervasoni, & Nicolelis, 2006; Lin & Nicolelis, 2008).

**Distribution and Function of the Cholinergic Receptors**

ACh acts on both fast-acting ionotopic nicotinic receptors as well as slower-acting metabotropic muscarinic receptors in the brain and periphery. Both types of receptors are important for cognitive processes (Leblond, Beaufort, Delerue, & Durkin, 2002; Mirza & Stolerman, 2000; Ruotsalainen, Miettinen, MacDonald, Koivisto, & Sirvio, 2000; Spinelli, Ballard, Feldon, Higgins, & Pryce, 2006). However, the density of muscarinic receptors in the target sites of the basolocortical and septohippocampal pathways of the BF is much greater than that of nicotinic receptors (Mirza & Stolerman, 2000), and muscarinic receptor agonists have been shown to
excite a higher proportion of cholinergic neurons in the basolocortical pathway than nicotinic receptor agonists (Lamour, Dutar, Rascol, & Jobert, 1986). Thus, much of the pharmacological research to date that has investigated the influence of ACh on cognition has employed cholinergic antagonists, such as scopolamine, which specifically block muscarinic receptors.

Five muscarinic receptor subtypes have been identified, M₁ through M₅, all of which are expressed in the central nervous system. In general, the M₁ subtype regulates cortical excitability, the M₂ and M₃ subtypes modulate glandular secretion and cardiac and smooth muscle contraction, and the M₄ and M₅ subtypes modulate dopaminergic neurotransmission (Bymaster, McKinzie, Felder, & Wess, 2003; Caulfield, 1983; Wess et al., 2003). The M₁ subtype is the most densely distributed muscarinic receptor subtype in the cortex and hippocampus, comprising approximately 50% of the total muscarinic receptors expressed there, followed by the M₂ (~ 15-20%), M₄ (~ 15-20%), M₃ (~ 10%), and the M₅ (~ 1%) receptor subtypes (Levey, 1996; Levey, Kitt, Simonds, Price, & Brann, 1991; Wei, Walton, Milici, & Buccafusco, 1994). Experiments to date suggest that activity at the M₁ receptor subtype may predominately mediate the cognitive functions of ACh (Fisher, 2008; Wess et al., 2003), although there is also some equivocal evidence to implicate the M₂ receptor subtype in cognition as well (Carey et al., 2001; Lachowicz et al., 2001).

Support for a role for the M₁ receptor subtype in cognition has come from non-human animal studies demonstrating that the M₁-selective receptor antagonist pirenzepine impairs performance on a variety of cognitive tasks, including passive avoidance and spatial learning (Caulfield, Higgins, & Straughan, 1983; Hagan, Jansen, & Broekkamp, 1987; Ohnuki & Nomura, 1996; Worms, Gueudet, Perio, & Soubrie, 1989). Furthermore, lesions to the rat BF resulted in an up-regulation of M₁ receptors in the neocortex in response to the lesion-induced reduction of
cholinergic afferentation of the neocortex (Schliebs, Rossner, & Bigl, 1996) and enhancing the function of the M<sub>1</sub> receptor subtype was found to reverse scopolamine-induced cognitive impairments in mice (Espinosa-Raya, Espinoza-Fonseca, Picazo, & Trujillo-Ferrara, 2007). Most recently, it was demonstrated that activation of M<sub>1</sub> receptors is necessary for cholinergic facilitation of performance on a cued-detection task in mice, likely due to M<sub>1</sub> receptor excitement of cortical pyramidal neurons (Gulledge, Bucci, Zhang, Matsui, & Yeh, 2009).

Studies using genetic knockout mice that lack the gene that encodes the M<sub>1</sub> receptor provide further evidence for an important role for the M<sub>1</sub> receptor subtype in cognition as such mice were found to be impaired on a variety of cognitive tasks, including nonmatching-to-sample and working memory tasks (Anagnostaras et al., 2003; Miyakawa, Yamada, Duttaroy, & Wess, 2001). M<sub>1</sub> receptor knockout mice also showed reduced cortical plasticity following learning (Zhang, Hamilton, Nathanson, & Yan, 2006), as well as reduced neural synchronization in the gamma frequency range in the hippocampus, which is thought to be critical for cortical information processing (Fisahn et al., 2002). In light of the importance of the M<sub>1</sub> receptor subtype to cognition, M<sub>1</sub> receptor agonists as a potential pharmacological treatment for Alzheimer’s disease have become the focus of many studies in the Alzheimer’s disease (AD) literature (Fisher, 2008). Post-mortem histological analyses of neural tissue revealed that there was significantly reduced density of M<sub>1</sub> receptors in the cortex of AD patients (Flynn, Ferrari-DiLeo, Mash, & Levey, 1995; Shiozaki, Iseki, Hino, & Kosaka, 2001) as well as reduced coupling of M<sub>1</sub> receptors to G-proteins in the frontal cortex, which was correlated with cognitive decline (Tsang et al., 2006). While hypofunction of the cholinergic system has already been implicated in the cognitive decline associated with AD (Coyle, Price, & DeLong, 1983; Kobayashi, Miyazu, Fukutani, Nakamura, & Yamaguchi, 1991; Schliebs & Arendt, 2006), these recent findings linking AD with a reduction in the density and functionality of cortical M<sub>1</sub> receptors.
receptors further elucidate how disruption of the cholinergic system may contribute to cognitive decline. Support for a therapeutic role for $M_1$ receptor agonists has come from non-human animal studies showing that $M_1$ receptor agonists rescued the cognitive deficits of transgenic AD mice as well as reduced the amount of extracellular $\beta$-amyloid (A$\beta$)-containing plaques and intracellular neurofibrillary tangles, the primary neuropathological hallmarks of AD, in the neocortex and hippocampus (Caccamo et al., 2006). The potential for $M_1$ receptor agonists to be disease-modifying drugs that reduce the neuropathological hallmarks purported to cause cognitive dysfunction sets them apart from acetylcholinesterase inhibitors, the current treatment for AD, which have only been found to modestly reduce the cognitive symptoms of the disease (Fisher, 2008).

**Cholinergic Contributions to Cognition: Pharmacological Methods**

Scopolamine, a non-selective cholinergic muscarinic receptor antagonist, has been used to investigate the importance of ACh to cognition and to develop a cholinergic deficiency model that could explain the deterioration of cognitive functioning characterized by neurodegenerative disorders. Scopolamine shows near-equivalent affinity for each of the five muscarinic receptor subtypes (Bolden, Cusack, & Richelson, 1992). Scopolamine administered systemically to human participants and non-human animals was originally found to induce global amnesia (Beatty, Butters, & Janowsky, 1986; Flood & Cherkin, 1986; Safer & Allen, 1971). Later studies revealed that scopolamine has a far greater detrimental impact on information encoding and attention than information retrieval (Aigner, Walker, & Mishkin, 1991; Anagnostaras, Maren, & Fanselow, 1995; Caine, Weingartner, Ludlow, Cudahy, & Wehry, 1981; Dunne & Hartley, 1986; Evans, 1975; Jones & Higgins, 1995; Kirk, White, & McNaughton, 1988; Richmond, Nichols,
Deacon, & Rawlins, 1997; Rush, 1988; White & Ruske, 2002). For instance, while pre-training administration of scopolamine at moderate doses (0.3-3.0 mg/kg) yielded robust impairments in task acquisition, these doses did not result in any significant retention deficits in rats that were injected with scopolamine following training on a passive avoidance test (Rush, 1988). Similar results showing no significant effect of moderate doses of scopolamine on retrieval of well-learned tasks have been found in non-human animals using a Y-maze brightness discrimination task (Moss, Rogers, Deutsch, & Salome, 1981), visual delayed nonmatching-to-sample task (Aigner, Walker, & Mishkin, 1991), 8-arm radial maze (Toumane & Durkin, 1993), odor detection task (Doty, Bagla, Misra, Mueller, & Kerr, 2003) and contextual fear conditioning task (Anagnostaras, Maren, & Fanselow, 1995), and in human participants using a delayed visual recognition task (Rosier, Cornette, & Orban, 1998).

Cholinergic muscarinic blockade with scopolamine disrupts attentional performance in both non-human (Callahan, Kinsora, Harbaugh, Reeder, & Davis, 1993; Davidson, Cutrell, & Marrocco, 1999; Jones & Higgins, 1995; McGaughy, Turchi, & Sarter, 1994) and human animals (Brandeis, Naylor, Halliday, Callaway, & Yano, 1992; Broks et al., 1988; Ellis et al., 2006; Green et al., 2005; Levy, Parasuraman, Greenwood, Dukoff, & Sunderland, 2000; Mintzer & Griffiths, 2003). Scopolamine administered to non-human animals prior to the performance of attention-dependent tasks, such as the five-choice-serial-reaction time task or the cued target detection task, significantly reduced target-detection accuracy, increased the rate of omissions, and increased correct latency (Davidson, Cutrell, & Marrocco, 1999; Jones & Higgins, 1995). Scopolamine’s detrimental impact on performance was exacerbated when the attentional demands of the task were increased by adding distracting white noise or reducing the intensity of the target stimulus (Jones & Higgins, 1995). In neurologically intact human participants, oral administration of scopolamine impaired vigilance, sustained attention, and selective attention,
and disrupted acquisition of visual and verbal information with minimal impairment in the retrieval of well-learned information (Beatty, Butters, & Janowsky, 1986; Broks et al., 1988; Ghoneim & Mewaldt, 1977).

**Cholinergic Contributions to Cognition: Brain Lesioning Methods**

Brain lesioning techniques have the advantage of reducing cholinergic neurotransmission in specific projection pathways of the BF by destroying the neurons that provide the source of the cholinergic input. In the non-human literature, there have been two primary lesioning techniques used to reduce cholinergic functioning in the brain: excitotoxic and immunotoxic lesions. While both types of lesions involve the injection of a chemical directly into a brain region of interest to destroy neurons, excitotoxic lesions utilize a non-selective excitotoxin that indiscriminately destroys neurons of all neurochemical types, while immunotoxic lesions utilize a cholinergic-selective neurotoxin that specifically targets and destroys cholinergic neurons. Prior to the development of such cholinergic-selective immunotoxins in the mid-1990s, the use of non-selective excitotoxins constituted the primary means to destroy cholinergic neurons in the BF (McGaughy, Everitt, Robbins, & Sarter, 2000). Excitotoxins, such as quisqualic or ibotenic acid work by inducing excitotoxic cell death through hyper-stimulation of glutamate receptors (Waite & Thal, 1996). When injected into a BF region, excitotoxic lesions destroy both cholinergic and GABAergic neurons, in turn reducing both cholinergic and GABAergic input to the cortical targets of the BF projection pathway. This makes it difficult to conclude whether any cognitive impairment found following lesioning is solely due to destruction of cholinergic cells or rather to the combined damage of different cell types.
The invention of the cholinergic-selective immunotoxin, 192 Immunoglobulin G-saporin (192 IgG-saporin) has allowed for the selective destruction of cholinergic neurons in the BF without the concurrent loss of neurons of other neurochemical type. This advancement in lesion specificity has made it possible to selectively reduce cholinergic input to the cortical targets of a particular BF projection pathway. 192-IgG is a monoclonal antibody to the low affinity p75 nerve growth factor (NGF) receptor that is located on the cell bodies and axon terminals of most cholinergic neurons (Waite et al., 1994). Only those cholinergic neurons that project to the amygdala and thalamus do not express this NGF receptor and they are thus spared by saporin injections (McGaughy, Everitt, Robbins, & Sarter, 2000). The NGF antibody (192-IgG) is coupled to the ribosome-inactivating protein, saporin, which enters the neuron via endocytosis, stops protein synthesis, and induces cell death (Waite et al., 1994).

**Cholinergic Contributions to Learning and Memory**

ACh is also critically involved in the structural and functional remodeling of cortical circuits necessary for learning in many mammalian species (Gu, 2002, 2003; Liljenstrom & Hasselmo, 1995). For instance, cholinergic agonists enhance long-term potentiation (LTP) in the hippocampus, piriform cortex, and neocortex (Blitzer, Gil, & Landau, 1990; Brocher, Artola, & Singer, 1992; Hasselmo & Barkai, 1995; Patil, Linster, Lubenov, & Hasselmo, 1998), reduce neuronal adaptation of cortical pyramidal neurons receiving afferent input such that they fire for longer in response incoming sensory stimulation (Hasselmo, Anderson, & Bower, 1992; Hasselmo, Schnell, & Barkai, 1995; Liljenstrom & Hasselmo, 1995; Vogt & Regehr, 2001), and suppress intrinsic excitatory neurotransmission within the cortex that may interfere with the encoding of afferent sensory input. Furthermore, human and non-human animal work has shown
that cholinergic input from the BF is critical for activity-dependent synaptic plasticity in the hippocampus, visual, somatosensory, auditory, and motor cortices (Baskerville, Schweitzer, & Herron, 1997; Dotigny, Ben Amor, Burke, & Vaucher, 2008; Gu, 2002, 2003; Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007; McKenna, Ashe, & Weinberger, 1989; Metherate, Cox, & Ashe, 1992). Metherate, Cox, and Ashe (1992) have shown that tetanic stimulation of the NBM in rats induces high frequency low amplitude neural oscillations in the cortex, and changes the discharge mode of cortical neurons from bursting activity to self-sustained spiking activity. Such self-sustained neural activity is believed to be necessary during the encoding of new information (Hasselmo & McGaughy, 2004). Interestingly, at least in the hippocampus and visual cortex, induction of synaptic plasticity may be mediated by the activation of M₁ muscarinic receptors (Gu & Singer, 1993; Luo et al., 2008; Shinoe, Matsui, Taketo, & Manabe, 2005). For instance, the M₁-selective muscarinic receptor antagonist pirenzepine abolished the normal shift in ocular dominance normally seen following monocular deprivation in cats (Gu & Singer, 1993).

As previously discussed, while systemic cholinergic muscarinic blockade with scopolamine can induce mnemonic deficits, its effects on information acquisition and attention are very robust (Aigner, Walker, & Mishkin, 1991; Anagnostaras, Maren, & Fanselow, 1995; Caine, Weingartner, Ludlow, Cudahy, & Wehry, 1981; Dunne & Hartley, 1986; Evans, 1975; Jones & Higgins, 1995; Kirk, White, & McNaughton, 1988; Richmond, Nichols, Deacon, & Rawlins, 1997; Rush, 1988; White & Ruske, 2002). Furthermore, while a role for the basalocortical BF projection pathway in memory was once strongly promoted, its role in memory has been called into question with the advent of the highly specific cholinergic immunotoxin, 192 IgG-saporin (Blockland, 1996; McGaughy, Everitt, Robbins, & Sarter, 2000). Studies using non-selective excitotoxins, which destroy all cell types, not just those that are cholinergic, have demonstrated deficits in working, reference, and spatial memory following their infusion into the NBM of non-
human animals (Altman, Crosland, Jenden, & Berman, 1985; Berman, Crosland, Jenden, & Altman, 1988; Connor, Langlais, & Thal, 1991; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Mayo, Kharouby, Le Moal, & Simon, 1988; Santucci & Haroutunian, 1989; Vale-Martinez et al., 2002). However, attempts at replicating such mnemonic impairments using cholinergic-selective immunotoxic lesions of the NBM with saporin have failed (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995; Baxter et al., 1996; Blockland, 1996; Dornan et al., 1997; Galani et al., 2002; McGaughy, Everitt, Robbins, & Sarter, 2000; Waite & Thal, 1996). This suggests that damage to non-cholinergic neurons in the NBM, such as GABAergic neurons, or the combined damage of cholinergic and non-cholinergic neurons may have been responsible for these excitotoxin-induced memory impairments. Evidence in support of this hypothesis has come from studies in which systemic administration of GABA receptor agonists reversed the scopolamine-induced mnemonic deficits of mice (Sharma & Kulkarni, 1993).

Encoding versus Retrieval Dissociation

The ability of ACh to induce a cortical synaptic state that enables neuroplasticity, enhances the detection and processing of incoming sensory information, and suppresses the processing of potentially interfering information makes it a likely candidate for facilitating information acquisition. In light of ACh’s influences on cortical synaptic circuits, a model of cholinergic function has been proposed that can account for the consistent findings from pharmacological and lesion work implicating ACh in information encoding, but not retrieval. This model proposes that ACh may play a role in switching the functional dynamics of the cortex from an information-encoding state to an information-retrieval state (Hasselmo & Bower, 1993). An active cholinergic system is conducive to the encoding of new information, while a hypoactive cholinergic system is conducive to the consolidation and retrieval of previously-learned
information (Hasslemo & McGaughy, 2004). When there are high levels of cholinergic activity in the cortex, afferent input from external sensory stimuli is enhanced, while intrinsic processing within cortices is reduced. Intrinsic processing within cortices is associated with the reactivation of neural connections representing previously-learned information. Such a reduction in intrinsic intracortical feedback due to high levels of ACh would thus prevent the recall of previously-learned information from interfering with the encoding of new information.

Conversely, when there are low levels of cholinergic activity in the cortex, the strength of afferent input from sensory stimuli is reduced, while the intrinsic intracortical processing of recently-encoded information is increased. Such a state allows for the consolidation of recently-encoded information into stable neural representations, and facilitates the retrieval of previously-learned information. In support of this model, scopolamine increased proactive interference from previously-learned odor- and word-pair associations during the encoding of novel associations in non-human and human animals, respectively (Atri et al., 2004; De Rosa & Hasselmo, 2000; De Rosa, Hasselmo, & Baxter, 2001). Furthermore, functional magnetic resonance imaging (fMRI) studies have shown that boosting cholinergic neurotransmission in humans with the anticholinesterase physostigmine improves performance of a visual working memory task by enhancing perceptual processing during the encoding stage (Furey, Pietrini, & Haxby, 2000). Conversely, administration of the muscarinic receptor antagonist scopolamine abolished repetition priming in a face recognition paradigm (Thiel, Henson, & Dolan, 2002), suggesting that sufficient cortical ACh levels are necessary for repeating faces to be encoded and neurally recognized as familiar.
**Cholinergic Contributions to Attention**

As noted above, the synaptic changes induced by cholinergic activity enhance the signal-to-noise ratio of cortical neurons such that they are primed to acquire information from the environment; this is similar to the psychological construct of attention. ACh’s role in modulating attention has been extensively studied in both human and non-human animals using pharmacological, brain lesioning, *in vivo* microdialysis, electrochemistry, and functional neuroimaging techniques, and these variety of techniques across species have consistently revealed a critical role for ACh in attention (Himmelheber, Sarter, & Bruno, 2000; Sarter & Bruno, 1997; Sarter, Bruno, & Givens, 2003; Sarter, Hasselmo, Bruno, & Givens, 2005; Thiel, 2003). Given that ACh boosts the neural signal of incoming sensory information, while suppressing the neural signal of information that is not currently relevant, it is likely to facilitate attentional processes that require the detection and processing of a stimulus in a complex environment that contains a substantial amount of potentially distracting information.

Non-human animal experiments that have disrupted the basalocortical BF projection pathway with cholinergic-selective immunotoxic lesions of the NBM using 192 IgG-saporin have revealed substantial post-surgical deficits in sustained, selective, and divided-attention tasks (Burk, Lowder, & Altemose, 2008; Butt, Noble, Rogers, & Rea, 2002; Chiba, Bucci, Holland, & Gallagher, 1995; Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; McGaughy, Kaiser, & Sarter, 1996; Risbrough, Bontempi, & Menzaghi, 2002; Turchi & Sarter, 1997). Importantly, the nature of the attentional deficits incurred is consistent with a reduction in the signal-to-noise ratio. For instance, bilateral saporin-induced lesions of the NBM/SI impaired the ability of rats to increase their attention to a salient conditioned stimulus, but not their ability to decrease their attention to a less salient conditioned
stimulus (Chiba, Bucci, Holland, & Gallagher, 1995). Furthermore, this same type of bilateral cholinergic-selective lesion of the NBM/SI significantly impaired the ability of rats to detect signals, but not their ability to correctly reject non-signals on a visual vigilance task (McGaughy, Kaiser, & Sarter, 1996). Such findings suggest that cholinergic deafferentation of the neocortex attenuates the neural signal of incoming sensory information, while disinhibiting the neural signals that represent irrelevant information, thereby disrupting attention.

Human neuropsychological and functional neuroimaging studies along with non-human animal lesion studies have implicated a frontoparietal cortical network in attentional processing (Behrmann, Geng, & Shomstein, 2004; Bucci, 2008; Constantinidis, 2006; Corbetta, 1998; Naghavi & Nyberg, 2005; Reep & Corwin, 2009; Wardak, Olivier, & Duhamel, 2004). In vivo microdialysis studies of freely behaving animals have revealed that cholinergic neurotransmission in frontoparietal cortical regions is important for attentional processing (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002; Himmelheber, Sarter, & Bruno, 1997, 2000, 2001; Kozak, Bruno, & Sarter, 2005; Passetti, Dalley, O'Connell, Everitt, & Robbins, 2000). Increasing the attentional demands of a visuospatial vigilance task by introducing a distracting stimulus resulted in a substantial increase in ACh efflux in the frontal and parietal cortices of rats (Himmelheber, Sarter, & Bruno, 2000), suggesting that high levels of cortical ACh are needed to support the increased attentional demands of the task. Importantly, while it was found that performance of low-attentional-load control tasks increased ACh efflux in the frontal and parietal cortices, performance of an attentionally-demanding task was associated with a much larger increase in cortical ACh release (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002). Such findings are consistent with the notion that sufficient cortical levels of ACh are necessary to boost afferent sensory input for learning in general, but much higher cortical ACh levels are needed for tasks that tax the attentional system. Furthermore, Orsetti, Casamenti, and Pepeu (1996) have
shown that while naïve rats acquiring an operant task show robust cortical ACh release, well-trained animals performing the same task show minimal cortical ACh release, which is consistent with the notion that low levels of ACh are associated with information retrieval (Hasselmo & McGaughy, 2004).

Lesion work as well as microelectrode recording studies have revealed that cholinergic input to frontoparietal cortices is critical for attentional processing in non-human animals (Broussard, Karelina, Sarter, & Givens, 2009; Broussard, Sarter, & Givens, 2006; Bucci, 2009; Bucci & Chess, 2005; Bucci, Holland, & Gallagher, 1998; Bucci & Macleod, 2007; Dalley et al., 2004; Maddux, Kerfoot, Chatterjee, & Holland, 2007). Injection of the cholinergic-selective immunotoxic 192 IgG-saporin directly into the ventromedial prefrontal cortex of rats resulted in cholinergic deafferentation to this area of the cortex and impaired performance on a visual attention task, decreasing the ability of rats to detect a visual target when the attentional demands of the task were increased (Dalley et al., 2004). Direct cortical injection of 192 IgG-saporin revealed that cholinergic deafferentation of the posterior parietal cortex impaired the ability of rats to increase attention to a conditioned stimulus following a change in its predictive value (Bucci, Holland, & Gallagher, 1998). Immediate early-gene expression in the posterior parietal cortex of rats increased when the predictive value of a conditioned stimulus was increased and more attention paid to it, further implicating this region in mediating incremental changes in attention (Bucci & Macleod, 2007).

Maddux, Kerfoot, Chatterjee, and Holland (2007) have dissociated the attentional function of cholinergic input to the medial prefrontal and posterior parietal cortices in rats. Cholinergic deafferentation of the medial prefrontal cortex impaired performance on a visual vigilance task, but did not impair rats’ ability to increase attention to changes in the predictive value of cues.
The opposite pattern of results was found for cholinergic deafferentation of the posterior parietal cortex. The authors contend that the frontal and parietal components of the frontoparietal cortical attentional network may mediate different aspects of attentional processing, with the former being more critically involved in the attentional guidance of action and the latter more critically involved in the allocation of attention to relevant stimuli for learning (Maddux, Kerfoot, Chatterjee, & Holland, 2007).

Consistent with this hypothesis, recent single-unit recording studies have shown that neuronal activity in the posterior parietal cortex predicts the allocation of attention to stimuli relevant to learning (Broussard, Karelina, Sarter, & Givens, 2009; Broussard, Sarter, & Givens, 2006), which is consistent with a recently proposed hypothesis that the posterior parietal cortex encodes the salience and relevance of stimuli in the environment (Gottlieb, 2007). In rats performing a visual sustained attention task, the correct detection of signals was predicted by increased neuronal activity in the posterior parietal cortex (Broussard, Sarter, & Givens, 2006), and this cue-detection-related activity was subsequently suppressed by injection of the cholinergic-selective immunotoxin 192 IgG-saporin directly into the posterior parietal cortex (Broussard, Karelina, Sarter, & Givens, 2009). Interestingly, following cholinergic deafferentation to the area, neuronal activity increased in the posterior parietal cortex in response to a distractor stimulus, while cue-detection-related activity was further suppressed, indicating a reduction in the signal-to-noise ratio (Broussard, Karelina, Sarter, & Givens, 2009).

Human fMRI studies provide further support for an important role for cortical cholinergic neurotransmission in modulating attention and activity in frontoparietal cortical networks. Administration of the cholinergic-enhancing drug physostigmine to neurologically intact human participants reduced response time and improved accuracy on a visual working memory task.
Such behavioural findings were accompanied by augmented activation in sensory cortices and decreased activation in frontoparietal cortical networks (Furey, Pietrini, Alexander, Schapiro, & Horwitz, 2000; Furey, Pietrini, & Haxby, 2000; Furey, Ricciardi, Schapiro, Rapoport, & Pietrini, 2008). In light of ACh’s established role in modulating the signal-to-noise ratio, these neuroimaging findings suggest that increased cortical ACh levels boosted the neural signal of task-relevant visual information during encoding, while suppressing the neural signal of task-irrelevant information, thereby facilitating attention and reducing demands on the frontoparietal cortical network.

**Cholinergic Hypothesis of Feature Binding**

In light of ACh’s established role in modulating attention, it can be inferred that ACh may also be important for feature binding given the attention-dependent nature of this cognitive process and the potential facilitation a boost of the signal-to-noise ratio would have on the binding of features. We know that attention must be deployed to a relevant object in the environment for its features to be bound, and it could be that cortical cholinergic neurotransmission supports the attentional processes needed for feature binding by enhancing the neural signal of a relevant stimulus and its features. While there is no direct evidence implicating ACh in feature binding, indirect evidence to support a cholinergic feature binding hypothesis can be gleaned from neuropsychological studies with Alzheimer’s disease (AD) patients, pharmacological work with neurologically intact human participants, and pharmacological and lesion work with non-human animals (Butt, Noble, Rogers, & Rea, 2002; Foster, Behrmann, & Stuss, 1999; Rodriguez, Kallenbach, Singer, & Munk, 2004; Tales et al., 2002).
AD is associated with neurodegeneration of cholinergic nuclei in the BF resulting in a hypoactive cholinergic system and reduced cholinergic input to the neocortex, including frontoparietal cortical regions critical for attention (Schliebs & Arendt, 2006). Attentional processing is one of the primary domains of cognitive functioning that is disrupted in AD and such patients are impaired on a variety of attentional tasks (Perry & Hodges, 1999). Importantly, recent work has revealed that AD patients are impaired on the conjunctive, but not feature search trials of visual search, only the former of which require feature binding (Foster, Behrmann, & Stuss, 1999; Tales et al., 2002). AD patients made more errors and took significantly longer to locate targets during conjunctive search trials, but were unimpaired relative to age-matched control participants on feature search trials (Foster, Behrmann, & Stuss, 1999) even when the difficulty of the feature search trials was increased by reducing the discriminability of the stimuli (Tales et al., 2002). There was a linear relationship between the number of distractor stimuli present and the reaction time for all participants on conjunction trials, but the slope of the regression line was significantly greater for AD patients than control participants. This increase in slope was found to be greater than that expected by age-related cognitive slowing (Foster, Behrmann, & Stuss, 1999). Thus, similar to patients with attentional deficits due to parietal cortex damage, AD patients are impaired at feature binding suggesting that cortical cholinergic neurotransmission may be important for this cognitive process. Furthermore, functional neuroimaging work has revealed reduced activation in frontoparietal cortical regions of Alzheimer’s patients performing conjunctive, but not feature search trials of visual search (Hao et al., 2005).

Pharmacological work with neurologically intact human participants has shown that indirectly boosting the cholinergic system with caffeine, a chemical that blocks cortical adenosine receptors that normally inhibit ACh release, improves visual feature binding (Colzato, Fagioli,
Erasmus, & Hommel, 2005). Such a facilitation in feature binding performance was not found following transdermal administration of nicotine, suggesting that increased binding at muscarinic cholinergic receptors may be mediating caffeine’s facilitatory effect on feature binding (Colzato et al., 2005). Indirect suppression of the cholinergic system with moderate alcohol consumption resulted in an impairment in visual feature binding (Colzato, Erasmus, & Hommel, 2004). However, given that both caffeine and alcohol have effects on multiple neurotransmitter systems it is not possible to conclude from these studies that modulation of cholinergic activity directly affected feature binding performance.

Non-human animal work has shown that the muscarinic cholinergic system modulates gamma frequency neural synchronization in the cortex (Rodriguez, Kallenbach, Singer, & Munk, 2004), which, according to the neural synchronization hypothesis, is necessary for feature binding. Multi-unit recordings were taken from neuronal clusters of the visual cortex while cats viewed synchrony-inducing light stimuli (moving gratings presented for 4500 ms and repeated every 10 sec). When the muscarinic receptor antagonist scopolamine was injected directly into the visual cortex, there was an immediate and significant reduction in the occurrence and strength of gamma frequency oscillations and action potential synchronization. Conversely, when the cholinergic receptor agonist carbachol was injected, there was delayed but significant long-term facilitation of light-stimulus-induced gamma frequency oscillations and synchronization in the visual cortex. The incidence and strength of gamma frequency oscillations and neural synchronization doubled following carbachol administration. Such findings support the hypothesis that ACh plays a role in the temporal synchronization of neural responses in the cortex, which may be necessary for feature binding.
Lastly, Butt, Noble, Rogers, and Rea (2002) demonstrated that bilateral cholinergic-selective immunotoxin 192 IgG-saporin lesions of the NBM impaired the ability of rats to acquire a configural association learning task that required crossmodal sensory integration, while sparing acquisition of a simple discrimination task not requiring crossmodal sensory integration. In the simple discrimination task, rats were reinforced with food for lever pressing during the presentation of light (L+), and not reinforced for lever pressing during the presentation of a tone (T-). In the configural association task, rats were reinforced for lever pressing during the presentation of either the L+ or T+ was presented alone, but were not reinforced for lever pressing when both stimuli were presented simultaneously (LT-). Although crossmodal integration between the visual and auditory sensory domains was required for rats to learn the configural association task, the auditory and visual cues (light and tone) presented were separate stimuli, rather than features of a single stimulus. Thus, the task was not a true test of feature binding, and the authors do not implicate a feature binding impairment as the cause of the configural association learning decrement. Rather, they suggest that reduction of cortical cholinergic neurotransmission impaired the ability of rats to divide attention between the two stimulus modalities of the compound. Nonetheless, this study does provide evidence to suggest that cortical cholinergic neurotransmission is important for sensory integration, a necessary component of feature binding.

**Cholinergic Influences on Crossmodal Feature Binding in Rats**

A rodent model of attention using feature binding would allow for a direct investigation of ACh’s contribution to this attention-dependent cognitive process given the availability of techniques that can precisely manipulate the functioning of the cholinergic system. Thus, to
determine whether one role of ACh in attention is to facilitate feature binding, we designed a novel feature binding task, faithful to the Feature Integration Theory (Treisman & Gelade, 1980), for rats that utilized crossmodal stimuli and capitalized on rats’ natural tendency to dig for food (Botly & De Rosa, 2007). In this task, rats were simultaneously presented with two digging bowls on every trial: a crossmodal odor-texture bowl that was covered with a texture and scented with an odor, and a blank bowl with no odor or texture components. We used these two types of digging bowls, odor-texture or blank, to create a forced-choice paradigm with two trial types: Target and Distractor. On Target trials, a sweet cereal reward was always found in the odor-texture bowl, not the blank bowl, while on Distractor trials, a sweet cereal reward was always found in the blank bowl, not the odor-texture bowl. Rats had to use the crossmodal features of the presented odor-texture bowl to determine which bowl to dig in to obtain a food reward.

We designed two different tasks using forced-choice digging, a Feature-Conjunction (FC) task that required crossmodal feature binding and a Feature-Singleton (FS) task that did not require feature binding. In the FC task, the crossmodal features of the Target and Distractor bowls overlapped such that each individual odor and texture was associated with both a Target and a Distractor bowl. Thus, no single odor or texture could be used for correct bowl selection and rats had to feature bind to determine the correct bowl choice. Conversely, in the FS task, the crossmodal features of the Target and Distractor bowls did not overlap such that each individual odor and texture was associated with either a Target or a Distractor bowl. Thus, feature binding was not required for correct bowl selection and rats could use a single odor or texture or the distinct combination of the two features to determine the correct bowl choice. The learning-based nature of this feature binding task (i.e., acquisition of stimulus sets across sessions of training) allowed us to dissociate the encoding and retrieval stages of feature binding, which to our knowledge had not been done before in the human cognitive literature.
Using a within-subjects pharmacological design, we tested rats’ ability to acquire and retrieve the FC task as well as to acquire the FS task under the muscarinic receptor antagonist, scopolamine (0.2 mg/kg), relative to two control drug conditions, methylscopolamine (0.2 mg/kg), a peripheral muscarinic receptor antagonist that does not readily cross into the brain, and physiological saline, an injection control. Relative to the two control drug conditions, scopolamine selectively impaired the ability of rats to acquire the crossmodal FC task, but not their ability to acquire the crossmodal FS task. In addition, scopolamine left the retrieval of previously-learned FC stimuli intact (Botly & De Rosa, 2007). These findings indicate that muscarinic receptors in the brain are needed in crossmodal feature binding at the encoding stage. Given the highly attention-dependent nature of feature binding and the well-established role of ACh in modulating attention, we proposed that muscarinic cholinergic neurotransmission is necessary for feature binding at encoding because it supports the attentional processes needed to form conjunctive stimuli. In support of this contention, rats under the influence of scopolamine were not impaired at acquiring the FS task. Neuropsychological and neuroimaging work with human participants also shows that FS tasks are much less attentionally demanding than FC tasks (Bernstein & Robertson, 1998; Corbetta, Shulman, Miezin, & Petersen, 1995; Foster, Behrmann, & Stuss, 1999; Treisman & Gelade, 1980).

Our retrieval findings are consistent with a well-supported model of cholinergic function proposing that high levels of cortical ACh set the stage for encoding, whereas low cortical ACh levels set the stage for retrieval (Hasselmo & McGaughy, 2004). They further suggest that once a conjunctive stimulus is well learned, a bound and stable neural representation is formed in memory, reducing the need for an attentionally-demanding feature binding process during retrieval. This agrees with human cognitive research showing that disrupting attention selectively
impairs the encoding, relative to the retrieval, of episodic memories (Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Naveh-Benjamin, Craik, Guez, & Dori, 1998).

A Cross-species Examination of Cholinergic Influences on Feature Binding

The following series of experiments set out to further investigate a functional role for the cholinergic system in feature binding using the digging task discussed above and a human visual analog of the rat task (Experiments 1-3), as well as a rat analog of the human visual search paradigm (Experiment 4).

Experiment 1

Using scopolamine and the digging-based rat feature binding task, we have previously demonstrated that ACh acting at muscarinic receptors in the brain is critical for crossmodal feature binding during the encoding stage, and we proposed that it was through an attentional mechanism that ACh facilitated the formation of conjunctive stimuli (Botly & De Rosa, 2007). If blockade of the muscarinic cholinergic system with scopolamine impairs feature binding at encoding by disrupting attention, then an attentional challenge alone should result in a similar feature binding impairment.

To test this attentional hypothesis and to translate our rodent feature binding task for use with humans, we designed intramodal versions of the digging-based FC and FS tasks for rats using odors and an intramodal analog for humans using coloured shapes. As feature binding is traditionally assessed intramodally in humans, FC and FS tasks using intramodal stimuli would provide a stronger test of our hypothesis and would also test the validity of our rodent feature binding paradigm in the intramodal sensory domain. We conducted a cross-species study to
assess whether muscarinic cholinergic blockade with scopolamine in rats results in a similar impairment in intramodal feature binding in neurologically intact human participants when their attention is divided. During performance of the FC and FS tasks, we challenged the cholinergic system of rats (Experiment 1A) using the muscarinic receptor antagonist scopolamine and challenged the attentional system of humans (Experiment 1B) using a concurrent task. We predicted that both manipulations would impair acquisition of the FC task, leaving retrieval of the FC task and acquisition the FS task relatively intact. Further support for our prediction of intact FC retrieval performance in human participants comes from research showing that disrupting attention is selectively detrimental to the encoding, relative to the retrieval, of episodic memories in humans (Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Logie, Della Sala, MacPherson, & Cooper, 2007; Naveh-Benjamin, Craik, Guez, & Dori, 1998; Naveh-Benjamin, Craik, Perretta, & Tonev, 2000; Naveh-Benjamin, Kilb, & Fisher, 2006), suggesting that minimal attentional resources are required for information retrieval.

**Experiment 2**

While the human cognitive literature has yielded substantial insights into the cognitive mechanisms and broad neocortical systems involved in feature binding, the specific neuroanatomical contributions to feature binding remain unknown. Patients with attentional impairments are impaired at FC, but not FS tasks (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Tales et al., 2002), and functional neuroimaging work has implicated frontoparietal cortical networks in feature binding (Constantinidis, 2006; Corbetta, Shulman, Miezin, & Petersen, 1995; Esterman, Verstynen, & Robertson, 2007; Luck & Ford, 1998; Reynolds & Desimone, 1999).
We hypothesized that the NBM of the BF would be critical for feature binding given its demonstrated importance to a variety of forms of attention, including sustained, selective, and divided attention (Butt, Noble, Rogers, & Rea, 2002; Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; Pang, Williams, Egeth, & Olton, 1993), and its widespread afferent projections to regions of the neocortex important for attentional processing, including the frontal and parietal cortices (Mesulam, Mufson, Wainer, & Levey, 1983). To determine whether the NBM plays an important functional role in feature binding, we bilaterally lesioned the NBM of rats using the excitotoxin quisqualic acid, and compared the ability of NBM-lesioned rats to that of sham-lesioned rats to: (1) retrieve a FC stimulus set learned prior to surgery; (2) acquire a novel FC stimulus set, and (3) acquire a FS stimulus set using the crossmodal digging-based task previously discussed (Botly & De Rosa, 2007).

We predicted that NBM lesions would impair the ability of rats to acquire the attentionally-demanding FC task, while sparing their ability to acquire the FS task. However, whether excitotoxic NBM lesions would spare the ability of rats to retrieve a set of FC stimuli learned prior to surgery was not clear. Quisqualic acid is a non-selective excitotoxin that indiscriminately destroys neurons irrespective of neurochemical type. When injected into the NBM, where cholinergic neurons are co-localized with a substantial population of GABAergic neurons, quisqualic acid destroys both cholinergic and GABAergic neurons, in turn reducing both cholinergic and GABAergic input to the cortical targets of the basalo-cortical BF projection pathway. While there is considerable evidence to suggest that ACh is not necessary for information retrieval (Hasselmo & McGaughy, 2004), previous work with non-selective excitotoxins has revealed mnemonic deficits following their injection into the NBM (Altman, Crosland, Jenden, & Berman, 1985; Berman, Crosland, Jenden, & Altman, 1988; Connor,
Langlais, & Thal, 1991; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Mayo, Kharouby, Le Moal, & Simon, 1988; Santucci & Haroutunian, 1989; Vale-Martinez et al., 2002). Importantly, there is evidence to suggest that this may be due to the destruction of GABAergic neurons in the NBM (Sharma & Kulkarni, 1993). Thus, a FC retrieval deficit following quisqualic acid-induced lesions of the NBM was a possible result.

**Experiment 3**

While our previous pharmacological work with scopolamine has implicated ACh acting on muscarinic receptors in feature binding at encoding (Botly & De Rosa, 2007), the neural source of the critical cholinergic neurotransmission has yet to be identified. While Experiment 2 set out to investigate a functional role for the NBM of the BF in feature binding irrespective of its neurochemistry, the aim of Experiment 3 was to determine whether it is specifically cholinergic input to the neocortex from the NBM that is critical for feature binding, and whether such cortical cholinergic input is essential only for the encoding stage of feature binding as suggested by our previous crossmodal pharmacological work (Botly & De Rosa, 2007). Basalocortical cholinergic projections from the NBM provide 90% of the cholinergic input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983), including frontoparietal cortical regions implicated in attentional processing (Behrmann, Geng, & Shomstein, 2004; Bucci, 2008; Bucci & Chess, 2005; Constantinidis, 2006; Donner et al., 2002). Cholinergic-selective lesions of the NBM have been shown to impair the performance of non-human animals on a variety of attention-dependent tasks (Chiba, Bucci, Holland, & Gallagher, 1995; Chiba, Bushnell, Oshiro, & Gallagher, 1999; Lehmann, Grottick, Cassel, & Higgins, 2003; McGaughy, Kaiser, & Sarter, 1996), and increased ACh efflux in frontoparietal cortical regions is correlated with increased attentional effort (Himmelheber, Sarter, & Bruno, 2000; Pepeu & Giovannini, 2004). We thus hypothesized that
the NBM of the BF may provide cholinergic input to the cortex that is critical for feature binding.

To test this hypothesis, we bilaterally lesioned the NBM of rats using the cholinergic-selective immunotoxin, 192 IgG-saporin, and compared the ability of ACh-NBM-lesioned rats to that of sham-lesioned rats to: (1) retrieve a FC stimulus set learned prior to surgery; (2) acquire a novel FC stimulus set, and (3) acquire two FS stimulus sets, one of greater difficulty than the other, but neither requiring feature binding, using the crossmodal digging-based task previously discussed and employed (Botly & De Rosa, 2007).

If cortical cholinergic neurotransmission is necessary to support the attentional processes needed for feature binding, then reducing cholinergic afferentation of the neocortex via cholinergic-selective lesions of the NBM should impair the ability of rats to acquire the attentionally-demanding FC task, while sparing their ability to acquire the FS tasks. We also predicted that ACh-NBM-lesioned rats would not be impaired at retrieving a previously-learned set of FC stimuli, which would be consistent with our previous pharmacological findings (Botly & De Rosa, 2007), and with a well-supported model of cholinergic function implicating ACh in information encoding, but not retrieval (Hasselmo & McGaughy, 2004).

While the FS and FC tasks both required rats to discriminate between four odor-texture bowls, the FS task presented rats with twice the amount of feature information (4 odors and 4 textures) than the FC task (2 odors and 2 textures) because each odor-texture FS bowl was characterized by a distinct odor and texture with no overlap across bowls. However, despite the greater amount of feature information associated with the FS task, it could still be argued that any unimpaired acquisition of the FS task by ACh-NBM-lesioned rats was simply due to the FS task being less difficult than the FC task given the lack of feature overlap across odor-texture FS bowls. To
address this potential criticism, rats acquired a FS Enhanced-Difficulty stimulus set in addition to acquiring a FS stimulus set. Although both FS tasks did not require feature binding, as rats could rely on a single feature (odor or texture) for correct bowl selection, the FS Enhanced-Difficulty task also required rats to learn when to rely on odor and when to rely on texture as one odor and one texture were associated with both the correct and incorrect bowl choices, resulting in partial feature overlap across the odor-texture bowls. While we predicted that both ACh-NBM-lesioned and sham-lesioned rats would not perform as well on the FS Enhanced-Difficulty task relative to the FS task, we did not anticipate a lesion effect given that feature binding was not a requirement of the FS or FS Enhanced-Difficulty tasks, which should have kept cholinergically-mediated attentional demands relatively low during acquisition.

*Experiment 4*

If ACh is critical for feature binding, as suggested by our initial pharmacological work with scopolamine and the odor-texture digging-based feature binding task (Botly & De Rosa, 2007), then its modulatory influence on this cognitive process should generalize to other tests of feature binding. As previously discussed, in the human cognitive literature the standard test of feature binding is the visual search task (see introductory section). A rodent analog of the human visual search paradigm would allow for an important test of our hypothesis that cholinergic input to the neocortex from the NBM of the BF is important for feature binding. While visual search tasks comparable to those employed in the human cognitive literature have been designed and successfully tested in pigeons (Blough, 1984) and monkeys (Latto, 1971), to our knowledge visual search has not been attempted with rats. Despite olfaction being the dominant sensory modality of rodents, studies have demonstrated that rats of the Long-Evans strain possess the visual acuity necessary to make fine achromatic visual discriminations based on shape, pattern,
and orientation (Bushnell, 1999; Prusky, Harker, Douglas, & Wishaw, 2002), and the use of touchscreen-based cognitive assessment in rodents is increasing in the behavioural neuroscience literature (Bussey et al., 2008; Bussey, Saksida, & Rothblat, 2001; Cook, Geller, Zhang, & Gowda, 2004; Markham, Butt, & Dougher, 1996). Based on this existing research, we predicted that rats would be capable of performing a touchscreen-based visual search task.

In this experiment, rats were trained to perform a touchscreen-based visual search task using set-sizes of four, six, and eight stimuli. Each session was counterbalanced for target and distractor positions, trial-type (Conjunctive or Feature Search trials), and stimulus set-size. The target stimulus was always a white square, and the distractor stimuli were a black square, white triangle, and black triangle. On Conjunctive Search trials, the target stimulus was presented along with distractor stimuli that differed from the target on two feature dimensions, shape and pattern, such that feature binding was required to locate the target. On Feature Search trials, the target stimulus was presented along with distractor stimuli that differed from the target on only a single feature dimension, shape or pattern, such that feature binding was not required to locate the target. It was predicted that on the visual search task, rats’ latency to locate the target stimulus would increase as the stimulus set-size increased on Conjunctive, but not Feature Search trials, as previously found in humans (Treisman & Gelade, 1980).

Following pre-surgical acquisition of the visual search task, we bilaterally lesioned the NBM of rats using the cholinergic-selective immunotoxin, 192 IgG-saporin, and compared the ability of ACh-NBM-lesioned rats to that of sham-lesioned rats to perform the visual search task using the same target and distractor stimuli rats were trained on prior to surgery. If cortical cholinergic neurotransmission is necessary to support the attentional processes needed for feature binding, then reducing cholinergic afferentation of the neocortex via cholinergic-selective lesions of the
NBM should impair performance on visual search. Relative to sham-lesioned controls, ACh-NBM-lesioned rats were expected to take longer to locate the target stimulus on Conjunctive, but not Feature Search trials, as only the former require an attentionally-demanding feature binding process. It was also predicted that any lesion-induced increase in conjunctive search latency would significantly interact with stimulus set-size, such that the detrimental impact of cholinergic-selective NBM lesions on search latency would be greatest during Conjunctive Search trials with the largest number of distractor stimuli present.

While the digging-based feature binding tasks employed in Experiments 1-3 allowed us to dissociate the encoding and retrieval stages of feature binding (Botly & De Rosa, 2008, 2009a, 2009b), the visual search paradigm is characterized by negligible mnemonic demands. The locations of the target and distractor stimuli change unpredictably across trials, such that a “visual search” for the target is always necessary. Because the identity of the target stimulus remains constant across all trials and sessions, no other information must be held in memory, making visual search tasks a relatively pure test of the attentional demands of feature binding.

Consistent with the model that high levels of ACh are not needed for the retrieval of previously-learned information (Hasselmo & McGaughy, 2004), our previous work has shown that rats are unimpaired at retrieving well-learned crossmodal conjunctive stimuli following systemic muscarinic blockade with scopolamine (Botly & De Rosa, 2007). Given that the target and distractor stimuli were well learned by rats prior to surgery, we thus did not anticipate a difference in the ability of sham-lesioned and ACh-NMB-lesioned rats to accurately locate the target stimulus on Feature or Conjunctive Search trials of visual search post surgery.
Chapter 1

Experiment 1:

A cross-species investigation of acetylcholine, attention, and feature binding

*Contents of this chapter have been published in Psychological Science: Botly, L.C.P. & De Rosa, E. (2008). A cross-species investigation of acetylcholine, attention, and feature binding. Psychological Science, 19(11), 1185-1193.*
Abstract

The binding problem is the brain's fundamental challenge to integrate sensory information to form a unified representation of a stimulus. A recent non-human animal model suggests that acetylcholine serves as the neuromodulatory substrate for feature binding. We hypothesized that this animal model of cholinergic contributions to feature binding may be an analog of human attention. To test this hypothesis, we conducted a cross-species study in which rats and humans learned comparable intramodal feature-conjunction (FC) and feature-singleton (FS) tasks. We challenged the cholinergic system of rats using the muscarinic receptor antagonist scopolamine (0.2 mg/kg) and challenged the attentional system of humans by dividing attention. The two manipulations yielded strikingly similar patterns of behavior, impairing FC acquisition, while sparing FS acquisition and FC retrieval. These cross-species findings support the hypothesis that cholinergically driven attentional processes are essential to feature binding at encoding, but are not required for retrieval of neural representations of bound stimuli.
1.1 Experiment 1A

Introduction

The mammalian brain is organized in a modular fashion such that distinct regions are primarily responsible for processing the different features of a stimulus, such as its colour and shape. Feature binding, the cognitive process by which a unified neural representation of a stimulus is formed, has been shown to depend on attention (Treisman & Gelade, 1980). Although feature-singleton (FS) tasks require only the processing of single features, feature-conjunction (FC) tasks require the binding of multiple features. Patients with attentional impairments are impaired at FC, but not FS, tasks (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Tales et al., 2002), and functional magnetic resonance imaging (fMRI) studies have implicated frontoparietal cortical networks in feature binding (Corbetta, Shulman, Miezin, & Petersen, 1995; Luck & Ford, 1998; Reynolds & Desimone, 1999; Treisman, 1998).

Although the cognitive mechanisms and functional neuroanatomy of feature binding have been well examined in the literature on human cognition, the neurochemistry of feature binding remains unknown. Research on non-human animals suggests that the neurotransmitter acetylcholine (ACh) may be critical to feature binding given its presumed role in modulating attention (Sarter, Hasselmo, Bruno, & Givens, 2005). Recent work showed that the muscarinic receptor antagonist scopolamine selectively impaired the ability of rats to learn a crossmodal odor-texture FC task, but not their ability to learn an FS task. In addition, scopolamine left the retrieval of previously learned FC stimuli intact (Botly & De Rosa, 2007). Such an encoding-retrieval dissociation is consistent with a model in which high cortical ACh levels set the stage
for encoding, whereas low cortical ACh levels set the stage for retrieval (Hasselmo & McGaughy, 2004).

We hypothesized that this animal model of cholinergic contributions to feature binding may be an analog of human attention. As feature binding is traditionally assessed intramodally in humans, a test with intramodal stimuli would provide a stronger test of this hypothesis. Accordingly, we designed intramodal versions of the FC and FS tasks for rats (using odors) and a analog for humans (using coloured shapes), and conducted a cross-species study to assess whether muscarinic cholinergic blockade in rats is an appropriate model for impairment in human attention.

During performance of the FC and FS tasks, we challenged the cholinergic system of rats (Experiment 1A) using the muscarinic receptor antagonist scopolamine and challenged the attentional system of humans (Experiment 1B) using a concurrent task. If the animal model of cholinergic contributions to feature binding is analogous to human attention, then challenging the attention of human participants behaviourally with a divided-attention task and challenging the cholinergic system of rats with scopolamine should result in similar effects on feature binding. We predicted that both manipulations would impair acquisition of FC stimuli, leaving acquisition of FS stimuli relatively intact. In addition, our hypothesis predicted a novel behavioural dissociation in feature binding performance in humans: Under diminished attention, encoding of FC stimuli should be impaired, but retrieval of previously bound FC stimuli should remain intact.

Methods

Participants. Eight experimentally naive adult male Long-Evans rats (Charles River, Quebec, Canada) were maintained at 90% of ad libitum free-feeding weight for the duration of the
experiment. This study was approved by the University of Toronto’s Institutional Animal Care Committee.

Stimuli. The stimuli were presented in bowls containing the granular commercial bedding Bed-o’cobs (The Andersons, Maumee, OH). At the bottom of each odor-odor bowl was a small metal cap with small holes. Each metal cap contained cotton gauze, which was injected with 0.1 ml of the appropriate scented mineral oil each day (Aveda®, Blain, MN; The Body Shop®, Wake Forest, NC). This oil constituted one odor stimulus. The second odor stimulus was provided by mixing ground herb or spice with the bedding and filling the bowl with this mixture. Table 1 lists the odorants from which the experimental stimuli were created.

On each trial, rats were simultaneously presented with two digging bowls in a testing arena: an odor-odor bowl and a blank bowl containing Bed-o’cobs bedding (Figure 1). On target trials, the reward (half piece of Kellogg's Froot Loops cereal) was buried in the odor-odor bowl, and on distractor trials, the reward was buried in the blank bowl. Finely ground pieces of Froot Loops cereal were added to the bedding of all bowls to mask the location of the food reward.

Each Feature-Conjunction (FC) stimulus set contained four conjunction odor-odor bowls, along with the blank bowl. Two of the odor-odor bowls were designated target bowls (T1 and T2) and were presented on target trials. The remaining two odor-odor bowls were designated distractor bowls (D1 and D2) and were presented on distractor trials. In a forced-choice design, rats were allowed only one bowl choice on each trial. Binding of odors was required to determine the correct bowl choice as each individual odor was associated with either the target or the distractor, depending on its feature pairing, and the different pairings occurred equally often across trials. That is, each odor was used in one target bowl and one distractor bowl, such that no single odor could be used to select the correct bowl (Figure 2).
Each Feature-Singleton (FS) stimulus set contained four nonconjunction odor-odor bowls, along with the blank bowl. Two of the odor-odor bowls were designated target bowls (T1 and T2), and the remaining two were designated distractor bowls (D1 and D2). Feature binding was not required to determine the correct bowl choice as each odor-odor bowl was characterized by two distinct odors. That is, rats could use a single odor or the distinct combination of two odors to select the correct bowl (Figure 2).

Pharmacological Manipulation. A within-subjects pharmacological design was used. Each rat participated in each of two drug conditions: scopolamine hydrobromide (0.2 mg/kg dissolved in sterile 0.9% physiological saline, pH = 7.4) and physiological saline (injection control). Rats were given an intraperitoneal injection 15 min prior to testing, and experimenters were blind to the drug condition. Given that rats in our previous crossmodal study performed comparably under the influence of the peripheral antagonist methylscopolamine (a control for the peripheral effects of scopolamine) and when injected with saline (Botly & De Rosa, 2007), we used only saline and scopolamine in the present study to best equate the drug conditions with the full- and divided-attention conditions used with the human participants in Experiment 2.

Training Procedure. Each session consisted of 12 trials, half of which were target trials (3 T1, 3 T2), and half of which were distractor trials (3 D1, 3 D2). Within a session, trials were presented in a pseudorandom order, such that no more than 3 consecutive trials were of the same type (target or distractor). First, the rats learned an initial FC stimulus set, which we refer to as the learning-to-learn set, without injections. The rats received one session per day until all rats reached a criterion of at least 5 out of 6 correct responses on target trials and 5 out of 6 correct responses on distractor trials for at least two nonconsecutive sessions. After acquiring the learning-to-learn set to criterion, the rats retrieved these same FC stimuli for two sessions under
each drug condition, given the rats' excellent retrieval performance. Next, the rats acquired novel FC stimuli under each drug condition until rats under the influence of scopolamine reached asymptotic performance, which took nine sessions. Finally, the rats acquired novel FS stimuli under each drug condition until rats under the influence of scopolamine reached asymptotic performance, which took six sessions. The sequence of drug conditions was always counterbalanced across rats.

Results

Statistical Analyses. Task accuracy was assessed using proportion of correct responses. For statistical analyses, acquisition data were binned into three-session blocks and retrieval data remained unblocked. All statistical analyses were conducted using SPSS Version 14 with an alpha level of .05.

Acquisition of FC Stimuli. Figure 3A depicts the results for FC acquisition. A two-way repeated measures analysis of variance (ANOVA) using drug condition (saline or scopolamine) and block as within-subjects factors revealed significant main effects of drug condition, F(1, 7) = 21.29, \( p < .01 \), \( \eta^2 = .75 \), and block, F(2, 14) = 28.90, \( p < .001 \), \( \eta^2 = .81 \), and a significant interaction, F(2, 14) = 4.92, \( p < .05 \), \( \eta^2 = .41 \). Within-subjects simple contrasts revealed that accuracy differed significantly between the saline and scopolamine conditions during all three blocks of acquisition (\( p < .05 \), \( \eta^2 = .49 \); \( p < .01 \), \( \eta^2 = .84 \); and \( p < .01 \), \( \eta^2 = .70 \), respectively).

Acquisition of FS Stimuli. Figure 3B depicts the results for FS acquisition. A two-way repeated measures ANOVA using drug condition (saline or scopolamine) and block as within-subjects factors revealed a significant main effect of block, F(2, 14) = 23.64, \( p < .001 \), \( \eta^2 = .77 \), but no significant effect of drug condition (F < 2.5, \( \eta^2 = .24 \)) and no significant interaction (F < 1, \( \eta^2 = .06 \)). A within-subjects ANOVA comparing starting performance (Block 1) on the FC and FS
tasks in the saline condition revealed that the effect of task was not significant ($F < 2, \eta^2 = .19$), which suggests that spared acquisition of the FS task under scopolamine was not simply due to the FS task being less difficult than the FC task at the outset of training.

Retrieval of FC Stimuli. Twelve sessions were required for all rats to reach criterion performance during initial drug-free acquisition of the learning-to-learn FC stimulus set. Accuracy during subsequent retrieval of these stimuli in the drug conditions was examined in a two-way repeated measures ANOVA using drug condition (saline or scopolamine) and session as within-subjects factors. This analysis revealed nonsignificant main effects of drug condition ($F < 2, \eta^2 = .22$) and session ($F < 1, \eta^2 = .01$) and no significant interaction ($F < 1, \eta^2 = .21$).

Effects of Scopolamine on Performance of the FC and FS Tasks. Figure 4A compares the effects of scopolamine on performance during the last block of FC acquisition, the last block of FS acquisition, and FC retrieval. The cost of scopolamine was computed by calculating a difference score (accuracy in the saline condition – accuracy in the scopolamine condition) for each task. A one-way repeated measures ANOVA using task (FC acquisition, FS acquisition, or FC retrieval) as a within-subjects factor revealed a significant effect of task, $F(2, 14) = 6.89, p < .01, \eta^2 = .50$. Within-subjects simple contrasts revealed a significant difference between the difference scores for FC acquisition and FC retrieval ($p < .05, \eta^2 = .61$), and between the difference scores for FC acquisition and FS acquisition ($p < .05, \eta^2 = .45$).

Discussion

Rats under the influence of scopolamine were significantly impaired at acquiring FC stimuli relative to acquiring FS stimuli and retrieving previously bound FC stimuli. These findings provide support for a critical role for the muscarinic cholinergic system in feature binding at encoding. We contend that this rat model of the role of ACh in feature binding at encoding may
be an analog of human attention. Experiment 1B tested this hypothesis using a human version of our rat feature binding task and a behavioural manipulation of attention.

1.2 Experiment 1B

Introduction

We predicted that if manipulations of the cholinergic system in rats are analogous to manipulations of human attention, then human participants in a divided-attention condition would exhibit impaired FC acquisition, but relative sparing of FS acquisition and FC retrieval, just as rats under the influence of scopolamine do.

Methods

Participants. One hundred two undergraduate students (mean age = 20.0 years, $SD = 3.82$; 57 females, 45 males) at the University of Toronto gave written informed consent to participate in return for course credit or remuneration. Participants were randomly assigned to one of six conditions: full-attention FC acquisition, divided-attention FC acquisition, full-attention FC retrieval, divided-attention FC retrieval, full-attention FS acquisition, and divided-attention FS acquisition. There were 17 participants in each condition.

Stimuli. On each trial, a computer screen simultaneously presented two visual stimuli: a coloured shape and a black star; the star served as the analog of the blank bowl in the rat paradigm (Figure 1). Both stimuli were presented within a centrally located rectangle ($12 \times 8.5$ cm); the coloured shape always appeared in the left half of the rectangle, and the black star always appeared in the right half of the rectangle. The stimuli subtended $2.5^\circ \times 2.5^\circ$ of visual angle. On target trials, the correct response was to select the coloured shape, and on distractor trials, the correct response
was to select the black star. Table 1 lists the colours and shapes from which the experimental stimuli were created.

The Feature-Conjunction (FC) stimulus set contained four coloured-shape stimuli, along with the black star. Two of the coloured shapes were designated target stimuli (T1 and T2) and were presented on target trials. The remaining two coloured shapes were designated distractor stimuli (D1 and D2) and were presented on distractor trials. In a forced-choice design, participants were allowed only one stimulus choice on each trial. Binding of colour and shape was required to determine the correct stimulus choice, as each individual colour and shape was associated with either the target or the distractor, depending on its feature pairing, and the different pairings occurred equally often across trials. That is, each colour and shape was used in one target bowl and one distractor bowl, such that neither colour nor shape could be used by itself to determine the correct stimulus choice (Figure 2).

The Feature-Singleton (FS) stimulus set contained four nonconjunction coloured-shape stimuli and the black star. Two of the coloured shapes were designated target stimuli (T1 and T2), and the remaining two were designated distractor stimuli (D1 and D2). Feature binding was not required for stimulus selection, as the colour and shape of each coloured shape were both unique. That is, participants could use either colour or shape alone or the distinct combination of the two features to determine which stimulus to choose (Figure 2).

**Attentional Manipulation.** In the concurrent letter-matching task, participants had to indicate whether two letters that flanked the centrally presented coloured shapes were the same or different. This task is similar to a classic method of dividing attention in studies of human cognition (Treisman & Schmidt, 1982).
Training Procedure. Each session consisted of at least 116 trials, half of which were target trials (29 T1, 29 T2), and half of which were distractor trials (29 D1 and 29 D2). Within a session, trials were presented in a pseudorandom order, such that no more than 3 consecutive trials were of the same type (target or distractor). A performance criterion of 18 correct responses in 20 consecutive trials (90%) was employed. If this criterion was not met by the 116th trial, the session continued until the criterion was met.

Full-Attention Conditions. Full-attention FC and FS trials commenced with a fixation cross presented for 250 ms in the middle of the screen. The FC or FS stimuli then appeared. Participants selected the coloured shape or black star, pressing one of two keyboard buttons using their right hand. The FC or FS stimuli disappeared from the screen as soon as a response was made, and were followed by a 25-ms coloured mask to clear any afterimages. If participants failed to respond within 1,000 ms, the coloured mask was presented, and then a screen prompting a response replaced the mask, remaining until a response was made. Participants received auditory feedback, a 500-ms high-pitched tone after every correct response and a low-pitched tone of the same duration after every incorrect response.

Divided–Attention Conditions. The trial structure of the divided-attention conditions was identical to that of the full-attention conditions except for the addition of a concurrent letter-matching task during which participants indicated whether two letters that flanked the centrally presented coloured shapes were the same or different. (One letter was uppercase, and the other was lowercase, to prevent simple visual matching.) Participants indicated their response by pressing one of two keyboard buttons using their left hand. Participants had to respond to the letter-matching task first in order for a response to the FC or FS stimuli to be counted. The FC or FS stimuli disappeared from the screen as soon as a response to the flanking letters was made,
and then the 25-ms coloured mask was presented. If there was no response to the letter-matching task within 1,000 ms, the coloured mask was presented, and then a screen prompting a response replaced the mask, remaining until a response was made. Following responses to the two tasks, feedback was provided to encourage strong performance on the divided-attention task; a percentage score indicating cumulative accuracy on the letter-matching task and an auditory tone reflecting performance on the FC or FS task were presented simultaneously for 500 ms.

**Practice Trials.** All participants practiced the divided-attention letter-matching task, and then they acquired a set of learning-to-learn FC stimuli, distinct from those used during subsequent FC acquisition and retrieval, until criterion performance was met (at least 116 trials).

**Acquisition of the FC or FS Stimuli.** After the completion of practice training, participants in the FC-acquisition and FS-acquisition conditions began training with novel FC or FS stimuli, under either full-attention or divided-attention conditions. Training continued until criterion performance was met (at least 116 trials).

**Retrieval of the FC Stimuli.** Participants in the FC-retrieval conditions completed practice training and subsequently acquired novel FC stimuli under full-attention conditions, continuing this training until they reached criterion performance (at least 116 trials). After a delay of 2 to 3 min, they retrieved these same FC stimuli, under either full-attention or divided-attention conditions, until criterion performance was met (at least 116 trials).

**Results**

**Statistical Analyses.** Task accuracy was assessed using proportion of correct responses. All statistical analyses were conducted using SPSS Version 14 with an alpha level of .05. We used only the first 90 trials of each session so that the data could be binned into 30-trial blocks for
ease of comparison with the rat data. Across all six conditions, there were no significant
differences between performance during the last 26 trials of a session (i.e., Trials 91–116) and
performance during the preceding 30 trials (i.e., Trials 61–90). Participants’ accuracy scores for
the last 30-trial block of each session, when performance was most stable, were transformed into
standardized z scores, and participants with z scores greater than +1.96 or less than −1.96 were
removed from analyses: 1 from the full-attention FS-acquisition condition, 2 from the divided-
attention FC-acquisition condition, 1 from the divided-attention FS-acquisition condition, and 2
from the divided-attention FC-retrieval condition.

Acquisition of FC Stimuli. Figure 3C depicts the results for FC acquisition. A two-way mixed
ANOVA using attention condition (full attention or divided attention) as a between-subjects
factor and block as a within-subjects factor revealed significant main effects of attention
condition, $F(1, 30) = 26.19, p < .001, \eta^2 = .47$, and block, $F(1.60, 47.88) = 13.61, p < .001, \eta^2 =
.31$, but no significant interaction ($F < 1, \eta^2 = .01$). At the end of training (Block 3), performance
was still impaired in the divided-attention condition, $t(30) = 4.57, p < .001, \eta^2 = .41$.

Acquisition of FS Stimuli. Figure 3D depicts the results for FS acquisition. A two-way mixed
ANOVA using attention condition (full attention or divided attention) as a between-subjects
factor and block as a within-subjects factor revealed significant main effects of attention
condition, $F(1, 30) = 16.91, p < .001, \eta^2 = .36$, and block, $F(2, 60) = 47.75, p < .001, \eta^2 = .61,$
and a significant interaction, $F(2, 60) = 7.29, p < .01, \eta^2 = .20$. Further analysis revealed a
significant difference between the full- and divided-attention conditions during the first block of
acquisition, $t(30) = 5.00, p < .001, \eta^2 = .46$; this difference diminished to nonsignificance by the
end of training (Block 3; $t < 2, \eta^2 = .01$). A one-way ANOVA comparing starting performance
(Block 1) on the FC and FS tasks in the full-attention condition revealed that the effect of task
was nonsignificant (F < 2, η² = .05); this suggests that spared acquisition of the FS task in the divided-attention condition was not simply due to the FS task being less difficult than the FC task at the outset of training.

**Retrieval of FC Stimuli.** To examine the effects of attention on FC retrieval, we conducted a two-way mixed ANOVA using attention condition (full attention or divided attention) as a between-subjects factor and block as a within-subjects factor. This analysis revealed a significant main effect of block, F(2, 60) = 10.64, p < .001, η² = .26, but no significant effect of attention condition (F < 3, η² = .10) and no significant interaction (F < 1, η² = .01). Because this was a between-subjects design, we conducted a two-way mixed ANOVA on FC acquisition using cohort (full-attention or divided-attention retrieval) as a between-subjects factor and block as a within-subjects factor, to ensure that there were no performance differences in participants’ initial acquisition (under full-attention conditions) of the FC stimulus set prior to its retrieval. The ANOVA confirmed a significant main effect of block, F(2, 58) = 9.55, p < .001, η² = .25, and revealed no significant effect of cohort (F < 1, η² = .01) and no significant interaction (F < 2, η² = .05).

**Effects of Divided Attention on Performance of the FC and FS Tasks.** To examine the performance of participants in the divided-attention condition during the last block of FC acquisition, the last block of FS acquisition, and FC retrieval, we conducted a one-way ANOVA using task (FC acquisition, FS acquisition, or FC retrieval) as a between-subjects factor. This analysis revealed a significant effect of task, F(2, 45) = 16.37, p < .001, η² = .42. Further analysis revealed a significant difference between performance in the divided-attention FC-acquisition condition and performance in the divided-attention FC-retrieval condition, t(30) = −2.40, p < .05, η² = .16, and between performance in the divided-attention FC-acquisition condition and
performance in the divided-attention FS-acquisition condition, \( t(30) = -5.65, p < .001, \eta^2 = .52 \).

Figure 4B compares the effect of divided attention on performance during the last block of FC acquisition, the last block of FS acquisition, and FC retrieval. For display purposes, the cost of divided attention was computed by calculating a mean difference score (mean accuracy of participants in the full-attention condition – mean accuracy of participants in the divided-attention condition) for each task.

**Letter-Matching Task.** Average accuracy on the concurrent divided-attention task was 90%, 92%, and 94% (collapsed across blocks) in the FC-acquisition, FS-acquisition, and FC-retrieval conditions, respectively. A two-way mixed ANOVA using task (FC acquisition, FS acquisition, or FC retrieval) as a between-subjects factor and block as a within-subjects factor revealed a significant main effect of block, \( F(2, 86) = 3.96, p < .05, \eta^2 = .08 \), but no significant effect of task \( (F < 2, \eta^2 = .06) \) and no significant interaction \( (F < 2, \eta^2 = .08) \).

**Discussion**

The effects of the attention manipulation mirrored those of the scopolamine manipulation in Experiment 1A. In the divided-attention condition, human participants were significantly impaired in acquiring intramodal FC stimuli, whereas both FS acquisition and retrieval of previously bound FC stimuli were relatively spared.

**General Discussion**

This cross-species study of feature binding suggests that cholinergically driven attentional processes are essential to feature binding at encoding. In a strikingly similar manner, rats under the influence of scopolamine and human participants under divided attention were impaired at acquiring FC stimuli, whereas their ability to acquire FS stimuli and to retrieve previously bound FC stimuli remained intact relative to performance under saline and full-attention conditions,
respectively. Our FS findings are consistent with those of studies demonstrating that single-feature processing constitutes a low attentional load for humans (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Luck & Ford, 1998; Treisman & Gelade, 1980). In addition, the invulnerability of the feature binding retrieval process to a cholinergic or divided-attentional challenge suggests that once a FC stimulus is well learned, it has a bound and stable neural representation, which reduces the need for an attentionally demanding feature binding process during retrieval.

Our retrieval findings are in agreement with a considerable body of research on human cognition showing that disrupting attention is selectively detrimental to the encoding, relative to the retrieval, of episodic memories (Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Logie, Della Sala, MacPherson, & Cooper, 2007; Naveh-Benjamin, Craik, Guez, & Dori, 1998; Naveh-Benjamin, Craik, Perretta, & Tonev, 2000; Naveh-Benjamin, Kilb, & Fisher, 2006). Furthermore, our data are consistent with a well-supported model of cholinergic function (Hasselmo & McGaughy, 2004) proposing that high levels of ACh facilitate encoding by boosting sensory input, whereas low levels facilitate retrieval by allowing reactivation of neural connections representing previously learned information (Anagnostaras, Maren, & Fanselow, 1995; McGaughy, Koene, Eichenbaum, & Hasselmo, 2005; Orsetti, Casamenti, & Pepeu, 1996; Pepeu & Giovannini, 2004; Safer & Allen, 1971; Schon et al., 2005; White & Ruske, 2002).

Although there is no direct evidence that dividing attention reduces cholinergic levels in humans, it has been shown that increasing cholinergic levels results in the behavioural and neural correlates of increased attention. Specifically, it has been demonstrated that administration of the cholinergic-enhancing drug physostigmine during fMRI results in enhanced visual working memory performance by augmenting activation of sensory cortices and decreasing reliance on
frontoparietal cortical networks (Bentley, Husain, & Dolan, 2004; Furey, Pietrini, Alexander, Schapiro, & Horwitz, 2000; Furey, Pietrini, & Haxby, 2000). There is, however, direct evidence from non-human animals to support the more general proposition that disruptions to the cholinergic system are akin to disruptions of attention. This evidence has come from studies in which pharmacological (Chiba, Bucci, Holland, & Gallagher, 1995; Mirza & Stolerman, 2000; Sarter, Bruno, & Givens, 2003) and in vivo microdialysis techniques were used to measure the amount of frontal cortical ACh efflux while animals performed attentional tasks (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002; Hata, Kumai, & Okaichi, 2007; Himmelheber, Sarter, & Bruno, 2000, 2001; Pepeu & Giovannini, 2004). Our cross-species data are consistent with the premise that the blockade of muscarinic cholinergic function in rats is analogous to imposition of an attentional load in humans (i.e., divided attention) and suggest that ACh may provide the attentional "glue" for feature binding.
Chapter 2

Experiment 2:

The nucleus basalis magnocellularis contributes to feature binding in the rat

*Contents of this chapter have been published in Physiology & Behavior:* Botly, L.C.P. & De Rosa, E. (2009). The nucleus basalis magnocellularis contributes to feature binding in the rat. 

*Physiology & Behavior, 97,* 313-320.
Abstract

The binding problem refers to the fundamental challenge of the central nervous system to integrate sensory information registered by multiple brain regions to form a unified neural representation of a stimulus. Although the human cognitive literature has yielded substantial insights into the attention-dependent nature and general cortical networks involved in feature binding, the specific downstream neuroanatomical modulatory contributions to feature binding remain unknown. We hypothesized that the nucleus basalis magnocellularis (NBM) of the basal forebrain would be critical for feature binding given the NBM’s widespread neuromodulatory projections to regions of the neocortex important for attentional processing, such as the frontal and parietal cortices. Accordingly, we tested the ability of rats with bilateral excitotoxic (quisqualic acid) lesions of the NBM to acquire a crossmodal Feature-Conjunction (FC) task that required feature binding and a Feature-Singleton (FS) task that did not require feature binding. Additionally, rats retrieved a FC stimulus set they had acquired prior to surgery. Relative to sham-lesioned controls, NBM-lesioned rats were significantly impaired at acquiring and retrieving the FC task, while their ability to acquire the FS task remained intact. These findings provide insight into the functional role of the NBM and establish the importance of this basal forebrain structure to the fundamental cognitive process of feature binding.
Introduction
We are capable of perceiving objects as a unified whole even though the mammalian brain is organized in a modular fashion; that is, distinct neural regions are primarily responsible for detecting and processing the different features of an object, such as its shape or colour. The unknown mechanism by which a unified neural representation of a stimulus is formed is referred to as feature binding (Robertson, 2003; Treisman & Gelade, 1980). Feature binding tasks have been widely employed in the human cognitive literature to investigate the cognitive processes and cortical networks involved in feature binding and such tasks typically involve the presentation of two different types of stimuli, Feature-Singleton (FS) and Feature-Conjunction (FC). While FS stimuli only require the processing of single features (e.g., find a yellow apple in a basket of green apples and pears), FC stimuli require the binding of multiple features (e.g., find a yellow apple within a basket of green apples and green and yellow pears). Patients with attentional impairments are impaired at FC, but not FS tasks (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Tales et al., 2002), and functional magnetic resonance imaging (fMRI) studies have implicated frontoparietal cortical networks in feature binding (Behrmann, Geng, & Shomstein, 2004; Constantinidis, 2006; Corbetta, Shulman, Miezin, & Petersen, 1995; Esterman, Verstynen, & Robertson, 2007; Luck & Ford, 1998; Reynolds & Desimone, 1999; Treisman, 1998).

While the human cognitive literature has yielded substantial insights into the cognitive mechanisms and broad neocortical systems involved in feature binding, the specific downstream neuroanatomical modulatory contributions to feature binding remain unknown. The basal forebrain (BF) projection system has a complex anatomical and neurochemical composition and is customarily defined by magnocellular cholinergic neurons. The BF projection system is
topographically organized, where, in simple terms, the anterior portion (medial septum/vertical limb of diagonal band of Broca; MS/VDB) projects primarily to the hippocampus and related structures and the posterior portion (nucleus basalis magnocellularis; NBM) projects to the neocortex. Hence, we hypothesized that the NBM would be critical for feature binding given its demonstrated importance to a variety of forms of attention, including sustained, selective, and divided attention (Butt & Bowman, 2002; Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; Lehmann, Grottick, Cassel, & Higgins, 2003; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; Pang, Williams, Egeth, & Olton, 1993; Wenk, 1997), and its widespread neuromodulatory input to regions of the neocortex important for attentional processing, including the frontal and parietal cortices (Mesulam, Mufson, Wainer, & Levey, 1983).

We set out to investigate whether the NBM was an important contributor to feature binding using a rodent feature binding task that employed the same forced-choice digging paradigm as that used in our previous systemic pharmacology work (Botly & De Rosa, 2007). To determine whether the NBM had a functional role in feature binding, we tested the ability of rats with bilateral quisqualic acid lesions of the NBM to acquire crossmodal FC and FS stimuli as well as retrieve a pre-surgery FC stimulus set. In our feature binding task, rats are simultaneously presented with two digging bowls on every trial: a crossmodal odor-texture bowl covered with a texture and scented with an odor, and a blank bowl with no odor or texture components. Only one of the diggings bowls is baited with a food reward on any given trial. In the FC task, rats must bind odor and texture features for correct bowl selection, while in the FS task they can rely on a single feature (odor or texture).
Methods

Participants. Participants were 30 experimentally naïve male Long-Evans rats (Charles River, Montreal, Quebec) that weighed 216-240 g at the start of the experiment. Rats were housed individually in 45 cm long x 25 cm wide plastic tub cages and maintained on a reversed 12 hr light – 12 hr dark cycle (lights off at 8 am) with testing occurring during the dark phase (between 10am-4pm). Rats were maintained at 90 % of ad libitum free-feeding weight for the duration of the experiment. This study was approved by the University of Toronto’s Institutional Animal Care Committee.

Apparatus. The training environment was a black Plexiglas chamber 30.5 cm high x 76.2 cm long x 45.7 cm wide. A black slide-in-door of the same material separated the chamber into two compartments (Figure 5). The door was positioned 25.4 cm from the back wall of the chamber creating a “start box” (25.4 cm long x 45.7 cm wide) and a “testing arena” (50.8 cm long x 45.7 cm wide). The experimental room was illuminated by a single 60-watt light bulb. The digging apparatus was positioned on a table next to a computer equipped with speakers that emitted white noise and ambient voices to mask any extraneous noises.

Odor-texture digging bowls. All digging bowls (8 cm deep x 4 cm diameter) were painted matte black and attached to heavy 10-cm-square 4-mm-thick black metal bases. The blank bowl had no additional sensory attributes. In contrast, the outside surface, rim, and metal base of each of the odor-texture bowls was covered by a textured cloth using silicon glue, which allowed for the easy removal of the textures. To minimize the tendency for rats to use visual cues to discriminate the textures, all textures were various shades of brown. Glued to the bottom interior surface of each odor-texture bowl was a small metal cap (8 cm in diameter x 1.1 cm high) containing cotton gauze. Each cap contained 32 small holes 3 mm in diameter. At the beginning of each training
day and after every four rats completed their sessions, the gauze was re-injected with 0.1 ml of
the appropriate scented undiluted aromatherapy oil (Aveda®, Blain, MN; The Body Shop®,
Wake Forest, NC). As an additional precaution, prior to beginning each rat’s session, every
odor-texture digging bowl was smelled by the experimenter to ensure that each odor was
detectible to the human nose and thereby detectible to the rats given their more sensitive
olfactory system. Although such an occurrence was rare, if an odor was not detected by the
experimenter, additional aromatherapy oil (0.1 ml at a time) was injected until the odor became
detectible. Before bowls were reused for novel stimuli, they were thoroughly soaked in rubbing
alcohol for three days and spray painted to remove any lingering odor. Table 1 lists the odorants
and textures from which the experimental stimuli were created.

*Forced-choice digging paradigm.* Figure 5 illustrates the two different trial types (a), Target and
Distractor, and (b) a typical session. On every trial, rats were simultaneously presented with two
digging bowls in the testing arena: an odor-texture bowl and a blank bowl, both of which were
filled approximately three-quarters of the way full of the granular commercial bedding Bed-
o’cobs (The Andersons, Maumee, OH). On target trials, the reward (half piece of Kellogg’s®
Froot Loops® cereal) was buried in the odor-texture bowl, and on distractor trials, the reward
was buried in the blank bowl. Rats were trained to dig in only one bowl one each trial, thus
requiring that they extract a rule for bowl selection. Finely ground pieces of Froot Loops cereal
were added to the bedding of all bowls to mask the location of the reward. The Froot Loop
reward was always buried approximately two-thirds of the way down into the bedding.

*Stimuli*

*Baseline Stimuli.* Due to the complexity of our forced-choice digging paradigm, rats were first
introduced to the different responses needed for the two trial types, Target and Distractor,
without the need for feature binding using two Baseline odor-texture bowls. Rats could use a single odor or texture or the distinct combination of the two features to determine the correct bowl choice as each Baseline stimulus was characterized by a distinct odor and texture. One of the odor-texture Baseline bowls was designated a Target bowl (B1) and the other odor-texture Baseline bowl was designated a Distractor bowl (B2). Although an odor-texture bowl and the blank bowl were present on every trial, on Target trials the rat had to dig in the odor-texture bowl and on Distractor trials the rat had to dig in the blank bowl to retrieve the reward. The same two Baseline odor-texture bowls were used throughout the experiment to measure the effect of lesion on general stimulus discrimination and digging performance.

*Feature-Conjunction Stimuli.* Each Feature-Conjunction (FC) stimulus set contained four conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As illustrated by Figure 6, crossmodal binding of odors and textures was required to determine the correct bowl choice as each individual odor and texture was associated with both a target and a distractor bowl such that no single odor or texture could be used for correct bowl selection. That is, the crossmodal features of the target and distractor bowls overlapped.

*Feature-Singleton Stimuli.* The Feature-Singleton (FS) stimulus set contained four non-conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As illustrated by Figure 6, feature binding was not required to determine the correct bowl choice as each individual odor and texture was associated with either a target or a distractor bowl such that rats could use a single odor or texture or the distinct
combination of the two features for correct bowl selection. That is, the crossmodal features of the
target and distractor bowls were distinct and did not overlap.

**Pre-Surgical Training Procedures**

**Habituation.** The testing arena was baited with 2 whole Froot Loops and rats were allowed to
explore the apparatus until they consumed at least one of the treats.

**Pretraining.** For two days, rats were trained to dig for a single whole Froot Loop buried at the
bottom of an aluminum metal food bowl in their home cages. Sessions lasted approximately 1
hour. The bowls used in their home cages were different from those used during subsequent
experimental training in the testing arena; they clipped onto the side of their home cage and
measured 4 cm deep x 8 cm in diameter.

**General forced-choice digging procedures.** At the start of every session, rats were placed in the
start box of the apparatus with the sliding door closed. During this time, the appropriate bowl
was baited with a single half Froot Loop and placed beside the unrewarded bowl in the testing
arena of the apparatus. The blank bowl was always positioned in the far left corner of the
chamber with the odor-texture bowl directly in front and flush against the blank bowl to counter
the rats’ natural strategy to first dig in the blank bowl and then over trials to acquire when to dig
in the odor-texture bowl. The sliding door was then lifted, and the rat was allowed to make a
bowl choice, after which it was gently guided back to the start box and allowed to eat the Froot
Loop (if obtained) with the door closed. A choice was defined as a dig if one or both paws
displaced the bedding of the chosen bowl, or if a rat put its nose half-way down into the bedding.
Rats remained in the start box between trials while the bowls were re-baited and replaced, which
took on average 30 sec. Rats received one session per day. The first few sessions were always
discovery sessions, during which rats were allowed to make as many choices as necessary to find
the buried reward, but only the first bowl choice counted towards accuracy. During all remaining sessions, rats were only allowed to make a single bowl choice. If rats did not make a choice within 2-3 min, then the Froot Loop was removed and placed on top of the bedding of the rewarded bowl for the rat to find and consume in the start box. In between sessions, any bedding that accumulated in the apparatus was vacuumed up and the walls and floor of the apparatus were wiped down with rubbing alcohol.

*Acquisition of Baseline stimuli.* Each session consisted of 14 trials, half of which were Target trials and half of which were Distractor trials. Within a session, trials were presented in a pseudorandom order, such that no more than 3 consecutive trials were of the same type (target or distractor). The first three sessions were discovery sessions. Baseline training continued until all rats reached a criterion of at least 6 out of 7 correct Target and 6 out of 7 correct Distractor trials for at least two nonconsecutive sessions.

*Acquisition of learning-to-learn Feature-Conjunction stimuli.* After the final Baseline training session, in the subsequent session, rats began feature binding training using the learning-to-learn FC stimulus set. FC sessions consisted of 16 trials, the first 4 of which were Baseline trials (2 Target and 2 Distractor trials) identical to those described above. The remaining 12 trials of a session were 6 Target trials (3 T1, 3 T2) and 6 Distractor trials (3 D1, 3 D2) presented in a pseudorandom order, such that no more than 3 consecutive trials of the same type (Target or Distractor) occurred in a session. The first three sessions were discovery sessions and only during training on this initial learning-to-learn stimulus set did rats receive correction trials in which an incorrect response was always followed by the same trial until a correct choice was made. Correction trials did not count towards accuracy.
Surgery

Rats were assigned to one of two surgical groups, sham-lesion (n = 14) or NBM-lesion (n = 16) equating the groups for pre-surgical Baseline and FC performance. Surgeries were performed under aseptic conditions. Rats were anesthetized with isoflurane (approximate maintenance dose was 2 % with 1 L/min of oxygen). A subcutaneous (s.c.) injection of the analgesic buprenorphine (0.03 mg/kg) and an intraperitoneal (i.p.) injection of atropine (0.05 mg/kg) were delivered immediately prior to surgery, the latter of which served to prevent fluid buildup in the lungs.

Four 1.0 mm holes were drilled at the following stereotaxic coordinates relative to bregma and the surface of the skull (Paxinos & Watson, 1998): anterior NBM: Anterior-Posterior (AP) -0.8 mm, Medial-Lateral (ML) ± 2.5 mm, Dorsal-Ventral (DV) - 8.2 mm; posterior NBM: AP -1.6 mm, ML ± 2.5 mm, DV – 7.6 mm. In comparison to other excitotoxins, quisqualic acid infused into the NBM results in the greatest reduction of cholinergic input to the neocortex, while causing minimal reductions in cholinergic input to the amygdala and leaving cholinergic neurons in the adjacent pallidal region of the basal ganglia relatively intact (Boegman, Cockhill, Jhamandas, & Beninger, 1992; Dunnett, Everitt, & Robbins, 1991; Muir, Page, Sirinathsinghji, Robbins, & Everitt, 1993; Robbins, Everitt, Marston et al., 1989; Robbins, Everitt, Ryan et al., 1989). There were a total of four intraparenchymal injection sites, two per hemisphere, of 0.8 µl sterile physiological saline (sham lesion) or 5.0 µg/µl quisqualic acid (Sigma Aldrich) dissolved in sterile physiological saline through a 26-gauge Hamilton syringe at 0.1 µl/min. The needle was left in place for 4 min after each injection. The body temperature of each rat was maintained with a homeothermic blanket throughout the surgery. After the injections were complete, the wound was closed with staples and EMLA topical analgesic ointment (2.5% lidocaine and 2.5% prilocaine) was liberally applied around the staples. To prevent dehydration, rats were given normal saline (0.9 % NaCl; 2 ml / 100 g body weight; s.c.) immediately post-surgery. All rats
received a minimum of 10 days of recovery with *ad libitum* food and water before being food restricted for subsequent testing.

*Post-Surgical Testing Procedures*

Experimenters were blind to the surgical group of the animals. All post-surgical FC and FS sessions consisted of 16 trials, the first 4 of which were Baseline trials (2 Target and 2 Distractor trials) identical to those described above to measure the effect of lesion on general stimulus discrimination and digging performance. The remaining 12 trials of a session were 6 Target trials (3 T1, 3 T2) and 6 Distractor trials (3 D1, 3 D2) presented in a pseudorandom order, such that no more than 3 consecutive trials of the same type (Target or Distractor) occurred in a session.

*Retrieval of Baseline Stimuli.* To ensure that rats could still perform the forced-choice digging task and discriminate the odors and textures post-surgery, rats retrieved the Baseline stimuli they had acquired prior to surgery. Baseline retrieval comprised 2 high-accuracy sessions.

*Retrieval of Feature-Conjunction (FC) Stimuli.* After the final Baseline retrieval session, in the subsequent session, rats began retrieval of the learning-to-learn FC stimulus set they had acquired prior to surgery. FC retrieval sessions continued until all rats reached asymptotic performance, which took 6 sessions.

*Acquisition of Feature-Conjunction Stimuli.* After the final FC retrieval session, in the subsequent session, rats began acquisition of novel FC stimuli. FC acquisition sessions continued until all rats reached asymptotic performance, which took 12 sessions.

*Acquisition of Feature-Singleton Stimuli.* After the final FC acquisition session, in the subsequent session, rats began acquisition of the FS stimuli. FS acquisition sessions continued until all rats reached asymptotic performance, which took 9 sessions.
Histological Analyses

Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and transcardially perfused with approximately 150 ml of ice-cold normal saline followed by approximately 150 ml of ice-cold 4% paraformaldehyde. Brains were extracted and immediately post-fixed in 4% paraformaldehyde for 2 hr at 4 °C, and then transferred to a solution of 20% sucrose in phosphate-buffered saline (0.1 M; pH 7.4) and stored for 2 weeks at 4 °C. Brains were sectioned at a thickness of 60 µm using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems, Canada). Adjacent sections were used for staining for acetylcholinesterase (AChE) histochemistry and choline acetyltransferase (ChAT) immunohistochemistry. AChE histochemistry was carried out according to the method described elsewhere (Paxinos & Watson, 1998), and was used to assess the extent of cholinergic fiber loss in target structures of the NBM. ChAT immunohistochemistry was carried out according to methods described elsewhere (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995; De Rosa, Hasselmo, & Baxter, 2001) to assess the extent of cholinergic cell body loss in the NBM and medial septum/diagonal band of Broca (MS/VDB). After all histological assays, brain slices were mounted on slides, dehydrated and cleared using an ascending ethanol and xylene series, coverslipped with DPX, and examined under a Leica light microscope (DM4000B, Ontario, Canada).

While we were able to complete AChE and ChAT cholinergic assays, due to an unanticipated loss of all of our remaining tissue sections within three weeks of sectioning, we were unable to complete parvalbumin immunohistochemistry to stain for GABAergic cell bodies in the NBM. As stated above, we presume damage to GABAergic cells co-localized in the NBM in our NBM-lesioned rats relative to our sham-lesioned rats. Importantly, both of our cholinergic assays were able to dissociate an intact MS/VDB and its efferents (hippocampus) from selective damage to the NBM and its efferents (frontal and parietal cortices).
Histological Quantification

ChAT-immunoreactive cells were counted in the NBM and MS/VDB as delineated by a rat brain atlas (Paxinos & Watson, 1998) using a Leica light microscope (DM4000B, Ontario, Canada) and Openlab image analysis software (Quorum Technologies, Ontario, Canada). Cell counts for each rat were taken from brain sections at AP = -0.80 mm relative to Bregma to assess damage to cholinergic cells in the NBM and at AP = + 0.60 mm relative to Bregma to assess damage to cholinergic cells in the MS/VDB. Anatomical landmarks were used to define the borders of the NBM cell-counting frames; the rectangular outlines superimposed on the rat brain coronal schematics (Paxinos & Watson, 1998) depicted in Figure 7 delineate the NBM and MS/VDB cell-counting frames used for histological quantification. ChAT-immunoreactive cells were easily identifiable and characterized by relatively large cell bodies with several extending dendrites and we only counted cells that were well distinguishable (i.e., well-defined borders) from the background.

Results

Statistical Analyses. All statistical analyses were conducted using SPSS Version 14 with an alpha level of .05.

Pre-Surgical Training. Rats required 12 training sessions to reach criterion performance on a Baseline task in which rats learned Target and Distractor trial response classification without the need for feature binding. They required 23 training sessions to reach criterion performance on the Feature-Conjunction (FC) task, which does depend on feature binding, using a learning-to-learn FC stimulus set.

Histological Analyses. We completed 16 NBM-lesion and 14 sham-lesion survival surgeries. Of the 30 rats, 1 sham-lesioned and 1 NBM-lesioned rat died post-surgery and histological analyses
revealed that 3 NBM-lesioned rats had received only unilateral lesions; behavioural and histological data from these 5 rats were removed from subsequent analyses, reducing the NBM-lesion sample size to 12 and the sham-lesion sample size to 13. While an equivalent number of choline acetyltransferase (ChAT)-immunoreactive cells was observed in the MS/VDB in both groups, there was a marked depletion of ChAT-immunoreactive cells in the NBM of the NBM-lesioned rats relative to sham-lesioned rats (Figure 7). Independent \( t \)-tests confirmed that there were significantly fewer ChAT-immunoreactive neurons in the NBM of NBM-lesioned compared to sham-lesioned rats \([t(24) = 11.97, p < .001, \eta^2 = .90; M_{\text{Sham}} = 107.1, SD = 4.6; M_{\text{NBM}} = 27.7, SD = 4.4]\). However, there was no significant difference between the number of such neurons in the MS/VDB of the two groups of rats \((t < 1.0, \eta^2 = .01; M_{\text{Sham}} = 174.6, SD = 5.9; M_{\text{NBM}} = 172.8, SD = 3.6)\), indicating that our lesions were specific to the NBM of the basal forebrain. Acetylcholinesterase (AChE) staining revealed a loss of cholinergic fibers in the frontal and parietal cortices, but not the hippocampus of NBM-lesioned rats relative to sham-lesioned rats (Figure 8).

**Post-Surgical Testing**

**Retrieval of Baseline Stimuli.** Rats in both surgical groups maintained very high accuracy on the Baseline task post-surgery. An analysis of variance (ANOVA) was performed on the Baseline retrieval data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Session as a within-subjects factor. The ANOVA revealed no significant main effect of Lesion Condition \((F < 2.0, \eta^2 = .05)\), a significant effect of Session \([F(3, 69) = 8.05 p < .001, \eta^2 = .26]\), and no significant interaction \((F < 2.0, \eta^2 = .11)\).

**Retrieval of Feature-Conjunction Stimuli.** Figure 9A depicts post-surgical retrieval of the FC task using the learning-to-learn FC stimulus set rats acquired prior to surgery with data binned
into 3-session blocks. An ANOVA was performed on the FC retrieval data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Block as a within-subjects factor. The ANOVA revealed a significant main effect of Lesion Condition \(F(1, 23) = 11.62, p < .01, \eta^2 = .34\) and Block \(F(1, 23) = 20.95, p < .001, \eta^2 = .48\], but no significant interaction \((F < 1.0, \eta^2 = .02)\). An ANOVA on Baseline performance during FC retrieval revealed non-significant effects of Lesion Condition \((F < 2.5, \eta^2 = .09)\) and Block \((F < 1.0, \eta^2 = .01)\), and no significant interaction \((F < 1.0, \eta^2 = .02)\).

\textit{Acquisition of Feature-Conjunction Stimuli.} Figure 9B depicts post-surgical acquisition of the FC task using a novel stimulus set with data binned into 3-session blocks. An ANOVA was performed on the FC acquisition data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Block as a within-subjects factor. The ANOVA revealed a significant main effect of Lesion Condition \(F(1, 23) = 9.39, p < .01, \eta^2 = .29\] and Block \(F(3, 69) = 31.49, p < .001, \eta^2 = .58\], but no significant interaction \((F < 1.0, \eta^2 = .02)\). An ANOVA on Baseline performance during FC acquisition revealed non-significant effects of Lesion Condition \((F < 1.0, \eta^2 = .01)\) and Block \((F < 1.0, \eta^2 = .01)\), and no significant interaction \((F < 2.5, \eta^2 = .10)\).

\textit{Acquisition of Feature-Singleton Stimuli.} Figure 9C depicts post-surgical acquisition of the FS task with data binned into 3-session blocks. An ANOVA was performed on the FS acquisition data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Block as a within-subjects factor. The ANOVA revealed no significant main effect of Lesion Condition \((F < 3.0, \eta^2 = .10)\), a significant effect of Block \([F(1.56, 35.90) = 66.47, p < .001, \eta^2 = .74]\], and no significant interaction \((F < 1.0, \eta^2 = .03)\). An ANOVA on Baseline performance during FS acquisition revealed non-significant effects of Lesion Condition \((F < 1.0, \eta^2 = .001)\) and Block \((F < 2.0, \eta^2 = .07)\), and no significant interaction \((F < 2.0, \eta^2 = .08)\).
Discussion

Relative to other neighbouring basal forebrain (BF) nuclei, the nucleus basalis magnocellularis (NBM) provides neuromodulatory input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983), including regions deemed critical for the attentional processes required for feature binding in humans, such as the frontal and parietal cortices (Behrmann, Geng, & Shomstein, 2004; Constantinidis, 2006; Donner et al., 2002). The NBM has been implicated in attentional processing in non-human animals (Lehmann, Grottick, Cassel, & Higgins, 2003; Muir, Page, Sirinathsinghji, Robbins, & Everitt, 1993; Pang, Williams, Egeth, & Olton, 1993; Voytko, 1996), and the attention-dependent nature of feature binding has been well established by the human cognitive literature (Botly & De Rosa, 2008; Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Treisman & Gelade, 1980). Thus, we hypothesized that the NBM would be a critical brain structure for feature binding.

Findings from the human cognitive literature provide support for the low-attentional load nature of single-feature processing. For instance, patients with attentional deficits who are impaired at encoding conjunctions of features remain fully capable of encoding single features (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Tales et al., 2002). Furthermore, human fMRI studies have revealed selective activation of parietal networks during tasks requiring feature binding, but not those requiring the processing of single features (Corbetta, Shulman, Miezin, & Petersen, 1995; Luck & Ford, 1998; Reynolds & Desimone, 1999). More recently, it was found that transcranial magnetic stimulation of the intraparietal sulcus influenced the binding of colour and form, but not the detection or processing of single features (Esterman, Verstynen, & Robertson, 2007). Accordingly, we predicted that the single-feature processing of the FS task would demand relatively less attentional resources and would thus be less dependent on the attentional
modulatory influences from the NBM than the FC task. Consistent with this hypothesis, NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at acquiring crossmodal Feature-Conjunction (FC) stimuli, while their ability to acquire Feature-Singleton (FS) stimuli remained intact.

Unexpectedly, NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at retrieving the crossmodal FC stimuli they had acquired prior to surgery. Such a retrieval deficit is inconsistent with research suggesting a more crucial role for the NBM in encoding and attention than retrieval and memory (Bailey & Lee, 2007; Galani et al., 2002; Gonzalez, Miranda, Gutierrez, Ormsby, & Bermudez-Rattoni, 2000; Miranda & Bermudez-Rattoni, 1999; Montero-Pastor, Vale-Martinez, Guillazo-Blanch, & Marti-Nicolovius, 2004), and it is also inconsistent with our previous cross-species work demonstrating the intact FC retrieval performance of rats and human participants when their attention was challenged (Botly & De Rosa, 2008). However, there is one important methodological difference between our cross-species study and the present NBM-lesion study. In the former, rats and human participants retrieved a well-learned FC stimulus set following a very short retention interval (1-day or 2-3 minutes, respectively), while in the present study rats retrieved the FC stimulus set following a 10-day surgical recovery period. It is possible that following such a longer retention interval, a certain degree of re-binding was necessary during retrieval of the FC stimulus set resulting in the poorer retrieval performance demonstrated by the NBM-lesioned rats relative to the sham-lesioned controls. Consistent with such a re-binding hypothesis, the FC retrieval performance of rats from both lesion groups gradually improved across the six sessions of retrieval, suggestive of re-acquisition. Moreover, during the first few sessions of retrieval, sham-lesioned rats were only performing at 70-75% accuracy compared to the very high accuracy levels of 90-95% demonstrated by control rats in our previous cross-species study.
Quisqualic acid infused into the NBM resulted in large reductions in cholinergic input to the neocortex as evidenced by significant cholinergic cell body destruction in the NBM, but not the MS/VDB and loss of cholinergic fibers in the neocortex of NBM-lesioned rats relative to sham-lesioned rats. However, quisqualic acid is a non-selective excitotoxin that destroys neurons irrespective of neurochemical type and cholinergic neurons in the NBM are co-localized with a substantial population of non-cholinergic neurons; γ-aminobutyric acid (GABAergic) neurons represent the main population of non-cholinergic neurons in the BF (Gritti, Mainville, & Jones, 1993; Gritti, Mainville, Mancia, & Jones, 1997; Sarter & Bruno, 2002; Zaborszky & Duque, 2000). Furthermore, it has been shown that cholinergic drugs (e.g., scopolamine) which act at muscarinic receptors can influence GABAergic neurotransmission (Kawaguchi, 1997; Sim & Griffith, 1996), and recent research has implicated non-cholinergic basal forebrain neurons in attentional processing (Lin, Gervasoni, & Nicolelis, 2006; Lin & Nicolelis, 2008). Such findings suggest the potential contribution of both cholinergic and noncholinergic (e.g., GABAergic) neuromodulation from the NBM to our feature binding findings. For instance, the impaired post-surgical FC retrieval performance of our NBM-lesioned rats may have been due to the quisqualic acid-induced destruction of non-cholinergic (e.g., GABAergic) neurons within the NBM. 

There is evidence from the non-human animal literature to suggest that while non-selective lesions of the NBM result in both mnemonic and attentional deficits, immunotoxic lesions that selectively destroy cholinergic neurons within the NBM solely result in attentional deficits (Baxter et al., 1996; McGaughy, Everitt, Robbins, & Sarter, 2000; Waite & Thal, 1996).

The findings from the current study provide insight into the functional role of the NBM and establish the importance of this basal forebrain structure to the fundamental cognitive process of feature binding. The NBM provides 90% of the cholinergic input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983) and our previous pharmacology work with this feature binding
paradigm has revealed that ACh acting at muscarinic receptors provides the neuromodulatory support for crossmodal (Botly & De Rosa, 2007) and intramodal feature binding (Botly & De Rosa, 2008). The present excitotoxic lesion findings thus permit us to probe the neurochemical underpinnings of the NBM’s role in feature binding using the cholinergic-selective immunotoxin 192 IgG-saporin to determine whether it is cortical cholinergic input from the NBM that makes this basal forebrain region essential for feature binding. Future work must also concentrate on delineating the specific neocortical targets (frontal vs. parietal cortices) NBM efferents must reach for successful feature binding to occur.
Experiment 3:

Cholinergic deafferentation of the neocortex using 192 IgG-Saporin impairs feature binding in rats

Abstract

The binding problem refers to the fundamental challenge of the central nervous system to integrate sensory information registered by distinct brain regions to form a unified neural representation of a stimulus. While the human cognitive literature has established that attentional processes in frontoparietal cortices support feature binding, the neurochemical and specific downstream neuroanatomical contributions to feature binding remain unknown. Using systemic pharmacology in rats, it has been shown that the neurotransmitter acetylcholine is essential for feature binding at encoding, but the neural source of such critical cholinergic neurotransmission has yet to be identified. Cholinergic efferents from the nucleus basalis magnocellularis (NBM) of the basal forebrain provide the majority of the cholinergic input to the neocortex. Accordingly, it was hypothesized that the NBM is the neural source that provides the critical neuromodulatory support for feature binding. To test this hypothesis, rats received bilateral 192 IgG-saporin lesions of the NBM and their feature binding performance was tested using a forced-choice digging paradigm. Relative to sham-lesioned rats, ACh-NBM-lesioned rats were significantly impaired at acquiring a crossmodal Feature-Conjunction (FC) stimulus set that required feature binding, while their ability to retrieve a FC stimulus set and to acquire two crossmodal Feature-Singleton (FS) stimulus sets, one of greater difficulty than the other but neither requiring feature binding, remained intact. These behavioural findings, along with histological analyses demonstrating positive relationships between feature binding acquisition and markers of cholinergic activity in frontoparietal cortical regions, reveal the importance of neocortical cholinergic input from the NBM to feature binding at encoding.
Introduction

We perceive objects as unified wholes, but the mammalian brain is organized such that distinct neural regions are primarily responsible for detecting and processing the different features of an object, such as its shape or colour. The unknown mechanism by which unified neural representations of objects are formed is referred to as the binding problem (Treisman & Gelade, 1980). While the human cognitive literature has established that attentional processes in frontoparietal cortices support feature binding, (Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Esterman, Verstynen, & Robertson, 2007; Friedman-Hill, Robertson, & Treisman, 1995; Robertson, 2003), the neurochemical and specific downstream neuroanatomical contributions to feature binding remain unknown. It was hypothesized that acetylcholine (ACh) would be critical to feature binding given this neurotransmitter’s presumed role in modulating attention (Sarter, Hasselmo, Bruno, & Givens, 2005), and the well-established importance of attention to feature binding (Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Tales et al., 2002).

In support of this hypothesis, previous data have shown that systemic muscarinic cholinergic blockade with scopolamine impaired the ability of rats to acquire Feature-Conjunction (FC) stimuli that require feature binding, while leaving their ability to acquire Feature-Singleton (FS) stimuli that do not require binding intact (Botly & De Rosa, 2007). Moreover such muscarinic blockade selectively impaired feature binding at encoding, leaving the retrieval of previously-learned FC stimuli intact. Such an encoding-retrieval dissociation is consistent with a well-supported model of cholinergic function (Hasselmo & McGaughy, 2004).

Based on these pharmacological findings, it was hypothesized that ACh provides the neurochemical support for the attentional processes critical for feature binding. Evidence for this
proposition has come from a recent cross-species study using an intramodal version of this paradigm that showed that dividing the attention of humans with a concurrent task yielded a similar pattern of impairment in feature binding to muscarinic blockade in rats, impairing the acquisition of an intramodal FC task, while leaving FC retrieval and FS acquisition intact (Botly & De Rosa, 2008).

While these data implicate ACh in feature binding at encoding, the neural source of such critical cholinergic neurotransmission has yet to be identified. Thus, we hypothesized that the nucleus basalis magnocellularis (NBM) of the basal forebrain would be critical for feature binding given that its cholinergic efferents provide 90% of the cholinergic input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983), including frontoparietal cortical regions implicated in attentional processing (Behrmann, Geng, & Shomstein, 2004; Bucci, 2008; Bucci & Chess, 2005; Constantinidis, 2006; Donner et al., 2002).

To test this hypothesis, we bilaterally lesioned the NBM of rats using the cholinergic-selective immunotoxin, 192 IgG-saporin, and compared their ability to that of sham-lesioned rats to: (1) retrieve a crossmodal FC stimulus set learned prior to surgery; (2) acquire a novel crossmodal FC stimulus set, and (3) acquire two crossmodal FS stimulus sets, one of greater difficulty than the other, but neither requiring feature binding. We employed the same forced-choice digging paradigm as that used in our previous pharmacology work (Botly & De Rosa, 2007, 2008).

Methods

Participants. Participants were 20 experimentally naïve male Long-Evans rats (Charles River, Montreal, Quebec) that weighed 211-230 g at the start of the experiment. Rats were housed individually in 45 cm long x 25 cm wide plastic tub cages and maintained on a reversed 12 hr light – 12 hr dark cycle (lights off at 8 am) with testing occurring during the dark phase (between
Rats were maintained at 90% of ad libitum free-feeding weight for the duration of the experiment. This study was approved by the University of Toronto’s Institutional Animal Care Committee.

**Apparatus.** The training environment was a black Plexiglas chamber 30.5 cm high x 76.2 cm long x 45.7 cm wide. A black slide-in-door of the same material separated the chamber into two compartments (Figure 10). The door was positioned 25.4 cm from the back wall of the chamber creating a “start box” (25.4 cm long x 45.7 cm wide) and a “testing arena” (50.8 cm long x 45.7 cm wide). The experimental room was illuminated by a single 60-watt light bulb. The digging apparatus was positioned on a table next to a computer equipped with speakers that emitted white noise and ambient voices to mask any extraneous noises.

**Odor-texture digging bowls.** All digging bowls (8 cm deep x 4 cm diameter) were painted matte black and attached to heavy 10-cm-square 4-mm-thick black metal bases. The blank bowl had no additional sensory attributes. In contrast, the outside surface, rim, and metal base of each of the odor-texture bowls was covered by a textured cloth using silicon glue, which allowed for the easy removal of the textures. To minimize the tendency for rats to use visual cues to discriminate the textures, all textures were various shades of brown. Glued to the bottom interior surface of each odor-texture bowl was a small metal cap (8 cm in diameter x 1.1 cm high) containing cotton gauze. Each cap contained 32 small holes 3 mm in diameter. At the beginning of each training day and after the first four rats completed their sessions, the gauze was re-injected with 0.1 ml of the appropriate scented undiluted aromatherapy oil (Aveda®, Blain, MN; The Body Shop®, Wake Forest, NC). However, prior to beginning each rat’s sessions, the strength of the odor in each of the digging bowls was checked by the experimenter and additional aromatherapy oil injected if necessary to ensure consistent odor potency across rats. Before bowls were reused for
novel stimuli, they were thoroughly soaked in rubbing alcohol for three days and spray painted to remove any lingering odor. Table 1 lists the odorants and textures from which the experimental stimuli were created.

**Forced-choice digging paradigm.** Figure 10 illustrates the two different trial types (a), Target and Distractor, and (b) a typical session. On every trial, rats were simultaneously presented with two digging bowls in the testing arena: an odor-texture bowl and a blank bowl, both of which were filled approximately three-quarters of the way full of the granular commercial bedding Bed-o’cobs (The Andersons, Maumee, OH). On target trials, the reward (half piece of Kellogg's® Froot Loops® cereal) was buried in the odor-texture bowl, and on distractor trials, the reward was buried in the blank bowl. Rats were trained to dig in only one bowl one each trial, thus requiring that they extract a rule for bowl selection. Finely ground pieces of Froot Loops cereal were added to the bedding of all bowls to mask the location of the reward. The Froot Loop reward was always buried approximately two-thirds of the way down into the bedding.

**Stimuli**

**Baseline Stimuli.** Due to the complexity of our forced-choice digging paradigm, rats were first introduced to the different responses needed for the two trial types, Target and Distractor, without the need for feature binding using two Baseline odor-texture bowls. Rats could use a single odor or texture or the distinct combination of the two features to determine the correct bowl choice as each Baseline stimulus was characterized by a distinct odor and texture. One of the odor-texture Baseline bowls was designated a Target bowl (B1) and the other odor-texture Baseline bowl was designated a Distractor bowl (B2). Although an odor-texture bowl and the blank bowl were present on every trial, on Target trials the rat had to dig in the odor-texture bowl
and on Distractor trials the rat had to dig in the blank bowl to retrieve the reward. The same two Baseline odor-texture bowls were used throughout the experiment to measure the effect of lesion on general stimulus discrimination and digging performance.

*Feature-Conjunction Stimuli.* Each Feature-Conjunction (FC) stimulus set contained four conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As illustrated by Figure 11, crossmodal binding of odors and textures was required to determine the correct bowl choice as each individual odor and texture was associated with both a target and a distractor bowl such that no single odor or texture could be used for correct bowl selection. That is, the crossmodal features of the target and distractor bowls overlapped.

*Feature-Singleton Stimuli.* The Feature-Singleton (FS) stimulus set contained four non-conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As illustrated by Figure 11, feature binding was not required to determine the correct bowl choice as each individual odor and texture was associated with either a target or a distractor bowl such that rats could use a single odor or texture or the distinct combination of the two features for correct bowl selection. That is, the crossmodal features of the target and distractor bowls were distinct and did not overlap.

*Feature-Singleton Enhanced-Difficulty Stimuli.* The FS Enhanced-Difficulty stimulus set contained four non-conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As was the case for the FS stimuli, feature
binding was not required as rats could use a single odor or texture or the distinct combination of the two features to determine the correct bowl choice. However, as illustrated by Figure 11, the difficulty of the FS Enhanced-Difficulty stimuli was greater than that of the FS stimuli because rats had the additional requirement of learning when to rely on odor and when to rely on texture to determine the correct bowl choice. That is, one odor (Odor 7) and one texture (Texture 8) were associated with both a target and a distractor bowl, forcing the rats to use the particular crossmodal features of the odor-texture bowl to determine whether to use odor or texture for correct bowl selection.

Pre-Surgical Training Procedures

Habituation. The testing arena was baited with 2 whole Froot Loops and rats were allowed to explore the apparatus until they consumed at least one of the treats.

Pretraining. For two days, rats were trained to dig for a single whole Froot Loop buried at the bottom of an aluminum metal food bowl in their home cages. Sessions lasted approximately 1 hour. The bowls used in their home cages were different from those used during subsequent experimental training in the testing arena; they clipped onto the side of their home cage and measured 4 cm deep and 8 cm in diameter.

General forced-choice digging procedures. At the start of every session, rats were placed in the start box of the apparatus with the sliding door closed. During this time, the appropriate bowl was baited with a single half Froot Loop and placed beside the unrewarded bowl in the testing arena of the apparatus. The blank bowl was always positioned in the far left corner of the chamber with the odor-texture bowl directly in front and flush against the blank bowl to counter the rats’ natural strategy to first dig in the blank bowl and then over trials to acquire when to dig in the odor-texture bowl. The sliding door was then lifted, and the rat was allowed to make a
bowl choice, after which it was gently guided back to the start box and allowed to eat the Froot Loop (if obtained) with the door closed. A choice was defined as a dig if one or both paws displaced the bedding of the chosen bowl, or if a rat put its nose half-way down into the bedding. Rats remained in the start box between trials while the bowls were re-baited and replaced, which took on average 30 sec. Rats received one session per day. The first few sessions were always discovery sessions, during which rats were allowed to make as many choices as necessary to find the buried reward, but only the first bowl choice counted towards accuracy. During all remaining sessions, rats were only allowed to make a single bowl choice. If rats did not make a choice within 2-3 min, then the Froot Loop was removed and placed on top of the bedding of the rewarded bowl for the rat to find and consume in the start box. In between sessions, any bedding that accumulated in the apparatus was vacuumed up and the walls and floor of the apparatus were wiped down with rubbing alcohol.

Acquisition of Baseline stimuli. Each session consisted of 14 trials, half of which were Target trials and half of which were Distractor trials. Within a session, trials were presented in a pseudorandom order, such that no more than 3 consecutive trials were of the same type (target or distractor). The first three sessions were discovery sessions. Baseline training continued until all rats reached a criterion of at least 6 out of 7 correct Target and 6 out of 7 correct Distractor trials for at least two nonconsecutive sessions.

Acquisition of learning-to-learn Feature-Conjunction stimuli. After the final Baseline training session, in the subsequent session, rats began feature binding training using the learning-to-learn FC stimulus set. FC sessions consisted of 16 trials, the first 4 of which were Baseline trials (2 Target and 2 Distractor trials) identical to those described above. The remaining 12 trials of a session were 6 Target trials (3 T1, 3 T2) and 6 Distractor trials (3 D1, 3 D2) presented in a
pseudorandom order, such that no more than 3 consecutive trials of the same type (Target or Distractor) occurred in a session. The first three sessions were discovery sessions and only during training on this initial learning-to-learn stimulus set did rats receive correction trials in which an incorrect response was always followed by the same trial until a correct choice was made. Correction trials did not count towards accuracy. To ensure that stable and high levels of performance were attained on the FC task prior to surgery, a performance criterion of 5 out of 6 correct FC Target and 5 out of 6 correct FC Distractor trials was used. Training continued until all rats reached criterion performance for at least 10 consecutive sessions.

Surgery

Rats were assigned to one of two surgical groups, sham-lesioned (n = 8) or ACh-NBM-lesioned (n = 11) equating the groups for pre-surgical Baseline and FC performance. One rat became ill and was euthanized prior to surgery, reducing the ACh-NBM-lesioned sample size to 11. Surgeries were performed under aseptic conditions. Rats were anesthetized with isoflurane (approximate maintenance dose was 2 % with 1 L/min of oxygen). A subcutaneous (s.c.) injection of the analgesic buprenorphine (0.03 mg/kg) and an intraperitoneal (i.p.) injection of atropine (0.05 mg/kg) were delivered immediately prior to surgery, the latter of which served to prevent fluid buildup in the lungs. Four 1.0 mm holes were drilled at the following stereotaxic coordinates relative to bregma and the surface of the skull (Paxinos & Watson, 1998): anterior NBM: Anterior/Posterior (A/P) -0.8 mm, Medial/Lateral (M/L) ± 2.5 mm, Dorsal/Ventral (D/V) - 8.2 mm; posterior NBM: A/P -1.6 mm, M/L ± 2.5 mm, D/V – 7.6 mm. It should be noted that our anterior lesion site bordered the most dorsal and lateral extent of the substantia innominata (SI); however, we refer to our lesions as NBM lesions given that our injection sites were primarily restricted to the anatomical confines of the NBM as verified by immunohistochemical analyses of the extent of our lesions (see Results). There were a total of four intraparenchymal
injection sites, two per hemisphere, of 0.2 µl sterile 0.1 M (pH 7.4) phosphate-buffered saline (sham-lesion) or 0.2 µg/µl 192 IgG-saporin (Advanced Targeting Systems, San Diego, California, lot# 41-105) dissolved in sterile 0.1 M (pH 7.4) phosphate-buffered saline through a 26-gauge Hamilton syringe at 0.1 µl/min. The needle was left in place for 3 min after each injection. Choice of coordinates for lesioning and dose of immunotoxin were based on pilot surgeries performed in our laboratory. The body temperature of each rat was maintained with a homeothermic blanket throughout the surgery. After the injections were complete, a small piece of sterile gelfoam was applied over the exposed skull to control any bleeding and the wound was closed with staples and EMLA topical analgesic ointment (2.5% lidocaine and 2.5% prilocaine) was liberally applied around the staples. To prevent dehydration, rats were given normal saline (0.9 % NaCl; 2 ml / 100 g body weight; s.c.) immediately post-surgery. All rats received a minimum of 14 days of recovery with *ad libitum* food and water before being food restricted for subsequent testing.

**Post-Surgical Testing Procedures**

Experimenters were blind to the surgical group of the animals. All post-surgical FC and FS sessions consisted of 16 trials, the first 4 of which were Baseline trials (2 Target and 2 Distractor trials) identical to those described above to measure the effect of lesion on general stimulus discrimination and digging performance. The remaining 12 trials of a session were 6 Target trials (3 T1, 3 T2) and 6 Distractor trials (3 D1, 3 D2) presented in a pseudorandom order, such that no more than 3 consecutive trials of the same type (Target or Distractor) occurred in a session. The same odor-texture pairings were used for rats in both the NBM- and sham-lesioned groups across all stimulus sets to ensure that differences between particular odors and textures did not influence any effect of lesion.
Retrieval of Baseline Stimuli. To ensure that rats could still perform the forced-choice digging task and discriminate the odors and textures post-surgery, rats retrieved the Baseline stimuli they had acquired prior to surgery. Baseline retrieval comprised 2 high-accuracy sessions.

Retrieval of Feature-Conjunction Stimuli. After the final Baseline retrieval session, in the subsequent session, rats began retrieval of the learning-to-learn FC stimulus set they had acquired prior to surgery. FC retrieval sessions continued until all rats reached asymptotic performance, which took 6 sessions.

Acquisition of Feature-Conjunction Stimuli. After the final FC retrieval session, in the subsequent session, rats began acquisition of novel FC stimuli. FC acquisition sessions continued until all rats reached asymptotic performance, which took 16 sessions.

Acquisition of Feature-Singleton Stimuli. After the final FC acquisition session, in the subsequent session, rats began acquisition FS stimuli. FS acquisition sessions continued until all rats reached asymptotic performance, which took 12 sessions.

Acquisition of Feature-Singleton Enhanced-Difficulty Stimuli. After the final FS acquisition session, in the subsequent session, rats began acquisition of the FS Enhanced-Difficulty stimuli. FS Enhanced-Difficulty acquisition sessions continued until all rats reached asymptotic performance, which took 12 sessions.

Histological Analyses

Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and transcardially perfused with approximately 150 ml of ice-cold normal saline followed by approximately 150 ml of ice-cold 4% paraformaldehyde. Brains were extracted and immediately post-fixed in 4% paraformaldehyde for 2 hr at 4 °C, and then transferred to a solution of 20% sucrose in
phosphate-buffered saline (0.1 M; pH 7.4) and stored for 2 weeks at 4 °C. Brains were sectioned at a thickness of 60 µm using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems, Canada). Adjacent sections were used for staining for acetylcholinesterase (AChE) histochemistry, choline acetyltransferase (ChAT) immunohistochemistry, and parvalbumin immunohistochemistry. AChE histochemistry was carried out according to the method described by Paxinos and Watson (1998), and was used to confirm cholinergic fiber loss in target neocortical structures of the NBM. ChAT and parvalbumin immunohistochemistry were carried out according to methods described in Baxter, Bucci, Gorman, Wiley, and Gallagher, (1995) and De Rosa, Hasselmo, and Baxter (2001) to assess the extent of cholinergic and GABAergic cell body loss in the NBM and medial septum/vertical limb of the diagonal band of Broca (MS/VDB), respectively. After completion of all histological assays, brain slices were mounted on slides, dehydrated and cleared using an ascending ethanol and xylene series, coverslipped with DPX, and examined under a Leica light microscope (DM4000B, Ontario, Canada).

**Histological Quantification**

*Cell counting.* ChAT- and parvalbumin-immunoreactive cells were counted bilaterally in the NBM and MS/VDB as delineated by Paxinos and Watson (1998) for each rat across brain sections 300 µm apart using a Leica light microscope (DM4000B, Ontario, Canada) and Openlab image analysis software (Quorum Technologies, Ontario, Canada). For the NBM, cell counts were taken from brain sections at approximately the following A/P (anterior/posterior) coordinates relative to bregma: A/P = -0.84 mm, -1.32 mm, and -1.56 mm. The rectangular outlines superimposed on the rat brain coronal schematics depicted in Figure 12 (Paxinos & Watson, 2007) delineate the NBM cell-counting frames used for histological quantification. For the MS/VDB, approximately the following A/P coordinates relative to bregma were used for cell
counting: A/P = +0.84 mm, +0.72 mm, and +0.60 mm. The rectangular outlines superimposed on the rat brain schematics depicted in Figure 13 (Paxinos & Watson, 2007) delineate the MS/VDB cell-counting frames used for histological quantification. ChAT- and parvalbumin-immunoreactive cells were easily identifiable and characterized by relatively large cell bodies with several extending dendrites, and we only counted cells that were well distinguishable (i.e., well-defined borders) from the background.

AChE Densitometry. To quantify the reduction of cortical cholinergic input induced by our NBM cholinergic lesions, estimates of optical density in the frontal and parietal cortices and hippocampus were obtained from photomicrographs of AChE-stained brain sections using the software package Scion (Scion Corporation, Maryland, USA). For each rat, optical density values obtained from the three target brain regions were then normalized to raw striatal optical density values to eliminate the potential influence of different staining intensities across animals [see similar methods used by Vuckovich, Semel, and Baxter (2004)]. Raw optical density values from the striatum did not differ between the NBM- and sham-lesioned groups (see Results). The rectangular outlines superimposed on the rat brain schematics depicted in Figure 14 (Paxinos & Watson, 2007) delineate the boundaries used for obtaining optical density values in the frontal and parietal cortices and hippocampus.

Results

Statistical Analyses. All statistical analyses were conducted using SPSS Version 14 with an alpha level of .05.

Pre-Surgical Training. Thirteen training sessions were required for all rats to reach criterion performance on the Baseline task, and 79 training sessions were required for all rats to reach
criterion performance on the Feature-Conjunction (FC) task using the learning-to-learn FC stimulus set.

**Histological Analyses**

Of the 20 rats, one became ill and was euthanized prior to surgery and two sham-lesioned rats became ill and were euthanized post-surgery. Post-mortem assessment revealed that all three of these rats had become diabetic. Accordingly, behavioural and histological data from these three rats were removed from statistical analyses for a total of 17 rats. Quantitative histological analyses revealed that two ACh-NBM-lesioned rats had statistically insufficient reductions of choline acetyltransferase (ChAT)-immunoreactive cells in the NBM as indicated by a ChAT-immunoreactive cell count greater than two standard deviations above the mean of the ACh-NBM-lesioned group and non-significantly different from the mean of the sham-lesioned group. Moreover, the acetylcholinesterase (AChE) optical density values obtained from the frontal and parietal cortices of these two animals were non-significantly different from the means of the sham-lesioned group. Accordingly, behavioural and histological data from these two rats were removed from statistical analyses. The sham-lesion sample was thus reduced to six and the ACh-NBM-lesioned sample size was reduced to nine.

**Cell counting.** There was significant depletion of ChAT-immunoreactive cells in the NBM of the ACh-NBM-lesioned rats relative to sham-lesioned rats (Figure 12), while an equivalent number of ChAT-immunoreactive cells were observed in the neighboring MS/VDB nuclei of both groups of rats (Figure 13). Independent *t*-tests confirmed that there were significantly fewer ChAT-immunoreactive cells in the NBM of ACh-NBM-lesioned compared to sham-lesioned rats [*t*(13) = 15.05, *p* < 0.001; *M*$_{\text{Sham}}$ = 405.50, *SD* = 41.27; *M*$_{\text{NBM}}$ = 129.56, *SD* = 30.05]. However, there was no significant difference between the two groups of rats in the number ChAT-
immunoreactive cells in the MS/VDB ($t < 2.0; M_{\text{Sham}} = 251.67, SD = 19.12; M_{\text{NBM}} = 238.11, SD = 11.78$). An equivalent number of parvalbumin-immunoreactive cells were observed in the NBM (Figure 12) and MS/VDB (Figure 13) of both sham- and ACh-NBM-lesioned rats.

Independent $t$-tests confirmed that there were no significant differences, $t < 1.0$, between the two groups of rats in the number of parvalbumin-positive cells in the NBM ($M_{\text{Sham}} = 161.33, SD = 28.24; M_{\text{NBM}} = 161.44, SD = 24.13$) and MS/VDB ($M_{\text{Sham}} = 228.33, SD = 27.28; M_{\text{NBM}} = 221.22, SD = 28.90$).

**AChE Densitometry.** AChE staining revealed a loss of cholinergic fibers in the frontal and parietal cortices, but not the hippocampus of ACh-NBM-lesioned rats relative to sham-lesioned rats (Figure 14). This was confirmed by independent $t$-tests comparing the optical density values (normalized to raw striatal optical density values) from the three target brain regions between the two groups of rats. The raw optical density values from the striatum (used for normalization) did not differ between the two groups ($t < 1.0; M_{\text{Sham}} = 240.13, SD = 4.91; M_{\text{NBM}} = 240.05, SD = 6.77$). There was significantly less AChE reactivity as measured by optical density in the frontal [$t(13) = 9.09, p < 0.001; M_{\text{Sham}} = 0.53, SD = 0.03; M_{\text{NBM}} = 0.38, SD = 0.03$] and parietal [$t(13) = 6.59, p < 0.001; M_{\text{Sham}} = 0.47, SD = 0.02; M_{\text{NBM}} = 0.38, SD = 0.03$] cortices of ACh-NBM-lesioned compared to sham-lesioned rats. However, there was no significant difference between the two groups of rats in AChE reactivity in the hippocampus as measured by optical density ($t < 1.0; M_{\text{Sham}} = 0.60, SD = 0.05; M_{\text{NBM}} = 0.61, SD = 0.06$).

**Post-Surgical Testing**

**Retrieval of Baseline Stimuli.** Rats in both lesion conditions maintained very high accuracies on the Baseline stimuli during the first post-surgical retrieval session ($M_{\text{Sham}} = 85\%, SD = 0.20; M_{\text{NBM}} = 88\%, SD = 0.11$). An analysis of variance (ANOVA) was performed using Lesion
Condition (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor and Session as a within-subjects factor. The ANOVA revealed no significant effect of Lesion Condition ($F < 1.0$, $\eta^2 = 0.06$), a significant effect of Session [$F(1, 13) = 6.38, p < 0.05, \eta^2 = 0.33$], and no significant interaction ($F < 1.0, \eta^2 = 0.001$).

*Retrieval of Feature-Conjunction Stimuli.* Figure 15 depicts the performance of rats in the NBM- and sham-lesioned groups on the learning-to-learn FC stimulus set during the last six sessions prior to surgery and the subsequent retrieval of these same FC stimuli during the six post-surgical sessions. A two-way mixed ANOVA was performed using Lesion Condition (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor and Performance Period (pre-surgery or post-surgery) and Session as within-subjects factors. The ANOVA revealed no significant effect of Lesion Condition ($F < 1.0, \eta^2 = 0.03$) or Performance Period ($F < 1.0, \eta^2 = 0.04$), a significant effect of Session [$F(5, 65) = 8.03, p < 0.001, \eta^2 = 0.38$], and a significant Session X Performance Period interaction [$F(5, 65) = 8.03, p < 0.001, \eta^2 = 0.38$]. All other interactions were found to be non-significant ($F < 2.5, \eta^2 < 0.15$). An ANOVA on Baseline stimuli performance during post-surgical FC retrieval revealed non-significant effects of Lesion Condition ($F < 1, \eta^2 = 0.04$) and Session ($F = 1.0, \eta^2 = 0.08$), and no significant interaction ($F = 1.0, \eta^2 = 0.08$).

*Acquisition of Feature-Conjunction Stimuli.* Figure 16A depicts post-surgical acquisition of the FC task using a novel stimulus set with data binned into 4-session blocks. An ANOVA was performed using Lesion Condition (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor and Block (first or last) as a within-subjects factor. The ANOVA revealed a significant effect of Lesion Condition [$F(1, 13) = 4.69, p = 0.05, \eta^2 = 0.27$], a significant effect of Block [$F(1, 13) = 136.22.43, p < 0.001, \eta^2 = 0.91$], and no significant interaction ($F = 2.0, \eta^2 = 0.13$).
An ANOVA on Baseline stimuli performance during FC acquisition revealed non-significant effects of Lesion Condition \((F < 1.0, \eta^2 = 0.06)\) and Block \((F < 1.5, \eta^2 = 0.09)\), and no significant interaction \((F < 1.5, \eta^2 = 0.09)\).

**Acquisition of Feature-Singleton Stimuli.** Figure 16B depicts post-surgical acquisition of the FS task with data binned into 4-session blocks. An ANOVA was performed using Lesion Condition (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor and Block (first or last) as a within-subjects factor. The ANOVA revealed no significant effect of Lesion Condition \((F < 1.0, \eta^2 = 0.06)\), a significant effect of Block \([F(1, 13) = 58.82, p < 0.001, \eta^2 = 0.82]\), and no significant interaction \((F < 1.0, \eta^2 = 0.03)\). An ANOVA on Baseline stimuli performance during FS acquisition revealed non-significant effects of Lesion Condition \((F < 1.5, \eta^2 = 0.10)\) and Block \((F < 2.5, \eta^2 = 0.15)\), and no significant interaction \((F < 2.0, \eta^2 = 0.01)\).

**Acquisition of Feature-Singleton Enhanced-Difficulty Stimuli.** Figure 16C depicts post-surgical acquisition of the FS Enhanced-Difficulty task with data binned into 4-session blocks. An ANOVA was performed on the acquisition data using Lesion Condition (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor and Block (first or last) as a within-subjects factor. The ANOVA revealed no significant effect of Lesion Condition \((F < 1.0, \eta^2 = .02)\), a significant effect of Block \([F(1, 13) = 38.64, p < 0.001, \eta^2 = 0.75]\), and no significant interaction \((F < 2.0, \eta^2 = 0.13)\). An ANOVA on Baseline stimuli performance during FS Enhanced-Difficulty acquisition revealed non-significant effects of Lesion Condition \((F < 1.0, \eta^2 = 0.001)\) and Block \((F < 1.0, \eta^2 = 0.06)\), and no significant interaction \((F < 1.0, \eta^2 = 0.02)\).

Indicative of its greater difficulty, sham-lesioned rats performed worse on the FS Enhanced-Difficulty task than the FS task during acquisition. This was confirmed by performing a within-subjects ANOVA on sham-rat acquisition data using Task (FS or FS Enhanced-Difficulty) and
Block (first or last) as within-subjects factors. The ANOVA revealed a significant effect of Block \([F(1,5) = 306.82, p < 0.001, \eta^2 = 0.98]\) and Task \([F(1, 5) = 31.57, p < 0.01, \eta^2 = 0.86]\), and no significant interaction \((F < 1.5, \eta^2 = 0.21)\).

*Correlations between task performance and ChAT immunoreactivity and AChE reactivity.*

The results of the correlational analyses between post-surgical task performance and ChAT immunoreactivity as measured by the number of ChAT-positive cells in the NBM, and neocortical AChE reactivity as measured by the optical density of AChE staining in the frontal and parietal cortices, are shown in Table 2. Accuracy during FC acquisition significantly and positively correlated with both ChAT immunoreactivity in the NBM and with AChE reactivity in the frontal cortex. All other correlations were found to be non-significant \((p > 0.05)\); however, there was a weak trend towards significance found for the positive relationship found between FC acquisition and AChE reactivity in the parietal cortex. The scatterplots depicted in Figure 17 illustrate these three relationships. There were no significant correlations found between post-surgical task performance and AChE reactivity in the hippocampus \((r < 0.15, p > 0.15)\), ChAT immunoreactivity in the MS/VDB \((r < 0.15, p > 0.30)\), and parvalbumin immunoreactivity in the NBM \((r < 0.23, p > 0.20)\) or MS/VDB \((r < 0.15, p > 0.20)\).

**Discussion**

We contend that acetylcholine (ACh) provides the neuromodulatory support for the attentional processes critical for feature binding. In support of this hypothesis, previous work using systemic pharmacology demonstrated the importance of ACh to crossmodal feature binding at encoding (Botly & De Rosa, 2007), and more recent cross-species work revealed striking parallels between the effects of modulations of attention in humans and modulations of the muscarinic cholinergic system in rats on intramodal feature binding (Botly & De Rosa, 2008). However, the
neural source of the cholinergic neurotransmission critical for feature binding has yet to be determined. Human neuropsychological and functional magnetic resonance imaging (fMRI) studies have implicated frontoparietal cortices in the attentional processing necessary for feature binding (Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Donner et al., 2002; Esterman, Verstynen, & Robertson, 2007; Friedman-Hill, Robertson, & Treisman, 1995). Cholinergic efferents originating from the nucleus basalis magnocellularis (NBM) of the basal forebrain (BF), relative to other BF nuclei clustered nearby, provide 90% of the cholinergic input to the neocortex, including frontoparietal cortical regions (Mesulam, Mufson, Wainer, & Levey, 1983). We hypothesized that the NBM may be the neural source of the cholinergic neurotransmission critical for feature binding, and thus reducing cholinergic cortical afferentation via cholinergic immunotoxic lesioning of the NBM would impair feature binding in a similar manner to systemic muscarinic cholinergic blockade in rats.

The crossmodal Feature-Conjunction (FC) and Feature-Singleton (FS) tasks employed a forced-choice digging paradigm that required rats to decide in which of two bowls to dig to obtain a food reward. In the FC task, rats had to bind odor and texture features to determine the correct bowl choice, while in the FS tasks there was no requirement to bind odor and texture features and the rats could rely on a single feature (odor or texture). We predicted that the FS tasks would be less dependent on cortical cholinergic neurotransmission than the FC task because processing single features should be less attentionally demanding than processing conjunctions of features. Consistent with this hypothesis, in the present study, rats with 192 IgG-saporin-induced lesions of NBM were significantly impaired relative to sham-lesioned control rats at acquiring FC stimuli, while their ability to acquire FS stimuli remained intact. Additionally, the number of cholinergic (ChAT-positive) cells in the NBM and the degree of neocortical, but not hippocampal AChE reactivity were found to positively correlate only with post-surgical FC
acquisition performance, signifying the importance of neocortical cholinergic input from the NBM to feature binding at encoding.

Findings from the human cognitive literature support the low-attentional load nature of single-feature processing. Patients with attentional deficits who are impaired at encoding conjunctions of features remain fully capable of encoding single features (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Tales et al., 2002), and human fMRI studies have revealed selective activation of parietal attentional networks during tasks requiring feature binding, but not those requiring single-feature processing (Corbetta, Shulman, Miezin, & Petersen, 1995; Luck & Ford, 1998; Reynolds & Desimone, 1999). More recently, it was found that transcranial magnetic stimulation of the intraparietal sulcus influenced the binding of colour and form, but not the detection or processing of single features (Esterman, Verstynen, & Robertson, 2007).

Although the FS and FC tasks required the same number of odor-texture bowl discriminations (i.e., four), it could be argued that ACh-NBM-lesioned rats’ spared acquisition of FS stimuli was simply due to the FS task being less difficult than the FC task. To address this potential criticism, rats acquired a FS Enhanced-Difficulty stimulus set in addition to acquiring a FS stimulus set. Although both FS tasks did not require feature binding, as rats could rely on a single feature (odor or texture) for correct bowl selection, the FS Enhanced-Difficulty stimuli required rats to also learn when to rely on odor and when to rely on texture as one odor and one texture were associated with both the correct and incorrect bowl choices. Indicative of its greater difficulty, sham-lesioned rats performed worse while acquiring the FS Enhanced-Difficulty task than the FS task. However, there was no significant difference between the performance of rats in the two lesions groups during FS or FS Enhanced-Difficulty acquisition. This suggests that the feature
binding requirement of the FC task increased the attentional load of the task over and above any increase induced by greater task difficulty. Findings from the human cognitive literature support this proposition: even after controlling for target-distractor similarity, which makes both FS and FC tasks more attentionally demanding, feature binding in itself requires additional attentional resources over and above those needed for making fine perceptual discriminations (Carter, 1982; Duncan & Humphreys, 1989; Foldi et al., 2005; von Grunau, Dube, & Galera, 1994).

Lastly, ACh-NBM-lesioned rats were not impaired at retrieving a FC stimulus set they had acquired prior to surgery. This is consistent with previous pharmacological (Botly & De Rosa, 2007) and cross-species findings (Botly & De Rosa, 2008), and provides further evidence to suggest that once a conjunction stimulus is well-learned, a bound and stable neural representation is formed, thereby eliminating the need for an attentionally-demanding feature binding process during retrieval. Such dissociative encoding-retrieval findings are consistent with a model of cholinergic function proposing that an active cholinergic system is conducive to the encoding of new information, while a hypoactive cholinergic system is conducive to the consolidation and retrieval of previously-learned information (Hasselmo & McGaugh, 2004). When there are high levels of cholinergic neurotransmission in the cortex, afferent input from external sensory stimuli is enhanced, while intrinsic processing within cortices is reduced.

Intrinsic processing is associated with the reactivation of neural connections representing previously-learned information, and a reduction in such processing would prevent the recall of previously-learned information from interfering with the encoding of new information. This model of cholinergic function is well supported by both the human and non-human animal literatures (Aigner, Walker, & Mishkin, 1991; Anagnostaras, Maren, & Fanselow, 1995; Everitt & Robbins, 1997; Miranda & Bermudez-Rattoni, 1999; Orsetti, Casamenti, & Pepeu, 1996;
Pepeu & Giovannini, 2004; Safer & Allen, 1971; White & Ruske, 2002). For instance, scopolamine increased proactive interference from previously-learned odor- and word-pair associations during the encoding of novel associations in non-human and human animals, respectively (Atri et al., 2004; De Rosa & Hasselmo, 2000; De Rosa, Hasselmo, & Baxter, 2001). Furthermore, fMRI studies have shown that boosting cholinergic neurotransmission in humans with the anticholinesterase physostigmine improves memory performance by enhancing perceptual processing during the encoding stage (Furey, Pietrini, & Haxby, 2000).

Given past research implicating frontoparietal cortical cholinergic input from the NBM in attention (Bucci, Holland, & Gallagher, 1998; Dalley et al., 2004), we contend that it is likely cholinergic neurotransmission in a frontoparietal cortical network that is critical for feature binding, and the results of our correlational analyses between cortical AChE densitometry and post-surgical task performance are in support of this hypothesis. We found a significant positive correlation between FC acquisition and AChE reactivity in the frontal cortex and a weak trend towards significance in the parietal cortex. These findings are consistent with previous microdialysis work demonstrating positive associations between task-related attentional effort and ACh efflux in frontoparietal cortices in rats (Himmelheber, Sarter, & Bruno, 2000, 2001). More recent work using a novel enzyme-based amperometric technique, which allows for the monitoring of cortical ACh release on multiple timescales in freely behaving animals, has revealed both tonic and phasic attention-dependent changes in cortical cholinergic activity (Parikh, Kozak, Martinez, & Sarter, 2007; Parikh & Sarter, 2008). This work demonstrated that cortex-wide tonic ACh activity was associated with efficacious attentional processing and was positively correlated with attentional effort, thus we would hypothesize greater increases in tonic frontoparietal cortical ACh activity while rats performed our more attentionally-demanding FC versus FS tasks. Additionally, we expect that these tonic ACh increases would be specific to the
task-dependent regions that support the required attentional processes, and therefore would not expect to observe such increases in task-independent cortical regions such as the hippocampus. Further research is needed to determine the specific roles of tonic versus phasic attention-dependent cortical ACh activity, thus we cannot yet speculate on what these two forms may specifically contribute to feature binding.

The present study provides insight into the functional role of the NBM and extends previous systemic pharmacology work to provide further support for a critical role for the neurotransmitter ACh in the attention-dependent process of feature binding. Using the cholinergic immunotoxin 192 IgG-saporin to lesion the NBM of rats, it was shown that reducing cholinergic neocortical afferentation impairs crossmodal feature binding at encoding, while sparing its retrieval and the acquisition of crossmodal tasks only requiring single-feature processing. Such destruction of cholinergic neurons in the NBM resulted in reduction of cholinergic input to the entire cortical mantle, and our histological densitometry analyses revealed positive relationships between post-surgical feature binding acquisition performance and cortical AChE staining in frontoparietal cortical regions. Accordingly, future work should concentrate on delineating the specific cortical targets cholinergic input must reach for successful feature binding to occur.
Chapter 4

Experiment 4:

Using visual search to examine cholinergic contributions to feature binding in the rat
Abstract

Feature binding refers to the fundamental challenge of the central nervous system to integrate sensory information registered by distinct brain regions to form a unified neural representation of a stimulus. We have previously demonstrated that the neurotransmitter acetylcholine critically supports the attentional processes required for both crossmodal (Botly & De Rosa, 2007) and intramodal (Botly & De Rosa, 2008) feature binding using a novel digging-based rat feature binding task and human visual analog of the task. Moreover, we have demonstrated that the nucleus basalis magnocellularis (NBM) of the basal forebrain is the neural source of the cortical cholinergic neurotransmission necessary for feature binding in the rat (Botly & De Rosa, 2009a).

We have now translated the standard test of human feature binding, visual search, for rats. In the present study, sixteen male Long-Evans rats were trained to perform visual search using touchscreen-equipped operant chambers and black-and-white shapes. Testing sessions comprised Feature Search (no feature binding required) and Conjunctive Search (feature binding required) trials using set sizes of four, six, and eight stimuli. Following acquisition of the visual search task, eight rats received bilateral cholinergic-selective lesions of the NBM using the immunotoxin 192 IgG-saporin to reduce cholinergic afferentation of the neocortex. As expected, relative to sham-lesioned rats, ACh-NBM-lesioned rats took significantly longer to locate the target stimulus on Conjunctive Search but not Feature Search trials, thus reflecting less efficient visual search. These data confirm that cholinergic contributions from the NBM support feature binding using a rat analog of the visual search paradigm.
Introduction
We perceive objects as unified wholes, but the mammalian brain is organized such that distinct neural regions are primarily responsible for detecting and processing the different features of an object, such as its shape or colour. The unknown mechanism by which unified neural representations of objects are formed is referred to as feature binding (Treisman & Gelade, 1980). While the human cognitive literature has established the attention-dependent nature of feature binding, (Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Esterman, Verstynen, & Robertson, 2007; Friedman-Hill, Robertson, & Treisman, 1995; Robertson, 2003), the neurochemical contributions to feature binding remain unknown. We hypothesized that the neurotransmitter acetylcholine (ACh) would play a critical role in feature binding given its well-established role in modulating attention (Sarter & Bruno, 1997; Sarter, Hasselmo, Bruno, & Givens, 2005).

In support of this hypothesis, using a digging-based feature binding task, we have shown that systemic muscarinic cholinergic blockade with scopolamine impairs the ability of rats to acquire crossmodal Feature-Conjunction (FC) stimuli that required the binding of odor and texture stimuli, while leaving their ability to acquire Feature-Singleton stimuli that did not require crossmodal feature binding intact (Botly & De Rosa, 2007). Our recent cross-species work has demonstrated that human participants performing a visual intramodal analog of our rat feature binding task using coloured shapes under divided-attention yielded a similar pattern of impairment in feature binding at encoding to that of rats under the influence of scopolamine, suggesting that ACh supports the attentional processes necessary for feature binding (Botly & De Rosa, 2008). Most recently, we have shown that cortical cholinergic input from the nucleus
basalis magnocellularis (NBM) of the basal forebrain is critical for rats to efficiently acquire our crossmodal odor-texture FC task (Botly & De Rosa, 2009a).

While our previous rat work has established the importance of cholinergically-driven attentional processes to feature binding using odors and textures, in the human cognitive literature, the standard test of feature binding is visual search (Corbetta, Shulman, Miezin, & Petersen, 1995; Nobre, Coull, Walsh, & Frith, 2003; Treisman, 1998). In this paradigm, participants must find a visual target stimulus embedded in an array of distractor stimuli. On feature search trials, feature binding is not required to find the target because participants can look for a single feature (e.g., look for the colour green to find a green apple in a basket of red apples). However, on conjunctive search trials, feature binding is required because participants must look for a conjunction of two features to find the target (e.g., look for the colour green and the shape of an apple to find a green apple in a basket of red apples and red and green pears). During feature search, the demand on attention is minimal as targets appear to “pop out” regardless of the number of distractors present, while during conjunctive search, an attentionally-demanding serial search for the target is necessary, as demonstrated by a significant increase in target detection time with increasing numbers of distractors (Treisman & Gelade, 1980). It has been revealed that patients diagnosed with Alzheimer’s disease have attentional deficits because these patients are selectively impaired on conjunctive, but not feature search trials of visual search (Foster, Behrmann, & Stuss, 1999; Tales et al., 2002), and functional neuroimaging work with neurologically intact human participants has revealed selective activation of frontoparietal cortical attentional networks during conjunctive, but not feature search trials of visual search (Corbetta, 1998; Corbetta, Shulman, Miezin, & Petersen, 1995; Nobre, Coull, Walsh, & Frith, 2003).
If ACh is critical for feature binding, as suggested by our pharmacological and lesion work that utilized odor and texture stimuli with our digging-based feature binding task (Botly & De Rosa, 2007, 2008, 2009a), then its modulatory influence on this cognitive process should generalize to other tests of feature binding. In order to extend the validity of our previous findings and to integrate our cholinergic feature binding hypothesis with the human cognitive literature, we designed a rodent visual search paradigm. A rodent analog of the human visual search paradigm would allow for an important test of our hypothesis that cholinergic input to the neocortex from the NBM of the basal forebrain is important for feature binding. Basalocortical cholinergic projections from the NBM provide 90% of the cholinergic input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983), including frontoparietal cortical regions implicated in attentional processing (Behrmann, Geng, & Shomstein, 2004; Bucci, 2008; Bucci & Chess, 2005; Constantinidis, 2006; Donner et al., 2002).

While visual search tasks comparable to those employed in the human literature have been designed and successfully tested in pigeons (Blough, 1984) and monkeys (Latto, 1971), to our knowledge visual search has not been attempted with rats. Despite olfaction being the dominant sensory modality of rodents, studies have demonstrated that rats of the Long-Evans strain possess the visual acuity necessary to make fine achromatic visual discriminations based on shape, pattern, and orientation (Bushnell, 1999; Prusky, Harker, Douglas, & Wishaw, 2002), and the use of touchscreen-based cognitive assessment in rodents is on the rise in the behavioural neuroscience literature (Bussey et al., 2008; Bussey, Saksida, & Rothblat, 2001; Cook, Geller, Zhang, & Gowda, 2004; Markham, Butt, & Dougher, 1996). Based on this existing research, we predicted that rats would be capable of performing a touchscreen-based visual search task.
In the present study, rats were trained to perform a touchscreen-based visual search task using set-sizes of four, six, and eight stimuli. Each session was counterbalanced for target and distractor positions, trial-type (Conjunctive or Feature Search trials), and stimulus set-size. The target stimulus was always a white square, and the distractor stimuli were a black square, white triangle, and black triangle. On Conjunctive Search trials, the target stimulus was presented along with distractor stimuli that differed from the target on two feature dimensions, shape and pattern, such that feature binding was required to locate the target. On Feature Search trials, the target stimulus was presented along with distractor stimuli that differed from the target on only a single feature dimension, shape or pattern, such that feature binding was not required to locate the target. It was predicted that rats’ performance on the visual search task would yield the classic pattern of a significant increase in the latency to locate the target stimulus as the stimulus set-size increased on Conjunctive, but not Feature Search trials.

Following pre-surgical acquisition of the visual search task, we bilaterally lesioned the NBM of rats using the cholinergic-selective immunotoxin, 192 IgG-saporin, and compared the ability of ACh-NBM-lesioned rats to that of sham-lesioned rats to perform the visual search task using the same target and distractor stimuli rats were trained on prior to surgery. If cortical cholinergic neurotransmission is necessary to support the attentional processes needed for feature binding, then reducing cholinergic afferentation of the neocortex via cholinergic-selective lesions of the NBM should yield a performance pattern reflective of less efficient visual search. Relative to sham-lesioned controls, ACh-NBM-lesioned rats were expected to take longer to locate the target stimulus on Conjunctive, but not Feature Search trials, as only the former require an attentionally-demanding feature binding process. It was also predicted that any lesion-induced increase in conjunctive search latency would significantly interact with stimulus set-size, such
that the detrimental impact of cholinergic-selective NBM lesions on search latency would be greatest during Conjunctive Search trials with the largest number of distractor stimuli present.

While the digging-based feature binding task employed in our previous work allowed us to dissociate the encoding and retrieval stages of feature binding (Botly & De Rosa, 2008, 2009a, 2009b), the visual search paradigm is characterized by negligible mnemonic demands as the sole task of participants is to locate a well-learned target stimulus amongst an array of distractor stimuli. The locations of the target and distractor stimuli change unpredictably across trials, such that a “visual search” for the target is always necessary. Because the identity of the target stimulus remains constant across all trials and sessions, no other information must be held in memory, making visual search tasks a relatively pure test of the attentional demands of feature binding. Consistent with a well-supported model of cholinergic function stating that high levels of ACh are not needed for the retrieval of previously-learned information (Hasselmo & McGaughy, 2004), our previous work has shown that rats are unimpaired at retrieving well-learned conjunctive stimuli following systemic muscarinic blockade with scopolamine (Botly & De Rosa, 2007) and cholinergic-selective lesions of the NBM (Botly & De Rosa, 2009a). Given that the target and distractor stimuli were very well learned by rats prior to surgery, we thus did not anticipate a difference in the ability of sham-lesioned and ACh-NMB-lesioned rats to accurately locate the target stimulus on Feature or Conjunctive Search trials of visual search post-surgery.

Methods

Participants. Participants were twenty experimentally naïve male Long-Evans rats (Charles River, Montreal, Quebec) aged 6 weeks and weighing 195-225 g at the start of the experiment. Rats were housed individually in 45 cm long x 25 cm wide plastic tub cages with food available
ad libitum. The vivarium was temperature and humidity controlled. Rats were maintained on a reversed 12 hr light – 12 hr dark cycle (lights off at 8 am) and training was conducted during the dark phase of the light: dark cycle between the hours of 10am and 5pm, 6-7 days a week. One week prior to the start of the experiment, rats were handled for 15 min per day for 7 days with water available *ad libitum*. Rats were water deprived 24 hr before the start of training. Throughout the experiment, rats received water during training and were given *ad libitum* access to water for 20-30 min each day following training.

*Apparatus.* Four custom-made operant chambers equipped with touchscreens (MED Associates Inc; ELO) were used. Figure 18 illustrates an overhead view of a single touchscreen-equipped operant chamber [41.2 cm x 41.2 cm x 29.2 cm (l x w x h)] housed inside a sound- and light-attenuating enclosure (74 cm x 60.5 cm x 60 cm) made of wood and equipped with a fan to provide ventilation and masking noise. The right and left operant chamber walls and ceiling were made of clear Plexiglas and the right wall was hinged creating an interior door for access to the chamber. The front and rear walls were made of stainless steel and the floor of the chamber was composed of steel rods (5 mm diameter) spaced 1.1 cm apart horizontally. The bottom of the touchscreen was level with the floor of the chamber. The touchscreens were 24.7 cm wide x 18.5 cm high and positioned in the center of the front wall of the chamber with 7.9 cm on either side. Touchscreens were inset 1.6 cm from the front wall of the chamber. A rectangular water-well (5 cm x 5 cm x 5 cm) was located in the center of the rear wall of the chamber. A water pump equipped with a 25 ml syringe dispensed 0.05 ml of water at a time. A circular white light 2.5 cm in diameter was located 2.2 cm above the water well and acted as a cue to the availability of water. Each chamber contained three pairs of infrared (IR) emitters and detectors. Two pairs were located on the right and left walls of the chamber and were positioned 4 cm above the floor to detect movement. One of these pairs was located 5.5 cm from the back wall of the chamber.
(back IR beam) and the other was equidistant between the rear and front wall of the chamber (middle IR beam). The third pair was positioned on either side of the water-well (water IR beam) to detect drinking. Each chamber was equipped with a speaker so that warning tone stimuli could be emitted to indicate the presentation of a stimulus. A computer located in the testing room that detected responses and presented stimuli on the touchscreen, controlled each chamber. A single computer located outside of the testing room controlled all the components of the operant boxes and recorded data via a control interface (MED Associates Inc.).

*Visual Stimuli.* A set of black-and-white computer-generated stimuli was created: 1 target stimulus (white square) and 3 distractor stimuli (black square, white triangle, black triangle). Each stimulus measured 5 X 5 cm (Figure 19).

*Pre-training Procedures*

*Habituation.* Prior to water restriction, each rat was exposed to the inside of an operant chamber for 15 min per day for 2 days.

*Light-water training.* Twenty-four hours following the start of water restriction, rats began light-water training using a Variable Interval-60 sec (15 seconds variance) schedule of reinforcement. The light above the water-well indicated the availability of water during which rats could insert their nose into the water-well to break the IR-beam and activate the water pump to receive a reward of 0.05 ml of water. Rats received 30 min sessions per day until criterion performance of breaking the IR-beam in the water-well on average less than 2 seconds after the onset of the water-light occurred within a session for all rats.

*Touch-light-water training.* Rats were required to touch the touchscreen with their nose or front paws to activate the water light and then break the water IR-beam to receive a reward of 0.05 ml
of water. To discourage rats from directing their touches to the corners of the touchscreen, a 22.9 cm wide x 15.2 cm high central response area was used during all phases of the experiment. Surrounding the central response area was 1.1 cm of non-responsive touchscreen on either side, and 1.59 cm on the top and bottom. An inter-trial interval (ITI) of 2 sec was employed, which was approximately the same length of time spent by rats in the water-well following the offset of the water light. Rats received 40 min of training or 72 trials per session (whichever came first) until criterion performance of touching the touchscreen on average less than 10 sec from the offset of the ITI occurred within a session for all rats.

Single-stimulus training. Rats were trained to touch a white square stimulus presented in the center of the touchscreen to activate the water light and then break the water IR-beam to receive 0.05 ml of water. The area of the touchscreen surrounding the centrally-presented stimulus was inactivated to facilitate training, and thus incorrect responses were not possible. The stimulus remained on the screen until a response was made and it disappeared immediately following a response. An inter-trial interval (ITI) of 2 sec was employed. In order for a trial to be initiated, the back IR beam had to be broken for 2 sec, but the water IR and middle IR beams had to be intact during this time interval. Hence, rats had to be at the back of the chamber for a stimulus to be presented, which ensured that rats did not impulsively run up to the touchscreen before a stimulus was presented. As soon as the IR requirements for trial initiation were met, a 10 KHz warning tone was presented for 2 sec. The stimulus was presented 1 sec after the onset of the warning tone. Presentation of the warning tone was intended to signal to the animal that a stimulus was about to be presented. If the middle or water-well IR beams were broken during presentation of the first second of the warning tone, no stimulus was presented and rats had to break the back IR beam again to initiate the next trial. To facilitate training, during the first few sessions, a small amount of cocoa paste (mixture of cocoa powder and water) was applied to the
center of the stimulus on the touchscreen using a cotton-tipped applicator. Application of cocoa paste was done prior to the start of a session and before a rat was placed into the chamber. Rats received 40 min of training or 72 trials per session (whichever came first) until criterion performance of touching the stimulus on average less than 10 sec from stimulus onset occurred within a session for all rats.

*Follow-stimulus training.* The touchscreen was divided up into a 2 X 4 grid and rats were trained to touch the white square stimulus which could appear in any one of the 8 locations on the touchscreen (4 locations on the top half and 4 locations on the bottom half of the touchscreen) to activate the water light and then break the water IR-beam to receive a reward of 0.05 ml of water. To facilitate the touching of stimuli presented on top half of the touchscreen, a clear Plexiglas perch 30 cm x 0.5 cm x 1.0 cm (l x w x h) was adhered with Velcro to the front of the touchscreen so that rats could rear and place their paws onto it when reaching for stimuli, thus preventing them from inadvertently touching the lower half of the touchscreen in the process. The perches were positioned such that they did not obscure the view of stimuli presented on the lower half of the touchscreen. The perches were left in the chambers for the remainder of the experiment. The same general trial procedures as those discussed above were used. All areas of the touchscreen other than the location of the stimulus were inactivated. Rats received 72 trials per session and the location of the stimulus was counterbalanced within a session such that the stimulus was presented 9 times in each location per session in a pseudorandom order. Training continued until criterion performance of touching the stimulus on average less than 10 sec from stimulus onset occurred within a session for all rats.

*Simultaneous-discrimination training.* The touchscreen was divided up into a 2 X 2 grid (2 stimulus locations on the top half and 2 stimulus locations on the bottom half of the touchscreen)
and rats were trained to touch the white square stimulus, from now on referred to as the “target” stimulus, when presented along with 1 of 3 distractor stimuli (black square, white triangle, black triangle). The same general trial procedures as those discussed above were used. Rats first received training using only the bottom two locations of the touchscreen. All areas of the touchscreen other than the locations of the two stimuli (1 target, 1 distractor) were inactivated. Rats received 72 trials per session and the location of the target stimulus was counterbalanced for left/right position within a session and each of the 3 distractors was presented 24 times within a session in a pseudorandom order. The stimuli remained on the touchscreen until a response was made. If rats made an incorrect response by touching the distractor stimulus instead of the target stimulus, both stimuli disappeared immediately from the screen following a response and rats received a 10-sec time-out period during which the entire touchscreen turned white to signal a mistake. If rats made a correct response by touching the target stimulus and not the distractor stimulus, this activated the water light and rats could then break the water IR-beam to receive a reward of 0.05 ml of water. Following an incorrect response, rats received correction trials whereby the exact same trial was presented until a correct response was made. Correction trials continued to be implemented until rats achieved two sessions during which a criterion level of performance was met. Criterion was defined as 18/20 correct responses anywhere within the 72-trial session. Once rats achieved two sessions of criterion-level performance without the assistance of correction trials, they moved onto simultaneous-discrimination training using only the top two stimulus locations of the touchscreen. The same trial and counterbalancing procedures as those discussed above were employed. Following successful criterion-level performance with and without the assistance of correction trials using only the top two stimulus locations of the touchscreen, rats moved onto simultaneous-discrimination training using both the bottom and top stimulus locations. Thus, the target and
distractor stimuli appeared together equally often on either the top-half (36 times) or bottom-half (36 times) of the touchscreen within each 72-trial session in a pseudorandom order. The same trial and counterbalancing procedures as those discussed above were employed. Training continued until criterion-level performance with and without the assistance of correction trials was attained.

Visual Search Training Procedures

Visual Search Set-Size 4 training. The touchscreen was divided up into a 2 X 2 grid (2 stimulus locations on the top half and 2 stimulus locations on the bottom half of the touchscreen) and rats were trained to touch the target stimulus (white square) when presented along with 3 distractor stimuli (black square, white triangle, black triangle). Rats received 72 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. For the 36 Feature Search trials, the target stimulus location was counterbalanced for position, with each of the 4 possible positions repeated 9 times per session in a pseudorandom order. On every Feature Search trial, the target stimulus was presented in one of the 4 possible stimulus locations and the remaining 3 stimulus locations were occupied by 1 of the 3 distractor stimuli. Each distractor stimulus was repeated 12 times per session in a pseudorandom order. For the 36 Conjunctive Search trials, the target stimulus location was counterbalanced for position, with each of the 4 possible positions repeated 9 times per session in a pseudorandom order. On every Conjunctive Search trial, the target stimulus was presented in one of the 4 possible stimulus locations and the remaining 3 stimulus locations were occupied by 1 of each of the 3 distractor stimuli, counterbalanced for position within a session. Training continued until criterion-level performance was attained with and without the assistance of correction trials. Criterion was defined as 18/20 correct responses anywhere within the 72-trial session. The same general trial procedures as those discussed above were employed. Throughout
Visual Search Set-Size 4 training and for the remainder of the experiment, rats were always allowed two touches of the touchscreen to make a correct response. This was employed to ensure that rats did not become frustrated during sessions as they were prone to inadvertently touching adjacent distractor stimuli while in the process of touching the target stimulus. However, on trials in which rats touched the target stimulus on its first touch, this first touch was rewarded. Only on trials in which a rat made an incorrect touch first did they receive a second chance to make a correct response.

*Visual Search Set-Size 6 training.* The touchscreen was divided up into a 2 X 3 grid (3 stimulus locations on the top half and 3 stimulus locations on the bottom half of the touchscreen) and rats were trained to touch the target stimulus (white square) when presented along with 5 distractor stimuli (black square, white triangle, black triangle). Rats received 72 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. For the 36 Feature Search trials, the target stimulus location was counterbalanced for position, with each of the 6 possible positions repeated 6 times per session in a pseudorandom order. On every Feature Search trial, the target stimulus was presented in one of the 6 possible stimulus locations and the remaining 5 stimulus locations were occupied by 1 of the 3 distractor stimuli. Each distractor stimulus was repeated 12 times per session in a pseudorandom order. For the 36 Conjunctive Search trials, the target stimulus location was counterbalanced for position, with each of the 4 possible positions repeated 9 times per session in a pseudorandom order. On every Conjunctive Search trial, the target stimulus was presented in one of the 6 possible stimulus locations and the remaining 5 stimulus locations were occupied by the distractor stimuli, counterbalanced for position within a session. Because there were 5 stimulus locations for distractor stimuli to occupy, but only 3 different distractor stimuli, one of the distractor stimuli had to occupy 2 stimulus locations on each Conjunctive Search trial, while
the others occupied 1 stimulus location. The selection of distractor stimuli for occupying 1 or 2 stimulus locations on Conjunctive Search trials was pseudorandomized within a session. The same general trial procedures as those discussed above were employed. Training continued until criterion-level performance (18/20 correct responses anywhere within session) was attained with and without the assistance of correction trials.

*Visual Search Set-Size 8 training.* The touchscreen was divided into a 2 X 4 grid (4 stimulus locations on the top half and 4 stimulus locations on the bottom half of the touchscreen) and rats were trained to touch the target stimulus (white square) when presented along with 7 distractor stimuli (black square, white triangle, black triangle). Rats received 96 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. For the 48 Feature Search trials, the target stimulus location was counterbalanced for position, with each of the 8 possible positions repeated 6 times per session in a pseudorandom order. On every Feature Search trial, the target stimulus was presented in one of the 8 possible stimulus locations and the remaining 7 stimulus locations were occupied by 1 of the 3 distractor stimuli. Each distractor stimulus was repeated 16 times per session in a pseudorandom order. For the 48 Conjunctive Search trials, the target stimulus location was counterbalanced for position, with each of the 8 possible positions repeated 6 times per session in a pseudorandom order. On every Conjunctive Search trial, the target stimulus was presented in one of the 8 possible stimulus locations and the remaining 7 stimulus locations were occupied by the distractor stimuli, counterbalanced for position within a session. Because there were 7 stimulus locations for distractor stimuli to occupy, but only 3 different distractor stimuli, one of the distractor stimuli had to occupy 3 stimulus locations on each Conjunctive Search trial, while the others occupied 2 stimulus locations. The selection of distractor stimuli for occupying 2 or 3 stimulus locations on Conjunctive Search trials was pseudorandomized within a session. The
same general trial procedures as those discussed above were employed. Training continued until
criterion-level performance (18/20 correct responses anywhere within session) was attained with
and without the assistance of correction trials.

*Visual Search All Set-Sizes training.* Rats received 144 trials per session, half of which were
Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom
order. Of the 72 Feature Search trials per session, 24 were Feature Search Set-Size 4 trials, 24
were Feature Search Set-Size 6 trials, and 24 were Feature Search Set-Size 8 trials, presented in a
pseudorandom order. Both target stimulus location and distractor stimuli were counterbalanced
within each type of Feature Search. Of the 72 Conjunctive Search trials per session, 24 were
Conjunctive Search Set-Size 4 trials, 24 were Conjunctive Search Set-Size 6 trials, and 24 were
Conjunctive Search Set-Size 8 trials, presented in a pseudorandom order. Both target and
distractor stimuli locations were counterbalanced within each set-size of Conjunctive Search
trials. Correction trials were not employed. The same general trial procedures as those discussed
above were employed. To establish a stable level of performance prior to advancing to the final
Visual Search task, rats received 12 Visual Search All Set-Sizes training sessions.

*Visual Search task.* The final version of the visual search paradigm detailed below was the last
training program given to rats prior to surgery and would subsequently be used to assess rats’
visual search performance following surgery. Figure 19 illustrates the different trial-types and
stimulus set-sizes of the Visual Search task. To establish a stable level of performance prior to
surgery, rats received 12 sessions of training on the Visual Search task, which consisted of 144
trials per session, half of which were Feature Search and the remaining half Conjunctive Search
trials presented in a pseudorandom order. Of the 72 Feature Search trials per session, 24 were
Feature Search 4 trials, 24 were Feature Search 6 trials, and 24 were Feature Search 8 trials,
presented in a pseudorandom order. To equate for the heterogeneity of the Conjunctive Search displays, we introduced heterogeneous Feature Search trials in which the distractor stimuli presented were not identical. Of the 24 Feature Search 4 trials, half were homogeneous (3 identical distractors presented) and half were heterogeneous (two black triangles and 1 black square presented OR two black triangles and 1 white triangle presented). Of the 24 Feature Search 6 trials, half were homogeneous (5 identical distractors presented) and half were heterogeneous (three black triangles and 2 black squares presented OR three black triangles and 2 white triangles presented). Of the 24 Feature Search 8 trials, half were homogeneous (7 identical distractors presented) and half were heterogeneous (four black triangles and 3 black squares presented OR four black triangles and 3 white triangles presented). One of the two types of heterogeneous Feature Search trials required discrimination of the target from the distractors based on pattern and the second based on shape. Both target and distractor stimuli locations were counterbalanced within each set-size of Feature Search trials. Of the 72 Conjunctive Search trials per session, 24 were Conjunctive Search 4 trials, 24 were Conjunctive Search 6 trials, and 24 were Conjunctive Search 8 trials, presented in a pseudorandom order. Both target and distractor stimuli locations were counterbalanced within each set-size of Conjunctive Search trials. The same general trial procedures as those discussed above were employed. Correction trials were not employed.

Surgery

Of the 20 rats, two did not successfully acquire the pre-training tasks and were removed from the study. The remaining 18 rats were assigned to one of two surgical groups, sham-lesioned (n = 8) or ACh-NBM-lesioned (n = 10) equating the groups for pre-surgical visual search performance based on accuracy and latency to locate the target stimulus on correct trials. Surgeries were performed under aseptic conditions. Rats were anesthetized with isoflurane (approximate
maintenance dose was 2% with 1 L/min of oxygen). A subcutaneous (s.c.) injection of the analgesic buprenorphine (0.03 mg/kg) and an intraperitoneal (i.p.) injection of atropine (0.05 mg/kg) were delivered immediately prior to surgery, the latter of which served to prevent fluid buildup in the lungs. Sterotaxic coordinates for NBM lesioning and dose of immunotoxin were the same as those employed in our crossmodal feature binding study (Botly & De Rosa, 2009a). Four 1.0 mm holes were drilled at the following stereotaxic coordinates relative to bregma and the surface of the skull (Paxinos & Watson, 1998): anterior NBM: Anterior/Posterior (A/P) -0.8 mm, Medial/Lateral (M/L) ± 2.5 mm, Dorsal/Ventral (D/V) - 8.2 mm; posterior NBM: A/P -1.6 mm, M/L ± 2.5 mm, D/V – 7.6 mm. There were a total of four intraparenchymal injection sites, two per hemisphere, of 0.2 µl sterile 0.1 M (pH 7.4) phosphate-buffered saline (sham-lesion) or 0.2 µg/µl 192 IgG-saporin (Advanced Targeting Systems, San Diego, California, lot# 41-105) dissolved in sterile 0.1 M (pH 7.4) phosphate-buffered saline through a 26-gauge Hamilton syringe at 0.1 µl/min. The needle was left in place for 3 min after each injection. The body temperature of each rat was maintained with a homeothermic blanket throughout the surgery. After the injections were complete, a small piece of sterile hemostatic gelfoam was applied over the exposed skull to control any bleeding and the wound was closed with staples and EMLA topical analgesic ointment (2.5% lidocaine and 2.5% prilocaine) was liberally applied around the staples. To prevent dehydration, rats were given normal saline (0.9 % NaCl; 2 ml / 100 g body weight; s.c.) immediately post-surgery. All rats received a minimum of 14 days of recovery with ad libitum food and water before being water restricted for subsequent testing.

Post-Surgical Testing Procedures

Experimenters were blind to the surgical group of the animals. Rats received 12 Visual Search task testing sessions identical to those described above to establish a stable level of post-surgical performance.
Histological Analyses

Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and transcardially perfused with approximately 150 ml of ice-cold normal saline followed by approximately 150 ml of ice-cold 4% paraformaldehyde. Brains were extracted and immediately post-fixed in 4% paraformaldehyde for 2 hr at 4 °C, and then transferred to a solution of 20% sucrose in phosphate-buffered saline (0.1 M; pH 7.4) and stored for 2 weeks at 4 °C. Brains were sectioned at a thickness of 60 µm using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems, Canada). Adjacent sections were used for staining for acetylcholinesterase (AChE) histochemistry, choline acetyltransferase (ChAT) immunohistochemistry, and parvalbumin immunohistochemistry. AChE histochemistry was carried out according to the method described by Paxinos and Watson (1998), and was used to confirm cholinergic fiber loss in target neocortical structures of the NBM. ChAT and parvalbumin immunohistochemistry were carried out according to methods described in (Botly & De Rosa, 2009a) to assess the extent of cholinergic and GABAergic cell body loss in the NBM and medial septum/vertical limb of the diagonal band of Broca (MS/VDB), respectively. After completion of all histological assays, brain slices were mounted on slides, dehydrated and cleared using an ascending ethanol and xylene series, coverslipped with DPX, and examined under a Leica light microscope (DM4000B, Ontario, Canada).

Histological Quantification

Cell counting. ChAT- and parvalbumin-immunoreactive cells were counted bilaterally in the NBM and MS/VDB as delineated by Paxinos and Watson (1998) for each rat across brain sections 300 µm apart using a Leica light microscope (DM4000B, Ontario, Canada) and Openlab image analysis software (Quorum Technologies, Ontario, Canada). For the NBM, cell counts
were taken from brain sections at approximately the following A/P (anterior/posterior) coordinates relative to bregma: A/P = -0.84 mm, -1.32 mm, and -1.56 mm. The rectangular outlines superimposed on the rat brain coronal schematics depicted in Figure 21 (Paxinos & Watson, 2007) delineate the NBM cell-counting frames used for histological quantification. For the MS/VDB, approximately the following A/P coordinates relative to bregma were used for cell counting: A/P = +0.84 mm, +0.72 mm, and +0.60 mm. The rectangular outlines superimposed on the rat brain schematics depicted in Figure 22 (Paxinos & Watson, 2007) delineate the MS/VDB cell-counting frames used for histological quantification. ChAT- and parvalbumin-immunoreactive cells were easily identifiable and characterized by relatively large cell bodies with several extending dendrites, and we only counted cells that were well distinguishable (i.e., well-defined borders) from the background.

_AChE Densitometry._ To quantify the reduction of cortical cholinergic input induced by our NBM cholinergic lesions, estimates of optical density in the frontal and parietal cortices and hippocampus were obtained from photomicrographs of AChE-stained brain sections using the software package Scion (Scion Corporation, Maryland, USA). For each rat, optical density values obtained from the three target brain regions were then normalized to raw striatal optical density values to eliminate the potential influence of different staining intensities across animals [(see similar method used by Vuckovich, Semel, and Baxter, (2004)]. Raw optical density values from the striatum did not differ between the NBM- and sham-lesioned groups (see Results). The rectangular outlines superimposed on the rat brain schematics depicted in Figure 23 (Paxinos & Watson, 2007) delineate the boundaries used for obtaining optical density values in the frontal and parietal cortices and hippocampus.
Results

Statistical Analyses. All statistical analyses were conducted using SPSS Version 15 with an alpha level of .05.

Pre-surgical Performance

Pre-training. Rats required 3 sessions of Light-water training, 4 sessions of Touch-light-water training, 7 sessions of Single-stimulus training, 16 sessions of Follow-stimulus training, and a total of 28 sessions of Simultaneous-discrimination training for criterion performance for all rats to be met.

Visual Search training. Rats required 12 sessions of Visual Search Set-Size 4 training, 10 sessions of Visual Search Set-Size 6 training, and 18 sessions of Visual Search Set-Size 8 training for criterion performance for all rats to be met. Rats required 12 sessions of Visual Search All Set-Sizes training for stable performance levels to be attained.

Visual Search task. Figure 20 illustrates the pre-surgical performance of rats as measured by (A) accuracy and (B) correct latency on the Visual Search task. Performance has been binned across the 12 sessions of training, and the performance of rats on the two different types of Feature Search trials, homogeneous and heterogeneous, has been collapsed to yield a single Feature Search score for accuracy and correct latency. A within-subjects analysis of variance (ANOVA) was performed using Session, Set-Size (4, 6, and 8 stimuli), and Trial-Type (Feature Search and Conjunctive Search) as within-subjects factors for both accuracy and correct latency on the Visual Search task. The accuracy ANOVA revealed significant main effects of Set-Size \(F(1.14, 17.10) = 69.26, p < .001, \eta^2 = .82\) and Trial-Type \(F(1, 15) = 179.63, p < .001, \eta^2 = .92\), as well as a significant Set-Size X Trial-Type interaction \(F(1.43, 21.39) = 15.13, p < .001, \eta^2 = .50\).

The correct latency ANOVA revealed a significant main effect of Session \(F(2.67, 40.04) = 1.96,\)
$p < .05, \eta^2 = .12$], a trend towards significance for Trial-Type [$F(1, 15) = 3.37, p = .09, \eta^2 = .18$], but no significant Set-Size X Trial-Type interaction ($F < 1.0, \eta^2 = 0.04$). Two additional ANOVAs were conducted to confirm that the rats assigned to the two surgical conditions, sham-lesioned and ACh-NBM-lesioned, did not differ with respect to pre-surgical visual search accuracy ($F < 1.0, \eta^2 = 0.01$) and correct latency ($F < 1.0, \eta^2 = 0.02$).

**Histological Analyses**

Quantitative histological analyses revealed that one ACh-NBM-lesioned rat had a statistically insufficient reduction of choline acetyltransferase (ChAT)-immunoreactive cells in the NBM as indicated by a ChAT-immunoreactive cell count greater than two standard deviations above the mean of the ACh-NBM-lesioned group and non-significantly different from the mean of the sham-lesioned group. Moreover, the acetylcholinesterase (AChE) optical density values obtained from the frontal and parietal cortices of this animal were non-significantly different from the means of the sham-lesioned group. A second ACh-NBM-lesioned rat was found to have a significant reduction of ChAT-immunoreactive cells in the MS/VDB as indicated by a ChAT-immunoreactive cell count less than two standard deviations below the MS/VDB means of both the ACh-NBM-lesioned and sham-lesioned groups. Furthermore, the AChE optical density value obtained from the hippocampus of this animal was significantly lower than the means of both the ACh-NBM-lesioned and sham-lesioned groups. Accordingly, behavioural and histological data from these two rats were removed from pre- and post-surgical statistical analyses. The sham-lesioned sample size thus remained at eight rats, while the ACh-NBM-lesioned sample size was reduced from ten to eight rats.

**Cell counting.** There was significant depletion of ChAT-immunoreactive cells in the NBM of the ACh-NBM-lesioned rats relative to sham-lesioned rats (Figure 21), while an equivalent number
of ChAT-immunoreactive cells were observed in the neighboring MS/VDB nuclei of both groups of rats (Figure 22). Independent $t$-tests confirmed that there were significantly fewer ChAT-immunoreactive cells in the NBM of ACh-NBM-lesioned compared to sham-lesioned rats [$t(12) = 10.5, p < 0.001; M_{\text{Sham}} = 299.29, SD = 45.04; M_{\text{NBM}} = 147.71, SD = 20.33$]. However, there was no significant difference between the two groups of rats in the number ChAT-immunoreactive cells in the MS/VDB ($t < 2.5; M_{\text{Sham}} = 458.71, SD = 36.10; M_{\text{NBM}} = 448.43, SD = 21.55$). An equivalent number of parvalbumin-immunoreactive cells were observed in the NBM (Figure 21) and MS/VDB (Figure 22) of both sham- and ACh-NBM-lesioned rats. Independent $t$-tests confirmed that there were no significant differences, $t < 2.0$, between the two groups of rats in the number of parvalbumin-positive cells in the NBM ($M_{\text{Sham}} = 114.00, SD = 13.17; M_{\text{NBM}} = 124.71, SD = 8.46$) and MS/VDB ($M_{\text{Sham}} = 373.00, SD = 45.60; M_{\text{NBM}} = 397.43, SD = 48.01$). It should be noted that the neural tissue of one sham-lesioned and one ACh-NBM-lesioned rat was too fragile due to insufficient paraformaldehyde tissue penetration to endure immunohistochemical analyses. Thus, for these two animals, histological analyses and quantification was based solely on AChE histochemistry and densitometry.

**AChE Densitometry.** AChE staining revealed a loss of cholinergic fibers in the frontal and parietal cortices, but not the hippocampus of ACh-NBM-lesioned rats relative to sham-lesioned rats (Figure 23). This was confirmed by independent $t$-tests comparing the optical density values (normalized to raw striatal optical density values) from the three target brain regions between the two groups of rats. The raw optical density values from the striatum (used for normalization) did not differ between the two groups ($t < 1.0; M_{\text{Sham}} = 247.33, SD = 12.31; M_{\text{NBM}} = 251.38, SD = 1.32$). There was significantly less AChE reactivity as measured by optical density in the frontal [$t(14) = 4.62, p < 0.001; M_{\text{Sham}} = 0.50, SD = 0.07; M_{\text{NBM}} = 0.37, SD = 0.03$] and parietal [$t(14) = 5.34, p < 0.001; M_{\text{Sham}} = 0.48, SD = 0.05; M_{\text{NBM}} = 0.35, SD = 0.04$] cortices of ACh-NBM-
lesioned compared to sham-lesioned rats. However, there was no significant difference between the two groups of rats in AChE reactivity in the hippocampus as measured by optical density ($t < 2.0; M_{\text{Sham}} = 0.52, SD = 0.04; M_{\text{NBM}} = 0.48, SD = 0.04$).

Post-surgical Performance

An ANOVA was performed using Session, Set-Size (4, 6, and 8 stimuli), and Trial-Type (Feature Search and Conjunctive Search) as within-subjects factors and Lesion (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor for both post-surgical accuracy and correct latency on the Visual Search task. The accuracy ANOVA revealed significant main effects of Session [$F(11, 154) = 4.55, p < .001, \eta^2 = .25$], Set-Size [$F(1.33, 18.57) = 67.43, p < .001, \eta^2 = .83$] and Trial-Type [$F(1, 14) = 110.03, p < .001, \eta^2 = .89$], as well as a significant Set-Size X Trial-Type interaction [$F(1.83, 25.66) = 32.51, p < .001, \eta^2 = .70$], but no significant effect of Lesion ($F < 1.0, \eta^2 = 0.07$). The correct latency ANOVA did not reveal any significant main effects or interactions.

Figure 24 illustrates a trial-type breakdown of the post-surgical performance of sham- and ACh-NBM-lesioned rats on the Visual Search task as measured by accuracy and correct latency. The graphs in the top row show rats’ accuracy on the (A) Conjunctive and (B) Feature Search trials, while the graphs in the bottom row show rats’ correct latency on the (C) Conjunctive and (D) Feature Search trials. Due to the large amount of variance in the post-surgical correct latency data, two separate ANOVAs were performed to better elucidate any influence of lesion: one for Conjunctive Search trials and one for Feature Search trials. For these ANOVAs, Session and Set-Size (4, 6, and 8 stimuli) were within-subjects factors and Lesion (sham-lesioned or ACh-NBM-lesioned) was the between-subjects factor. The Conjunctive Search ANOVA revealed significant main effects of Set-Size [$F(2, 26) = 3.91, p < .05, \eta^2 = .23$] and Lesion [$F(1, 13) = 3.89, p = .05,$}
$\eta^2 = .23]$. Between-subjects contrasts revealed significant differences between the conjunctive search correct latency of sham- and ACh-NBM-lesioned rats at set-sizes of six [$F(1,13) = 5.12 \quad .05 \eta^2 = .28$] and eight [$F(1,13) = 2.70, p = .05, \eta^2 = .18$] stimuli, but not four stimuli ($F < 1.0, \eta^2 = 0.05$). The Feature Search ANOVA revealed a significant main effect of Session [$F(3.14, 44.04) = 1.96, p < .05, \eta^2 = .12$], but no significant effect of Lesion ($F < 1.5, \eta^2 = 0.10$).

Comparable trial-type-independent ANOVAs were also performed using post-surgical accuracy and revealed non-significant effects of Lesion for both Conjunctive Search ($F < 1.5, \eta^2 = 0.09$) and Feature Search ($F < 1.0, \eta^2 = 0.04$) trials.

**Discussion**

If ACh is critical for feature binding, as suggested by our pharmacological and lesion work that utilized our digging-based rat feature binding task (Botly & De Rosa, 2007, 2008, 2009a), then its modulatory influence on this cognitive process should generalize to other tests of feature binding. A rodent analog of the standard test of feature binding from the human cognitive literature, visual search, would allow for an important test of our cholinergic hypothesis of feature binding. Thus, a visual search task was designed for rats using touchscreen-equipped operant chambers and achromatic stimuli. If cortical cholinergic neurotransmission is necessary to support the attentional processes needed for feature binding as suggested by our previous lesion work (Botly & De Rosa, 2009a), then reducing cholinergic afferentation of the neocortex via cholinergic-selective lesions of the NBM should yield a performance pattern reflective of less efficient visual search.

Importantly, rats were very successful at performing the visual search task, which to our knowledge has not been demonstrated before. While the analyses conducted on the pre-surgical correct latency data revealed only a weak trend towards significance with regard to the time it took for rats to locate the target stimulus on Conjunctive versus Feature Search trials,
comparable analyses conducted on the pre-surgical accuracy data revealed that rats were significantly more accurate at locating the target stimulus on Feature Search than Conjunctive Search trials. Importantly, stimulus set-size was found to significantly interact with trial-type such that as the number of distractors increased, rats’ accuracy at locating the target stimulus decreased on Conjunctive Search trials, while remaining relatively more stable on Feature Search trials. This accuracy-based pattern of performance demonstrated by rats resembles the classic latency-based performance pattern typically observed in human participants, and suggests that the feature binding requirement of Conjunctive Search trials made it more challenging for rats to accurately locate the target stimulus, especially as the number of distractors increased. This is consistent with findings from the human cognitive literature demonstrating that feature binding errors are more likely to occur under conditions of high attentional load, such as when conjunctive search with large stimulus set-sizes is required (Cinel, Humphreys, & Poli, 2002; Treisman & Schmidt, 1982). However, it should be noted that even during Conjunctive Search trials with the largest number of distractors present, rats’ accuracies at locating the target stimulus were consistently above 80%, indicating that they were still very good at locating the target stimulus during trials that were the most attentionally demanding.

As previously discussed, the NBM of the BF provides 90% of the cholinergic input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983), and our previous lesion work has revealed a critical role for cortical cholinergic input from the NBM in supporting the attentional processes necessary for feature binding (Botly & De Rosa, 2009a). We thus predicted that the attentional disruption induced by cholinergic deafferentation of the neocortex would lead to selective performance decrements on the visual search task. Our previous work has shown that rats are unimpaired at retrieving well-learned conjunctive stimuli following systemic muscarinic blockade with scopolamine (Botly & De Rosa, 2007) and cholinergic-selective lesions of the
NBM (Botly & De Rosa, 2009a). Given that the target and distractor stimuli were very well learned by rats prior to surgery, we thus did not anticipate a difference in the ability of sham-lesioned and ACh-NMB-lesioned rats to accurately locate the target stimulus on Feature or Conjunctive Search trials of visual search post-surgery. However, we did predict that ACh-NBM-lesioned rats would be less efficient at visual search and thus take longer than sham-lesioned rats to locate the target stimulus on Conjunctive, but not Feature Search trials due to the greater attentional load of Conjunctive Search trials.

Consistent with these predictions, there was no significant effect of lesion on accuracy for locating the target stimulus for either Feature or Conjunctive Search trials post-surgery, which is congruent with a well-supported model of cholinergic function stating that high levels of ACh are not needed for the retrieval of well-learned information (Hasselmo & McGaughy, 2004). Such equivalent post-surgical accuracy performance by rats in both surgical groups allowed us to attribute any lesion-induced difference in correct latency to inefficiency at visual search rather than a simple inability of ACh-NBM-lesioned rats to perform the task.

As predicted, ACh-NBM-lesioned rats took significantly longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search, but not Feature Search trials. Furthermore, this significant lesion-induced increase in correct latency was found only during Conjunctive Search trials with stimulus set-sizes of six or eight. ACh-NBM-lesioned rats took approximately 400-500 ms longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search trials when five or seven distractors were present while the difference was almost half as much (~ 275 ms) when only three distractors were present. This is consistent with the notion that as the number of distractors increased on Conjunctive Search trials, a more extensive serial search was necessary to locate the target which required attentional resources that were not available to
ACh-NBM-lesioned rats. Previous work has shown that cholinergic-selective lesions of the NBM significantly impaired the ability of rats to perform attentionally-demanding tasks (Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; Lehmann, Grottick, Cassel, & Higgins, 2003; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002), and our previous lesion work has revealed that sufficient cortical cholinergic activity is necessary for rats to efficiently acquire a feature binding task (Botly & De Rosa, 2009a). Based on such findings, it can be argued that reducing cholinergic afferentation of the neocortex in the current experiment disrupted the attentional processes needed for rats to efficiently find the target stimulus on the feature-binding dependent Conjunctive Search trials, thus leading to an increase in latency.

Use of this rat analog of the standard test of human feature binding allowed for a critical test of the hypothesis that cholinergic input to the neocortex from the NBM of the BF is important for feature binding. In support of this hypothesis, it was found that reducing cortical cholinergic neurotransmission via cholinergic-selective lesions of the NBM decreased the efficiency of visual search in rats as evidenced by the greater time required for ACh-NBM-lesioned rats relative to sham-lesioned rats to locate the target stimulus on Conjunctive, but not Feature Search trials. This study further highlights the importance of cholinergic contributions to feature binding using a rat analog of the visual search paradigm.
General Discussion

An important question in psychology and neuroscience is how the brain forms unified neural representations of objects when distinct neural regions are primarily responsible for detecting and processing an object’s features; this unknown mechanism of feature integration is referred to as feature binding (Reynolds & Desimone, 1999; Treisman & Gelade, 1980). While the human cognitive literature has established that attentional processing in frontoparietal cortical networks supports feature binding (Corbetta, Shulman, Miezin, & Petersen, 1995; Friedman-Hill, Robertson, & Treisman, 1995), the neurochemical contributions to feature binding remain unknown. Using systemic pharmacological manipulations and a novel digging-based feature binding task for rats that used odor and texture stimuli, we previously demonstrated that acetylcholine (ACh) acting at muscarinic receptors in the brain is critical for crossmodal feature binding during the encoding stage, and we proposed that it was through an attentional mechanism that ACh facilitated the formation of neural representations of conjunctive stimuli (Botly & De Rosa, 2007).

This series of experiments set out to further investigate a functional role for ACh and the cholinergic basal forebrain (BF) in feature binding. In Experiment 1, a cross-species experimental design was employed in which rats under the systemic influence of the muscarinic receptor antagonist scopolamine and human participants under divided-attention performed comparable intramodal feature binding tasks using odor stimuli for rats and coloured-shape visual stimuli for humans. Given the comparable feature binding acquisition impairment demonstrated by both species, Experiment 1 suggested that ACh acting at muscarinic receptors supports the attentional processes necessary for feature binding at encoding. Experiments 2-4 investigated the potential neuroanatomical source for ACh to support feature binding using
bilateral quisqualic acid excitotoxic (Experiment 2) and 192 IgG-saporin cholinergic immunotoxic (Experiments 3 and 4) brain lesions. Using the previous crossmodal digging-based rat feature binding task that employed odor and texture stimuli, Experiment 2 revealed that the nucleus basalis magnocellularis (NBM) of the BF is critically involved in feature binding, and Experiment 3 revealed that cholinergic neurons in the NBM are necessary for feature binding at encoding. Lastly, in Experiment 4 a rat version of visual search, the standard test of feature binding used in the human cognitive literature, was designed using touchscreen-equipped operant chambers to determine whether ACh’s modulatory influences on feature binding would generalize to a well-established test of feature binding. Here it was also revealed that cholinergic neurons in the NBM of the BF are critical for efficient visual search.

Experiment 1

Our previous crossmodal pharmacological study revealed a critical role for muscarinic cholinergic neurotransmission in feature binding at encoding (Botly & De Rosa, 2007). In light of ACh’s well-established role in modulating attention, it was hypothesized that ACh may support the attentional processes necessary for feature binding. We reasoned that if blockade of the muscarinic cholinergic system with scopolamine impaired feature binding at encoding by disrupting attention in our crossmodal study, then an attentional challenge alone should result in a similar feature binding impairment. Thus, in Experiment 1, a cross-species study was conducted in which rats and humans learned comparable FC and FS tasks. Intramodal versions of the digging-based FC and FS tasks were designed for rats using odors and an intramodal analog of the rat task was created for humans using coloured shapes. During performance of the FC and FS tasks, we challenged the cholinergic system of rats (Experiment 1A) using the
muscarinic receptor antagonist scopolamine and challenged the attentional system of humans (Experiment 1B) using a concurrent divided-attention task.

In a strikingly similar manner, rats under the influence of scopolamine and human participants under divided attention were significantly impaired at acquiring the FC tasks, whereas their ability to acquire the FS tasks and to retrieve previously-bound FC stimuli remained comparatively intact relative to performance under saline and full attention, respectively. These findings are in agreement with our previous crossmodal pharmacological findings (Botly & De Rosa, 2007) and extend the validity of this feature binding task to the intramodal domain.

Furthermore, the performance of human participants on visual analogs of the rat tasks has confirmed the attention-dependent nature of this feature binding task; behaviourally disrupting attention significantly impaired FC acquisition, with minimal impact on FS acquisition. By the final stage of acquisition, human participants under divided-attention were performing at comparable accuracy levels to that of participants under full-attention on the FS task, but not the FC task.

Intact FS performance under divided-attention is consistent with previous findings from the human cognitive literature demonstrating that single-feature detection and processing constitutes a low attentional load as evidenced by the intact feature-detection performance of patients with parietal damage and Alzheimer’s disease (Bernstein & Robertson, 1998; Corbetta, Shulman, Miezin, & Petersen, 1995; Foster, Behrmann, & Stuss, 1999). Conversely, the detection and processing of conjunctions of features is much more attentionally demanding as evidenced by the impaired performance of patients with parietal damage and Alzheimer’s disease on tasks requiring feature binding and also evidenced by functional magnetic resonance imaging (fMRI)
work revealing selective activation of frontoparietal cortical networks during FC but not FS tasks (Corbetta, Shulman, Miezin, & Petersen, 1995; Nobre, Coull, Walsh, & Frith, 2003).

In contrast to the strong FS performance yielded by rats under the influence of scopolamine and human participants under divided attention by the final stage of acquisition, they remained significantly impaired on the FC tasks relative to control performance and made only negligible improvements in FC performance across training. This significant and parallel detrimental impact of cholinergic blockade and divided attention on FC acquisition suggests that muscarinic cholinergic blockade impairs feature binding at encoding by disrupting attentional processing during learning. This contention is consistent with pharmacological and cholinergic-selective lesion work suggesting that ACh’s predominant function in cognition is to modulate attention for learning (Baxter et al., 1996; McGaughy, Everitt, Robbins, & Sarter, 2000; Sarter & Bruno, 1997; Waite & Thal, 1996). Specifically, previous work suggests that the synaptic changes induced by cholinergic activity enhance the signal-to-noise ratio of cortical neurons such that they are primed to acquire information from the environment. ACh boosts the neural signal of incoming sensory information while suppressing the neural signal of previously-acquired and distracting information, thereby facilitating the detection and processing of a relevant stimulus in a complex environment (Hasselmo, Anderson, & Bower, 1992; Hasselmo, Schnell, & Barkai, 1995; Liljenstrom & Hasselmo, 1995; Vogt & Regehr, 2001).

These convergent cross-species behavioural findings suggest that disruption of the muscarinic cholinergic system is akin to disruption of the attentional system given that both manipulations yielded comparable impairments in feature binding on analogous tasks. In support of this assertion, previous work has shown that scopolamine administered to both human and non-human animals yields substantial impairments in attention that are further exacerbated by
increasing the attentional demands of the task (Brandeis, Naylor, Halliday, Callaway, & Yano, 1992; Broks et al., 1988; Callahan, Kinsora, Harbaugh, Reeder, & Davis, 1993; Davidson, Cutrell, & Marrocco, 1999; Ellis et al., 2006; Green et al., 2005; Levy, Parasuraman, Greenwood, Dukoff, & Sunderland, 2000; McGaughy, Turchi, & Sarter, 1994; Mintzer & Griffiths, 2003). Furthermore, in vivo microdialysis work from non-human animals has shown that dividing attention modulates cholinergic levels: increased ACh efflux in frontoparietal cortical attentional regions of the brain is correlated with increased attentional effort (Himmelheber, Sarter, & Bruno, 2000; Pepeu & Giovannini, 2004). Specifically, while sufficient cortical levels of ACh are necessary to boost afferent sensory input for learning in general, much higher cortical ACh levels are needed for tasks that greatly tax the attentional system (Himmelheber, Sarter, & Bruno, 2000; Pepeu & Giovannini, 2004). Consistent with this notion, while reducing the activity of the cholinergic system by blocking muscarinic receptors impaired rats’ ability to acquire the attentionally-demanding FC task in the current experiment, rats were still capable of successfully acquiring the much less attentionally-demanding FS task despite decreased binding of ACh to muscarinic receptors. Importantly, both the FC and FS tasks required the same number of stimulus discriminations, but only the FC task required feature binding.

While a relationship between attentional effort and cortical cholinergic efflux has not been demonstrated in humans, fMRI work with neurologically intact human participants has revealed that administration of the cholinergic-enhancing drug physostigmine augmented neuronal activation in sensory cortices and decreased activation in frontoparietal cortical networks while improving performance on a visual working memory task (Furey, Pietrini, & Haxby, 2000; Furey, Ricciardi, Schapiro, Rapoport, & Pietrini, 2008). This suggests that increased cortical ACh levels boosted the neural signal of task-relevant visual information during encoding, while
suppressing the neural signal of task-irrelevant information, thereby facilitating attention and reducing demands on the frontoparietal cortical network.

The learning-based nature of this feature binding task allowed us to dissociate the encoding and retrieval stages of feature binding, which to our knowledge has not been done before in the human cognitive literature. Consistent with our previous crossmodal pharmacological findings (Botly & De Rosa, 2007), both rats under the influence of scopolamine and human participants under divided attention were unimpaired at retrieving a previously-learned set of FC stimuli. This invulnerability of the feature binding retrieval process to a cholinergic or divided-attentional challenge suggests that once a FC stimulus is well learned, it has a bound and stable neural representation which reduces the need for an attentionally-demanding feature binding process during retrieval. Consistent with this hypothesis, research from the human cognitive literature has shown that disrupting attention is selectively detrimental to the encoding, relative to the retrieval, of episodic memories (Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Naveh-Benjamin, Craik, Guez, & Dori, 1998). Furthermore, consistent findings from the non-human animal literature using pharmacological and lesion techniques have implicated ACh in information encoding, but not retrieval (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995; Baxter et al., 1996; Blockland, 1996; Dorman et al., 1997; Galani et al., 2002; McGaughy, Everitt, Robbins, & Sarter, 2000; Waite & Thal, 1996).

In light of these findings and given ACh’s influences on cortical synaptic circuits, a model of cholinergic function has been proposed and contends that levels of ACh may play a role in switching the functional dynamics of the cortex from an information-encoding state to an information-retrieval state (Hasselmo & Bower, 1993). High levels of ACh facilitate information encoding by boosting afferent sensory input and suppressing intrinsic cortical activation
representing previously-learned information, whereas low levels facilitate retrieval by allowing activation of intrinsic neural connections (Hasselmo & McGaughy, 2004). In support of this model, Orsetti, Casamenti, and Pepeu (1996) have shown that while naïve rats acquiring an operant conditioning task show robust cortical ACh release, well-trained animals performing the same task show minimal cortical ACh release, which is consistent with the notion that low levels of ACh are associated with information retrieval (Hasselmo & McGaughy, 2004).

A cellular-based theory of feature binding has recently come to the forefront, proposing that temporal synchronization of neuronal activity in the gamma frequency range is responsible for feature binding (Engel, Fries, & Singer, 2001; Senkowski, Schneider, Foxe, & Engel, 2008). Our results do not preclude a role for ACh in the modulation of feature binding via an enhancement of neural synchronization in the cortex. In both human and non-human animals, the occurrence of such high-frequency gamma-range neural oscillations is limited to states of alertness (Engel & Singer, 2001) and is correlated with cognitive activities requiring attention (Muller, Gruber, & Keil, 2000). Selective spatial attention has been shown to enhance gamma-band synchronization in the human cortex during visual feature binding (Muller, Gruber, & Keil, 2000; Tiitinen et al., 1993). Furthermore, Rodriguez, Kallenbach, Singer, and Munk (2004) have shown that modulation of the cortical muscarinic cholinergic system influences gamma-band neural synchronization in the cortex (Engel, Kreiter, Konig, & Singer, 1991; Friedman-Hill, Maldonada, & Gray, 2000; Gray & Singer, 1989). Thus, the attention and neural synchronization hypotheses of feature binding do not appear to be mutually exclusive and ACh may even support the attentional processes needed for cortical synchronization. Further research is needed to understand how attention, ACh, and neural synchronization relate to each other and to feature binding.
It is important to note that these convergent cross-species findings demonstrating the comparable impact on feature binding performance of scopolamine in rats and divided attention in humans are correlational in nature. Human participants were not administered scopolamine and the attention of rats was not behaviourally divided with a concurrent task such that direct comparisons across manipulations and species could be made. A follow-up study in which human participants are administered a comparable dose of scopolamine to that of rats prior to performance of the FC and FS tasks would thus be informative.

In conclusion, Experiment 1 complements our previous crossmodal pharmacological work (Botly & De Rosa, 2007) and provides confirmatory correlational evidence from the intramodal domain that ACh acting at muscarinic receptors in the brain is critical for feature binding at encoding. The performance of human participants on visual analogs of our rat digging-based tasks has confirmed that our feature binding task requires attention in humans. Lastly, our translational cross-species results provide support for the hypothesis that blockade of muscarinic cholinergic function in rats is analogous to the imposition of an attentional load in humans (i.e., divided attention), suggesting that ACh provides the attentional "glue" for feature binding.

Experiment 2

Experiment 1 (Botly & De Rosa, 2008), coupled with our previous crossmodal pharmacological work (Botly & De Rosa, 2007), has provided support for an important role for ACh in facilitating feature binding at the encoding stage, and suggests that ACh acting at muscarinic receptors in the brain supports the attentional processes necessary for acquisition of a feature binding task. While the human cognitive literature has yielded substantial insights into the broad neocortical systems involved in feature binding (Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen,
1995; Friedman-Hill, Robertson, & Treisman, 1995), the specific neuroanatomical contributions to this cognitive process remain unknown.

It was hypothesized that the nucleus basalis magnocellularis (NBM) of the BF would be critical for feature binding given its demonstrated importance to a variety of forms of attention, including sustained, selective, and divided attention (Butt, Noble, Rogers, & Rea, 2002; Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; Pang, Williams, Egeth, & Olton, 1993), and its widespread afferent projections to regions of the neocortex important for attentional processing, including the frontal and parietal cortices (Mesulam, Mufson, Wainer, & Levey, 1983).

To determine whether the NBM plays an important functional role in feature binding, we bilaterally lesioned the NBM of rats using the excitotoxin quisqualic acid, and compared the ability of NBM-lesioned rats to that of sham-lesioned rats to: (1) retrieve a FC stimulus set learned prior to surgery; (2) acquire a novel FC stimulus set, and (3) acquire a FS stimulus set using the crossmodal digging-based task previously discussed and employed (Botly & De Rosa, 2007). We predicted that NBM lesions would impair the ability of rats to acquire the attentionally-demanding FC task, while sparing their ability to acquire the FS task. However, whether NBM lesions induced by quisqualic acid would spare the ability of rats to retrieve a set of FC stimuli learned prior to surgery was not clear given that previous studies using excitotoxins to lesion the NBM have resulted in both attentional and mnemonic deficits in non-human animals (Altman, Crosland, Jenden, & Berman, 1985; Berman, Crosland, Jenden, & Altman, 1988; Connor, Langlais, & Thal, 1991; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Mayo, Kharouby, Le Moal, & Simon, 1988; Santucci & Haroutunian, 1989; Vale-Martinez et al., 2002).
Consistent with our hypothesis, NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at acquiring the crossmodal FC task, while their ability to acquire the FS task remained intact. By the final stage of acquisition, NBM-lesioned rats were performing at comparable accuracy levels to that of sham-lesioned rats on the FS task, but were significantly impaired relative to sham-lesioned rats on the FC task. In light of the results of Experiment 1, which confirmed the attention-dependent nature of this FC task (Botly & De Rosa, 2008), the impaired FC acquisition of NBM-lesioned rats suggests that the NBM supports the attentional processes necessary for the formation of neural representations of conjunctive stimuli. Previous lesion work with non-human animals implicating the NBM in attention further strengthens this argument (Butt, Noble, Rogers, & Rea, 2002; Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; Pang, Williams, Egeth, & Olton, 1993).

Interestingly, NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at retrieving the crossmodal FC stimuli they had acquired prior to surgery. Such a retrieval deficit is inconsistent with recent research suggesting a more crucial role for the NBM in encoding and attention than retrieval and memory (Bailey & Lee, 2007; Galani et al., 2002; Gonzalez, Miranda, Gutierrez, Ormsby, & Bermudez-Rattoni, 2000; Miranda & Bermudez-Rattoni, 1999; Montero-Pastor, Vale-Martinez, Guillazo-Blanch, & Marti-Nicolovius, 2004), and it is also inconsistent with the results of Experiment 1 which demonstrated intact FC retrieval performance by human participants when their attention was challenged (Botly & De Rosa, 2008).

Histological analyses revealed that quisqualic acid infused into the NBM resulted in large reductions in cholinergic input to the neocortex as evidenced by significant cholinergic cell body
destruction in the NBM, but not the medial septum and vertical limb nucleus of the diagonal band of Broca (MS/VDB). Furthermore, there was a significant loss of cholinergic fibers in the neocortex of NBM-lesioned rats relative to sham-lesioned rats. Importantly, however, quisqualic acid is a non-selective excitotoxin that indiscriminately destroys neurons irrespective of neurochemical type. When injected into the NBM, where cholinergic neurons are co-localized with a substantial population of cortically-projecting GABAergic neurons, quisqualic acid presumably destroyed both cholinergic and GABAergic neurons, and in turn reduced both cholinergic and GABAergic input to the cortical targets of the basalcortical BF projection pathway in NBM-lesioned rats. While there is considerable evidence to suggest that ACh is not necessary for information retrieval (Hasselmo & McGaughy, 2004), previous work with non-selective excitotoxins has revealed mnemonic deficits following their injection into the NBM (Altman, Crosland, Jenden, & Berman, 1985; Berman, Crosland, Jenden, & Altman, 1988; Connor, Langlais, & Thal, 1991; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Mayo, Kharouby, Le Moal, & Simon, 1988; Santucci & Haroutunian, 1989; Vale-Martinez et al., 2002). Importantly, there is evidence to suggest that this may be due to the destruction of GABAergic neurons in the NBM as non-selective lesions of the NBM result in both mnemonic and attentional deficits, while lesions that selectively destroy cholinergic neurons within the NBM solely result in attentional deficits (Baxter et al., 1996; McGaughy, Everitt, Robbins, & Sarter, 2000; Sharma & Kulkarni, 1993; Waite & Thal, 1996). Such findings suggest that destruction of cortically-projecting GABAergic neurons in the NBM may have primarily contributed to the deficit in feature binding at retrieval demonstrated by NBM-lesioned rats in Experiment 2.

It is important to address the potential criticism that non-selective lesions of the NBM induced a general lesion effect resulting in deficits in motor behaviour, exploration, or stimulus discrimination. However, there is evidence to suggest that this was not the case in Experiment 2.
Firstly, NBM-lesioned rats performed comparably to that of sham-lesioned rats at acquiring the FS stimulus set, indicating that NBM-lesioned rats were not impaired at discriminating digging bowls using odor or texture cues. Secondly, although reaction time data were not included in the results of Experiment 2 given that this behavioural measure did not significantly differ between the tasks (FC, FS), it is important to note that NBM-lesioned rats could not be differentiated from sham-lesioned rats in terms of latency to dig, suggesting that non-selective lesions of the NBM did not adversely impact motor behaviour during task performance or tendency to explore the digging bowl stimuli.

In conclusion, Experiment 2 was an essential first step in establishing the functional importance of the NBM of the BF to the fundamental cognitive process of feature binding using the same digging-based task employed in Experiment 1. The next step was to probe the neurochemical underpinnings of the NBM’s role in feature binding to determine whether it is cortical cholinergic input from the NBM that makes this BF region essential for feature binding.

*Experiment 3*

While our previous systemic pharmacological work has implicated ACh acting on muscarinic receptors in feature binding at encoding (Botly & De Rosa, 2007), the neural source and targets of such critical cholinergic neurotransmission have yet to be identified. Experiment 2 investigated a functional role for the NBM of the BF in feature binding irrespective of its neurochemistry; thus, the aim of Experiment 3 was to determine whether cholinergic input to the neocortex from the NBM is critical for feature binding, and whether such cortical cholinergic input is essential only for the encoding stage of feature binding as suggested by Experiment 1 (Botly & De Rosa, 2008) and our previous crossmodal pharmacological study (Botly & De Rosa, 2007). The basalocortical cholinergic projections from the NBM provide 90% of the cholinergic
input to the neocortex, including frontoparietal cortices implicated in attentional processing (Behrmann, Geng, & Shomstein, 2004; Bucci, 2009; Bucci & Chess, 2005; Donner et al., 2002; Mesulam, Mufson, Wainer, & Levey, 1983). Cholinergic-selective lesions of the NBM have been shown to impair the performance of non-human animals on a variety of attention-dependent tasks (Chiba, Bucci, Holland, & Gallagher, 1995; Chiba, Bushnell, Oshiro, & Gallagher, 1999; Lehmann, Grottick, Cassel, & Higgins, 2003; McGaughy, Kaiser, & Sarter, 1996), and increased ACh efflux in frontoparietal cortices is correlated with increased attentional effort (Himmelheber, Sarter, & Bruno, 2000; Pepeu & Giovannini, 2004). We thus hypothesized that the NBM of the BF may provide cholinergic input to the cortex that is critical for feature binding.

To test this hypothesis, we bilaterally lesioned the NBM of rats using the cholinergic-selective immunotoxin, 192 IgG-saporin, and compared the ability of ACh-NBM-lesioned rats to that of sham-lesioned rats to: (1) retrieve a FC stimulus set learned prior to surgery; (2) acquire a novel FC stimulus set, and (3) acquire two FS stimulus sets, one of greater difficulty than the other, but neither requiring feature binding, using the crossmodal digging-based task previously discussed and employed in Experiment 2 (Botly & De Rosa, 2009b). If cortical cholinergic neurotransmission is necessary to support the attentional processes needed for feature binding, then reducing cholinergic afferentation of the neocortex via cholinergic-selective lesions of the NBM should impair the ability of rats to acquire the attentionally-demanding FC task, while sparing their ability to acquire the FS tasks and to retrieve a previously-learned set of FC stimuli.

Consistent with this hypothesis, ACh-NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at acquiring the crossmodal FC task, while their ability to acquire the FS tasks remained intact. By the final stage of acquisition, ACh-NBM-lesioned rats were performing at
comparable accuracy levels to that of sham-lesioned rats on both FS tasks, but were significantly impaired relative to sham-lesioned rats on the FC task. In light of the cross-species results of Experiment 1, which confirmed the attention-dependent nature of this FC task at encoding (Botly & De Rosa, 2008), the impaired FC acquisition of ACh-NBM-lesioned rats suggests that cortical cholinergic input from the NBM supports the attentional processes necessary for the formation of neural representations of conjunctive stimuli.

While the FS and FC tasks both required rats to discriminate between four odor-texture bowls, the FS task presented rats with twice the amount of feature information (4 odors and 4 textures) than the FC task (2 odors and 2 textures) because each odor-texture FS bowl was characterized by a distinct odor and texture with no overlap across bowls. However, despite the greater amount of feature information associated with the FS task, it could still be argued that the unimpaired acquisition of the FS task by ACh-NBM-lesioned rats was simply due to the FS task being less difficult than the FC task given the lack of feature overlap across odor-texture FS bowls. To address this potential criticism, rats acquired a FS Enhanced-Difficulty stimulus set in addition to acquiring a FS stimulus set. Although both FS tasks did not require feature binding, as rats could rely on a single feature (odor or texture) for correct bowl selection, the FS Enhanced-Difficulty task also required rats to learn when to rely on odor and when to rely on texture as one odor and one texture were associated with both the correct and incorrect bowl choices, resulting in partial feature overlap across the odor-texture bowls.

While we predicted that both ACh-NBM-lesioned and sham-lesioned rats would not perform as well on the FS Enhanced-Difficulty task relative to the FS task, we did not anticipate a lesion effect given that feature binding was not a requirement of the FS or FS Enhanced-Difficulty tasks. Consistent with this prediction and indicative of its greater difficulty, by the final block of
acquisition, sham-lesioned rats were performing significantly worse on the FS Enhanced-Difficulty task relative to the FS task. However, there was no significant difference between the performances of rats in the two lesion groups during FS or FS Enhanced-Difficulty acquisition. This suggests that the feature binding requirement of the FC task increased the attentional load of the task over and above any increase induced by greater task difficulty. This is consistent with findings from the human cognitive literature showing that even after making it more difficult to discriminate a target from surrounding distractors in visual search tasks, feature binding in itself requires additional attentional resources over and above those needed for making fine stimulus discriminations (Carter, 1982; Duncan & Humphreys, 1989; Foldi et al., 2005; Treisman, 1991; von Grunau, Dube, & Galera, 1994). The intact FS Enhanced-Difficulty acquisition performance of ACh-NBM-lesioned rats is an important piece of evidence as it suggests that the intact FS acquisition performance of rats under the influence of scopolamine and human participants under divided attention in Experiment 1 (Botly & De Rosa, 2008) was not simply due to the task being less difficult than the FC task.

As in Experiment 2, it is also important to address the potential criticism that cholinergic-selective lesions of the NBM induced a general lesion effect resulting in deficits in motor behaviour, exploration, or stimulus discrimination. However, there is ample evidence to suggest that this was not the case in Experiment 3. Firstly, ACh-NBM-lesioned rats performed comparably to that of sham-lesioned rats at acquiring the FS and FS Enhanced-Difficulty stimulus sets, indicating that ACh-NBM-lesioned rats were not impaired at discriminating digging bowls using odor or texture cues. Furthermore, ACh-NBM-lesioned rats performed comparably to that of sham-lesioned rats at retrieving a previously-learned FC stimulus set indicating that they were not impaired at discriminating compound odor-texture stimuli that required the use of both odor and texture cues. The lesioned-induced deficit only emerged during
FC acquisition when rats had to bind novel odor and texture cues. Secondly, ACh-NBM-lesioned rats were not entirely devastated at acquiring the novel FC stimulus set, but rather they were much less efficient at acquiring it relative to sham-lesioned rats. While sham-lesioned rats reached an average of 93% accuracy by the final block FC acquisition, ACh-NBM lesioned rats reached only 80% accuracy. This indicates that ACh-NBM-lesioned rats were still capable of acquiring the FC task, but they were less efficient at acquiring it relative to sham-lesioned rats. Lastly, although reaction time data were not included in the results of Experiment 3 given that this behavioural measure did not significantly differ between the tasks (FC, FS, FS Enhanced-Difficulty), it is important to note that ACh-NBM-lesioned rats could not be differentiated from sham-lesioned rats in terms of latency to dig, suggesting that cholinergic-selective lesions of the NBM did not adversely impact motor behaviour during task performance or tendency to explore the digging bowl stimuli.

Consistent with the cross-species findings of Experiment 1, which revealed that the retrieval of conjunctive stimuli requires neither substantial attentional resources nor maximal binding of ACh to muscarinic receptors (Botly & De Rosa, 2008), ACh-NBM-lesioned rats were not impaired relative to sham-lesioned rats at retrieving a set of FC stimuli they had acquired prior to surgery. This finding provides further support for the argument that once a conjunctive stimulus is well learned it has a bound and stable neural representation, which reduces the need for a cholinergic-dependent attentionally demanding feature binding process during retrieval. This is consistent with the model of cholinergic function previously discussed which proposes that high levels of ACh are conducive to the encoding of new information, while lower levels are conducive to the consolidation and retrieval of previously-learned information (Hasselmo & McGaughy, 2004). It is important to note that in contrast to the rats that received non-selective lesions of the NBM with quisqualic acid in Experiment 2, ACh-NBM-lesioned rats in
Experiment 3 were not impaired at retrieving a previously-learned FC stimulus set. We had suggested in Experiment 2 that the impaired FC retrieval deficit of NBM-lesioned rats may have been due to forgetting of the FC stimuli during the 10-day post-surgical recovery period, resulting in the need for an attentionally-demanding re-binding process during retrieval. However, the results of Experiment 3 do not support this re-binding hypothesis, as ACh-NBM-lesioned rats received a post-surgical recovery period of similar length to that received by NBM-lesioned rats in Experiment 2 (14-days) and they were not impaired relative to sham-lesioned rats at retrieving a previously-learned FC stimulus set. These contrasting findings suggest that the impaired FC retrieval performance of NBM-lesioned rats in Experiment 2 was due to excitotoxin-induced neuronal death of co-localized non-cholinergic neurons in the NBM, likely of GABAergic neurochemical type. This would be consistent with previous research showing that excitotoxic lesions of the NBM typically result in both mnemonic and attentional deficits (Altman, Crosland, Jenden, & Berman, 1985; Berman, Crosland, Jenden, & Altman, 1988; Connor, Langlais, & Thal, 1991; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Mayo, Kharouby, Le Moal, & Simon, 1988; Santucci & Haroutunian, 1989; Vale-Martinez et al., 2002), while cholinergic-selective lesions of the NBM primarily impact attentional performance (Baxter et al., 1996; McGaughy, Everitt, Robbins, & Sarter, 2000; Sharma & Kulkarni, 1993; Waite & Thal, 1996).

Given past human neuroimaging work implicating neural activity in frontoparietal cortices in feature binding (Corbetta, Shulman, Miezin, & Petersen, 1995; Nobre, Coull, Walsh, & Frith, 2003) and non-human animal work demonstrating the importance of frontoparietal cortical cholinergic input from the NBM to attention (Bucci, Holland, & Gallagher, 1998; Dalley et al., 2004), we contend that cholinergic neurotransmission in frontoparietal cortices supports the attentional processes necessary for the formation of conjunctive stimuli. The results of our
correlational analyses examining the relationship between cortical cholinergic activity and post-surgical task performance are in support of this hypothesis. A significant positive correlation between FC acquisition and cholinergic activity, as measured by AChE reactivity, was found in the frontal cortex and a weak trend towards significance was found in the parietal cortex. These findings are also consistent with in vivo microdialysis work demonstrating positive associations between task-related attentional effort and ACh efflux in frontoparietal cortices in rats (Himmelheber, Sarter, & Bruno, 2000, 2001). Furthermore, the number of cholinergic cells in the NBM and the degree of cholinergic activity in the neocortex positively correlated with postsurgical FC acquisition performance, but not FS acquisition or FC retrieval performance, signifying the selective importance of cortical cholinergic input from the NBM to the feature binding encoding process. This is consistent with the findings from Experiment 1, which demonstrated that only FC acquisition required substantial attentional resources in human participants (Botly & De Rosa, 2007).

It should be noted that our correlational analyses also revealed that despite significant reductions in the number of cholinergic NBM cells and cortical cholinergic activity, three ACh-NBM-lesioned rats reached accuracy levels comparable to those of sham-lesioned rats by the final block of FC acquisition. Thus, a closer examination of these three rats’ NBM lesions was conducted to determine whether any differences in lesion extent could be detected and possibly explain these aberrant behavioural findings. The cholinergic-selective immunotoxin 192 IgG-saporin was injected into both the anterior and posterior subdivisions of the NBM of ACh-NBM-lesioned rats. The anterior subdivision projects primarily to the frontal and temporal cortices, while the posterior subdivision projects primarily to the parietal cortex in rats (Saper, 1984), and differences in the extent to which the different subdivisions were lesioned may influence postsurgical performance. Although not statistically significant, a larger number of cholinergic cells
(~50) was found in the posterior subdivision of the NBM of two of the three ACh-NBM-lesioned rats in question relative to the other ACh-NBM-lesioned rats, suggesting that a greater proportion of parietal cortex-projecting NBM neurons remained intact in those two rats relative to the other ACh-NBM-lesioned rats. However, no statistical difference in the degree of parietal cholinergic activity as measured by AChE optical density was found between those two rats and the remaining ACh-NBM-lesioned rats. While only speculative conclusions can be drawn from such histological findings, greater cholinergic input reaching the parietal cortex and leading to better acquisition of the FC task is consistent with research suggesting that the parietal cortex is involved in the allocation of attention to relevant stimuli for learning (Maddux, Kerfoot, Chatterjee, & Holland, 2007), and with recent single-unit recording studies showing that neuronal activity in the posterior parietal cortex predicts the allocation of attention to stimuli relevant to learning (Bucci, 2009; Broussard, Karelina, Sarter, & Givens, 2009; Broussard, Sarter, & Givens, 2006).

It is important to distinguish feature binding from the hippocampal-dependent mnemonic binding that is associated with the formation of episodic memories. Research has shown that the hippocampus is critically involved in integrating the different components of memories, such as the “what, where, and when” elements of episodic memories (Barry & Doeller, 2010; Dayawansa et al., 2006; Eichenbaum & Fortin, 2009; Eichenbaum & Bunsey, 1995; Komorowski, Manns, & Eichenbaum, 2009). While much of this research has focused on the hippocampus’ role in encoding spatial information, recent research has revealed that the hippocampus is also important for encoding other elements of memory, such as context, and integrating them with spatial information (Barry & Doeller, 2010). For example, Komorowski et al. (2009) recently showed that the hippocampus is involved in item-place conjunctive encoding. In this experiment, rats learned to obtain a food reward from a digging bowl scented with odor A
and containing digging medium B in environmental context 1, while in environmental context 2, rats learned to obtain a food reward from a digging bowl scented with odor C and containing digging medium D. Using *in vivo* tetrode-based recording from hippocampal neurons, the authors found that individual hippocampal neurons fired in response to the specific item-context conjunction by the end of training, revealing that the hippocampus was encoding and integrating multiple associative elements. However, it is important to note that the digging-based item-context conjunctive task used by Komorowski et al. (2009) did not require feature binding. Unlike our FC task in which the odor and texture features of the digging bowls completely overlapped such that rats could not rely on any single feature (odor or texture) to locate their food reward, in the task used by Komorowski et al. (2009), the odor and digging medium features used were not overlapping, such that rats could form conjunctions using the distinct odor or bedding or context elements. Furthermore, it is important to note that our cholinergic-selective immunotoxic lesions were restricted to the NBM of the BF and thus left cholinergic innervation of the hippocampus via the septohippocampal BF projection pathway intact, as verified by histological and immunohistological analyses. Therefore, intact cholinergic afferentation of the hippocampus was not sufficient for undisrupted acquisition of our crossmodal FC task, indicating that the feature binding needed for rats to accurately acquire our FC task was dependent on cortical cholinergic innervation.

While the results of Experiment 1 (Botly & De Rosa, 2008) and our previous crossmodal pharmacological work (Botly & De Rosa, 2007) have directly implicated muscarinic receptors in feature binding at encoding, the cholinergic-selective NBM lesions employed in Experiment 3 likely reduced both muscarinic and nicotinic neurotransmission in the neocortex. Thus, we cannot conclude that reducing muscarinic cholinergic afferentation of the neocortex was solely responsible for the impaired ability of rats to acquire the FC task. However, as illustrated by
summary Figure 25, divided attention in human participants (Experiment 1), muscarinic cholinergic blockade with scopolamine in rats (Experiment 1), and cholinergic-selective NBM lesions (Experiment 3) all yielded the same performance pattern on comparable FC and FS tasks. Furthermore, the magnitude of impairment in FC acquisition was equivalent for all three manipulations.

These congruent findings suggest that it is cholinergic input from the NBM acting on muscarinic receptors in the neocortex that supports the attentional processes necessary for the formation of conjunctive stimuli. Further support for this assertion can be gleaned from previous work showing that the density of muscarinic receptors in the cortical targets of the basalocortical projection from the NBM is much greater than that of nicotinic receptors (Mirza & Stolerman, 2000). This suggests that the destruction of cortically-projecting cholinergic neurons in the NBM would result in a greater reduction of ACh binding at muscarinic than nicotinic receptors in the cortex. Furthermore, while pharmacological studies from both the human and non-human animal literature have shown that nicotinic receptor agonists improve performance on attention-dependent tasks, blockade of nicotinic receptors with mecamylamine has been shown to disrupt attention much less dramatically than muscarinic receptor antagonists, such as scopolamine (Ellis et al., 2006; Ruotsalainen, Miettinen, MacDonald, Koivisto, & Sirvio, 2000; Spinelli, Ballard, Feldon, Higgins, & Pryce, 2006). Interestingly, and consistent with the hypothesized role of ACh in boosting the signal of incoming sensory information for learning, research has implicated muscarinic activity in stimulus detection and modulation of neural activity in regions responsible for the processing of task-relevant information, while nicotinic activity appears to be more related to arousal and response control and modulates neural activity in regions responsible for maintaining attention on task (Greenwood, Lin, Sundararajan, Fryxell, & Parasuraman, 2009; Mentis et al., 2001; Ruotsalainen, Miettinen, MacDonald, Koivisto, & Sirvio, 2000). For
instance, while enhancement of nicotinic activity by administration of nicotine improved sustained attention in monkeys on a visual vigilance tasks, disruption of the muscarinic system by administration of scopolamine decreased target detection and increased omissions (Spinelli, Ballard, Feldon, Higgins, & Pryce, 2006).

In conclusion, Experiment 3 has shown that cortical cholinergic input from the NBM of the BF is critical for feature binding at encoding using the same digging-based feature binding task as that employed in Experiments 1 and 2. In light of the cross-species findings of Experiment 1, which confirmed the attention-dependent nature of our FC task (Botly & De Rosa, 2008), the impaired FC acquisition performance of ACh-NBM-lesioned rats in Experiment 3 suggests that the NBM is the neural source of the cortical cholinergic neurotransmission needed to support the attentional processes critical for the formation of neural representations of conjunctive stimuli. Furthermore, histological findings suggest that it is cholinergic activity in a frontoparietal cortical attentional network that facilitates feature binding at encoding.

**Experiment 4**

If ACh is critical for feature binding, as suggested by the findings of Experiments 1-3 (Botly & De Rosa, 2008, 2009a, 2009b), then its modulatory influence on this cognitive process should generalize to other tests of feature binding. A rodent analog of the standard test of feature binding from the human cognitive literature, visual search, would allow for an important test of our cholinergic hypothesis of feature binding. Thus, in Experiment 4, a visual search task was designed for rats using touchscreen-equipped operant chambers and achromatic stimuli. On any given visual search trial, the task of rats was always to locate and nose-poke a target stimulus (white square), which was embedded in an array of distractor stimuli (black square, white triangle, and black triangle). Each visual search session was counterbalanced for target and
distractor positions, trial-type (Conjunctive or Feature Search trials), and stimulus set-size (four, six, or eight stimuli). On Conjunctive Search trials, the target stimulus was presented along with distractor stimuli that differed from the target on two feature dimensions, shape and pattern, such that feature binding was required to locate the target. In contrast, on Feature Search trials, the target stimulus was presented along with distractor stimuli that differed from the target on only a single feature dimension, shape or pattern, such that feature binding was not required to locate the target.

Accuracy and latency to locate the target stimulus were used as measures of task performance. Following pre-surgical acquisition of the visual search task, the NBM of rats was bilaterally lesioned using the cholinergic-selective immunotoxin, 192 IgG-saporin, and the visual search performance of ACh-NBM-lesioned rats was compared to that of sham-lesioned rats using the same target and distractor stimuli rats were trained on prior to surgery. If cortical cholinergic neurotransmission is necessary to support the attentional processes needed for feature binding, as suggested by Experiment 3, then reducing cholinergic afferentation of the neocortex via cholinergic-selective lesions of the NBM should yield a performance pattern reflective of less efficient visual search.

A classic visual search performance pattern that has been well documented by the human cognitive literature is a significant increase in the latency to locate the target stimulus as the number of distractors increases on conjunctive, but not feature search trials (Treisman, 1998; Treisman & Gelade, 1980). The positive slope of the conjunctive search latency curve is considered to be due to the occurrence of a serial search for the target. During conjunctive search, participants are required to feature bind in order to locate a particular conjunction of features and this requires a visual inspection of each stimulus in the array until the target is
located. Thus, increasing the number of distractors on conjunctive search trials has a substantial effect on the latency to locate the target. In contrast, the relatively flat slope that typically characterizes the feature search latency curve is termed the “pop-out” effect: the single feature differentiating the target stimulus from the distractors is highly noticeable, eliminating the need for a serial search for the target. Thus, increasing the number of distractors on feature search trials has a negligible effect on the latency to locate the target. As previously discussed, behavioural, neuropsychological, and neuroimaging work with humans has revealed that locating a target stimulus during conjunctive, but not feature search trials requires substantial attentional resources (Corbetta, Shulman, Miezin, & Petersen, 1995; Foster, Behrmann, & Stuss, 1999; Tales et al., 2002).

While it was predicted that rats’ pre-surgical performance on the visual search task would yield the classic latency-based pattern discussed above that is typically observed in humans (Treisman, 1998), analyses conducted on the pre-surgical correct latency data revealed only a weak trend towards significance with regard to the time it took for rats to locate the target stimulus on Conjunctive versus Feature Search trials. Given that rats received a tremendous amount of visual search training using the same stimuli prior to surgery, it was possible that such over-training may have significantly attenuated any trial-type differences in correct latency, which has been shown to occur in pigeons (Vreven & Blough, 1998). A closer examination of our visual search training data failed to reveal any initial significant differences between correct latency during Feature and Conjunctive Search trials. It should be noted that in order to keep the size of the stimuli large enough for rats to accurately discriminate, our largest stimulus set-size comprised only eight stimuli, which is much smaller than the set-sizes of approximately twenty stimuli typically employed with human participants (Nobre, Coull, Walsh, & Frith, 2003). Thus, our maximum stimulus set-size may not have been large enough to yield significant interactions with
latex. Furthermore, an important difference between the human version of the visual search task and our rat analog that likely led to greater variance in our correct latency data is that human participants typically indicate that they have located the target stimulus with a keyboard button press rather than physically touching the target stimulus. Rats had to travel from the back to the front of the operant chamber to touch the target stimulus and the variance associated with this action would be much greater than that associated with a button press. Lastly, in light of our past experience with training rats to perform touchscreen-based visual tasks which revealed that rats stay much more task focused when there is no time limit for them to respond, during all visual search trials, the target and distractor stimuli remained on the touchscreen until a response was made. This necessity of giving rats an unlimited amount of time to nose-poke the target stimulus resulted in a large amount of variance in the correct latency data due to significant variability in target touch-times both within and across sessions. Unfortunately, outlier analyses were unsuccessful at eliminating this large amount of variance, which reduced the chances of finding a significant difference between correct latency during Feature and Conjunctive Search trials.

Nonetheless, analyses conducted on the pre-surgical accuracy data revealed that rats were significantly more accurate at locating the target stimulus on Feature Search than Conjunctive Search trials. Importantly, stimulus set-size was found to significantly interact with trial-type such that as the number of distractors increased, rats’ accuracy at locating the target stimulus decreased on Conjunctive Search trials, while remaining relatively more stable on Feature Search trials. This accuracy-based pattern of performance demonstrated by rats resembles the classic latency-based performance pattern typically observed in human participants, and suggests that the feature binding requirement of Conjunctive Search trials made it more challenging for rats to accurately locate the target stimulus, especially as the number of distractors increased. This is consistent with findings from the human cognitive literature demonstrating that feature binding
errors are more likely to occur under conditions of high attentional load, such as when conjunctive search with large stimulus set-sizes is required (Cinel, Humphreys, & Poli, 2002; Treisman & Schmidt, 1982). Furthermore, rats’ accuracy on the visual search task is consistent with that of pigeons (Cook, 1992). In a cross-species study in which pigeons and human participants performed visual search using the same search displays, pigeons primarily showed feature-conjunctive differences in accuracy while human participants showed feature-conjunction differences in latency (Cook, 1992). However, like humans, pigeons also yielded latency-based feature-conjunctive differences in target detection time when large stimulus set-sizes of twenty or more stimuli were used (Blough, 1989). Thus, rats may also demonstrate such latency-based feature-conjunctive differences if larger stimulus set-sizes were employed. 

As previously discussed, the NBM of the BF provides 90% of the cholinergic input to the neocortex (Mesulam, Mufson, Wainer, & Levey, 1983), and Experiment 3 revealed a critical role for cortical cholinergic input from the NBM in supporting the attentional processes necessary for feature binding at encoding (Botly & De Rosa, 2009a). We thus predicted that the attentional disruption induced by cholinergic deafferentation of the neocortex would lead to selective performance decrements on the visual search task. While the digging-based feature binding tasks employed in Experiments 1-3 allowed us to dissociate the encoding and retrieval stages of feature binding (Botly & De Rosa, 2008, 2009a, 2009b), visual search is characterized by negligible mnemonic demands. Because the identity of the target stimulus remains constant across all trials and sessions, no other information must be held in memory, making visual search tasks a relatively pure test of the attentional demands of feature binding. The results of Experiments 1 and 3 have shown that rats are unimpaired at retrieving well-learned conjunctive stimuli following systemic muscarinic blockade with scopolamine (Botly & De Rosa, 2007) and cholinergic-selective lesions of the NBM (Botly & De Rosa, 2009a). Given that the target and
distractor stimuli were very well learned by rats prior to surgery, we did not anticipate a difference in the ability of sham-lesioned and ACh-NMB-lesioned rats to accurately locate the target stimulus on Feature or Conjunctive Search trials post-surgery. However, we did predict that ACh-NBM-lesioned rats would be less efficient at visual search and thus take longer than sham-lesioned rats to locate the target stimulus on Conjunctive, but not Feature Search trials due to the greater attentional load of Conjunctive Search trials.

Consistent with these predictions, there was no significant effect of lesion on accuracy for locating the target stimulus for either Feature or Conjunctive Search trials post-surgery. This is congruent with the intact FC retrieval findings of Experiments 1 (Botly & De Rosa, 2007) and 3 (Botly & De Rosa, 2009a) and with a well-supported model of cholinergic function stating that high levels of ACh are not needed for the retrieval of previously-learned information (Hasselmo & McGaughy, 2004). Importantly, equivalent post-surgical accuracy performance by rats in both surgical groups allowed us to attribute any lesion-induced difference in correct latency to inefficiency at visual search rather than a simple inability of ACh-NBM-lesioned rats to perform the task.

As predicted, ACh-NBM-lesioned rats took significantly longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search, but not Feature Search trials. Furthermore, this significant lesion-induced increase in correct latency was found only during Conjunctive Search trials with stimulus set-sizes of six or eight. ACh-NBM-lesioned rats took approximately 400-500 ms longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search trials when five or seven distractors were present while the difference was almost half as much (~ 275 ms) when only three distractors were present. This is consistent with the notion that as the number of distractors increased on Conjunctive Search trials, a more extensive serial search was
necessary to locate the target which required attentional resources that were not available to ACh-NBM-lesioned rats. Previous work has shown that cholinergic-selective lesions of the NBM significantly impaired the ability of rats to perform attentionally-demanding tasks (Harati, Barbelivien, Cosquer, Majchrzak, & Cassel, 2008; Lehmann, Grottick, Cassel, & Higgins, 2003; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002), and Experiment 3 revealed that sufficient cortical cholinergic activity is necessary for rats to efficiently acquire a feature binding task. Based on such findings, it can be argued that reducing cholinergic afferentation of the neocortex in Experiment 4 disrupted the attentional processes needed for rats to efficiently find the target stimulus on the feature-binding dependent Conjunctive Search trials, thus leading to an increase in latency. Given ACh’s known effects on cortical dynamics, it can be reasoned that sufficient cortical cholinergic neurotransmission facilitates efficient visual search by boosting the afferent sensory input representing the target stimulus, while suppressing the sensory input representing the distractor stimuli (Hasselmo, Anderson, & Bower, 1992; Hasselmo, Schnell, & Barkai, 1995; Liljenstrom & Hasselmo, 1995; Vogt & Regehr, 2001).

Functional neuroimaging work with neurologically intact human participants showing that frontoparietal cortical networks are selectively activated during conjunctive search trials of visual search (Corbetta, Shulman, Miezin, & Petersen, 1995; Nobre, Coull, Walsh, & Frith, 2003) has been used as evidence that attentional processes in frontoparietal cortices support feature binding. Consistent with this theory, in Experiment 3, significant positive relationships between feature binding acquisition performance in rats and histological markers of cholinergic activity in frontoparietal cortical regions were found (Botly & De Rosa, 2009a). Unfortunately, no significant relationships between histological markers of cholinergic activity and performance on the visual search task were found in Experiment 4. This may have been due to the large amount of between-subject and within-session variance that characterized the correct latency
data, which reduced the likelihood of finding significant correlations between histological markers and behavioural measures of performance. Although doing so may have a detrimental impact on stimulus discriminability, for future studies it may be necessary to significantly increase the stimulus set-sizes used in the rat visual search task so that differences of greater magnitude may be found between correct latencies during Feature and Conjunctive Search trials. While previous experience has shown that giving rats as long as they need to respond to a visual stimulus on a touchscreen is ideal, it may also be necessary to implement a response time-limit in future studies to help reduce variance within the correct latency visual search data.

Experiment 4 has demonstrated that rats are capable of successfully performing a visual search task, which to our knowledge, has not been done before. This rat visual search paradigm can now be used in future studies to further characterize the importance of cortical cholinergic neurotransmission to efficient visual search, such as in determining the relative importance of cholinergic input to frontal versus parietal cortices. Furthermore, this rodent visual search paradigm could be adapted for use as an additional cognitive paradigm for testing rodent models of Alzheimer’s disease (AD). Numerous transgenic rodent models of AD have been created over the past decade, which have been invaluable in further understanding the causes and pathological course of AD (Liu et al., 2008; McGowan, Eriksen, & Hutton, 2006; Morrissette, Parachikova, Green, & LaFerla, 2009). While these transgenic rodents exhibit both the neuropathological hallmarks (extracellular β-amyloid (Aβ)-containing plaques, neuronal atrophy), as well as the age-dependent deficits in cognitive functioning typically seen in human AD patients, the simple tests used to measure their cognitive functioning greatly differ from the more cognitively-complex tests used with human AD patients (Eriksen & Janus, 2007; Morris et al., 2006).
Using a well-established cognitive test from the human literature, such as visual search to measure attentional dysfunction in transgenic AD rodents would be warranted given that attentional processing is one of the primary domains of cognitive functioning that is disrupted in AD (Perry & Hodges, 1999). Furthermore, as previously discussed, recent work has revealed that AD patients are selectively impaired on conjunctive, but not feature search trials of visual search (Foster, Behrmann, & Stuss, 1999; Tales et al., 2002), which was also the pattern of impairment observed in rats with cholinergic-selective lesions of the NBM in the current study. Interestingly, there is evidence to suggest that AD patient’s attentional impairments may be due to cortical cholinergic hypofunction as a result of degeneration of the BF, in particular the NBM (Auld, Kornecook, Bastianetto, & Quirion, 2002; Doucette, Fisman, Hachinski, & Mersky, 1986; Growdon, 1999; Hall, Moore, Lopez, Kuller, & Becker, 2008; Iraizoz, de Lacalle, & Gonzalo, 1991; Kihara & Shimohama, 2004; Rinne, Paljarvi, & Rinne, 1987; Schliebs & Arendt, 2006; Teipel et al., 2005; Vogels et al., 1990). Two neuropathological hallmarks of the disease that have been found to be dense within the NBM, extracellular beta-amyloid-containing plaques and intracellular neurofibrillary tangles, are detrimental to cortical cholinergic neurotransmission (Arendt, Taubert, Bigl, & Arendt, 1988; Auld, Kornecook, Bastianetto, & Quirion, 2002), and functional neuroimaging work has revealed reduced activation in frontoparietal cortices of AD patients performing conjunctive search trials (Hao et al., 2005). If the feature binding impairments observed in human AD patients are in fact due to reduced cholinergic afferentation of the neocortex, then using a visual search paradigm to measure attentional function in transgenic AD rodents would be a good choice for testing the efficacy of novel pharmacological treatments for AD, such as cholinergic agonists that selectively target the M₁ muscarinic receptor subtype (Fisher, 2008).
In conclusion, use of this rat analog of the standard test of human feature binding in Experiment 4 allowed for a critical test of the hypothesis that cholinergic input to the neocortex from the NBM of the BF is important for feature binding. In support of this hypothesis, it was revealed that reducing cortical cholinergic neurotransmission via cholinergic-selective lesions of the NBM decreased the efficiency of visual search in rats as evidenced by the greater time required for ACh-NBM-lesioned rats relative to sham-lesioned rats to locate the target stimulus on Conjunctive, but not Feature Search trials. Experiment 4 has thus extended the validity of the results of Experiments 1-3 by demonstrating that ACh’s modulatory influences on feature binding generalize to a well-established test of this fundamental attention-dependent cognitive process.

**Limitations, Concluding Remarks, and Future Directions**

The learning-based nature of the feature binding tasks used in Experiments 1-3 (Botly & De Rosa, 2008, 2009a, 2009b) allowed us to dissociate the encoding and retrieval stages of feature binding, which to our knowledge has not been done before even in the human cognitive literature. Furthermore, while human participants under divided attention were impaired at acquiring a visual analog of the rat feature binding task, they were not impaired at retrieving previously-learned conjunctive stimuli. This is in agreement with a considerable body of research from the human cognitive literature showing that disrupting attention is selectively detrimental to the encoding, relative to the retrieval, of episodic memories (Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Naveh-Benjamin, Craik, Guez, & Dori, 1998). Taken together, these findings suggest that once a conjunctive stimulus is well learned it has a bound
and stable neural representation, which reduces the need for a cholinergic-dependent attentionally demanding feature binding process during retrieval.

The histological analyses conducted in Experiment 3 revealed that cholinergic input to frontoparietal cortical regions may support feature binding at encoding (Botly & De Rosa, 2009a). Further research is needed to determine the relative importance to feature binding of cholinergic input to frontal versus parietal cortices. This could be accomplished by directly injecting the cholinergic-selective immunotoxin 192 IgG-saporin into the frontal or parietal cortices of rats. Through retroactive neuronal transport, the immunotoxin would travel back to cholinergic cell bodies in the NBM and selectively destroy frontal- or parietal-cortex projecting cholinergic neurons (Bucci, Holland, & Gallagher, 1998; Dalley et al., 2004; Newman & McGaughy, 2008; Ross, McGaughy, & Eichenbaum, 2005). Comparisons could then be made between the performances of rats with cholinergic deafferentation of the parietal versus frontal cortices on our digging- and visual search-based feature binding tasks. Predictions regarding the relative importance of cholinergic input to frontal versus parietal cortices to feature binding can be made in light of recent work which has shown that the frontal and parietal components of the frontoparietal cortical attentional network may mediate different aspects of attentional processing. While the frontal cortex appears to be more critically involved in the attentional guidance of action, the parietal cortex appears to be more critically involved in the allocation of attention to relevant stimuli for learning (Maddux, Kerfoot, Chatterjee, & Holland, 2007). These findings suggest that cholinergic neurotransmission in the parietal cortex may be more critical to feature binding given that efficient formation or detection of conjunctive stimuli would require attentional allocation to relevant stimuli and features in the environment. This potential parietal cortex-mediated attentional mechanism is consistent with the notion that ACh facilitates
attention and information encoding by boosting the neural signal of incoming sensory information, while suppressing the neural signal of information that is not currently relevant.

In our original crossmodal feature binding experiment (Botly & De Rosa, 2007) as well as in Experiment 1 (Botly & De Rosa, 2008), we selectively blocked muscarinic receptors in the brains of rats using scopolamine. However, the cholinergic-selective brain lesioning techniques used in Experiments 3 (Botly & De Rosa, 2009a) and 4 reduced both muscarinic and nicotinic neurotransmission in the neocortex by destroying cortically-projecting cholinergic neurons in the NBM of the BF. Thus, we cannot conclude from Experiments 3 and 4 that ACh acting at muscarinic receptors in the neocortex was essential for feature binding. However, it should be noted that muscarinic cholinergic blockade with scopolamine (Botly & De Rosa, 2007) and cholinergic-selective NBM lesions (Botly & De Rosa, 2009a) in rats yielded the same magnitude of impairment in feature binding acquisition using the same digging-based FC task. This suggests that cortical muscarinic neurotransmission may be more critical to feature binding, and there is evidence to suggest that muscarinic receptors may play a more important role in attentional processing than nicotinic receptors (Ellis et al., 2006; Mirza & Stolerman, 2000; Ruotsalainen, Miettinen, MacDonald, Koivisto, & Sirvio, 2000; Spinelli, Ballard, Feldon, Higgins, & Pryce, 2006).

It would be valuable to directly test the relative importance of nicotinic versus muscarinic neurotransmission to feature binding by directly injecting receptor-selective antagonists, such as scopolamine or mecamylamine into the neocortex of rats to selectively reduce muscarinic or nicotinic neurotransmission, respectively. Comparisons could then be made between the performances of rats with hypoactive muscarinic or nicotinic neurotransmission on our digging- and visual search-based feature binding tasks. Further work could also be undertaken to
determine the relative importance of the different muscarinic receptor subtypes to feature binding, with a particular focus on the M₁ receptor in light of its purported role in mediating the cognitive functions of ACh (Fisher, 2008; Wess et al., 2003).

It is important to address the potential impact on task performance of compensatory cholinergic mechanisms that were likely engaged following chronic administration of the muscarinic receptor antagonist scopolamine in Experiment 1 and the destruction of cholinergic neurons in the NBM of the BF in Experiments 3 and 4. As previously discussed, scopolamine is a non-selective muscarinic receptor antagonist that binds indiscriminately to all five muscarinic receptor subtypes. Blockade of M₂ autoreceptors by scopolamine would have decreased the negative feedback regulating ACh release from pre-synaptic terminals resulting in increased release of ACh onto synapses throughout the brain. Furthermore, during acquisition of the intramodal FC and FS stimuli in Experiment 1, rats were injected daily with scopolamine 15 min prior to their testing sessions. The impact of such chronic muscarinic receptor blockade may have resulted in the up-regulation/sensitization of muscarinic receptors on post-synaptic terminals, thereby increasing the likelihood that ACh would bind to post-synaptic muscarinic receptors. However, rats under the influence of scopolamine were found to be impaired relative to performance under saline at acquiring intramodal FC stimuli despite the potential increase in pre-synaptic ACh release and up-regulation/sensitization of post-synaptic muscarinic receptors. This suggests that these compensatory cholinergic mechanisms were not enough to buffer the decrease in ACh binding to post-synaptic muscarinic receptors and allow for efficient acquisition of the FC task. Following the destruction of cholinergic neurons in the NBM in Experiments 3 and 4, the release of ACh in the cortex was reduced as confirmed by our histological analyses. However, such a reduction in cholinergic input to the cortex likely resulted in decreased binding of ACh to M₂ autoreceptors on the pre-synaptic terminals of cortically-projecting cholinergic
NBM neurons located throughout the cortex. This decrease in the negative feedback regulating the release of ACh from pre-synaptic terminals likely enhanced the basal firing rate of cholinergic NBM neurons, in turn increasing their release of ACh onto synapses in the cortex. Furthermore, the decreased cortical cholinergic input due to destruction of cholinergic NBM neurons may have resulted in a compensatory up-regulation/sensitization of post-synaptic cholinergic receptors in the cortex, increasing the likelihood that ACh would bind to post-synaptic receptors. Compensatory cholinergic effects similar to these were noted by De Rosa, Hasselmo, & Baxter (2001) who demonstrated that rats with cholinergic-selective lesions of the BF nuclei were hypersensitive to the effects of oxotremorine, a non-selective muscarinic receptor agonist, suggesting that the decreased release of ACh in target structures of the BF resulted in a compensatory up-regulation/sensitization of post-synaptic muscarinic receptors. With regard to Experiments 3 and 4, it is important to note that despite the action of these potential compensatory mechanisms, ACh-NBM-lesioned rats were less efficient than sham-lesioned rats at acquiring crossmodal FC stimuli and performing the visual search task. This suggests that these compensatory cholinergic mechanisms were not enough to buffer the decrease in ACh binding to post-synaptic receptors in the cortex and allow for efficient feature binding performance.

Although not all of the possible stimulus modality combinations were tested, strikingly, these four experiments have yielded converging evidence from multiple sensory modalities that ACh is important for feature binding. Intramodal binding of olfactory and visual stimuli was required by rats in Experiments 1 and 4, while crossmodal binding of olfactory and texture stimuli was required by rats in Experiments 2 and 3. Disruption of the cholinergic system was found to impair all of these forms of feature binding in rats, and disruption of the attentional system in humans yielded comparable intramodal visual feature binding impairments in Experiment 1.
Such convergent findings suggest that a common cholinergically-mediated attentional mechanism may be required for feature binding regardless of sensory modality. There is evidence that synchronization of cortical neuronal firing in the gamma frequency range may be needed for feature binding (Engel, Fries, & Singer, 2001; Senkowski, Schneider, Foxe, & Engel, 2008) and both attention (Muller, Gruber, & Keil, 2000; Tiitinen et al., 1993) and ACh have been shown to modulate such gamma frequency synchronization in the cortex (Engel, Kreiter, König, & Singer, 1991; Friedman-Hill, Maldonada, & Gray, 2000; Gray & Singer, 1989; Rodriguez, Kallenbach, Singer, & Munk, 2004). Further research is needed to determine whether cholinergically-driven synchronization of neuronal firing in the cortex is a common neural mechanism for feature binding.

It is important to consider the role other cholinergic BF nuclei may play in feature binding. As previously discussed, research has shown that the hippocampus is critically involved in configural learning and integrating the different components of memories, such as the “what, where, and when” elements of episodic memories (Barry & Doeller, 2010; Dayawansa et al., 2006; Eichenbaum & Fortin, 2009; Eichenbaum & Bunsey, 1995; Komorowski, Manns, & Eichenbaum, 2009). Recently, Iordanova, Burnett, Aggleton, Good, and Honey (2009) revealed that the hippocampus of rats was needed for the encoding and retrieval of configural memories characterized by auditory, time-of-day, and visual contextual information, but it was not needed for the encoding or retrieval of memories characterized by single elements. It is important to note that the episodes encoded and retrieved by rats in the Iordanova et al. (2009) study did not require feature binding given that the elements rats had to integrate were not associated with a single stimulus. Nonetheless, the hippocampus was found to be essential for integrating the multiple associative elements of an episode, and the neural processes involved in such
integration may be similar to those involved in the feature integration associated with feature binding.

The MS/VDB of the BF provides cholinergic input to the hippocampus via the septohippocampal projection pathway and whether disruption of this pathway would adversely impact feature binding as measured by our digging- and visual search-based feature binding tasks warrants discussion. The septohippocampal BF pathway has been primarily implicated in hippocampal-dependent forms of learning and memory (Chang & Gold, 2004; Frielingsdorf, Thal, & Pizzo, 2006; Janisiewicz, Jackson, Firoz, & Baxter, 2004; Pang & Nocera, 1999; Xu, Datta, Wu, & Alreja, 2004), and thus cholinergic input to the hippocampus from the MS/VDB would be predicted to be important for configural learning. However, Gibbs and Johnson (2007) found that cholinergic-selective lesions of the MS/VDB failed to impair the ability of rats to acquire a configural learning task, while cholinergic-selective lesions of the NBM were successful at impairing rat’s configural learning. Consistent with this finding, Butt, Noble, Rogers, and Rea (2002) demonstrated that cholinergic-selective lesions of the NBM impaired the ability of rats to acquire a crossmodal configural association learning task, while sparing acquisition of an elemental association task. These findings indicate that the cognitive processes needed for rats to successfully acquire the putative hippocampal-dependent configural learning task were dependent on cholinergic input to the cortex rather than the hippocampus. The authors of the Butt et al. (2002) study suggested that the lesion-induced reduction of cholinergic afferentation of the cortex disrupted the attention of rats such that they could not sufficiently allocate their attention to the crossmodal elements during acquisition of the configural task.

The series of experiments presented in this dissertation have argued that attention is the critical component necessary for the acquisition and performance of our digging- and visual-search-
based feature binding tasks and that ACh and the NBM of the BF support the attentional processes needed for efficient feature binding. The histological results of Experiments 3 and 4 indicate that intact cholinergic afferentation of the hippocampus was not sufficient for undisrupted performance of our digging- and visual search-based feature binding tasks, revealing that the cognitive processes needed for rats to efficiently perform these feature binding tasks was dependent on cortical cholinergic innervation arising from the NBM. Experiment 1 provided correlational evidence to suggest that the impaired feature binding acquisition performance of rats given a cholinergic challenge was due to disrupted attentional processes (Botly & De Rosa, 2008), and Experiment 3 associated less efficient acquisition of a feature binding task with reduced cholinergic innervation of cortical areas known to be important to attention, namely frontoparietal cortices (Botly & De Rosa, 2009a). In light of the importance of attention and the frontoparietal cortices to feature binding suggested by Experiments 1 and 3 and supported by substantial evidence from the human cognitive literature (Bernstein & Robertson, 1998; Cohen & Rafal, 1991; Corbetta, Shulman, Miezin, & Petersen, 1995; Foster, Behrmann, & Stuss, 1999; Friedman-Hill, Robertson, & Treisman, 1995; Luck & Ford, 1998; Reynolds & Desimone, 1999; Tales et al., 2002; Treisman, 1998), we would predict that cholinergic input to the hippocampus is not critical to feature binding. While there is some weak evidence to support this hypothesis, namely that cholinergic input to the hippocampus was not necessary for the sensory integration associated with configural associative learning (Butt, Noble, Rogers, & Rea, 2002; Gibbs & Johnson, 2007), further research is needed to conclusively determine whether the MS/VDB of the BF plays a role in feature binding.

While this series of experiments focused on investigating cholinergic influences on feature binding, the role that other neurotransmitter systems may play in feature binding remains unknown. Similar to ACh, norepinephrine (NE) is a neurotransmitter that innervates broad
regions of the cortex via ascending pathways originating in a brainstem nucleus called the locus coeruleus. Importantly, NE has been implicated in attention and learning (Beane, & Marrocco, 2004; Carli, Robbins, Evenden, & Everitt, 1983; Dalley et al., 2001; Guerin, Peace, Didier, Linster, & Cleland, 2008; Mandairon, et al., 2008; McGaughy, Ross, & Eichenbaum, 2008; Tait & Brown, 2008; Yu & Dayan, 2005) and has been shown to have a similar influence on cortical circuits to that of ACh, namely that it enhances the neural signal of afferent sensory information (Brocher, Artola, & Singer, 1992; Gu, 2002; Kobayashi, 2000). Lesion work using non-human animals (McGaughy, Ross, & Eichenbaum, 2008; Tait & Brown, 2008) along with computational modeling (Yu & Dayan, 2005) have begun to dissociate the different roles that ACh and NE may play in attentional processing. McGaughy, Ross, and Eichenbaum (2008) demonstrated that noradrenergic, but not cholinergic, deafferentation of the medial prefrontal cortex of rats impaired their ability to shift attention away from a previously-relevant stimulus dimension (e.g. odor) to a newly-relevant dimension (e.g. texture) in order to obtain a food reward. Importantly, the authors found that noradrenergic deafferentation of the medial prefrontal cortex did not impair rats’ ability to ignore irrelevant stimuli. Consistent with these findings, Tait and Brown (2008) demonstrated that cholinergic deafferentation of the entire cortex using cholinergic-selective lesions of the NBM of the BF failed to result in a deficit in the attentional set-shifting task in rats.

Such findings are consistent with what is known about ACh’s role in attention, namely that it is critical for enhancing the signal-to-noise ratio during information encoding. Disrupting the cholinergic system impairs the detection of signals, but not the correct rejection of non-signals (McGaughy, Kaiser, & Sarter, 1996), increases proactive interference during learning (Atri et al., 2004; De Rosa & Hasselmo, 2000), and increases neuronal activity in response to a distractor stimulus, while decreasing neuronal activity in response to a target stimulus (Broussard,
Karelina, Sarter, & Givens, 2009). Given such findings indicating that cholinergic disruption widens the scope of attention, it is not surprising that cholinergic deafferentation of the cortex was found to have no detrimental impact on shifting attentional set to a newly-relevant stimulus dimension (McGaughy, Ross, & Eichenbaum, 2008; Tait & Brown, 2008).

It has been proposed that NE’s role in attention involves the detection of abrupt environmental changes, such as changes in the predictive relationship between instrumental action and reinforcement (Beane, & Marrocco, 2004; Dalley et al., 2001; Yu & Dayan, 2005). This theory is consistent with the previously-discussed study which showed that disruption of the noradrenergic system impaired the ability of rats to shift their attention to a newly-relevant stimulus dimension to obtain a food reward (McGaughy, Ross, & Eichenbaum, 2008). Using computational modeling, Yu and Dayan (2005) proposed that NE signals unexpected uncertainty, while ACh signals expected uncertainty. Attentional set-shifting tasks require the detection of unexpected uncertainty as the relevance of the stimulus dimensions to task completion and food reward are changed unpredictably by the experimenter across sessions of training. In contrast, ACh is proposed to signal expected uncertainty, which Yu and Dayan (2005) contend is involved in tasks such as covert orienting. In this attentional task, a visual orienting cue can either correctly or incorrectly predict the location at which a target stimulus will appear, such that participants expect that the orienting cue may not be predictive of target location and should thus not be relied upon during performance of the task. Disruption of the cholinergic system increases reliance on the unpredictable orienting cue (Chiba, Bushnell, Oshiro, & Gallagher, 1999; Phillips, McAlonan, Robb, & Brown, 2000; Witte, Davidson, & Marrocco, 1997).

The noradrenergic system has also been implicated in learning, in particular perceptual learning (Guerin, Peace, Didier, Linster, & Cleland, 2008; Mandairon, et al., 2008). Recently, Mandairon
et al. (2008) showed that manipulating noradrenergic activity in the olfactory bulb of rats influenced odor discrimination learning. Specifically, discrimination of chemically-similar odors was impaired following blockade of noradrenergic receptors in the olfactory bulb. Furthermore, deafferentation of noradrenergic input to the olfactory bulb impaired the ability of rats to remember an odor over a delay period and decreased their ability to discriminate chemically-similar odors, while NE infused directly into the olfactory bulb rescued these deficits (Guerin et al., 2008). Interestingly, cholinergic input to the olfactory bulb and piriform cortex has also been shown to be critical for olfactory discrimination as rats with cholinergic-selective lesions of the HDB of the BF were found to be impaired at discriminating chemically-similar odors (Linster, Hasselmo, Garcia, & Baxter, 2001). Furthermore, blockade of cholinergic receptors directly in the olfactory bulb impaired the ability of rats to discriminate chemically-similar odors (Mandairon et al., 2006). Most recently, enhancing cholinergic neurotransmission in the olfactory bulb of rats was found to sharpen the receptive field of olfactory bulb neurons and improved the ability of rats to discriminate chemically-similar odors (Chaudhury, Escanilla, & Linster, 2009). This suggests that ACh facilitates difficult odor discriminations by refining the receptive field of olfactory neurons such that generalization between chemically-similar odors is reduced.

In light of the research implicating both ACh and NE in attention and learning, it is important to consider whether NE may play an important role in feature binding. The intramodal and crossmodal feature-binding dependent FC tasks employed in Experiments 1-3 required rats to discriminate between digging bowls characterized by overlapping odor and texture features. Such feature-overlap increased the perceptual similarity of the digging bowls such that perceptually-challenging discriminations were required. Given the importance of NE to the discrimination of perceptually-similar stimuli, such as chemically-similar odors (Guerin, Peace,
Didier, Linster, & Cleland, 2008; Mandairon, et al., 2008), it would be interesting and important to determine whether noradrenergic input to sensory cortices (i.e. piriform and somatosensory cortices) is needed to acquire our digging-based intramodal and crossmodal FC tasks.

Lastly, given ACh’s demonstrated role in facilitating perceptual learning through the refinement of neuronal receptive fields (Chaudhury, Escanilla, & Linster, 2009; Fletcher & Wilson, 2002; Kilgard & Merzenich, 1998; Mandairon et al., 2006; McKenna, Ashe, & Weinberger, 1989; Miasnikov, Chen, & Weinberger, 2008; Wilson, 2001; Wilson, Fletcher, & Sullivan, 2004), a potential neural mechanism by which ACh may facilitate feature binding at encoding can be theorized. We have proposed that ACh’s role in boosting the neural signal of incoming sensory information, while suppressing the signal of previously-learned and irrelevant information facilitates attention, and in turn, feature binding. More specifically, ACh may facilitate the learning of our digging-based FC stimuli by sharpening the receptive fields of the sensory neurons encoding the intramodal or crossmodal stimuli such that despite their feature overlap, distinct neural representations of the FC stimuli are efficiently formed. By disrupting cholinergic activity via the muscarinic receptor antagonist scopolamine in Experiment 1 (Botly & De Rosa, 2008) or cholinergic-selective lesions of the NBM of the BF in Experiment 3 (Botly & De Rosa, 2009a), the efficiency with which refined neural representations of feature-overlapping FC stimuli could be formed may have been reduced, in turn increasing stimulus generalization and thus making it more difficult for rats to discriminate between and thus learn the FC stimuli. In contrast, disrupting cholinergic activity was found to leave the retrieval of previously-learned FC stimuli intact which would be expected if the receptive fields of the sensory neurons encoding the stimuli had already been refined during acquisition. Importantly, challenging the attentional system of human participants in Experiment 1 resulted in a similar FC acquisition impairment to that of cholinergic disruption (Botly & De Rosa, 2008) suggesting that sufficient
attentional processes may be necessary to carry out such receptive field refinement. Further research is needed to determine whether ACh facilitates the acquisition of our digging-based FC tasks by refining the neuronal receptive fields of sensory neurons.

In conclusion, using cross-species behavioural, pharmacological, and brain-lesioning methods along with stimuli from multiple sensory modalities, this series of experiments has provided important insights into the neurochemical and neuroanatomical contributions to the fundamental attention-dependent process of feature binding. Experiment 1 suggested that ACh acting at muscarinic receptors supports the attentional processes necessary for intramodal feature binding at encoding and Experiments 2-4 revealed that cholinergic input to the cortex from the NBM of the BF is necessary for efficient crossmodal and intramodal feature binding using two different feature binding tasks. Future research must focus on identifying the specific neural mechanisms by which ACh facilitates the attention-dependent feature binding process.
References


Table 1

Experiment 1: List of odorants, colours, and shapes from which the experimental stimuli were created

<table>
<thead>
<tr>
<th>Experiment 1A</th>
<th>Experiment 1B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oils</td>
<td>Herbs and spices</td>
</tr>
<tr>
<td>Vanilla</td>
<td>Cumin</td>
</tr>
<tr>
<td>Peach</td>
<td>Ginger</td>
</tr>
<tr>
<td>Ylang-ylang</td>
<td>Cinnamon</td>
</tr>
<tr>
<td>Geranium</td>
<td>Sage</td>
</tr>
<tr>
<td>Tangerine</td>
<td>Cocoa</td>
</tr>
<tr>
<td>Peppermint</td>
<td>Nutmeg</td>
</tr>
<tr>
<td>Bergamot</td>
<td>Garlic</td>
</tr>
<tr>
<td>Musk</td>
<td>Coffee</td>
</tr>
<tr>
<td>Passion fruit</td>
<td>Coriander</td>
</tr>
<tr>
<td>Jasmine</td>
<td>Oregano</td>
</tr>
<tr>
<td>Sandalwood</td>
<td>Mustard</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Dried coconut</td>
</tr>
<tr>
<td>Chamomile</td>
<td>Dill</td>
</tr>
<tr>
<td>Lilac</td>
<td>Turmeric</td>
</tr>
<tr>
<td>Lavender</td>
<td>Rosemary</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>Ground almonds</td>
</tr>
<tr>
<td>Tea tree</td>
<td>Onion</td>
</tr>
<tr>
<td>Frankincense</td>
<td>Basil</td>
</tr>
<tr>
<td>Papaya</td>
<td>Curry</td>
</tr>
<tr>
<td>Tobacco flower</td>
<td>Paprika</td>
</tr>
<tr>
<td>Melon</td>
<td>Celery salt</td>
</tr>
</tbody>
</table>
Table 2. Experiment 2: List of odorants and textures from which the experimental stimuli were created.

<table>
<thead>
<tr>
<th>Aromatherapy Oils</th>
<th>Textures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patchouli</td>
<td>Masking tape</td>
</tr>
<tr>
<td>Geranium</td>
<td>Faux fur</td>
</tr>
<tr>
<td>Ylang ylang</td>
<td>Sandpaper</td>
</tr>
<tr>
<td>Lavender</td>
<td>Foam</td>
</tr>
<tr>
<td>Tea-tree</td>
<td>Cardboard</td>
</tr>
<tr>
<td>Tangerine</td>
<td>Packing tape</td>
</tr>
<tr>
<td>Musk</td>
<td>Velcro</td>
</tr>
<tr>
<td>Vanilla</td>
<td>Suede</td>
</tr>
<tr>
<td>Peach</td>
<td>Terry cloth</td>
</tr>
<tr>
<td>Peppermint</td>
<td>Carpet</td>
</tr>
</tbody>
</table>
Table 3. Experiment 3: List of odorants and textures from which the experimental stimuli were created.

<table>
<thead>
<tr>
<th>Aromatherapy Oils</th>
<th>Textures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patchouli</td>
<td>Masking tape</td>
</tr>
<tr>
<td>Geranium</td>
<td>Faux fur</td>
</tr>
<tr>
<td>Ylang ylang</td>
<td>Sandpaper</td>
</tr>
<tr>
<td>Lavender</td>
<td>Foam</td>
</tr>
<tr>
<td>Tea-tree</td>
<td>Cardboard</td>
</tr>
<tr>
<td>Tangerine</td>
<td>Packing tape</td>
</tr>
<tr>
<td>Almond</td>
<td>Velcro</td>
</tr>
<tr>
<td>Vanilla</td>
<td>Suede</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Terry cloth</td>
</tr>
<tr>
<td>Jasmine</td>
<td>Duct Tape</td>
</tr>
<tr>
<td>Bergamot</td>
<td>Chiffon</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Velvet</td>
</tr>
<tr>
<td>Chamomile</td>
<td>Silk</td>
</tr>
</tbody>
</table>
Table 4. Experiment 3: Correlations between task performance and ChAT immunoreactivity and AChE reactivity.

<table>
<thead>
<tr>
<th>Task</th>
<th>ChAT Immunoreactivity</th>
<th>AChE Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBM</td>
<td>Frontal cortex</td>
</tr>
<tr>
<td>FC Retrieval</td>
<td>$r = 0.18$</td>
<td>$r = 0.20$</td>
</tr>
<tr>
<td>FC Acquisition</td>
<td>$r = 0.56^*$</td>
<td>$r = 0.45^*$</td>
</tr>
<tr>
<td>FS Enhanced-Difficulty Acquisition</td>
<td>$r = 0.08$</td>
<td>$r = 0.19$</td>
</tr>
</tbody>
</table>

Correlations (Pearson’s $r$ values) between performance and ChAT immunoreactivity in the NBM (as measured by the number of ChAT-positive cells) and neocortical AChE reactivity (as measured by optical density). The correlations were conducted using post-surgical performance data from FC retrieval and the last block of FC and FS Enhanced-Difficulty acquisition. ChAT = choline acetyltransferase; NBM = nucleus basalis magnocellularis; AChE = acetylcholinesterase. ($p < 0.05$; $p = 0.10$)
Figure Captions

Figure 1. Experiment 1: Illustration of the two different trial types, Target and Distractor, of the cross-species forced-choice tasks. On Target trials, the rewarded (+) stimulus was the odor-odor bowl (for rats) or the coloured shape (for humans), shown here in white. On Distractor trials, the rewarded stimulus was the blank bowl (for rats) or the black star (for humans).

Figure 2. Experiment 1: Illustration of the features defining the intramodal Feature-Conjunction (FC) and Feature-Singleton (FS) stimuli. Solid lines indicate pairings of features on Target trials, and dashed lines indicate pairings of features on Distractor trials. The FC task required feature binding because the features of target and distractor FC stimuli overlapped. In contrast, the FS task did not require feature binding because each FS stimulus was a combination of two unique odors (for rats) or a unique colour and a unique shape (for humans). The FC stimuli in each stimulus set comprised four distinct features, and the FS stimuli in each stimulus set comprised eight distinct features so that the number of feature pairs to be learned was equated (i.e., four).

Figure 3. Experiment 1: Acquisition of the Feature-Conjunction (FC) and Feature-Singleton (FS) tasks in Experiment 1a (rats) and Experiment 1b (humans). The graphs in the top row show rats' accuracy on the (A) FC and (B) FS tasks in the saline and scopolamine conditions. The graphs in the bottom row show humans' accuracy on the (C) FC and (D) FS tasks in the full-attention and divided-attention conditions. Results are shown as a function of block (rats: three-session blocks for the FC task and two-session blocks for the FS task; humans: 30-trial blocks). The boxes highlight the final level of performance attained by rats and humans on the FC and FS tasks.

Figure 4. Experiment 1: Performance costs of the cholinergic and attentional challenges on the Feature-Conjunction (FC) and Feature-Singleton (FS) tasks. The cost of the cholinergic
challenge \((A)\) was calculated by subtracting rats' accuracy in the scopolamine condition from their accuracy in the saline condition during the final block of training; the cost of the attentional challenge \((B)\) was calculated by subtracting humans' performance in the divided-attention condition from their performance in the full-attention condition during the final block of training. Results are shown separately for acquisition of the FC stimuli, acquisition of the FS stimuli, and retrieval of the FC stimuli.

*Figure 5.* Experiment 2: *A.* Illustration of the two different trial types, Target and Distractor, of the forced-choice rat digging tasks. On Target trials, the rewarded \((+)\) stimulus was the odor-texture bowl, while on Distractor trials, the rewarded stimulus was the blank bowl. *B.* Illustration of a typical session. On every trial, rats were simultaneously presented with two digging bowls: an odor-texture bowl and the blank bowl. Half of the trials were Target \((T)\) trials and the remaining half were Distractor \((D)\) trials presented in a pseudorandom order. Rats had to use the crossmodal features of the presented odor-texture bowl to determine the correct bowl choice.

*Figure 6.* Experiment 2: Illustration of the features defining the crossmodal Feature-Conjunction \((FC)\) and Feature-Singleton \((FS)\) stimuli. Solid lines indicate pairings of features in Target bowls, and dashed lines indicate pairings of features in Distractor bowls. For the FC stimuli, feature binding was required for correct bowl selection as each individual odor and texture was associated with both a target and a distractor bowl. For the FS stimuli, feature binding was not required as rats could rely on a single feature (odor or texture) for correct bowl selection as each individual odor and texture was associated with either a target or a distractor bowl.

*Figure 7.* Experiment 2: Choline acetyltransferase immunohistochemistry of the NBM and MS/VDB. Displayed are a typical sham-lesioned rat on the left and a typical NBM-lesioned rat on the right (magnification 10x). Two schematics of the rat brain in coronal section (A/P...
stereotaxic coordinates: bregma -0.84 mm and +0.60 mm) highlight the NBM and MS/VDB cell-counting frames (Paxinos & Watson, 2007). The number of ChAT-immunoreactive cells in the NBM (A and B) was significantly reduced in the NBM-lesioned rats (C). There was no significant difference in the number of ChAT-immunoreactive cells between the two lesion groups (D and E) in the MS/VDB (F). ChAT = choline acetyltransferase; MS/VDB = medial septum/vertical limb of diagonal band of Broca; NBM = nucleus basalis magnocellularis; +/-SEM; *p < 0.05.

**Figure 8.** Experiment 2: Acetylcholinesterase histochemistry. AChE staining in the frontal (A and B) and parietal cortices and hippocampus (C and D) of a typical sham-lesioned (left) and NBM-lesioned (right) rat. A marked depletion of AChE-positive fibers is evident in the cortex of the NBM-lesioned rat. AChE = acetylcholinesterase; NBM = nucleus basalis magnocellularis.

**Figure 9.** Experiment 2: Post-surgical (A) retrieval and (B) acquisition of Feature-Conjunction (FC) stimuli and (C) acquisition of Feature-Singleton (FS) stimuli. Accuracy has been binned into 3-session blocks. The boxes highlight the final level of performance attained by rats on the tasks.

**Figure 10.** A. Experiment 3: Illustration of the two different trial types, Target and Distractor, of the forced-choice rat digging tasks. On Target trials, the rewarded (+) stimulus was the odor-texture bowl, while on Distractor trials, the rewarded stimulus was the blank bowl. B. Illustration of a typical session. On every trial, rats were simultaneously presented with two digging bowls: an odor-texture bowl and the blank bowl. Half of the trials were Target (T) trials and the remaining half were Distractor (D) trials presented in a pseudorandom order. Rats had to use the crossmodal features of the presented odor-texture bowl to determine the correct bowl choice.
**Figure 11.** Experiment 3: Illustration of the features defining the crossmodal Feature-Conjunction (FC), Feature-Singleton (FS), and FS Enhanced-Difficulty stimuli. Solid lines indicate pairings of features in Target bowls, and dashed lines indicate pairings of features in Distractor bowls. For the FC stimuli, feature binding was required for correct bowl selection as each individual odor and texture was associated with both a target and a distractor bowl. For both types of FS stimuli, feature binding was not required as rats could rely on a single feature (odor or texture) for correct bowl selection. However, for the FS Enhanced-Difficulty stimuli, rats had the additional requirement of learning when to rely on odor and when to rely on texture to determine the correct bowl choice as one odor (Odor 7) and one texture (Texture 8) were associated with both a target and a distractor bowl.

**Figure 12.** Experiment 3: Choline acetyltransferase and parvalbumin immunohistochemistry of the NBM. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 10x). The rectangular outlines superimposed on the rat brain coronal schematics delineate the NBM cell-counting frames and the solid gray fill illustrates the typical extent of ChAT-immunoreactive cell loss. The number of ChAT-immunoreactive cells in the NBM (A and B) was significantly reduced in the ACh-NBM-lesioned rats (C). There was no significant difference in the number of parvalbumin-immunoreactive cells between the two groups (D and E) in the NBM (F). ChAT = choline acetyltransferase; NBM: nucleus basalis magnocellularis; A/P: anterior/posterior; +/- SEM; *p < 0.05. Rat brain schematics adapted from Paxinos and Watson (2007).

**Figure 13.** Experiment 3: Choline acetyltransferase and parvalbumin immunohistochemistry of the MS/VDB. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 10x). The rectangular outlines superimposed on the rat
brain coronal schematics delineate the MS/VDB cell-counting frames. There was no significant
difference in the number of ChAT-immunoreactive cells between the two groups (A and B) in the
MS/VDB (C), and there was no significant difference in the number of parvalbumin-
immunoreactive cells between the two groups (D and E) in the MS/VDB (F). ChAT = choline
acetyltransferase; MS/VDB = medial septum/vertical limb of diagonal band of Broca; A/P:
anterior/posterior; +/- SEM. Rat brain schematics adapted from Paxinos and Watson (2007).

Figure 14. Experiment 3: Acetylcholinesterase histochemistry. Displayed are a typical sham-
lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 1.25x).
The rectangular outlines superimposed on the rat brain coronal schematics delineate the
boundaries used for obtaining optical density values (normalized to raw striatal optical density
values) in the frontal and parietal cortices and hippocampus. The degree of AChE-positive
staining as measured by optical density in the frontal (A and B) and parietal cortices (D and E)
was significantly reduced in the ACh-NBM-lesioned rats (C and F). There was no significant
difference in the amount of AChE-positive staining in the hippocampus as measured by optical
density (G and H) between the ACh-NBM- and sham-lesioned groups (I). AChE =
acetylcholinesterase; +/- SEM; *p < 0.05. Rat brain schematics adapted from Paxinos and
Watson (2007).

Figure 15. Experiment 3: Feature-Conjunction (FC) performance as measured by accuracy
during the last six sessions prior to surgery and the subsequent retrieval of these same FC stimuli
during the six post-surgical sessions.

Figure 16. Experiment 3: Post-surgical acquisition of (A) novel Feature-Conjunction (FC)
stimuli, (B) Feature-Singleton (FS) stimuli, and (C) FS Enhanced-Difficulty stimuli. Accuracy
has been binned into 4-session blocks. The boxes highlight the final level of performance attained by rats on the three tasks.

*Figure 17.* Experiment 3: Scatterplots illustrating the relationships between Feature-Conjunction (FC) acquisition performance and NBM ChAT immunoreactivity and neocortical AChE reactivity. Linear regression of the data revealed significant positive relationships between postsurgical performance during the final block of FC acquisition and the number of ChAT-positive cells in the NBM (A) and AChE optical density in the frontal cortex (B). A weak trend towards significance was found for the positive relationship between FC acquisition performance and AChE optical density in the parietal cortex (C). *p < 0.05; +p = 0.10

*Figure 18.* Experiment 4: Overhead view of a touchscreen-equipped operant chamber housed inside a sound- and light-attenuating enclosure.

*Figure 19.* Experiment 4: Illustration of the two different trial-types, Feature Search and Conjunctive Search, and the three different stimulus set-sizes (4, 6, and 8 stimuli) used in the Visual Search task. The target stimulus was always the white square and rats were rewarded with access to water for touching the target. Each Visual Search task session comprised a total of 144 trials counterbalanced for target and distractor positions, trial-type (Feature Search or Conjunctive Search), and stimulus set-size. There were two different types of Feature Search trials, homogeneous and heterogeneous. On homogeneous trials, all distractors presented were identical, while on heterogeneous trials, the distractors presented were not identical. One type of heterogeneous Feature Search trial required discrimination of the target from the distractors based on pattern and the other type required discrimination based on shape.
**Figure 20.** Experiment 4: Pre-surgical performance of rats on the Visual Search task as measured by (A) accuracy and (B) correct latency. Performance has been binned across the 12 sessions of training, and the performance of rats on the two different types of Feature Search trials, homogeneous and heterogeneous, has been collapsed to yield a single Feature Search score for accuracy and correct latency.

**Figure 21.** Experiment 4: Choline acetyltransferase and parvalbumin immunohistochemistry of the NBM. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 10x). The rectangular outlines superimposed on the rat brain coronal schematics delineate the NBM cell-counting frames and the solid gray fill illustrates the typical extent of ChAT-immunoreactive cell loss. The number of ChAT-immunoreactive cells in the NBM (A and B) was significantly reduced in the ACh-NBM-lesioned rats (C). There was no significant difference in the number of parvalbumin-immunoreactive cells between the two groups (D and E) in the NBM (F). ChAT = choline acetyltransferase; NBM: nucleus basalis magnocellularis; A/P: anterior/posterior; +/- SEM; *p < 0.05. Rat brain schematics adapted from Paxinos and Watson (2007).

**Figure 22.** Experiment 4: Choline acetyltransferase and parvalbumin immunohistochemistry of the MS/VDB. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 10x). The rectangular outlines superimposed on the rat brain coronal schematics delineate the MS/VDB cell-counting frames. There was no significant difference in the number of ChAT-immunoreactive cells between the two groups (A and B) in the MS/VDB (C), and there was no significant difference in the number of parvalbumin-immunoreactive cells between the two groups (D and E) in the MS/VDB (F). ChAT = choline
acetyltransferase; MS/VDB = medial septum/vertical limb of diagonal band of Broca; A/P: anterior/posterior; +/- SEM. Rat brain schematics adapted from Paxinos and Watson (2007).

Figure 23. Experiment 4: Acetylcholinesterase histochemistry. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 1.25x). The rectangular outlines superimposed on the rat brain coronal schematics delineate the boundaries used for obtaining optical density values (normalized to raw striatal optical density values) in the frontal and parietal cortices and hippocampus. The degree of AChE-positive staining as measured by optical density in the frontal (A and B) and parietal cortices (D and E) was significantly reduced in the ACh-NBM-lesioned rats (C and F). There was no significant difference in the amount of AChE-positive staining in the hippocampus as measured by optical density (G and H) between the NBM- and sham-lesioned groups (I). AChE = acetylcholinesterase; +/- SEM; *p < 0.05. Rat brain schematics adapted from Paxinos and Watson (2007).

Figure 24. Experiment 4: Trial-type breakdown of the post-surgical performance of sham- and ACh-NBM-lesioned rats on the Visual Search task as measured by accuracy and correct latency. The graphs in the top row show rats’ accuracy on the (A) Conjunctive and (B) Feature Search trials, while the graphs in the bottom row show rats’ correct latency on the (C) Conjunctive and (D) Feature Search trials. +/- SEM; *p < 0.05

Figure 25. Comparison of the impact of divided attention, scopolamine, and cholinergic-selective lesions of the NBM on the acquisition and retrieval of comparable Feature-Conjunction (FC) and Feature-Singleton (FS) tasks. The performance of human participants under divided attention and rats under the influence of scopolamine represents data from Experiment 1, while the performance of ACh-NBM-lesioned rats represents data from Experiment 3.
Figure 1
Figure 2
Figure 3
Figure 4
A. Trial Type

Target (T)  Distractor (D)

Test Arena

Start Box

B. Session

Trial 1  Trial 2  Trial 3  Trial...

Figure 5
Figure 6
Figure 7
Figure 8
Figure 9
A. Trial Type

**Target (T)**

- Odor-texture Bowl
- Blank Bowl

**Distractor (D)**

- Odor-texture Bowl
- Blank Bowl

Test Arena

Start Box

B. Session

- **T**
  - Trial 1
- **D**
  - Trial 2
- **D**
  - Trial 3
- **T**
  - Trial...

Figure 10
Figure 11
Figure 12
Figure 13
Figure 14
Figure 15
Figure 16
Figure 17
Figure 18
Figure 19
Figure 20
Figure 22
Figure 23
Figure 24
Figure 25
Copyright Acknowledgements

**Experiment 1:**


**Experiment 2:**


**Experiment 3:**