The Role of Cholinergic Cortical Modulation from the Nucleus Basalis Magnocellularis in Visual and Olfactory Attention using the 5-Choice Serial Reaction Time Task

by

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Abstract

To date, research using rodent models has primarily dealt with the visual aspects of attention, while in the present research, we examined neurochemical modulation of attentional processes using both a visual and an olfactory five choice serial reaction time task (5-CSRTT). The nucleus basalis magnocellularis (NBM) in the basal forebrain is the primary source of cortical cholinergic input, which is thought to have an important modulatory effect on rat attentional processes. Thus, after successful acquisition of the task, the rats were subjected to either a cholinergic immunotoxic or sham lesion surgery of the NBM. Cortical cholinergic deafferentation of the cortical mantle was induced by bilaterally infusing 0.2 µg/µl of the cholinergic immunotoxin, 192 IgG-saporin, into the NBM (saporin-lesion). Reduction of cortical cholinergic modulation led to comparable attentional impairments in the saporin-lesion group, relative to the sham-lesioned group, on both visual and olfactory versions of the 5-CSRTT.
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Chapter 1
Introduction

1.1 Basal forebrain

Cortical areas that generally contribute to cognition, such as learning, memory, and attention, receive widespread cholinergic modulation (Bigl, Woolf, & Butcher, 1982; Sarter & Bruno, 1997). The majority of this cortical acetylcholine (ACh) originates from the collection of nuclei in the anterior ventral portion of the brain, collectively termed the basal forebrain (BF). Mesulam and colleagues classified the rat (Mesulam, Mufson, Levey, et al., 1983a) and primate (Mesulam, Mufson, Levey, et al., 1983b) basal forebrain nuclei, distinguishing between different cell groups on the basis of the origin and the specific targets of its cells. According to this classification, the anterior portion of the BF includes the medial septum (MS) and vertical limb of the diagonal band of Broca (VDB), which project cholinergic neurons to the hippocampus, hypothalamus, cingulate cortex, and to lesser extent to the olfactory bulb. The posterior portion of the BF, called nucleus basalis magnocellularis (NBM) in rodents or nucleus basalis of Meynert in primates, projects widely to the neocortical mantle. Finally, the horizontal limb nucleus of the diagonal band (HDB) provides cholinergic innervation of the olfactory bulb and piriform cortex. ACh from the NBM has been shown to play a role in modulating attentional processes (Botly & De Rosa, 2009; Harati, Barbelivien, & Cosquer, 2008; Lehman, Grottick, Cassel, et al., 2003; McGaughy, Kaiser, & Sarter, 1996; McGaughy, Dalley, Morrison, et al., 2002; Risbrough, Bontempi, & Menzaghi, 2002; Turchi & Sarter, 1997).

1.2 Cholinergic modulation of attention

Many decades ago, it was observed that the magnitude of degeneration of the BF nuclei and disrupted function of cortical cholinergic projections originating from the BF strongly correlate with the magnitude of cognitive decline observed in patients with Alzheimer’s disease (Bowen, Smith, White, et al., 1976; Perry, Gibson, Blessed, et al., 1977). These findings motivated a program of research, either with Alzheimer’s patients or using animal (primarily rodent) models, which examined whether deficits in learning and memory may be attributable to dysfunction of BF cholinergic system. To date this line of study has not yielded conclusive evidence that the loss of cortical cholinergic input primarily causes impairments in learning and memory. However, there is a growing body of evidence, in both the human and animal literature, in the
support of the hypothesis that various attentional functions, such as detection and selection, are
dependent on the activity of the cortical cholinergic projections (for review see Everitt &

Pharmacological manipulations with various agents that act by increasing or diminishing activity
at cholinergic synapses, in particular at the muscarinic receptors, were used as a tool in the early
investigations of cholinergic contribution to attentional processes. For instance, in humans,
systemic administration of muscarinic cholinergic antagonists, such as scopolamine, can cause
transient deficits in sustained attentional performance (Broks, Preston, Traub, et al., 1988; Ellis,
Ellis, Bartholomeusz, et al., 2006), stimulus detection and vigilance (Wesnes & Warburton,
1983), and selective attention (Mintzer & Griffiths, 2003). On the other hand, administration of
the acetylcholinesterase inhibitor physostigmine, which causes prolonged activation at ACh
synapses, causes improvements in attentional performance, presumably by cholinergic
modulation of activity in the brain areas responsible for the processing of relevant stimuli
(Bentley, Vuilleumier, Thiel, et al., 2003a; Bentley, Vuilleumier, Thiel, et al., 2003b; Furey,
Pietrini, & Haxby, 2000).

Pharmacological manipulation of cortical cholinergic activity in rodents has provided results
similar to findings from the human studies (Muir, 1996). For example, scopolamine
administration has been shown to cause impaired rat performance on a variety of tasks measuring
different aspects of attention such as sustained attention (Jones & Higgins, 1995) and stimulus
orienting (Philips, McAlonan, Robb, et al., 2000). Also, it has been demonstrated that
behavioural consequences of the systemic scopolamine exposure are comparable to the
behavioural manifestations of left visual hemi-neglect, with the difference that scopolamine
caused inattention in both left and right visual field (Hoff, van Oostenbrugge, Liedenbaum, et al.,
2007).

Additional evidence that cholinergic circuit dysfunction underlies attentional impairments came
from a series of investigations by Muir and colleagues. In order to investigate cortical ACh
function, they used different methods of cortical cholinergic denervation, such as the infusion of
a GABA agonist muscimol into BF (Muir, Robbins, & Everitt, 1992), the intracerebrovascular
injection of choline uptake blocker hemicholinium (Muir, Dunnett, Robbins, et al., 1992), or
AMPA-induced excitotoxic lesions of the BF (Muir, Everitt, Robbins, 1994; Muir, Everitt,
Robbins, 1995). In this series of studies, authors used the five choice serial reaction time test (5-
CSRTT), a behavioural paradigm that, in its basic form, measures sustained attention and also allows for an assessment of divided attention. This is because the rat is required to respond to a brief and spatially unpredictable stimulus in one of five locations across many trials. In each study they observed impaired performance on the 5-CSRTT, i.e., lowered accuracy and increased correct response latencies, as a consequence of the reduced cholinergic cortical input. Importantly, they also demonstrated the cholinergic specificity of these deficits by observing improvements in attentional performance following either a systemic administration of physostigmine (Muir, Everitt, Robbins, 1995; Muir, Everitt, Robbins, 1994; Muir, Robbins, & Everitt, 1992) or following a transplant of the embryonic BF cholinergic cells into the rat cortex (Muir, Dunnett, Robbins, et al., 1992).

Several experiments have examined the characteristics of ACh release into the rat neocortex, using in vivo microdialysis coupled with high-performance liquid chromatography with electrochemical detection. This technique enables investigation of the dynamics of cholinergic cortical efflux in animals performing various attentional tasks. Increased cholinergic efflux was measured in this manner in the rat medial prefrontal cortex during the performance on 5-CSRTT (Dalley, McGaughy, O'Connell, et al., 2001; Passetti, Dalley, O'Connell, et al., 2000) as well as in the frontoparietal cortical regions during the performance on sustained attention task (Himmelheber, Sarter, & Bruno, 2001). However, this group of studies failed to find a correlation between the attentional demands of a task, task performance, and the magnitude of cortical cholinergic release. Additional investigations by Himmelheber, Sarter, and Bruno (2000) and Arnold, Burk, Hodgson et al. (2002) addressed this issue. Namely, Himmelheber and colleagues (2000) introduced a distractor into the sustained attention paradigm, which increased attentional demands of the task and initially caused impaired performance. But, as the task session progressed, the improvement in rat performance on this modified task was observed, and this change was associated with the increase in frontoparietal ACh efflux. Arnold et al. (2002) compared cholinergic frontoparietal efflux observed in rats during sustained attention task with the ACh efflux observed on two control tasks which had similar sensory, motor, and motivational characteristics but lacked the explicit attentional component. Performance on both groups of tasks, sustained attention task versus control tasks, was associated with increased cortical ACh efflux as compared to baseline levels, but the magnitude of this increase was significantly greater in rats performing the sustained attention task. This evidence suggests that not only is the attentional performance dependent on cortical ACh efflux, but also that the
increased task demands and improvements in task performance may correlate with greater release of ACh into frontoparietal regions.

Even though the exact mechanism by which ACh contributes to the attentional processes is not known, Sarter, Hasselmo, Bruno et al. (2005) propose a model that accounts for some aspects of cholinergic modulatory influence. According to this model, the BF cholinergic system is recruited either by signal-driven (bottom-up) processes that are dependent on stimulus characteristics, such as salience or novelty, or by knowledge-driven (top-down) processes that are dependent on individuals’ knowledge, memory, practice, expectations or motivations. Activation of the BF causes release of the ACh into various cortical regions such as prefrontal cortex (PFC), as well as the primary sensory and sensory association cortices located within the occipital and parietal regions. Cortical release of the ACh in these target areas are presumed to lead to increased activation and enhanced processing efficiency in both anterior and posterior attentional systems (as defined by Posner & Petersen, 1990). Once activated, the anterior attentional system located in the PFC manipulates activity of both BF cholinergic system and posterior attentional system. This allows further top-down modulation of various attentional processes, which ultimately leads to the biased and enhanced stimulus processing and improved performance on tasks assessing attentional function (Sarter, Hasselmo, Bruno et al., 2005).

It has been proposed that ACh-induced changes in cortical circuit dynamics cause improved attentional performance by enhancing the afferent input processing relative to the internal feedback processing of previously formed cortical representations (Hasselmo & McGaughy 2004). This model is based on a series of electrophysiological studies that examined the effects of the ACh in various cortical areas, including the neocortex, piriform cortex, hippocampus, and thalamus. Hasselmo and colleagues proposed theoretical framework describing cholinergic enhancement of the feed-forward afferent input and suppression of the background activity resulting from three sets of effects: a) modulation of cortical pyramidal cell activity, b) changes in inhibitory interneuron activity, and c) selective modulation of excitatory synaptic transmission. The first set of effects relates to the fact that ACh reduces spike frequency accommodation in cortical pyramidal cells, thus enhancing their spiking response. Specifically, following a depolarization of pyramidal cell membrane, the activation of muscarinic ACh receptors causes blockage of outflowing potassium currents, thus causing a prolong state of membrane depolarization which allows the cell to maintain its high firing rates following the subsequent excitatory inputs (Barkai & Hasselmo, 1994). Further, presence of ACh at the
inhibitory feedback synapses causes lowered GABA release as well as depolarization of inhibitory interneurons. Computational modeling has shown that the equilibrium state of the network containing these inhibitory interneurons is such that it would produce an enhanced response (firing rates) to a strong afferent input and reduced response to a weak input in the presence of the ACh (Patil & Hasselmo, 1999). Finally, a series of investigations using slice preparations from piriform cortex, neocortex and hippocampus (for review see Hasselmo & Giacomo, 2006) has demonstrated that ACh selectively suppresses excitatory feedback but not afferent activity. Model described by Hasselmo and McGaughy (2004) proposes that the combination of previously described effects of ACh within cortical structures leads to enhanced sensitivity to relevant afferent input, suppression of a background activity, and reduced firing of internally activated circuits, thus providing beneficial contribution to attentional processing.

1.3 Neural substrates of the cholinergic modulation of attention

As was mentioned previously, all areas of cerebral cortex receive widespread cholinergic input from the BF, but the specific BF nuclei project to anatomically and functionally distinct cortical regions (Bigl, Woolf, & Butcher, 1982). For instance, the NBM provides cholinergic innervation of the neocortex (Mesulam, Mufson, Levey, et al., 1983a; Mesulam, Mufson, Levey, et al., 1983b), including the frontoparietal networks that are presumed to be the fundamental neural substrates of attention (Posner & Petersen, 1990). For that reason, the NBM has been a common brain region of interest in studies of cholinergic modulation of attention. Early investigations used a variety of non-specific excitotoxins, such as ibotenic acid, quisqualic acid, and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid), in order to produce the NBM lesion and induce cortical ACh depletion (Waite & Thal, 1996). These studies found behavioural impairments on a range of tasks assessing the attentional performance in rodents (McGaughy, Everitt, Robbins, et al., 2000) and primates (Voytko, Olton, Richardson, et al., 1994), but the interpretation of the results was often difficult due to the fact that the lesions lacked sufficient anatomical specificity and potency to induce cholinergic cell death (Waite & Thal, 1996).

In the late 1990s, the immunotoxin 192 IgG-saporin was introduced as a toxic agent that allowed more selective and more efficient lesion of cholinergic neurons in rats (Waite & Thal, 1996). 192 IgG is a monoclonal antibody that selectively binds to the p75 nerve growth factor (NGF) receptor found both on soma and axon terminals of the majority of cholinergic cells within the
central nervous system (Batchelor, Armstrong, Blaker, et al., 1989). Upon binding, receptor-ligand complex is internalized and transported into the cell body (Taniuchi & Johnson, 1985). Wiley, Oeltmann, and Lappi (1991) conducted one of the first studies where 192 IgG, coupled to a ribosome inactivating citotoxic protein saporin, was used to selectively destroy cholinergic neurons in the central nervous system. Further investigations provided additional evidence confirming both cholinergic selectivity and toxic efficiency of the 192 IgG-saporin complex, demonstrating its usefulness as a lesioning agent (Book, Wiley, & Schweitzer, 1992; Heckers, Ohtake, Wiley, et al., 1994; Holley, Wiley, Lappi, et al., 1994). It should be noted that the intraparenchymal injections into areas of interest appear to be more efficient and more selective method of saporin administration than the intracerebroventricular (icv) injection (Waite & Thal, 1996; Waite & Wardlow, 1999).

Studies employing behavioural tests of attentional performance in rodents coupled with the 192 IgG-saporin lesions of the NBM have provided ample evidence that the integrity of the cholinergic cortical projections from this nucleus is necessary for normal attentional functioning. Namely, it has been shown that the 192 IgG-saporin injection into the NBM, which correlated with the significant loss of cholinergic NBM neurons, produces impairments on tasks assessing divided attention (Turchi & Sarter, 1997), selective attention during associative learning (Chiba, Bucci, Holland, et al., 1995), vigilance (McGaughy, Keiser, & Sarter, 1996), signal detection and selection (Burke, Lowder, & Altemose, 2008), and sustained and spatial attention (Harati, Barbelivien, & Cosquer, 2008; Lehman, Grottick, Cassel, et al., 2003; McGaughy, Dalley, Morrison, et al., 2002; Risbrough, Bontempi, & Menzaghi, 2002). In addition, it has been demonstrated that saporin injection into the NBM cortical target area, specifically into the ventromedial PFC (vmPFC) (Dalley, Theobald, Bouger, et al., 2004) or into the posterior parietal cortex (PPC) (Bucci, Holland, & Gallagher, 1998), can have detrimental effects on attentional performance which was associated with the loss of cholinergic afferents in these cortical areas. In an interesting study which coupled saporin lesions of the NBM, in vivo microdialysis, and behavioural testing, McGaughy Dalley, Morrison et al. (2002) observed that the extent of cholinergic cortical depletion correlates with the degree of behavioural impairments. They used different doses of the immunotoxin to produce different levels of the NBM damage. At high dose of saporin, they observed an extensive loss of medial PFC (mPFC) cholinergic afferents that correlated with reduced mPFC ACh efflux and with the severity of the behavioural impairments on the 5-CSRTT. Lower doses of saporin, which produced lesser extent of cholinergic
deafferentation, failed to induce behavioural impairments in saporin-lesioned rats, as compared to the performance of sham-lesioned group, when they were tested under standard conditions. However, under the conditions of increased task demands, the performance of rats that underwent the lesion with a low-dose of saporin was significantly poorer than the performance of the control group.

It should be noted that the studies described in the previous section have investigated cholinergic contribution to attention primarily with the use of visual stimuli while present study examined the effects of the cholinergic lesion in the NBM on attentional processing of both visual and odor stimuli. One potential shortcoming of assessing the rat attentional performance following the cholinergic NBM lesion with visual stimuli only lies in the fact that the loss of cortical ACh can cause impaired visual processing. Namely, while loss of cholinergic NBM neurons causes reduced ACh input into frontoparietal regions responsible for attentional processing, it can also cause the reduction in cholinergic innervation of the primary and secondary visual cortices (for instance, see Lehman, Grottick, Cassel, et al., 2003). Such reduction would be independent of the inefficient attentional system, and therefore, any observed behavioural deficit following the cholinergic NBM lesion could be partially due to stimulus processing dysfunction, rather than being exclusively due to attentional dysfunction. On the other hand, with the use of odor stimuli, one would be able to avoid such problem. Namely, the brain regions involved in the processing of odor stimuli, such as the olfactory bulb and the piriform cortex (McNamara, Cleland, & Linster, 2004), receive cholinergic innervation from the VDB and the HDB respectively (Mesulam, Mufson, Levey, et al., 1983a). Cholinergic lesion in the NBM is not likely to cause reduced ACh innervation of these regions and, therefore, any behavioural deficits that are observed on an olfactory attentional task after the NBM lesion would be more likely due to attentional dysfunction only.

1.4 Present investigation

In the present study, we used performance on the 5-CSRTT as a behavioural measure of attentional functioning. To date, the 5-CSRTT has been exclusively used to assess the visual aspects of attention in rats. However, rats are nocturnal animals that do not rely heavily on vision (van Hooser & Nelson, 2006), but typically use olfaction as a primary sensory modality (Rouquier & Giorgi, 2007). The present study examined the rat attentional functions involved in
processing of both visual and odor stimuli, in an attempt to provide additional insights and understanding of cholinergic modulatory influences on attentional processes.

In the 5-CSRTT an animal is trained to respond to a brief and spatially unpredictable stimulus presented pseudorandomly in one of five locations (Robbins & Everitt, 2005). Upon stimulus presentation, the animal is required to respond to the stimulus by nose poking at the correct location within a limited time period in order to receive a reward. The error on the trial is registered if the animal: (1) nose-pokes at the incorrect location (commission error), (2) fails to respond within allocated time period (omission error), (3) responds prior to the stimulus presentation (premature response), or (4) responds to multiple locations within the same trial (perseverative response).

It has been hypothesized that the 5-CSRTT can be used to measure various aspects of attentional functioning (Bari, Dalley, & Robbins, 2008). Namely, the subject performing on the classic 5-CSRTT must allocate limited attentional resources between multiple spatial locations (selective attention) and attend to rare and unpredictably occurring events over extended periods of time (sustained attention). With some modifications, such as the introduction of a distracting stimulus, the 5-CSRTT can be used to assess divided attention as well (Jones & Higgins, 1995).

The primary goal of the present study was to examine the role of cholinergic cortical modulation of attention. To that purpose we examined the attentional processing capacities of animals with bilateral lesions within the NBM, which is the main source of cortical ACh, on both the visual and olfactory 5-CSRTT. By using both versions of the task we attempted to determine if the ACh from the NBM has a modulatory influence on attentional processing that is independent of sensory modality.

In the present research, rats underwent a lesion surgery with the 192 IgG-saporin injections into the NBM. Post-surgery, we assessed subjects’ attentional abilities by examining the dynamics of the re-acquisition of the baseline task performance as well as the effects of task parameter manipulations that taxed their attentional resources. Namely, we decreased the stimulus duration and introduced a within session variable inter-trial interval. We expected to observe behavioural impairments post-surgery on both visual and olfactory 5-CSRTT in the saporin-lesioned group when compared to sham-lesioned controls, especially when their attentional resources were taxed.
Chapter 2  
Methods

2.1 Subjects

Subjects were 14 experimentally naïve male Long-Evans rats (Charles River, Montreal, Quebec) that weighed 225-250 g at the start of the experiment. The rats were individually housed 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 behavioural testing occurring during the dark phase, typically between 10am and 4pm. Rats were water restricted to 25-30min daily access to water and received water as a reward during 5-CSRTT performance.

2.2 Apparatus

The five choice operant chamber, placed in a sound-isolating and ventilated wooden box, was used for all behavioural testing (Figure 1A). Five stimulus apertures (2.5cm x 2.5cm x 2.5cm), located at the front wall of the chamber, were arranged on the curved panel and raised 2.5cm from the grid floor. Stimulus apertures were equipped with a light-emitting diode (LED), a hole at the bottom through which an odor was released, and with a infrared (IR) beam that detected a nose poke. Another port was located at the back wall of the chamber, where a water reward was made available when a rat nose poked the IR beam after a correct response. Also on the back wall of the chamber was a light that indicated the availability of the reward, located above the water port, and a vacuum pump that removed the odor from the operant chamber, located above the reward light. Lastly, two IR beams passed across the width of the chamber and were placed at positions toward the front and back of the chamber to control the movement of the rats.

We also created a custom-made olfactometer (Figure 1B), which is an apparatus that releases odors into the testing chamber in a controlled fashion. It received the air supply from an air pump. After the air was dried, filtered, re-hydrated, it was directed into a vial where the odorant (10% citral dissolved in scentless mineral oil; 3,7-dimethylocta-2,6-dien-1-ol) was kept. The odor-enriched air was then directed toward one out of five three-way solenoid valves (each connected with one stimulus port) that either released the air into its stimulus port or toward the vacuum pump that removed the odor. The amount of odor enriched air that was released into the
stimulus port was controlled by the flow meter set at 1 LPM as well as the length of time its valve stayed open, which corresponded to stimulus duration parameter on the olfactory version of the task (1s, 0.5s, or 0.25s).

Figure 1. Apparatus. A. Illustration of the 5-CSRTT operant chamber (adapted from Bari, Dalley, & Robbins, 2008) with 5 stimulus apertures where light and/or odor stimuli are presented. B. Illustration of the part of the olfactometer that controls the supply of odor-enriched air into one stimulus port. Each stimulus port has an independent air supply.
2.3 Behavioural training

Throughout shaping and pre- and post-surgical 5-CSRTT testing, we used 0.05mL of water per trial as a reward for correct responses. During pre- and post-surgical 5-CSRTT testing we recorded the number of correct responses as well as four types of errors: a) incorrect response when rat nose poked into stimulus port where stimulus was not presented, b) omission when rat would fail to respond within allocated time, c) premature response when rats would nose poke into any of the apertures during the ITI, and d) perseverative response when rat nose poked into multiple apertures after the stimulus was presented. We also calculated response accuracy that was defined as following: accuracy = number of correct responses / (number of correct responses + number of incorrect responses). In addition, we recorded correct response latencies and water latencies.

After 3 20min long sessions of habituation to the operant chamber and before the behavioral training, the rats underwent shaping in four stages. (1) Light-to-Water conditioning, where a rat had to associate chamber illumination by the house light with the availability of water and respond to by nose-poking into the water port; rats were kept on this stage until water response time was stabilized at 2s. (2) Stimulus-to-Light-to-Water conditioning, where a rat had to learn to respond to the illuminated middle (third) stimulus port by a nose-poke, in order to receive the water reward. The behavioural criterion in this stage was a reaction time of 2s thus, from this point onward, the rat had to spend 2s breaking the IR beam toward the back width of the chamber to initiate the next trial. (3) Follow-Stimulus conditioning where stimulus was presented pseudorandomly across all five ports. In stages 2 and 3 of shaping, the stimulus was presented indefinitely and rats were allowed to make errors of commission and the behavioural criterion in the stage 3 was a reaction time of 2s. Lastly (4), in this stage we introduced feedback on the errors of commission. To introduce the errors of commission, the nose-poke into the incorrect stimulus port was punished with the 1s long 1 kHz tone and the water reward was not administered. The behavioural criterion at the final shaping stage was a reaction time of no more than 2s and 18 out of 20 correct trials at any point during the session. All habituation, shaping, and training sessions were limited to 20 min.

Behavioural training on 5-CSRTT started with the visual version of the task (LIGHTS ONLY) and with stimulus duration (SD) of 5s, inter trial interval (ITI) of 3s and limited hold (LH) period of 5s. In order to proceed to the next stage of training, the rat had to achieve criterion of having
18 out of 20 correct trials at any point during the session. The subsequent stages of behavioural training had a progressively shorter SD and progressively longer ITI, but the behavioural criterion stayed the same for each stage of training. Rats were considered to have completed the training for visual version of the task when they met the behavioural criterion during the session with following trial parameters: SD=1s, ITI=5s, and LH=5s (baseline conditions).

Behavioural training for the olfactory version of the task occurred after the rats achieved the visual 5-CSRT. It involved a coupled presentation of both visual and odor stimuli (LIGHTS+ODOR) under baseline conditions and proceeded for 5 days. After that, odor stimuli alone were presented (ODOR ONLY) and the rats continued training until they meet the behavioural criterion on the olfactory task (18 out of 20 correct trials during one session) under the baseline conditions of SD=1s, ITI=5s, and LH=5s. ODOR ONLY stage of training went on for 7 days. All sessions of the behavioural training were limited to 20min or 60 trials.

2.4 Surgery

At the end of the training, rats were assigned into either a sham-lesion (N=6) or saporin-lesion (N=8) surgical group. The groups were matched on pre-surgical performance using the percent of correct responses per session as a measure of attentional performance.

All surgeries were performed under aseptic conditions and using isoflurane as anesthetic (3% isoflurane with 1l/min oxygen for induction; approximately 2% isoflurane with 1l/min oxygen for maintenance of anesthesia). Immediately prior to the surgery, animals received a subcutaneous injection of analgesic buprenorphine (0.03mg/kg) and intraperitoneal injection of atropine (0.05mg/kg) to prevent the fluid buildup in the lungs. All cholinergic immunotoxic lesions were accomplished by the injections of 0.2 µl of 0.2 µg/µl 192 IgG-saporin (Advanced Targeting Systems, lot# 64-124) dissolved in sterile 0.1M (pH = 7.4) phosphate-buffered saline, while sham lesions involved the injection of 0.2 µl of sterile 0.1M (pH = 7.4) phosphate-buffered saline per site. Injection rate was 0.1 µl per minute through a 26-gauge Hamilton syringe, with an additional 3 min delay prior to the retraction of the injection. The NBM lesion was performed bilaterally at two sites: A/P -0.72 mm, M/L ±2.5 mm, D/V -8.3 mm, and A/P -1.32 mm, M/L ± 3.0 mm, D/V – 7.3 mm (Paxinos, & Watson, 2007). The choice of coordinates for lesioning and the 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.

2.5 Post-surgical testing procedures

Rats were given a two week post-surgery recovery period with access to ad libitum water and food. They were water restricted approximately 24 hours prior to the start of post-surgical testing. During the post-surgical testing, the experimenters were blind to the surgical group of the animal. Each session was 60 trials long. The testing involved four stages defined by the different parametric manipulations:

1. Testing at baseline conditions (SD=1s, ITI=5s, LH=5s) for 4 session on each version of the task (LIGHTS ONLY followed by ODOR ONLY)

2. Testing with SD=0.5s (ITI=5s and LH=5s) for 7 sessions on each version of the task

3. Testing with SD=0.25s (ITI=5s and LH=5s) for 7 sessions on each version of the task

4. Testing with within session variable ITIs (SD=1s and LH=5s) for 4 sessions on each version of the task; trials with ITI values of 2, 4, 6, or 8s were pseudorandomly distributed within each 60 trial session.

2.6 Histological analyses

Following the completion of post-surgical behavioural testing, the rats were anesthetized with sodium pentobarbital (60 mg/kg administered intraperitoneally), perfused with approximately 150 ml of ice-cold normal saline followed by approximately 150 ml of ice-cold 4%
parafomaldehyde. After the brain extraction, the brains were 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) where they were kept for 1 week at 4 °C.

The brains were sliced to sections 60 µm thick using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems, Canada) and the adjacent slices were used in either acetylcholinesterase (AChE) histochemistry, choline acetyltransferase (ChAT) immunohistochemistry, or parvalbumin immunohistochemistry essays. AChE histochemistry was carried out according to the method described by Paxinos and Watson (2007) to confirm the loss of cholinergic input into frontal and parietal lobes and preserved cholinergic input into hippocampus of the saporin-lesioned animals. ChAT and parvalbumin immunohistochemistry were carried out according to methods described in De Rosa, Hasselmo, and Baxter (2001) in order to determine the potential cholinergic and GABAergic cell body loss in the NBM and medial septum/vertical limb of the diagonal band of Broca (MS/VDB).

2.7 Histological quantification

All photographs of the target areas were taken using a Leica light microscope (DM4000B, Ontario, Canada) and Openlab image analysis software (Quorum Technologies). All photographs were taken with 5x magnification.

ChAT- and parvalbumin-immunoreactive cells were counted bilaterally on three brain sections containing the NBM at approximately following coordinates relative to bregma: AP -0.96mm, AP -1.20mm, and AP -1.44mm (Paxinos, & Watson, 2007). We defined cell counting frames in a following manner:

(1) At AP -0.96mm, the photograph was taken by positioning the bottom left (in the right hemisphere) or in the bottom right (in the left hemisphere) corner of the Openlab image window over the utmost dorsomedial point of the posterior part of the anterior commissure (ACP). All cells in the photograph were counted. Majority of the cells were positioned along the ventrolateral border of the internal capsule (IC).

(2) At AP -1.20mm, we used dorsal border of the ACP as the bottom border of the Openlab image window. The window was then moved toward medial parts of the brain
until medial-most border of the IC became visible. After photograph was taken, we used the Openlab ruler to exclude the regions that were within 400 measurement units along either left (for the right hemisphere) or right (for the left hemisphere) border of the photograph and 300 measurement units along the bottom border of the photograph. Remaining part of the photograph was used as the counting frame at this AP coordinate.

(3) At AP -1.44, we used the ventral border of the fornix as a bottom border of the Openlab image window. The window was then moved toward lateral parts of the brain as long as the medial-most border of the IC was visible. From the photograph taken in such way, we excluded the regions that were within 400 measurement units along the left (for the right hemisphere) or right (for the left hemisphere) border of the photograph. We also excluded the cells that were visible along dorsomedial border of the IC.

Cell counts from the MS/VDB were taken from three sections at approximately following coordinates: AP +1.20mm, AP +0.96mm, and AP +0.72mm relative to bregma (Paxinos, & Watson, 2007). At all three coordinates we took the photograph by positioning the middle point of the bottom border of the Openlab image window over the ventral-most point at which two hemispheres were still connected. Further, we counted the cells that were within 450 measurement units on both left and right side of the photograph axis.

Within each counting frame in both NBM and MS/VDB, we counted only cells that were clearly distinguishable from the background with well defined borders of the cell body. The cells with lighter staining and/or blurred borders were ignored.

AChE densitometry was conducted using the software package Scion (Scion Corporation, Maryland, USA). With this software we obtained the optical density (OD) values from the frontal lobe (at AP +1.32mm relative to bregma), parietal lobe (at AP -3.96mm relative to bregma), and from the hippocampus (at AP -3.24mm relative to bregma), as delineated by Paxinos and Watson (2007). These raw OD values we further divided by the OD value from the striatum of each rat. Ratios obtained in such way were used in the statistical analysis instead of the raw OD values in order to adjust for potential differences in staining intensity across different rats.
Chapter 3
Results

3.1 Histological quantification

Histological quantification revealed that one saporin-lesioned rat did not have sufficient decrease of the cholinergic cells in the NBM as indicated by the number of cells greater than two standard deviations above the mean NBM cell number of the saporin-lesioned group. This animal was removed from behavioural analysis leaving 7 rats in the saporin-lesioned group (SAP) and 6 controls (SHAM).

3.1.1 Cell counting

Independent \( t \)-tests showed that there was a significant decrease of ChAT-immunoreactive cells in the NBM of saporin-lesioned rats when compared to controls: \( t_{(13)}=7.49, \; p<0.005; \; M_{\text{control}}=530.50, \; SD_{\text{control}}=129.14; \; M_{\text{sap}}=249.43, \; SD_{\text{sap}}=70.71 \) (Figure 2A and 2B). The extent of the cholinergic cell loss in the NBM was, on average, 52.98%, ranging from 35.34% to 72.29%. There were no significant differences in the number of ChAT-immunoreactive cells in the MS/VDB between two groups: \( t_{(13)}<0; \; M_{\text{control}}=430.17, \; SD_{\text{control}}=36.49; \; M_{\text{sap}}=436.14, \; SD_{\text{sap}}=30.75 \) (Figure 3A and 3B), indicating that the lesion was restricted to target area of the NBM. In addition, there were no significant differences between groups in the number of parvalbumin-immunoreactive cells in the NBM (\( t_{(13)}<0; \; M_{\text{control}}=229.17, \; SD_{\text{control}}=67.57; \; M_{\text{sap}}=248.86, \; SD_{\text{sap}}=20.35 \) (Figure 2C and 2D) or in the MS/VDB (\( t_{(13)}<1; \; M_{\text{control}}=292.67, \; SD_{\text{control}}=48.80; \; M_{\text{sap}}=289.43, \; SD_{\text{sap}}=42.34 \) (Figure 3C and 3D) indicating cholinergic specificity of the lesion. Finally, we observed no signs of unspecific tissue damage within the brain regions that were investigated.
Figure 2. ChAT and parvalbumin immunohistochemistry of the nucleus basalis magnocellularis. The figure shows the photomicrographs of the ChAT- and parvalbumin-immunoreactive cells in the NBM of a saporin-lesioned (B,D) and sham-lesioned (A,C) rats. There is a marked decrease in the number of ChAT-IR cells in the NBM of the saporin-lesioned rats (B) when compared to sham-lesioned rats (A). The number of parvalbumin-IR cells in the NBM is comparable between the groups (C, D). The rectangular outlines superimposed on the rat brain coronal schematics approximately correspond to the NBM cell-counting frames. Rat brain schematics were adapted from Paxinos and Watson (2007) and displayed coordinates refer to the AP plane.

Figure 3. ChAT and parvalbumin immunohistochemistry of the medial septum/vertical branch of the diagonal band of Broca. The figure depicts the rat MS/VDB with either ChAT- (A,B) or parvalbumin-immunoreactive (C,D). Saporin-lesioned (B,D) and sham-lesioned (A,C) rats have a comparable numbers of both ChAT- and parvalbumin-immunoreactive cells within the MS/VDB.
3.1.2 AChE densitometry

The analysis (independent *t*-tests) of the adjusted optical density values (raw OD value divided by striatal OD value) obtained from the AChE-stained slices revealed that there was a significant decrease in the cholinergic input into frontal: \( t_{(13)} = 4.33, p < 0.005; M_{\text{control}} = 0.55, SD_{\text{control}} = 0.05; M_{\text{sap}} = 0.43, SD_{\text{sap}} = 0.06 \) (Figure 4A and 4B) and parietal: \( t_{(13)} = 5.70, p < 0.001; M_{\text{control}} = 0.55, SD_{\text{control}} = 0.02; M_{\text{sap}} = 0.47, SD_{\text{sap}} = 0.03 \) (Figure 4C and 4D) lobes of SAP group when compared to SHAM. Average decreases in the OD values from frontal and parietal lobes of the SAP rats were 23.01% and 14.40% respectively. However, we observed no difference between the groups in the amount of cholinergic input into the hippocampus: \( t_{(13)} < 1; M_{\text{control}} = 0.67, SD_{\text{control}} = 0.03; M_{\text{sap}} = 0.66, SD_{\text{sap}} = 0.04 \) (Figure 4E and 4F). In addition, we should note that the OD values from the striatum did not differ between two surgical groups either \( t_{(13)} < 1; M_{\text{control}} = 248.12, SD_{\text{control}} = 1.54; M_{\text{sap}} = 248.6, SD_{\text{sap}} = 0.76 \).

![Graphs showing AChE OD values for frontal, parietal, and hippocampal areas for control and saporin groups.](image)

Figure 4. Acetylcholinesterase histochemistry. The figure depicts the AChE staining in the frontal (A,B) and parietal (C,D) lobes, and in the hippocampus (E,F) of a typical sham-lesioned (A,C,E) and saporin-lesioned rat (B, D, F). The intensity of the staining is noticeably lower in the frontal (B) and parietal (D) lobes of the saporin-lesioned rats when compared to controls (A,C), indicating the loss of the cholinergic input into these areas in saporin-lesioned animals. However, there is no difference in the AChE staining intensity in their hippocampi (E,F). Rat brain schematics were adapted from Paxinos and Watson (2007) and displayed coordinates refer to the AP plane.
3.2 Post-surgical testing

3.2.1 Testing at baseline conditions

Post-surgical testing at baseline conditions revealed no significant differences between the two surgical groups on either version of the task. On LIGHTS ONLY 5-CSRTT, saporin- and sham-lesioned rats had a comparable percent of correct responses per session ($F_{(1, 11)} < 1; M_{control} = 67.71, SD_{control} = 4.48; M_{sap} = 66.25, SD_{sap} = 4.17$), with SAP rats having slightly increased percent of omissions ($F_{(1, 11)} = 1.18, p = 0.30; M_{control} = 15.42, SD_{control} = 3.50; M_{sap} = 20.60, SD_{sap} = 3.23$) (Figure 5). At this stage of testing, both groups maintained high accuracy of responding ($M_{control} = 88.29, SD_{control} = 3.06; M_{sap} = 87.90, SD_{sap} = 2.83$). SAP rats had a lower correct response latencies, however that difference was not statistically significant either ($F_{(1, 11)} = 2.76, p = 0.13; M_{control} = 1.06s, SD_{control} = 0.15s; M_{sap} = 1.41s, SD_{sap} = 1.40s$).

Similar results were obtained on ODOR ONLY version of the task (Figure 7). Namely, both groups maintained relatively high percent of correct responses per session ($F_{(1, 11)} < 1; M_{control} = 81.80, SD_{control} = 2.88; M_{sap} = 84.05, SD_{sap} = 2.67$), low percent of omissions ($F_{(1, 11)} < 1; M_{control} = 10.48, SD_{control} = 2.47; M_{sap} = 10.72, SD_{sap} = 2.28$), and high accuracy ($F_{(1, 11)} < 1; M_{control} = 95.72, SD_{control} = 1.23; M_{sap} = 96.64, SD_{sap} = 1.14$).

3.2.2 Behavioural challenge with SD=0.5s

After we increased the attentional demand of the task by lowering the stimulus duration, some differences between groups began to emerge. On LIGHTS ONLY version of the task, SAP animal performed somewhat poorer than the controls in terms of lower percentage of correct responses ($F_{(1, 11)} = 2.00, p = 0.19; M_{control} = 51.43, SD_{control} = 3.85; M_{sap} = 44.01, SD_{sap} = 3.57$) and more omissions committed per session ($F_{(1, 11)} = 2.05, p = 0.18; M_{control} = 23.10, SD_{control} = 4.60; M_{sap} = 32.20, SD_{sap} = 4.27$), however, neither of these differences were significant (Figure 5).

Similarly, on ODOR ONLY task, marginal and non-significant differences between the groups were again observed in the percent of correct responses ($F_{(1, 11)} = 3.91, p = 0.07; M_{control} = 81.70, SD_{control} = 2.88; M_{sap} = 73.95, SD_{sap} = 2.67$), and percent of omissions per session ($F_{(1, 11)} = 3.36, p = 0.09; M_{control} = 11.30, SD_{control} = 2.53; M_{sap} = 17.38, SD_{sap} = 2.35$) (Figure 7).
3.2.3 Behavioural challenge with SD=0.25s

Additional decrease in stimulus duration further deepened the distinction between the attentional performances of two surgical groups. On LIGHTS ONLY task, the differences in the percent of correct responses ($F(1, 11)=2.14$, $p=0.17$; $M_{control}=34.05$, $SD_{control}=3.90$; $M_{sap}=26.30$, $SD_{sap}=3.60$) and percent of omissions ($F(1, 11)=4.09$, $p=0.07$; $M_{control}=30.17$, $SD_{control}=5.07$; $M_{sap}=44.12$, $SD_{sap}=4.68$) were again non-significant, though (Figure 5). The number of cholinergic NBM cells correlated significantly with the percent of correct responses ($r=0.57; p=0.04$) as well as with the percent of omissions per session ($r=-0.60; p=0.03$) in this testing condition (Figure 6). These behavioral measures did not correlate with either frontal or parietal OD values.

![Figure 5](image1.png)

Figure 5. Rat performance on visual version of the task. The figure shows the effect of decreased stimulus duration on the % of correct responses (A) and % of omissions (B) on LIGHTS ONLY version of the task for both surgical groups.

![Figure 6](image2.png)

Figure 6. Correlation plots showing the relationship between the performance on visual version of the task with SD=0.25s with histological findings. The number of cholinergic NBM cells and has been positively correlated with the percent of correct responses (A) and negatively correlated with the percent of omissions (B) on the 5-CSRTT.
On ODOR ONLY 5-CSRTT with SD=0.25s, SAP rats had a significantly lower percent of correct responses ($F_{(1,11)}=7.23$, $p<0.05$; $M_{control}=84.28$, $SD_{control}=2.40$; $M_{sap}=75.48$, $SD_{sap}=2.23$) and made significantly more omissions per session ($F_{(1, 11)}=5.18$, $p<0.05$; $M_{control}=7.20$, $SD_{control}=2.03$; $M_{sap}=13.37$, $SD_{sap}=1.88$) (Figure 7). Percent of correct responses per session in this testing condition had a significant positive correlation with the number of cells in the NBM ($r=0.62$; $p=0.02$) as well as with the adjusted OD values from rat frontal ($r=0.58$; $p=0.04$) and parietal cortices ($r=0.66$; $p=0.01$) (Figure 8). Percent of omissions per session, on the other hand, had a statistically significant negative correlation with the number of cells in the NBM ($r=-0.64$; $p=0.02$) and with the adjusted OD values from rat parietal ($r=-0.58$; $p=0.04$), but not frontal cortex (Figure 9).

Figure 7. Rat performance on olfactory version of the task. The figure shows the effect of decreased stimulus duration on the % of correct responses ($A$) and % of omissions ($B$) on ODOR ONLY version of the task for both surgical groups. The saporin-lesioned rats have had significantly less correct responses and have committed more omission errors than sham-lesioned rats on ODOR ONLY task with SD=0.25s.

Figure 8. Correlation plots showing the relationship between the percent of correct responses on olfactory version of the task with SD=0.25s with histological findings. The loss of cholinergic NBM cells ($A$) and consequent loss of the cortical ACh input into frontal ($B$) and parietal lobes ($C$) has been correlated with the poorer performance on the 5-CSRTT.
Figure 9. Correlation plots showing the relationship between the percent of omissions on olfactory version of the task with SD=0.25s with histological findings. The loss of cholinergic NBM cells (A) and consequent loss of the cortical ACh input into parietal lobe (C) has been correlated with the poorer performance on the 5-CSRTT. However, correlation between the % of omissions and AChE optical density in the frontal lobe was nonsignificant.

3.2.4 Within session variable ITI

Attentional challenge with the within session variable ITI did not reveal any significant differences between the groups. Both groups of rats did have a tendency to commit more premature responses during the variable ITI sessions (SD=1s) when compared to baseline conditions where ITI was constant at 5s. However, none of these differences were significant (Table 1).

3.2.5 Remaining measures

During post-surgical testing we did not observe any significant differences between the groups on remaining 5-CSRTT measure. Namely, the numbers of incorrect and premature responses per session, response accuracy, correct response latency, and water times (the time the rats required to collect the water reward after a correct response) were similar between two surgical groups (Table 1). In addition, the number of perseverative responses per session was very low in all rats independent of the surgical group across all testing conditions ($M_{total} = 0.29$, $SD_{total} = 0.23$).
Table 1. Behavioural measures of the 5-CSRTT across different testing conditions.

<table>
<thead>
<tr>
<th>Behavioral measure (mean ± SEM) and surgical group</th>
<th>Sd=1s</th>
<th>Sd=0.5s</th>
<th>Sd=0.25s</th>
<th>Variable ITI</th>
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<tr>
<td></td>
<td>lights</td>
<td>odor</td>
<td>lights</td>
<td>odor</td>
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<tr>
<td>Incorrect responses (#)</td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>5.17±3.21</td>
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<td>10.29±2.33</td>
<td>2.02±1.25</td>
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<tr>
<td>Premature responses (#)</td>
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<tr>
<td>Control</td>
<td>4.92±2.37</td>
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<td>4.88±2.14</td>
<td>2.29±2.54</td>
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<td>Saporin</td>
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<td>2.90±2.05</td>
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<td>Water latencies (s)</td>
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<tr>
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<tr>
<td>Control</td>
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<tr>
<td>Response accuracy (%)</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
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<td>Saporin</td>
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<td>96.64±2.31</td>
<td>73.35±6.48</td>
<td>95.27±1.29</td>
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</table>
Chapter 4
Discussion

4.1 Olfactory and visual 5-CSRTT

In the present study we have demonstrated the feasibility and potential usefulness of an olfactory version of the 5-CSRTT. We have successfully trained a cohort of experimentally naïve rats on both visual and olfactory task and demonstrated that, along with the visual 5-CSRTT, the olfactory version of the task is also a potential tool for measurement of attentional function in rats.

After successful acquisition of the visual 5-CSRTT, animals needed total of 12 60-trial sessions of training to reach behavioural criterion on olfactory task: 7 sessions of coupled presentation of light and odor stimuli and 5 sessions with odor stimuli alone. It should be noted that initial presentation of odors led to a decrease in the percent of correct responses per session in the majority of rats, likely primarily due to neophobia toward the odor stimuli.

4.2 Attentional performance following the intrabasalis injection of 192 IgG-saporin

In the present study we have observed a pattern of mild attentional deficits in rats that underwent a cortical cholinergic deafferentation. Even though the NBM cholinergic lesioned rats performed similarly to the controls on both versions of the task under baseline conditions, group differences were observed after we increased the attentional demands of the task by lowering the stimulus duration. Two measures that appeared to provide the most consistent distinction between the groups, across different testing conditions, were percent of correct responses and percent of omissions per session. However, it should be noted that any observed differences between the groups were subtle and reached statistical significance only in one testing condition: ODOR ONLY task with SD=0.25s. There was a trend toward significance in the visual 5-CSRRRT, but this may have been due to a lack of statistical power. We are currently collecting data from N=9 saporin-lesion and N=4 sham-lesion rats to potentially add statistical power.

It has been argued that decreases in the percent of correct responses and increases in the percent of omissions per session on the 5-CSRTT reflects disrupted attentional function (Robbins, 2002), as long as sensory-motor capacities, executive function and motivation of animals are unaffected.
In the present study we did not conduct any additional tests assessing subjects’ sensory or motor function. However, we observed no group differences under baseline conditions, indicating that the saporin-lesioned rats did have the ability to perceive and respond to both light and odor stimuli normally, as long as the attentional demand of the task was low. Also, we did not observe any abnormal impulsive or compulsive behaviour, i.e., signs of disrupted executive function, in the saporin-lesioned rats as indicated by the low number of premature and perseverative responses, respectively. Finally, the time it took the rats to collect the reward (Water Time) did not differ between the groups across different testing conditions, indicating that the motivation level of SAP animals remained normal throughout the post-surgical testing. Therefore, we can conclude that the behavioural deficits we observed in saporin-lesioned rats were likely caused by the specific disruption to attentional function.

Response accuracy on 5-CSRTT has also been proposed as a measure of attentional function (Robbins, 2002). The present research, unlike some of the previous studies that employed 192 IgG-saporin lesion in the NBM coupled with the 5-CSRTT (Lehman, Grottick, Cassel, et al., 2003; McGaughy, Dalley, Morrison, et al., 2002), did not find any systematic group differences in the response accuracy. The pattern of behavioural deficits that we observed, however, is not uncommon. Namely, other studies that used similar methodology reported unaffected response accuracy following cholinergic NBM lesion (Harati, Barbelivien, & Cosquer, 2008; Risbrough, Bontempi, & Menzaghi, 2002). In fact, the only change in 5-CSRTT performance that has been systematically correlated with cortical cholinergic deafferentation, accomplished using the 192 IgG-saporin injection into the rat NBM, is the increased percent of omissions per session (Harati, Barbelivien, & Cosquer, 2008; Lehman, Grottick, Cassel, et al., 2003; McGaughy, Dalley, Morrison, et al., 2002; Risbrough, Bontempi, & Menzaghi, 2002).

As mentioned previously, the difference in the attentional performance between two surgical groups was fairly small or nonexistent across 8 testing conditions. One possible reason for this observation is the fact that 192 IgG-saporin injection into the site which we piloted in the present research was not particularly efficient in depleting cortical ACh in saporin-lesioned group. Namely, although we were able to cause reduction of cholinergic cells in the NBM of saporin-lesioned rats by 52.98% when compared to controls, the corresponding reduction of the frontal and parietal AChE activity was only 23.01% and 14.40% respectively. It is possible that the cholinergic system of the saporin-lesioned animals was able to compensate to some extent for the partial loss of cells in the NBM and still provide the frontoparietal regions with the substantial
level of cholinergic input. The possibility that NBM cholinergic system is able to do so has been reported previously (De Rosa, Hasselmo, & Baxter, 2001; McGaughy, Dalley, Morrison, et al., 2002).

4.3 Conclusion

Despite the low efficacy of the cholinergic NBM lesion, we did show that even marginal reduction in cortical ACh did have notable behavioural consequences, particularly if the attentional demands of the task were high. Specifically, the saporin-lesioned rats performed significantly poorer than controls on the ODOR ONLY version of the 5-CSRTT with SD=0.25s and this behavioural deficit did correlate with the loss of cholinergic cells in the NBM and with the lower ACh input into frontal and parietal lobes. On the LIGHTS ONLY version of the task, under the conditions of high attentional demand (SD=0.25s), saporin-lesioned animals had smaller percentage of correct responses and made more omissions, however the difference in the groups’ performance only trended to significance. Nevertheless, we can conclude that the cholinergic lesion in the NBM can produce relatively comparable behavioral deficits on the attentional task across multiple sensory modalities.

The primary goal of the present study was to examine the role of cholinergic cortical modulation of olfactory attention. To that purpose we used both the visual and olfactory 5-CSRTT to determine if the ACh from the NBM has a modulatory influence on attentional processing that is independent of sensory modality. Collectively, these findings indicate the existence of an amodal attentional system, whose functioning is dependent on the cholinergic innervation from the NBM.
References


