Nicotinic Receptor Activation in Perirhinal Cortex and Hippocampus
Facilitates Aspects of Object Memory

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ABSTRACT

NICOTINIC RECEPTOR ACTIVATION IN PERIRHINAL CORTEX AND HIPPOCAMPUS

FACILITATES ASPECTS OF OBJECT MEMORY

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University of Guelph, 2011
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This study investigated the role of nicotinic acetylcholine receptors (nAChR) in object recognition and spatial recognition memory using the spontaneous object recognition (SOR) and object-location (OL) tasks, respectively. Experiments 1 to 4, did not yield any consistent facilitative effects of systemic nAChR activation with nicotine using 24- and 48-hr delays. Using a 72-hr delay, experiments 5 and 8 revealed that systemic pre-sample nicotine dose-dependently facilitated SOR and OL performance, respectively. Experiments 6-7 and 9-10 investigated the potential involvement of the perirhinal cortex (PRh) and hippocampus (HPC) in these systemic effects, with activation of nAChR in both of these brain regions producing facilitative effects on SOR and OL performance. These results not only demonstrate that nAChR facilitate performance on SOR and OL memory tasks, but suggest these effects are mediated by nAChR action in both PRh and HPC. This study indicates that, even though PRh and HPC are functionally distinct, they can interact to enhance performance on tasks for which they are not entirely necessary.

Key words: nicotine, recognition, object, memory, perirhinal, hippocampus, rat
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Introduction

Animal Models of Declarative Memory

Declarative memory is defined as the conscious memory for facts and events and is often further divided into episodic memory (memory for personal events) and semantic memory (knowledge/facts) (Squire and Zola-Morgan, 1988; Squire and Zola, 1996). Our understanding of the link between the brain and memory—particularly episodic memory—was revolutionized by the case of the famous amnesic Henry G. Molaison (H.M.) (Squire, 2009). As a young child, H.M. sustained a head injury, which eventually resulted in epilepsy. In an attempt to alleviate this issue, H.M. underwent a bilateral medial temporal lobectomy. The surgery successfully reduced his epileptic episodes, but it also produced extensive anterograde and retrograde amnesia (Penfield and Milner, 1958). It was found, however, that some of H.M.’s memory capabilities were spared. For example, he was able to acquire and improve upon new motor skills even though he did not remember the training sessions (Scoville and Milner, 1957). These observations led researchers to suggest that there must be specific brain regions that are essential to declarative memory, and that these regions are likely located in the medial temporal lobe (MTL).

After examining patients with differential patterns of MTL damage, Milner (1970) deduced that the hippocampus might be the critical region that supports episodic memory. A memory testing procedure was later developed by researchers working with monkeys, called the delayed nonmatching-to-sample task (DNMS) (Mishkin and Delacour, 1975). This task has since been used to study the specific
mnemonic functions of the brain regions damaged by H.M.’s surgery, and therefore to determine which MTL areas are essential to episodic memory. The DNMS task consists of a sample phase, a variable delay period, and a choice phase. In the sample phase the monkey is presented with an object. Then, after a delay period, the monkey is presented with the sample object and a novel object, and the monkey is required to choose the novel object to receive a reward (Mishkin and Delacour, 1975). It is assumed that by choosing the novel object the monkey is indicating that it remembers being presented with the sample object previously. Only a single exposure to a particular stimulus is necessary for declarative memory to be established, which is the fundamental feature of DNMS and most other object recognition memory tests (Eichenbaum et al., 2007; Yonelinas, 2001). Because of this, and the fact that trial-unique stimuli can be used, such object recognition tasks are thought to be excellent models for examination of the neural substrates of aspects of mammalian declarative memory (Eichenbaum et al., 2007; Yonelinas, 2001).

Research with object recognition tasks has significantly advanced our understanding of medial temporal lobe memory functions, with specific contribution to new knowledge regarding perirhinal (PRh) and hippocampal (HPC) involvement in object memory processing (Winters et al., 2008). Adapted from the delayed non-matching-to-sample (DNMS) paradigm, the simpler spontaneous object recognition (SOR) task (Ennaceur and Delacour, 1988) is often used for evaluating elements of declarative memory in rodents and is traditionally conducted in an open field arena. The SOR tasks share a commonality with DNMS in that they are also driven by a
single exposure to a sample object and its subsequent recognition after a variable delay, with the SOR task exploiting the natural tendency of rats to explore novel stimuli in preference to familiar stimuli (Winters et al., 2008). The SOR task enables a relatively ‘pure’ approach to the study of object recognition memory without extensive pre-training or reinforcement complicating the interpretation of behavioural results (Winters et al., 2008). Controversial issues related to the use of the traditional open field, such as possible confounding influences by spatial or contextual factors, have prompted the introduction of a Y-shaped apparatus for testing SOR (Forwood et al., 2005; Winters et al., 2004). This “Y-apparatus” is a methodological variation that has revealed important dissociations between the roles of MTL structures in aspects of declarative memory (Fig. 1), and includes features such as high, homogenous white walls to minimize the influence of spatial and contextual factors and short, narrow arms to reduce locomotor exploration of the testing environment. In contrast, the traditional apparatus used for SOR is an open arena, usually 1 × 1 m with relatively low walls, which enables the rat to see the context of the room over the wall and also requires greater locomotor exploration of the testing area, making it difficult to determine whether patterns of spontaneous object exploration within the arena are influenced by these spatial and contextual factors (Winters, 2008). This is an important consideration because the types of information available to the animals at the time of memory encoding or testing may influence the brain circuitry used to perform the task. Therefore, it is imperative to properly control the type(s) of information available to the animal.
when investigating the specific MTL structures involved in certain aspects of declarative memory.

**Figure 1.** Illustration of the phases of the spontaneous object recognition task, as run in the Y-shaped apparatus. The nearest wall of the apparatus appears transparent for illustrative purposes. At the beginning of the sample or choice phase, the experimenter manually raises the guillotine door and the rat is released from the start box. During the sample phase the rat explores two identical objects, one at the end of each exploration arm, for a pre-determined amount of time. The rat is then removed from the apparatus for the variable retention delay. Following the retention delay, the rat is placed back in the apparatus to explore an identical copy of the sample object at the end of one exploration arm and a novel object in the other arm. Spatial information is not a confounding factor, as the side of the sample and novel objects is counterbalanced. Normal rats spend more time exploring the novel object. Figure adapted from Winters et al., 2004.

**Dissociating MTL Functions in Object Recognition**

The structures that have been suggested to constitute the “medial temporal lobe memory system” include the hippocampus, entorhinal, perirhinal, and parahippocampal cortices (Squire and Zola-Morgan, 1991); evidence suggests that the perirhinal cortex is the principle MTL structure involved in object recognition memory (Ennaceur et al., 1996; Bussey et al., 2000; Winters et al., 2004; Meunier et al., 1993; Eacott et al., 1994). Studies have revealed that deficits in DNMS in monkeys with perirhinal and parahippocampal lesions are analogous to deficits displayed in subjects with combined hippocampal and amygdala lesions produced
by aspiration (Mishkin, 1978; Zola-Morgan et al., 1989). Additionally, combined excitotoxic lesions of the hippocampus and amygdala that spare the surrounding cortex, do not produce impairments in DNMS performance in monkeys (Murray and Mishkin, 1998). These results, along with others (Murray and Mishkin, 1986; Buffalo et al., 1999; Horel et al., 1987), suggest that disruption of performance on the DNMS task can be observed even when the hippocampus is fully functional, thus indicating that the perirhinal and adjacent cortex plays an important role in object recognition memory. Indeed, perirhinal ablation in monkeys also yields DNMS deficits comparable to those observed with rhinal (including perirhinal and entorhinal) cortex lesions (Meunier et al., 1993); contrary to the effects of perirhinal lesions, entorhinal specific lesions produce more temporary deficits of lesser magnitude (Meunier et al., 1993). Therefore, it appears as though PRh damage is central to MTL lesion-induced object recognition deficits in non-human primates, and these results are supported by recent findings implicating PRh in human recognition memory (Buffalo et al., 1998; O’Neil, Cate, Kohler, 2009; Bowles et al., 2007; Holdstock, 2005), and reinforced by results yielded from rodent experiments.

Recent research in rats using the SOR task and transient inactivation of the PRh—through bilateral infusions of lidocaine (a sodium channel blocker) prior to the sample phase (acquisition), prior to the choice phase (retrieval), or within the retention delay following the sample phase (storage-consolidation)—has shown that inactivation at any point disrupts SOR performance, further demonstrating a role for the perirhinal cortex in various stages of object recognition memory (Winters and Bussey, 2005a). Another study by Winters and Bussey (2005b) using
transient blockade of AMPA or NMDA receptors in the PRh, demonstrated the importance of glutamatergic activity within the PRh for the interdependent but distinct stages of object recognition memory (encoding, retrieval, and consolidation). Together, the findings from monkey and rat studies suggest PRh function is critical for object recognition memory.

Although the role of the perirhinal cortex in object recognition memory is now widely established, controversy remains regarding the contribution of the hippocampus, due to the vast amount of inconsistent results (Mumby, 2001). Some of the most unambiguous evidence for the role of the hippocampus in object recognition memory comes from studies revealing functional dissociation between the perirhinal cortex and hippocampus. It has been shown that neurotoxic lesioning of the perirhinal cortex will produce deficits in object recognition memory in the SOR task while leaving performance on standard allocentric spatial memory tasks intact (Bussey et al., 2000; Bussey et al., 1999; Ennaceur et al., 1996). In addition, lesions of the fornix— the primary afferent system to the dorsal hippocampus — results in impaired spatial memory, but not object recognition memory, suggesting that normal hippocampal function is not essential for object recognition in the SOR task (Ennaceur et al., 1996; Bussey et al., 2000).

Further research clearly demonstrated this functional dissociation, with peri-postrhinal lesioned rats impaired on the SOR task and unimpaired in the radial maze spatial memory task, while rats with hippocampal lesions displayed opposite effects (Winters et al., 2004). This functional double dissociation was consistent with
immunohistochemistry investigations assessing neuronal activation via the expression of the immediate early gene c-fos, where it was found that activation of the PRh was significantly greater when rats were exposed to novel pictures of objects than familiar pictures, whereas the hippocampus was not responsive to the different object conditions. Conversely, the CA1 area of the hippocampus was significantly activated by pictures of novel spatial arrangements compared to familiar, while the PRh was not differentially activated (Wan et al., 1999). The results observed by Wan et al. (1999), along with others (e.g., Mumby et al., 1992; O’Brien et al., 2006; Piterkin et al., 2008), collectively provide a clearer picture of the specific role of the hippocampus in spatial recognition processes that are not essential to the recognition of object information per se, while simultaneously confirming the important role of the perirhinal cortex in object recognition memory (Winters et al., 2008). The fact that the hippocampus appears to be recruited when spatial and contextual information becomes pertinent to performance on a task highlights the importance of using the Y-apparatus for testing object recognition memory under conditions that minimize the influence of such factors to study the specific mechanisms involved in object representation and memory (Bussey and Aggleton, 2002). The potential contribution of spatial and contextual information to task performance may explain why hippocampal damage can disrupt object recognition in the SOR task when tested in the open field. Thus, reinforcing the assumption that PRh is the critical structure for object recognition memory function, while HPC is recruited when there is a contextual component involved in object encoding.
Although the perirhinal cortex and hippocampus are thus capable of functioning independently, these MTL structures may also interact during certain memory processes. Since strong anatomical connections between these brain regions exist (Burwell et al., 2008; Furtak et al., 2007), it would be logical to assume they interact to mediate more complex memory functions. There are SOR variants, such as object-location and object-in-place tasks, which incorporate a spatial component into the object recognition process. More specifically, the object location task requires the animal to explore two identical objects placed near two adjacent corners in an open field, and then following a variable retention delay the rat is placed back in the box to explore two identical copies of the original objects; however, only one object is in its original location and the other is in a new location (Barker et al., 2007). In contrast, the object-in-place task includes four different objects for the rat to explore, with one in each corner of the box; after a delay period the rat is placed back in the open field to explore identical copies of all four objects, but two of these objects have switched locations (Barker et al., 2007).

Even though these tasks likely have different neural substrates than ‘pure’ object recognition – likely involving a greater hippocampal contribution – it is reasonable to suggest that under such circumstances, MTL structures probably interact. In fact, HPC system damage as well as PRh damage – produced by fornix and peri-postrhinal lesions, respectively – have both been shown to disrupt object-in-place task performance (Bussey et al., 2000b); a result consistent with the suggestion that PRh-based object information and hippocampus-based spatial information must be integrated to perform such a task. Furthermore, Bussey et al.
(2001) went on to investigate this issue with a second experiment, which again required integration of object and space information, but this time included a rewarded, forced-choice task. Briefly, the experiment was conducted in a “double Y-maze” apparatus that enabled pairs of objects to be displayed at either end of a long runway, creating two “places” where the objects could be presented. Only two objects were used (A and B), one of which was correct, having a reward pellet hidden underneath, and the other being incorrect, thus hiding no reward pellet. The critical part of the design is that in one end of the arm, i.e. in one place, object A was correct and B was incorrect; conversely, at the other end of the arm—in the other place—object A was incorrect, whereas B was correct. Thus, proper performance on this task required the rats to integrate both object and place information.

Since previous research in monkeys has demonstrated this type of task is dependent on an intact HPC system (Gaffan and Harrison, 1988, 1989), Bussey et al. (2001) lesioned the PRh in rats to observe the effect it would have on this HPC-dependent task. The results revealed that PRh dysfunction leads to an inability to acquire this place-object task, which again leads to the conclusion that disruption of HPC or PRh function can produce deficits in tasks that require the integration of both object and place information (Bussey et al., 2001). It is important here to note that the same investigators who found evidence for PRh-HPC interactivity, have also demonstrated their independence, with PRh lesions producing different effects from fornix lesions on spatial and object memory tests (Gaffan, 1994a). This pattern of findings emphasizes that it is only under certain circumstances, in which object and
spatial information are integrated, that damage to either the PRh or HPC will result in diminished performance.

The HPC memory system also plays an important role in other spatial memory tasks – the Morris water maze, radial arm maze, T-maze, etc. – with lesions to the fornix and hippocampus producing major deficits in performance (Aggleton et al., 1986; Morris et al., 1982; Olton et al., 1979; Rasmussen et al., 1989). The Morris water maze encourages the rat – which is placed in a pool of water daily – to learn where a submerged escape platform is located. The rat is started in different locations each time, and thus must use distal spatial room cues to guide it to the platform location (Bussey and Aggleton, 2002). Morris et al. (1982) found that HPC-lesioned rats showed profound and lasting impairments on Morris water maze performance; conversely, combined peri- and postrhinal cortex lesions have no effect on the acquisition of this task (Bussey et al., 1999). The same pattern of results can be seen using the radial arm maze task, which generally requires the rat to forage the eight arms of the apparatus for a food reward placed at the end of each arm. An error is scored each time the rat visits an unbaited arm; good performance necessitates that the rat remembers which arm it previously visited during the trial (Bussey and Aggleton, 2002). Again, HPC system lesions produce detrimental effects to radial arm maze performance (Olton et al., 1979), while perirhinal plus postrhinal lesions had no effect on this hippocampus dependent task (Bussey, Muir, & Aggleton, 1999).
Contextual fear conditioning has also been shown to be affected by HPC system dysfunction. In one experiment, rats received three unsignaled footshocks, 3 min after being placed in the chamber (Maren and Fanselow, 1997); fear during training was measured by freezing, which is an associative fear response evoked by stimuli paired with aversive consequences (Fanselow, 1986). Twenty-four hours after training, the rats were returned to the conditioning chambers to measure conditioned fear to the context, which was determined by the amount of freezing that occurred. Rats with lesions of the fimbria/fornix or entorhinal cortex, both produced significantly less freezing to the context paired with shock, indicating contextual fear deficits. Furthermore, lesions of the dorsal hippocampus produced deficits of a similar magnitude. The lack of contextual fear conditioning in rats with HPC system damage provides further verification of its crucial role in spatial and contextual memory processes (Maren and Fanselow, 1997). Thus, the foregoing review presents robust evidence for the specific functional roles of PRh and HPC in object and spatial memory, respectively. Furthermore, a plethora of results indicate that input from the cholinergic system is strongly involved in these functions.

*Central role of Acetylcholine in Learning and Memory*

Extensive evidence has shown a definite association between the neurotransmitter acetylcholine (ACh) and cognitive function; however, the specific role of ACh in the various aspects of learning and memory formation is still uncertain (Gold, 2003; Sarter and Bruno, 1997; Everitt and Robbins, 1997; Hasselmo and Bower, 1993). The cell bodies of the cholinergic neurons are located primarily in
the striatum, nucleus basalis, medial septum, diagonal band nuclei, substantia innominata, pedunculopontine tegmental nucleus, and dorsolateral tegmental nucleus (Clementi, 2000). The basal forebrain cholinergic cell groups – which includes the nucleus basalis, substantia innominata, medial septal nucleus and the diagonal band nuclei – send fibers to all areas of the cortex—including perirhinal cortex—as well as to limbic system structures, such as the hippocampus and amygdala (Meyer & Quenzer, 2005).

Numerous experimental findings obtained during the past several decades support the view that forebrain ACh modulates a variety of cognitive functions (Gold, 2003; Hasselmo and Bower, 1993; Sarter and Bruno, 1997). These findings led to the idea that cholinergic dysfunction may be a major contributor to the severe cognitive deficits seen in Alzheimer’s Disease (AD), Schizophrenia, and Parkinson’s disease (PD) patients. Indeed the cholinergic hypothesis of memory dysfunction was also strongly influenced by the fact that cholinergic system efficacy is seriously compromised in AD (Bartus, 1982). Evidence for the central role of ACh in learning and memory comes from past experiments involving lesions of the basal forebrain, which were found to disrupt various cognitive processes (Aigner et al., 1984; Aigner et al, 1991; Robbins et al., 1989b; Everitt et al., 1987), including object recognition memory. Recently, studies that selectively lesioned the cholinergic basal forebrain input to the PRh have reported impaired object recognition memory in monkeys and rats, further implicating cholinergic neurons in normal cognitive function (Turchi et al., 2005; Winters & Bussey, 2005b).
Support for the fundamental role of ACh in modulating cognitive functions also stems from pharmacological studies, which show that disrupting cholinergic transmission generally causes deficits in learning and memory, while augmenting cholinergic functions usually facilitates learning and memory (Gold, 2003; Levin et al., 1990; Levin et al., 1992; Grigoryan and Gray; 1996). For example, several experiments have revealed that systemic administration of acetylcholinesterase (AChE) inhibitors facilitate performance on visual recognition tasks (Aigner & Mishkin, 1986; Furey et al., 2000; Scali et al., 1997a,b), providing further reinforcement that cholinergic input to MTL structures—specifically PRh and HPC—may be important for object recognition memory function.

Involvement of mAChR in Cognition

The facilitation observed by inhibiting AChE activity is likely due to stimulation of both cholinergic receptor subtypes – muscarinic (mAChR) and nicotinic (nAChR) receptors, although a substantially larger body of evidence supports the role of mAChR in learning and memory. mAChR are metabotropic ACh receptors that are more sensitive to activation by muscarine than nicotine, the ligand that activates their ionotropic nAChR counterparts. Memory studies involving systemic treatment with muscarinic cholinergic receptor antagonists have demonstrated recognition impairments in humans (Robbins et al., 1997), monkeys (Aigner & Mishkin, 1986; Aigner et al., 1991; Penetar & McDonough, 1983), and rats (Bartolini et al., 1996; Ennaceur & Meliani, 1992; Huston & Aggleton, 1987; Pitsikas
et al., 2001; Vannucchi et al., 1997), strongly implicating mAChR activation in at least certain aspects of ACh mediated learning and memory functions.

Further support for the essential role of ACh in learning and memory processes come from studying the role of mAChR in neural plasticity. For example, *in vivo* microdialysis in rhesus monkeys performing a visual recognition task showed that there was a 41% increase in ACh release in the PRh, compared with baseline levels (Tang & Aigner, 1996). Furthermore, the muscarinic receptor antagonist scopolamine disrupted the normally reduced activation of PRh neurons to familiar compared to novel pictures, while additionally blocking the production of long-term depression (LTD), but not long-term potentiation (LTP) of synaptic transmission in PRh tissue slices (Warburton et al., 2003). These decremental neuronal responses observed following exposure to familiar versus novel stimuli could be due to the decreases in synaptic efficacy occurring in such processes as LTD (Brown & Bashir, 2002; Cho et al., 2000). Interestingly it seems that activation of mAChR in rat PRh slices induce a form of protein synthesis-dependent LTD that is NMDA-dependent—another form of LTD in PRh requires activation of both NMDA and metabotropic glutamate receptors. Thus, a cholinergic mechanism of synaptic plasticity within PRh may play a role in the induction or expression of activity-dependent LTD (Winters et al., 2008). These findings offer biochemical evidence for cerebral cholinergic activation during recognition tasks in primates and are consistent with the view that such activation is important for visual recognition memory.
Collectively, findings such as the aforementioned implicate cholinergic input to the PRh in object recognition memory, and provide strong evidence for the involvement of the cholinergic system in synaptic plasticity processes within the PRh that may be necessary for recognition memory. However, such studies, though informative, do not always specify which type(s) of cholinergic receptors — mAChR and/or nAChR — within PRh are involved in these processes or at which stage(s) they exert their effects (Winters et al., 2008). Efforts to address these issues have lead to a variety of studies involving manipulations of the mAChR. Winters et al. (2006) examined the role of cortical ACh by infusing scopolamine, a mAChR antagonist, into the rat PRh at different phases of the SOR task. It was found that scopolamine infusions prior to the sample phase caused significant deficits in object recognition memory, whereas infusions immediately before the retrieval stage had no detectable effect on SOR performance. The results also demonstrated that post-sample infusions of scopolamine significantly facilitated OR memory, which appeared to be inconsistent with a direct function for cortical ACh in consolidation or retrieval of declarative memory. Further investigation of this phenomenon suggested that inhibiting the action of mAChR in the PRh may actually block the acquisition of interfering information, thus facilitating object recognition memory (Winters et al., 2007). Again, Warburton et al. (2003) demonstrated that mAChR antagonism disrupts LTD in the PRh—the mechanism implicated in the PRh response reductions that are believed to underlie familiarity-based recognition memory; however, they also show that antagonism of mAChR in the PRh impairs object recognition memory. Together these findings provide robust evidence...
supporting the role of mAChR in perirhinal-mediated object recognition memory, particularly the acquisition or encoding of object information. It is evident that many previous experiments have been devoted to studying the role of mAChR in object recognition; however, there is still limited information regarding the role of nAChR in this type of memory.

**Involvement of Nicotinic Acetylcholine Receptors in Cognition**

The involvement of mAChR in cognition is now well established, as the majority of previous research has focused on exploring their contribution to learning and memory. The focus on mAChR, however, has left a deficit in nicotinic ACh receptor (nAChR) research. Findings with AChE inhibitors (Prickaerts et al., 2004), 192 IgG/ME20.4-saporin induced cholinergic pathway lesions (Winters and Bussey, 2005b; Turchi et al., 2005), and in vivo microdialysis (Tang and Aigner, 1996) suggest a strong role for ACh in object recognition. Moreover, it cannot yet be concluded that the behavioural effects observed in such studies are exclusively due to action at mAChRs, as increases or decreases in ACh activity at nAChRs may be just as important. Furthermore, synergistic results may be caused by combined loss of muscarinic and nicotinic receptor activity. Thus my thesis research will seek a better understanding of the role of nAChR in object recognition memory.

Nicotinic acetylcholine receptors are cationic channels regulated by ACh and nAChR agonists and antagonists (Clementi, 2000). They belong to a large family of ligand-gated ion channels possessing a pentameric structure (Anand et al., 1991; Cooper et al., 1991). Nicotinic receptors are essential proteins for cholinergic
transmission in the somatic and autonomic divisions of the peripheral nervous
system, as well as in several brain areas including a variety of cortical areas, the
periaqueductal grey matter, basal ganglia, thalamus, hippocampus, cerebellum
(Clementi, 2000) and perirhinal cortex (Tribollet, 2004). It was previously believed
that cortical nAChR played a presynaptic modulatory role by regulating
acetylcholine release from cholinergic nerve terminals; however, recent findings
have revealed that ACh also acts on post-synaptic nAChRs to mediate rapid synaptic
transmission in the hippocampus and sensory cortex (Jones et al., 1999). nAChR are
either homomeric in structure, consisting of alpha (α7-α10) subunits, or
heteromeric in structure, consisting of both alpha (α2-α6) and beta (β2-β4) subunits
(Decker et al., 1995; Hogg et al., 2003; Jones et al., 1999; McGehee, 1999). nAChR
subunits are differentially distributed throughout the layers of the cortex, with the
hippocampus, for example, containing α3, α4, α5, α7, β2, β3, and β4 subunits (Jones
et al., 1999; Vailati et al., 1999). It is necessary to consider the distribution of
subunits when determining the function of particular receptor subtypes in various
brain regions, since the agonists and antagonists of the different subunits will likely
vary, along with the modality in which they exert their effects. Subunit distribution
is also important due to the fact that the receptor subunits are differently expressed
and distributed in human and rodent brains (Wada et al., 1989; Rubboli et al., 1994).
Thus, the receptor distribution must be considered when designing and analyzing
experiments that utilize intra-cranial administration of drugs. The majority of
studies to date have only focused on systemic administration of agonists and
antagonists of nAChR subtypes; however, knowing the distribution of receptors in
systemic studies is also very important since this will give an indication of where in the brain the drug may be exerting its effects. A recent study using autoradiography to map nAChR in the rat brain found that heteromeric and homomeric subtypes seem to be differentially distributed, with a moderate level of α7 homomeric nAChR found in the PRh, while both heteromeric and homomeric subtypes are distributed throughout the HPC (Tribollet, 2004), thus providing an indication of which nAChR subunits are likely contributing to cognitive processes in these brain areas.

Research involving human and non-human subjects has implicated nAChR in memory and other cognitive processes (Levin et al., 2006; Vesey et al., 2002; Picciotto et al., 2001), with much evidence indicating that they play a facilitative role in various types of memory and related attentional processes (Revani & Levin, 2001). Several studies have shown that nicotine administered by subcutaneous injections or via cigarette smoking can enhance attentiveness in smokers (Wesnes and Warburton 1983; 1984a; Peeke and Peeke 1984; Warburton et al., 1992; Warburton and Arnall, 1994; Bates et al., 1995), and non-smokers (Levin et al., 1998), as well as improve attention and attenuate symptomology of various diseases including schizophrenia (Levin et al., 1996a; Yang et al., 2002), AD (Sahakian and Jones, 1991; Jones et al., 1992; White and Levin, 1999), Parkinson's disease (O'Neill et al., 2002), Tourette's syndrome (Silver et al., 2001), and attention-deficit/hyperactivity disorder (ADHD) (Conners et al., 1996; Levin et al., 1996b; Shytle et al., 2002). Additionally, nicotine administered subcutaneously in mice was found to improve their performance on a 5-choice serial reaction-time task, which is used as a measure of sustained attention (Young et al., 2004). Experiments have also
shown that systemic administration of nicotine reduces the memory impairments induced by various brain lesions or cholinergic antagonists (Decker et al., 1992; Grigoryan et al., 1994). In a parallel manner, nicotinic agonists have been demonstrated to improve memory in intact rats or mice (Haroutunian et al., 1985; Sansone et al., 1991; Zarrindast et al., 1996). For example, nicotine improved both active and passive avoidance (Sansone et al., 1991) and increased the step-down latency in mice (Zarrindast et al., 1996), indicating that nicotine has a facilitative effect on memory in these tasks. Additionally, pretreatment with the nAChR antagonist mecamylamine attenuated the facilitative effect of nicotine on these memory tasks (Zarrindast et al., 1996). Past research has even shown that nicotine delivered through transdermal patches may prove to be a safe and effective treatment for cognitive impairments seen in AD, PD, and ADHD (Levin, 2002). This vast array of evidence provides strong support for the facilitative role of nAChR in cognitive processes.

Initial evidence that nAChR are involved in object recognition memory came from a study conducted by Puma et al. (1999), who reported that acute systemic nicotine enhances the acquisition, consolidation, and retrieval of object information by rats in an open field object recognition task. Since then, it has been found that homomeric α7 nAChRs seem to be involved in facilitating recognition memory. Systemic administration of an α7 nAChR agonist, ABBF, was shown to improve working and recognition memory in rats by increasing performance in a water maze repeated acquisition paradigm and improving object recognition memory (Boess et al, 2007). ABBF also improved social recognition memory in rats; this improvement
was subsequently blocked by intracerebroventricular administration of an $\alpha 7$ nAChR antagonist, methyllycaconitine (Boess et al., 2007). There is also some evidence that suggests a facilitative role for heteromeric $\alpha 4\beta 2$ nAChR activation in recognition memory function (Buccafusco, 2005). Oral administration of TC1734, a partial $\alpha 4\beta 2$ agonist, produces long-lasting cognitive enhancement in rats after a single dose (Gatto et al., 2004). Furthermore, systemic administration of the $\alpha 4\beta 2$ nAChR agonist, epibatidine, induces LTP in the intact mouse dentate gyrus (Matsuyama and Matsumoto, 2003). These results from behavioural studies indicate that $\alpha 7$ and $\alpha 4\beta 2$ nAChR are involved in various aspects of recognition memory function.

In addition to behavioural findings, several lines of evidence indicate potentially important roles for nAChRs in synaptic plasticity mechanisms that are thought to be required for long-term memory. Systemic administration of an $\alpha 7$ nAChR agonist, choline, and an $\alpha 4\beta 2$ agonist, epibatidine, appears to induce LTP in the rat dentate gyrus (Buccafusco, 2005). Specifically, in this region, the effects of epibatidine and choline appear to be additive, which would indicate that both $\alpha 4\beta 2$ and $\alpha 7$ receptor subtypes are involved in the full induction of this type of LTP (Buccafusco, 2005). Furthermore, Matsuyama and Matsumoto (2003) found that both $\alpha 4\beta 2$ and $\alpha 7$ nAChR activation appear to be essential for producing LTP of the same magnitude as nicotine-induced LTP. Systemic administration of epibatidine induced a long-lasting LTP similar to LTP induced by choline. The LTP induced by epibatidine or choline was suppressed by administration of mecamylamine, a non-
selective nAChR antagonist, prior to a series of recording electrode stimulations (pre-application), but not by administration following the stimulations (post-application). Furthermore, post-application of nicotine facilitated epibatidine-induced LTP to a similar magnitude as nicotine-induced LTP, but post-application of epibatidine did not have an effect on nicotine-induced LTP. Epibatidine-induced LTP was also increased by post-application of choline, and vice versa, reaching the same magnitude as nicotine-induced LTP. This evidence further suggests that both α7 nAChR and α4β2 nAChR play a role in synaptic plasticity, which may be important for the establishment of long term memory required for such processes as object recognition.

The Current Study

Investigation into the involvement of cholinergic receptors in learning and memory processes is still in progress, with a clear shortage in research regarding the specific role of nAChR. More specifically, previous studies have only focused on systemic administration of nAChR agonists, which has left much ambiguity in terms of where these receptors are exerting their effects during specific memory tasks, such as object recognition and object-location memory. Thus, the goal of the following study was to replicate and extend previous findings regarding the involvement of nAChR in learning and memory. To this end, we first examined the role of nAChR using systemic injections of nicotine preceding the sample phase in SOR and object-location tasks. Once the involvement of these receptors was confirmed systemically, subsequent experiments involved intra-cranial infusions of nicotine into the PRh
and HPC prior to memory acquisition in both the SOR and object-location tasks. This was done to further assess the functional dissociations between these two MTL regions and to examine the role of nAChR in these brain areas in mediating specific behavioural effects relating to object and spatial information processing. Since the PRh has previously been shown to be critical for performance on SOR tasks, whereas the HPC has been implicated in more spatial based memory tasks and is not thought to be crucial for object recognition memory per se, it is possible that there may be a dissociation between where nAChR are exerting their effects during the SOR and object-location tasks. However, it is also reasonable to assume that these MTL structures interact during complex memory functions; therefore PRh and HPC nAChR activity may be capable of contributing to performance on both recognition tasks when both structures are intact.

Methods

Subjects. The subjects were male Long Evans rats (Charles River Lab, St. Constant, Quebec), weighing ~ 250 to 350 g when they arrived. The animals were pair-housed in shoebox cages (48 cm L × 25 cm W × 20 cm H) in the colony room at an ambient temperature of 21ºC with a reversed 12-h light/12-h dark cycle (lights off 0700-1900 h). All behavioural testing was conducted during the dark part of the cycle. Rats were habituated to handling prior to testing. During each experiment the rats were fed ~ 15g of Teklad Global 18% Protein Rodent Diet (Harlan Laboratories, Inc.) daily after behavioural sessions to maintain weight at 85-90% of free-feeding body weight. Water was provided ad libitum throughout the duration of each experiment. All
experimental procedures adhered to the guidelines of the Canadian Council of Animal Care and were approved by the Animal Care Committee of the University of Guelph.

**Drugs.** All drugs were administered either intraperitoneally (i.p.; 1ml/kg) or intracranially (see specific Experimental methods below). The nicotinic acetylcholine receptor agonist, nicotine ((–)-Nicotine hydrogen tartrate salt; Sigma), was dissolved in physiological saline (0.9% NaCl, pH 7.0; Sigma). Physiological saline was utilized as a vehicle control in all experiments. All drug doses were based on previously established effective dosage levels (Puma et al., 1999; Sharifzadeh, 2005). For all experiments, animals received trials with each drug condition, in a within-subjects design. Additionally, drug administration was prior to the sample phase (pre-sample) for all experiments—experiments that utilized systemic administration of nicotine, drug administration (i.p) was 20 min prior to the sample phase, whereas experiments that involved intracranial administration of nicotine, drug administration was immediately prior to the sample phase. These doses were based upon previously established effective dosage levels (Puma et al., 1999). All rats received each of the four drug conditions, with physiological saline utilized for the control condition, and drug administration was counterbalanced for order. The experimenter was blind to the specific drug conditions during all phases of testing.

**Surgery.** Prior to testing, all rats for intracranial infusion experiments were implanted bilaterally with 22-gauge indwelling guide cannulas positioned in the PRh or HPC. Isoflurane was delivered to an induction chamber to deeply anaesthetize
each animal before surgery. The animals were left in the induction chamber for 7-10 min until fully anaesthetized and then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set at -3.2 mm. All animals were administered Metacam (Meloxicam; Boehringer Ingelheim) subcutaneously to reduce pain and inflammation for 24 hours post-surgery. The scalp was cut and retracted to expose the skull. Placement of the cannulas was determined according to the following coordinates, measured relative to the skull coordinates at bregma (Paxinos & Watson, 1998). For PRh cannula placement: anteroposterior -5.5 mm, mediolateral ±6.6 mm, dorsoventral -6.5 mm; for HPC cannula placement: anteroposterior -3.8 mm, mediolateral ±2.5 mm, dorsoventral -2.5 mm. The holes were drilled, and the guide cannulas were implanted, ending just dorsal to the main target area. The cannulas were secured to the skull using four jeweler screws and dental acrylic. Two sutures were placed at each end of the incision to close the area around the secured guide cannulas and prevent tearing of the tissue. Obturators designed to extend 1.1 mm beyond the tip of the guide cannulas, with an outer diameter of 0.36 mm were inserted into the guides and remained there except during infusions. Physiological saline (1 ml/200g) was administered i.p. to replenish fluids and aid in recovery. Animals were allowed to recover for at least 7 days prior to testing.

**Infusion procedure.** Infusate was delivered to the PRh or HPC of the rats. In experiments 6, 7, 9, and 10 rats received bilateral infusions of either nicotine (1.0, 2.0, 4.0 µg/µl) or 0.9% physiological saline immediately prior to the start of each
sample phase (pre-sample). All infusions were conducted in a separate room from the behavioural testing area. The rats were gently restrained while the infusion procedure took place. The obturators were removed and 28-gauge infusion cannulas, which were designed to project 1 mm beyond the tip of the guides, were inserted into the guides. A Harvard Apparatus precision syringe pump was used to expel the fluid from the syringe and deliver infusate to each hemisphere at a rate of 0.5 µl/min. For all experiments, drugs or saline were infused in a volume of 0.5 µl per hemisphere over 1 min. The infusion cannulas remained in the guide for an additional 1.5 min to allow diffusion of the infusate before removal. The obturators were then replaced. In all experiments, the habituation sessions prior to testing involved ‘mock’ infusions, which encompass all aspects of the infusion procedure described above, except that the injection cannulas contained no liquid.

**Histology.** Following the completion of each intracranial administration experiment, rats were anaesthetized by i.p. injection of 2 ml of Euthanol and perfused transcardially with 100 ml of phosphate buffered saline (PBS, pH 7.4), followed by 250 ml of 4% paraformaldehyde (PFA, pH 7.4). The animals were decapitated and the brains were removed and postfixed in 4% PFA at 4°C for 24 h and then immersed in 25% sucrose in PBS until they sank. Coronal sections (60 µm) were sliced on a cryostat freezing microtome through the extent of PRh or HPC, and every fifth section was mounted on a gelatin-coated glass slide and stained with thionin. Slides were examined under a light microscope to verify the cannula placements.
Apparatus

Y-apparatus. The spontaneous object recognition task was performed in a Y-shaped apparatus, as previously described by Winters et al. (2004) and Forwood et al. (2005). To explain briefly, the Y-shaped apparatus was constructed from Plexiglas and consisted of high (40 cm), homogenous, white walls to minimize the influence of external stimuli and maximize attention to the object stimuli. Each arm was 27 cm in length and 10 cm wide. The arm that contained the start box was equipped with a guillotine door 18 cm from the rear of the arm. This start box provided an area in which the rat could be restricted until the sample or choice phase for each trial began. Between each rat, the floors and walls of the Y-apparatus were wiped with a dry paper towel—no other cleaning of the apparatus occurred for the duration of the experiment. A video camera was mounted on a tripod above the apparatus to record all trials. The objects used ranged in height from 5 to 20 cm, and were made of plastic, metal, or glass. In between rats, the objects were wiped with a cloth sprayed with 50% isopropyl alcohol, to minimize the influence of any olfactory cues present. To prevent the objects from being displaced during a trial, they were affixed to the floor of the apparatus by odorless white tack. As well as can be determined, the objects had no natural significance for the rats and they were never associated with a reinforcer.

Open Field. The apparatus for the object location task was an open box made of corrugated white plastic cardboard (60 cm L × 60 cm W × 30 cm H). The objects to be discriminated were always placed in a designated area—in adjacent corners, with
the back of the object flush to the wall and the side of the object 4 inches from the wall—marked with permanent marker on the floor of the arena. The objects were from the same set as those used for the SOR task.

*General Procedure.* Habituation took place over two consecutive daily sessions in which all of the rats were administered mock injections or infusions (as described above) prior to placement in the object-free Y-apparatus, or open field, for a 5 min exploration period. When using the Y-apparatus, the rats were placed in the start box and the guillotine door was opened to allow the rat entry into the main area of the apparatus; the door was closed immediately once the rats had vacated the start box to prevent reentry into this area. Timing of the exploration session did not begin until the rat had exited the start box. When using the open field, the rats were placed in the designated starting area—a consistent location that allowed the animals to view all objects when placed in the apparatus—with timing of the exploration starting immediately after placement in the apparatus. Testing commenced 24 hr after the second habituation session. All testing took place at approximately the same time of day. Rats were given a series of daily test trials, with a minimum interval of 24 hr between trials. Each rat was tested with a different object pair every trial, with the order of exposure to the object pairs counterbalanced within and across groups. Additionally, designation of the sample and novel object for each pair was counterbalanced within and across groups. Through the use of video recordings, the duration of object exploration was measured for each rat on both the sample and choice phases of every trial. The data for each rat was collected by
scoring exploratory bouts using a custom personal computer program written in Visual Basic 6.0.

Object recognition test in the Y-apparatus. The test included a variable number of trials – depending on the number of drug conditions – with each trial consisting of a sample phase and a choice phase. The choice phase was conducted 24, 48, or 72-hr after the sample phase (see specific Experimental details below). During the sample phase, two identical objects (A1 and A2) were presented in the Y-apparatus at the end of each exploration arm. At the beginning of each exploration session, the rats were placed in the start box with the guillotine door closed. The video camera was then set to record and the guillotine door was then raised to allow the rat entry into the exploration area of the maze. Once the rat had fully exited the start box, the guillotine door was lowered to prevent reentry into this area, and the sample phase commenced. An experimenter viewing the rat’s behaviour on a video screen scored the duration of object exploration. Certain keys on the keyboard were designated to correspond to the object on a particular side. Additionally, pressing a given key on the keyboard indicated the beginning and end of exploratory bouts; the custom personal computer program calculated the cumulative duration of the exploratory bouts. Exploration of an object was defined as directing the nose to the object at a distance of <2 cm and/or touching it with the nose. Turning around or sitting on the object was not considered exploratory behaviour. The sample phase ended once the rat had completed 25 s of exploration of the sample objects. At the end of the sample phase the rat was returned to its home cage in the colony room for the duration of the retention delay.
After the retention delay, the rat was placed back in the start box of the Y-apparatus. The choice phase began once the rat entered the exploration area. The Y-apparatus now contained an identical copy of the sample (familiar) object (A3) in one arm, and a novel object (B) in the other arm. The presentation of the novel object in the arms of the Y-apparatus was counterbalanced between rats and across trials. During the choice phase, the rat was able to explore the objects in the Y-apparatus for a period of 2 min. Afterwards, the rat was returned to its home cage in the colony room. The amount of time spent exploring the novel and familiar objects were recorded for the duration of the choice session; however, analyses placed greater focus on the first minute exploration since this is when object discrimination is typically maximal (Dix and Aggleton, 1999). The discrimination ratio was calculated by dividing the difference in time spent exploring the novel and familiar objects by the total object exploration, for the first minute of the choice phase. This measure takes into account individual differences in total object exploration.

Object location task. All parameters were the same as SOR in the y-apparatus, except (1) the objects were presented in the two corners at one end of the open field and the rats began each testing session in a designated starting area at the opposite end of the field, across from the objects (2) during the choice phase, the objects remained the same and instead the position of one of the objects changed to the adjacent corner, on the opposite end of the field. Thus, both objects in the test phase were equally familiar, but one was in a new location. Each session began immediately after the rat was placed in the open field.
Experiment 1. Experiment 1 (n = 10) was conducted in the Y-apparatus and assessed the effects of systemic nicotine at 0.1, 0.2, and 0.4 mg/kg using a 24-hr delay between the sample and choice phase in the SOR task.

Experiment 2. Experiment 2 (n = 17) was conducted in the Y-apparatus and assessed the effects of systemic nicotine at 0.2 mg/kg using a 24-hr delay between the sample and choice phase in the SOR task.

Experiment 3. Experiment 3 (n = 20) was conducted in the Y-apparatus and assessed the effects of systemic nicotine at 0.1, 0.2, 0.4 mg/kg using a 24-hr delay between the sample and choice phase in the SOR task.

Experiment 4. Experiment 4 (n = 20) was conducted in the Y-apparatus and assessed the effects of systemic nicotine at 0.1, 0.2, 0.4 mg/kg using a 48-hr delay between the sample and choice phase in the SOR task.

Experiment 5. Experiment 5 (n = 10) was conducted in the Y-apparatus and assessed the effects of systemic nicotine at 0.1, 0.2, 0.4 mg/kg using a 72-hr delay between the sample and choice phase in the SOR task.

Experiment 6. Experiment 6 (n = 10) was conducted in the Y-apparatus and assessed the effects of infusing nicotine directly into the PRh at 1.0, 2.0, or 4.0 µg/µl using a 72-hr delay between the sample and choice phase in the SOR task.

Experiment 7. Experiment 7 (n = 6) was conducted in the Y-apparatus and assessed the effects of infusing nicotine directly into the HPC at 1.0, 2.0, and 4.0 µg/µl using a 72-hr delay between the sample and choice phase in the SOR task.
Experiment 8. Experiment 8 (n = 12) was conducted in the open field and assessed the effects of administering nicotine (ip) at 0.1, 0.2, and 0.4 mg/kg – using a 72-hr delay between the sample and choice phase – on the object-location task.

Experiment 9. Experiment 9 (n = 11) was conducted in the open field and assessed the effects of infusing nicotine directly into the PRh at 1.0, 2.0, and 4.0 µg/µl – using a 72-hr delay between the sample and choice phase – on the object-location task.

Experiment 10. Experiment 10 (n = 11) was conducted in the open field and assessed the effects of infusing nicotine directly into the HPC at 1.0, 2.0, and 4.0 µg/µl – using a 72-hr delay between the sample and choice phase – on the object-location task.

Data Analysis. For each experiment, the group means of the following four measures from object recognition and object-location testing were calculated: duration of the sample phase, total object exploration time in the sample phase, total object exploration time in the choice phase, and the discrimination ratio. Means for all measures were analyzed by a repeated measures ANOVA, using PASW Statistics 18 (SPSS). One-Sample t-tests against 0 (chance discrimination) were also used to further analyze the discrimination ratio data from all experiments. For post hoc analysis of significant main effects, paired-samples t-tests were utilized with a Bonferroni correction applied to the probability. All statistical analyses were conducted with a significance level of α = 0.05, except for the paired t-tests, which used a significance level of α = 0.0083 due to the six comparisons conducted for each experiment.
Results

*Experiment 1.* In experiment 1, the effect of systemic nicotine on object recognition in the Y-apparatus using a 24-hr delay between the sample and choice phase was examined. A repeated measures ANOVA showed no effect of nicotine on the discrimination ratio, $F(3, 27) = 2.417$, $ns$, suggesting that there was no difference in performance on the object recognition task across the drug conditions (Figure 1). Due to the trends observed in the drug conditions, however, single sample t-tests against 0 (i.e., no discrimination between novel and familiar objects) were used to further examine the effects of nicotine on object recognition. This analysis indicated that all conditions displayed a discrimination ratio that was significantly different from chance [0.1 mg/kg nicotine, $t(9) = 2.902$, $p = .009$; 0.2 mg/kg nicotine, $t(9) = 5.148$, $p < .001$; and 0.4 mg/kg nicotine, $t(9) = 5.914$, $p < .001$; and the saline control condition, $t(9) = 2.246$, $p = .026$ (Figure 1)]. There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(3, 27) = .345$, $ns$, total object exploration in the sample phase, $F(3, 27) = 0.758$, $ns$, or the choice phase, $F(3, 27) = 1.446$, $ns$ (Refer to Table 1).
Figure 1. Spontaneous object recognition performance in the Y-apparatus, with a 24-hr delay, in all drug conditions from Experiment 1 (*, p < .05; **, p < .01; ***, p < .001). There was indication of a facilitative effect with the 0.2 mg/kg dose of nicotine, but the difference was not statistically significant. Performance in all conditions was significantly above chance discrimination levels. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 1. Control measures from Experiment 1.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>113.21 ± 17.60</td>
<td>23.67 ± 1.02</td>
<td>17.35 ± 1.73</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>90.23 ± 12.58</td>
<td>24.90 ± 0.16</td>
<td>14.55 ± 1.81</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>113.35 ± 17.34</td>
<td>24.41 ± 0.69</td>
<td>13.43 ± 1.41</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td>95.90 ± 12.35</td>
<td>24.57 ± 0.52</td>
<td>14.96 ± 1.16</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.
Experiment 2. The data from Experiment 1 were suggestive, but not conclusively so, of a facilitative effect of the 0.2 mg/kg systemic dose of nicotine in the SOR task. Thus, in Experiment 2, the effect of systemic 0.2 mg/kg nicotine on object recognition in the Y-apparatus was reexamined using the same 24-hr delay between the sample and choice phase and a larger sample size (n = 20). A repeated measures ANOVA showed no effect of nicotine on the discrimination ratio, $F(1, 16) = 1.99, \text{ns}$, suggesting that there was no difference in performance on the object recognition task across the drug conditions (Figure 2). Single sample t-tests against 0 were used to further examine the effects of nicotine on object recognition. This analysis indicated that object recognition performance was significantly above chance in both the 0.2 mg/kg nicotine condition, $t(16) = 9.94, p < .001$, and the saline control condition, $t(16) = 5.94, p < .001$ (Figure 2). There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(1,16) = .556, \text{ns}$, total object exploration in the sample phase, $F(1,16) = 0.17, \text{ns}$, or the choice phase, $F(1,16) = 2.66, \text{ns}$ (Refer to Table 2).
Figure 2. Spontaneous object recognition performance in the Y-apparatus, with a 24-hr delay, in all drug conditions from Experiment 2 (*, p < .05; **, p < .01; ***, p < .001). There was no indication of a facilitative effect with the 0.2 mg/kg dose of nicotine. Performance in both conditions was significantly above chance. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 2. Control measures for Experiment 2.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>129.09 ± 11.14</td>
<td>23.24 ± 11.14</td>
<td>14.02 ± 1.15</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>139.60 ± 12.46</td>
<td>23.46 ± 12.46</td>
<td>11.74 ± 1.13</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 3. Due to the equivocal nature of the results from Experiments 1 and 2, we wished to perform a final dose-response analysis of nicotine in the SOR task with a 24-hr retention delay to see if a reliable facilitative effect was observable with these testing parameters. Thus, in Experiment 3, the effect of systemic nicotine on object recognition in the Y-apparatus using a 24-hr delay between the sample and choice phase was examined with a group of 20 naive rats. A repeated measures ANOVA showed no effect of nicotine on the discrimination ratio, $F(3, 57) = .714, ns$, suggesting that none of the drug conditions differed significantly on the object recognition task (Figure 3). Further analysis, using single sample t-tests against 0 indicated that object recognition performance was significantly different from chance for all conditions [0.1 mg/kg, $t(19) = 5.16, p < .001$; 0.2 mg/kg nicotine, $t(19) = 2.20, p = .020$; 0.4 mg/kg nicotine, $t(19) = 3.27, p = .002$; and the saline control condition, $t(19) = 2.104, p = .025$] (Figure 3). There were no significant effects on
general exploratory behaviour as indicated by sample phase duration, $F(3, 57) = .296, ns$, total object exploration in the sample phase, $F(3, 57) = .274, ns$, or the choice phase, $F(3, 57) = .188, ns$ (Refer to Table 3).

Figure 3. Spontaneous object recognition performance in the Y-apparatus, with a 24-hr delay, in all drug conditions from Experiment 3 (*, $p < .05$; **, $p < .01$; ***, $p < .001$). All groups were significantly different from chance. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 3. Control measures for Experiment 3.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>21.04 ± 1.34</td>
<td>138.55 ± 11.27</td>
<td>11.44 ± 1.30</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>21.35 ± 1.22</td>
<td>134.98 ± 11.71</td>
<td>12.35 ± 1.58</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>20.73 ± 1.44</td>
<td>127.43 ± 11.42</td>
<td>11.30 ± 1.12</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td>21.70 ± 1.31</td>
<td>139.12 ± 10.94</td>
<td>11.53 ± 1.16</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 4. Although there were indications from the first three experiments that systemic nicotine might facilitate object recognition memory in the SOR task, these findings were not robust or reliable. We thus sought to refine our task parameters to produce more consistent behavioral effects in both the drug and control conditions. In Experiment 4, the effect of systemic nicotine on object recognition in the Y-apparatus was therefore examined using a 48-hr delay between the sample and choice phases, again using a larger sample size. We predicted that lengthening the retention delay would be more likely to abolish object recognition memory in the control condition, thereby enhancing our ability to observe any nicotine-induced memory facilitation. A repeated measures ANOVA revealed no effect of nicotine on the discrimination ratio, $F(3, 57) = .540, ns$, suggesting that performance on the object recognition task was not significantly different across the drug conditions (Figure 4). Additionally, single sample t-tests against 0 were used to assess the presence of object recognition memory in each condition; results indicated that object recognition performance was significantly above chance in all conditions [0.1 mg/kg nicotine, $t(19) = 4.10, p < .001$; 0.2 mg/kg nicotine, $t(19) = 5.45, p < .001$; 0.4 mg/kg nicotine, $t(19) = 2.946, p = .004$; and the saline control, $t(19) = 2.86, p = .005$] (Figure 4). There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(3, 57) = 1.09, ns$, total object exploration in
the sample phase, $F(3, 57) = 1.08, ns$, or the choice phase, $F(3, 57) = .157, ns$ (Refer to Table 4).

![Figure 4. Spontaneous object recognition performance in the Y-apparatus, with a 48-hr delay, in all drug conditions from Experiment 4 (*, $p < .05$; **, $p < .01$; ***, $p < .001$). All groups were significantly different from chance. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.]

Table 4. Control measures from Experiment 4.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>136.50 ± 10.05</td>
<td>22.74 ± 0.96</td>
<td>10.64 ± 1.18</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>149.35 ± 10.04</td>
<td>21.35 ± 1.32</td>
<td>12.30 ± 1.35</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>127.05 ± 12.12</td>
<td>23.09 ± 0.81</td>
<td>11.58 ± 0.98</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td>145.27 ± 10.36</td>
<td>21.41 ± 1.15</td>
<td>9.19 ± 0.94</td>
</tr>
</tbody>
</table>
Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 5. As control performance was still found to be significantly above chance with a 48-hr retention delay, Experiment 5 tested the effect of systemic nicotine on object recognition in the Y-apparatus using a 72-hr delay between the sample and choice phases. A repeated measures ANOVA revealed a significant overall drug effect, $F(3, 27) = 3.23, p = .038$, (Figure 5), indicating that nicotine did have an effect on object recognition performance. Further analysis, using single sample t-tests against 0 indicated that nicotine significantly facilitated object recognition performance at 0.1 mg/kg, $t(9) = 2.27, p = .025$, 0.2 mg/kg, $t(9) = 3.99, p = .002$, and 0.4 mg/kg, $t(9) = 8.13, p < .001$; object recognition performance was not significantly different from chance in the saline control condition, $t(9) = 0.97, ns$ (Figure 5). Post hoc analyses conducted using a paired-samples t-test did not quite reach significance with Bonferonni corrections. There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(3, 27) = 0.58, ns$, total object exploration in the sample phase, $F(3, 27) = 1.17, ns$, or the choice phase, $F(3, 27) = 0.538, ns$ (Refer to Table 5).
Figure 5. Spontaneous object recognition performance in the Y-apparatus, with a 72-hr delay, in all drug conditions from Experiment 5 (*, p < .05; **, p < .01; ***, p < .001). Nicotine had a significant facilitative effect at 0.1 mg/kg, 0.2 mg/kg, 0.4 mg/kg; the saline control group was not significantly different from chance. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 5. Control measures for Experiment 5.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>74.56 ± 7.05</td>
<td>25.07 ± 0.01</td>
<td>24.10 ± 2.29</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>81.56 ± 12.74</td>
<td>25.05 ± 0.01</td>
<td>23.67 ± 2.25</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>81.34 ± 12.65</td>
<td>25.04 ± 0.01</td>
<td>19.70 ± 3.74</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td>65.89 ± 13.82</td>
<td>24.92 ± 0.13</td>
<td>22.88 ± 2.91</td>
</tr>
</tbody>
</table>
Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 6. Since Experiment 5 revealed a facilitative effect of systemic nicotine on SOR performance, Experiment 6 examined the effect of intra-perirhinal infusions of nicotine on object recognition using a 72-hr delay between the sample and choice phases. All rats included in the behavioural analyses had guide cannulae located bilaterally with injection needle tips terminating in PRh near the border between areas 35 and 36 within cortical layers 2-5 (Burwell, 2001). These cannulae placements were consistently located around 6.04 mm posterior to bregma (Figure 6a), the approximate midsection of the PRh rostral-caudal boundary.

Figure 6a. Cannulation of rat PRh. Schematic representation of the infusion cannula tip placements for all rats from Experiment 6 (n = 10). Cannulae were consistently located around 6.04 mm posterior to bregma.

A repeated measures ANOVA revealed a significant overall drug effect, $F(3, 27) = 3.05, p = .046$ (Figure 6b). Analysis with single sample t-tests against 0 indicated that nicotine significantly facilitated object recognition at 1.0 µg/µl, $t(9) = 10.32, p < .001$ and 4.0 µg/µl, $t(9) = 2.75, p = .01$; however, there was no facilitation observed at 2.0 µg/µl, $t(9) = 1.27, ns$. The saline control was not found to be significantly different
from chance, $t(9) = 0.30, ns$. Furthermore, post hoc analyses using a paired-samples t-test revealed that mean score for the 1.0 $\mu g/\mu l$ ($M = 0.37, SD = 0.11$) differs significantly from the saline ($M = 0.03, SD = 0.36$), $t(9) = -3.08, p = .002$, and 2.0 $\mu g/\mu l$ ($M = 0.12, SD = 0.29$), $t(9) = 3.00, p = .003$, conditions. There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(3, 27) = 0.69, ns$, total object exploration in the sample phase, $F(3, 27) = 2.18, ns$, or the choice phase, $F(3, 27) = 1.36, ns$ (Refer to Table 6).

Figure 6b. Spontaneous object recognition performance in the Y-apparatus, with a 72-hr delay, in all drug conditions from Experiment 6 (*, $p < .05$; **, $p < .01$; ***, $p < .001$). There was a significant overall effect of intra-PRh nicotine, with a facilitation observed in the 1.0 $\mu g/\mu l$ and 4.0 $\mu g/\mu l$ groups. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.
Table 6. Control measures for Experiment 6.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>163.42 ± 7.99</td>
<td>20.92 ± 1.91</td>
<td>6.75 ± 1.19</td>
</tr>
<tr>
<td>1.0 µg/µl</td>
<td>152.28 ± 11.72</td>
<td>20.50 ± 2.04</td>
<td>8.74 ± 1.36</td>
</tr>
<tr>
<td>2.0 µg/µl</td>
<td>164.18 ± 11.47</td>
<td>16.52 ± 2.03</td>
<td>9.57 ± 1.29</td>
</tr>
<tr>
<td>4.0 µg/µl</td>
<td>149.45 ± 11.62</td>
<td>20.37 ± 1.96</td>
<td>8.57 ± 1.01</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 7. In experiment 7, the effect of intra-hippocampal infusions of nicotine on object recognition using a 72-hr delay between the sample and choice phases was examined, to enable us to compare and contrast these effects with those observed in Experiment 6. All rats included in the behavioural analyses had guide cannulae located bilaterally with injection needle tips terminating in HPC. These placements were consistently located between 3.14 and 4.16 mm posterior to bregma (Figure 7a).
A repeated measures ANOVA indicated no effect of nicotine on the discrimination ratio, $F(3, 15) = .942, p = .445$ (Figure 7b). A single sample t-test against 0 was used to further examine the data, revealing that 1.0 μg/μl of nicotine produced a facilitation in object recognition performance, $t(5) = 2.32, p = .034$; all other conditions were not significantly different from chance [2.0 μg/μl nicotine, $t(5) = 1.66, p = .079$; 4.0 μg/μl nicotine, $t(5) = -.474, p = .328$; saline control condition, $t(5) = -.061, p = .477$], suggesting object memory was not intact in these conditions.

There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(3, 15) = 0.78, ns$, total object exploration in the sample phase, $F(3, 15) = 1.36, ns$, or the choice phase, $F(3, 15) = 1.75, ns$ (Refer to Table 7).
Figure 7b. Object recognition performance in all drug conditions from Experiment 7 (*, \( p < .05 \); **, \( p < .01 \); ***, \( p < .001 \)). There was no significant overall effect of nicotine; however, facilitation was observed in the 1.0 \( \mu \text{g}/\mu\text{l} \) condition, in which performance was significantly above chance. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 7. Control measures from Experiment 7.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>130.68 ± 18.91</td>
<td>23.15 ± 1.39</td>
<td>5.37 ± 1.14</td>
</tr>
<tr>
<td>1.0 ( \mu \text{g}/\mu\text{l} )</td>
<td>124.72 ± 18.90</td>
<td>23.16 ± 2.06</td>
<td>7.75 ± 1.61</td>
</tr>
<tr>
<td>2.0 ( \mu \text{g}/\mu\text{l} )</td>
<td>141.01 ± 8.98</td>
<td>25.04 ± 0.01</td>
<td>5.74 ± 1.09</td>
</tr>
<tr>
<td>4.0 ( \mu \text{g}/\mu\text{l} )</td>
<td>156.00 ± 19.31</td>
<td>22.20 ± 2.29</td>
<td>7.99 ± 1.41</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.
Experiment 8. Having demonstrated significant facilitative effects of systemic and intracranial nicotine in the SOR task, we then investigated whether similar effects could be observed in a test of spatial recognition, the object location task. Experiment 8 therefore examined the effect of systemic nicotine on object-location using a 72-hr delay between the sample and choice phases. A repeated measures ANOVA revealed a non-significant drug effect, $F(3, 33) = .964, ns$. Further analysis, however, using single sample t-tests against 0 showed that nicotine significantly facilitated performance at 0.2 mg/kg, $t(11) = 3.25, p < .01$. This drug effect contrasted with performance in the 0.4 mg/kg, $t(11) = 1.67, ns$, 0.1 mg/kg, $t(11) = 1.37, ns$, nicotine conditions, as well as the saline condition, $t(23) = .839, p = .205$, where there was no indication of successful spatial recognition. There were no significant effects on general exploratory behaviour as indicated by sample phase duration, $F(3, 33) = 1.06, ns$, or total object exploration in the sample phase, $F(3, 33) = 1.05, ns$. However, total object exploration in the choice phase was found to be significantly different, $F(3, 33) = 3.25, p = .034$ (Refer to Table 8); post hoc analyses of this effect revealed that the mean score for the saline condition ($M = 11.2, SD = 4.21$) was significantly different from the 0.2 mg/kg ($M = 14.7, SD = 2.86$), $t(11) = -2.29, p = .007$, and 0.4 mg/kg ($M = 14.3, SD = 3.82$) conditions, $t(11) = -2.42, p = .006$, and the mean scores for the 0.1 m/kg ($M = 11.6, SD = 3.29$) and 0.2 mg/kg ($M = 14.7, SD = 2.86$) conditions were also significantly different from each other, $t(11) = -2.22, p = .008$. 
Figure 8. Object-location performance in all drug conditions from Experiment 8 (*, p < .05; **, p < .01; *** p < .001). There was a significant overall effect of nicotine, with facilitation observed in the 0.2 mg/kg group. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 8. Control measures from Experiment 8.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>95.66 ± 7.86</td>
<td>72.87 ± 3.93</td>
<td>11.15 ± 1.27</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>114.8 ± 10.35</td>
<td>82.32 ± 5.11</td>
<td>11.57 ± 0.99</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>102.2 ± 7.52</td>
<td>76.16 ± 3.76</td>
<td>14.71 ± 0.86</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td>102.16 ± 8.74</td>
<td>75.61 ± 4.29</td>
<td>14.28 ± 1.15</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 9. In Experiment 9, the effect of intra-perirhinal infusions of nicotine on object location using a 72-hr delay between the sample and choice phase was
examined. All rats included in the behavioural analyses had guide cannulae located bilaterally with injection needle tips terminating in PRh near the border between areas 35 and 36 within cortical layers 2-5 (Burwell, 2001). These cannulae placements were consistently located between 4.16 and 6.04 mm posterior to bregma (Figure 9a), the approximate midsection of the PRh rostral-caudal boundary.

![Figure 9a. Cannulation of rat PRh. Schematic representation of the infusion cannula tip placements for all rats from Experiment 9 (n = 11). Cannulae were consistently located between 4.16 and 6.04 mm posterior to bregma. Some cannula tips overlap in the figure.](image)

A repeated measures ANOVA revealed that nicotine had no effect on the discrimination ratio, $F(3, 30) = 1.26, ns$, suggesting there was no significant difference in performance on the object location task across the drug conditions (Figure 9b). To examine the trends in the data, a single sample t-test against 0 was used, which indicated that the 1.0 µg/µl, $t(10) = 2.12, p = .03$, the 4.0 µg/µl, $t(10) = 7.85, p < .001$, and saline control, $t(10) = 2.61, p = .013$, groups were significantly
different from chance; however, the 2.0 µg/µl was not found to be significantly
different from chance $t(10) = 1.59$, ns. There were no significant effects on general
exploratory behaviour as indicated by sample phase duration, $F(3, 30) = 1.12$, $ns$,
total object exploration in the sample phase, $F(3, 30) = 1.66$, $ns$, or the choice phase,
$F(3, 30) = 1.48$, $ns$ (Refer to Table 9).

![Figure 9b](object_location_performance.png)

*Figure 9b. Object-location performance in all drug conditions from Experiment 9 (*, $p < .05$; **, $p < .01$; *** , $p < .001$). No significant effect of nicotine was observed. There was a
trend towards facilitation in the 1.0 µg/µl and 4.0 µg/µl groups; however, the saline
control performance was also significantly different from chance. Data are mean
discrimination ratio (from the first minute of the choice phase) +/- SEM.*

*Table 9. Control measures from Experiment 9.*
<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>143.12 ± 9.54</td>
<td>23.77 ± 0.98</td>
<td>10.29 ± 1.36</td>
</tr>
<tr>
<td>1.0 µg/µl</td>
<td>154.46 ± 10.40</td>
<td>20.97 ± 1.91</td>
<td>8.26 ± 1.04</td>
</tr>
<tr>
<td>2.0 µg/µl</td>
<td>133.66 ± 11.12</td>
<td>24.69 ± 0.39</td>
<td>9.88 ± 0.93</td>
</tr>
<tr>
<td>4.0 µg/µl</td>
<td>150.41 ± 10.73</td>
<td>22.89 ± 1.32</td>
<td>8.05 ± 0.91</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.

Experiment 10. In experiment 10, the effect of intra-hippocampal infusions of nicotine on object-location with a 72-hr delay between the sample and choice phase was examined. All rats included in the behavioural analyses had guide cannulae located bilaterally with injection needle tips terminating in HPC. These placements were consistently located between 3.14 and 4.16 mm posterior to bregma (Figure 10a).
Figure 10a. Cannulation of rat HPC. Schematic representation of the infusion cannula tip placements for all rats from Experiment 10 \((n = 11)\). Cannulae were consistently located between 3.14 and 4.16 mm posterior to bregma. Some cannula tips overlap in the figure.

A repeated measures ANOVA revealed that nicotine had a significant effect on the discrimination ratio, \(F(3, 30) = 5.22, p = .005\), indicating there was a significant difference in performance on the object-location task across the drug conditions (Figure 10b). Analysis of the data using a single sample t-test against 0, revealed that nicotine significantly facilitated performance at 1.0 \(\mu g/\mu l\), \(t(10) = 6.46, p < .001\), 2.0 \(\mu g/\mu l\), \(t(10) = 7.18, p < .001\), and 4.0 \(\mu g/\mu l\), \(t(10) = 4.90, p < .001\); the saline control performance was not found to be significantly different from chance, \(t(10) = 1.07, ns\).

Furthermore, post hoc comparisons using a paired-samples t-test showed that the mean score for the saline condition \((M = 0.09, SD = 0.29)\) was significantly different from the 1.0 \(\mu g/\mu l\) \((M = 0.36, SD = 0.19), t(10) = -2.57, p = .005, and 2.0 \(\mu g/\mu l\) \((M = 0.32, SD = 0.15), t(10) = -2.58, p = .005, conditions. Similarly, the means score for the 4.0 \(\mu g/\mu l\) condition \((M = 0.18, SD = 0.12)\) was significantly different from the 1.0 \(\mu g/\mu l\) \((M = 0.36, SD = 0.19), t(10) = 3.68, p < .001, and 2.0 \(\mu g/\mu l\) \((M = 0.32, SD = 0.15), t(10) = 2.45, p = .006, conditions. There were no significant effects on general exploratory behaviour as indicated by sample phase duration, \(F(3, 30) = 0.26, ns\), total object exploration in the sample phase, \(F(3, 30) = 1.20, ns\), or the choice phase, \(F(3, 30) = 0.74, ns\) (Refer to Table 10).
Figure 10b. Object-location performance in all drug conditions from Experiment 10 (*, p < .05; **, p < .01; ***, p < .001). There was a significant overall effect of nicotine, with facilitation observed in the 1.0, 2.0, and 4.0 µg/µl group. Data are mean discrimination ratio (from the first minute of the choice phase) +/- SEM.

Table 10. Control measures from Experiment 10.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Sample Phase Duration</th>
<th>Total Object Exploration Sample Phase</th>
<th>Total Object Exploration Choice Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>118.44 ± 7.83</td>
<td>24.08 ± 7.62</td>
<td>10.80 ± 1.29</td>
</tr>
<tr>
<td>1.0 µg/µl</td>
<td>126.30 ± 7.69</td>
<td>24.79 ± 7.84</td>
<td>10.21 ± 0.82</td>
</tr>
<tr>
<td>2.0 µg/µl</td>
<td>127.88 ± 8.82</td>
<td>24.03 ± 7.60</td>
<td>11.99 ± 1.12</td>
</tr>
<tr>
<td>4.0 µg/µl</td>
<td>123.79 ± 7.39</td>
<td>23.37 ± 7.39</td>
<td>11.66 ± 1.38</td>
</tr>
</tbody>
</table>

Data are expressed as mean (±SEM) of the total number of seconds spent exploring in the sample phase, the total number of seconds spent exploring the sample objects, and the total number of seconds spent exploring the choice objects.
Discussion

The present series of studies provides further evidence for the involvement of nAChR in learning and memory. More specifically, the results indicate that these receptors can be recruited during object recognition (OR) and object location (OL) memory tasks, and appear to have a facilitative effect on these two forms of memory. These results show that even though the HPC is not necessary for object recognition memory function, it may still be capable of interacting with PRh during object recognition processes. Similarly, although it has been demonstrated that PRh is not crucial for spatial memory function, it still appears to be capable of contributing to the object-location task. Taken together, these results indicate that even though there is a functional double dissociation between PRh and HPC in object recognition and spatial memory tasks, respectively, this does not preclude these brain areas from being recruited for complex memory processes that may benefit from their interaction.

Object recognition task.

Effects of systemic nicotine on SOR.

Experiments 1 to 5 developed a paradigm of object recognition that would allow for an assessment of the effects of nAChR activation on object recognition memory task performance. Since interpretation of the first three experiments was complicated by the fact that performance in the saline condition remained above chance, the experimental parameters were adjusted to allow for any facilitative effects to be more readily apparent. This required prolonging the delay period to 72-
hr, instead of 24- or 48-hr, at which point the vehicle performance was diminished, allowing the nicotinic facilitation to be observed in the drug-treated conditions. Therefore, systemic administration of nicotine was found to improve SOR performance. These findings indicate that nAChR activation is capable of facilitating the acquisition of long-term object recognition memory.

Effects of PRh infused nicotine on SOR.

Having confirmed a systemic effect of nicotine on SOR performance, we then infused nicotine directly into the PRh—the critical structure for object recognition memory—to determine whether nAChR action in this brain area contributes to the facilitation of SOR performance. Intra-PRh nicotine produced a facilitation of SOR performance, suggesting that PRh nAChR can positively influence object memory processing functions and thereby extend the period of time over which rats can remember a sample object. Further research will be required to determine whether nAChR contributions are limited to this facilitative effect of if they are normally required for OR memory acquisition. Very few studies clearly demonstrate impairments following administration of nAChR antagonists (Andrews et al., 1994; Bushnell et al., 1995; Deacon, 1991), which would indicate that even though nAChR may be involved in improving memory and synaptic plasticity, they may not be necessary for either under normal conditions.

Effects of HPC infused nicotine on SOR

Due to the nicotine-induced facilitation observed via systemic and intra-PRh administration, we infused nicotine directly into the HPC to determine whether it
also could contribute to the facilitation of object recognition memory. Although many studies now indicate that the HPC is not necessary for OR memory performance (Murray and Mishkin, 1998; Aggleton et al., 1986; Bachevalier et al., 1985b; Bussey et al., 2000; Casaday and Rawlins, 1995, 1997; Forwood et al., 2005), several previous findings have implicated the HPC in aspects of object memory processing (McKee and Squire, 1993; Zola-Morgan et al., 1986; Alvarez et al., 1995; de Lima et al., 2006; Mumby et al., 1992). We therefore investigated the possibility that activating nAChR in the HPC might facilitate long-term object recognition memory. These results indicate that nAChR in the HPC, when activated, can contribute to the processing of objects in such a way that facilitates their retention in long-term memory.

Collectively, these findings indicate that the nicotine-induced systemic facilitation of SOR performance observed in Experiment 5 was likely due to the contribution of nAChR in both the PRh and HPC.

Object-location task.

Effects of systemic nicotine on Object-location memory.

Using the paradigm developed for the SOR task—using a 72-hr retention delay between the sample and choice phase—systemic nicotine was found to facilitate performance on the object-location task. This task places an emphasis on spatial location memory, instead of object recognition memory; therefore, these results imply that nAChR are involved in facilitating the acquisition of long-term object-location memory.
Effects of PRh infused nicotine on Object-location memory

Having demonstrated the systemic effect of nicotine on object-location performance, we decided to infuse nicotine directly into the PRh to determine whether it is also involved in facilitating this type of memory. The data revealed a trend towards a facilitative effect; however, this data is difficult to interpret since performance in the saline control condition was also significantly better than chance. Further investigation indicates that nicotine-induced activation of PRh nAChR does produce a facilitative effect on object-location performance, although time constraints prevented the inclusion of these additional data in the current study.

Effects of HPC infused nicotine on Object-location memory

Due to the systemic effects of nicotine on object-location performance, HPC involvement in SOR performance, and the established role for HPC in spatial memory processes (Aggleton et al., 1986; Bussey et al., 1999; Bussey et al., 2000; Ennaceur et al., 1996, Morris et al., 1982; Mumby et al., 1992; Olton et al., 1979; Piterkin, 2008; Rasmussen et al., 1989; Wan et al., 1999), we decided to infuse nicotine directly into the HPC to determine whether nAChR in this brain area also contribute to object-location memory. This seemed likely given previous findings suggesting a role for the hippocampal system in object-location memory (e.g., Bussey et al., 2000), with the results demonstrating a facilitation of object-location performance.
Together, these findings point toward the involvement of PRh and HPC in both the SOR and OL tasks. This indicates that nAChR have an interesting ability to facilitate performance in two different aspects of object memory processing; that is they appear to be involved in memory for object identity as well as memory for object location.

**Functional differences in the MTL**

The results of the current study, being that PRh and HPC seem to contribute to the facilitation of both object recognition and object-location memory, are interesting, yet not contradictory, of the fact that several recent studies have demonstrated that the structures which constitute the MTL memory system have distinct and dissociable functions (Bussey et al., 1999; Bussey et al., 2000; Ennaceur et al., 1996; Meunier et al., 1993; Winters et al., 2004). Many of these studies have focused on comparing the effects of hippocampal dysfunction with dysfunction of the parahippocampal regions. Studies using lesions and pharmacological manipulations have evaluated the effects of disrupting hippocampal or parahippocampal function while rats perform different memory tasks. Studies using spatial memory tasks – Morris water maze, radial arm maze, etc. – have shown that the hippocampus is necessary for performance on these spatial tasks, but parahippocampal regions, such as the PRh, are not necessary (Aggleton et al., 1986; Bussey et al., 1999; Bussey et al., 2000b; Ennaceur & Aggleton, 1997; Glenn & Mumby, 1998; Kolb et al., 1994; Morris et al., 1982; Olton et al., 1979; Mumby & Glenn, 2000; Rasmussen et al., 1989). The opposite effects have been shown in
experiments using object recognition memory tasks – SOR, DNMS, delayed matching-to-sample – where the PRh appears to be necessary for these tasks, but the hippocampus is not (Aggleton et al., 1997; Bussey et al., 1999, 2000b; Meunier et al., 1993; Mumby & Pinel, 1994; Suzuki et al., 1993). Such studies provide a great amount of supporting evidence for the notion that the structures encompassing the MTL memory system are at times functionally distinct. However, this independence of MTL structures does not preclude their interaction for the execution of certain complex memory tasks. Indeed, it would be quite surprising to uncover the fact that these brain areas never interact, taking the anatomical connections between these structures into account (Burwell et al., 1995). Thus, it would be reasonable to believe that these MTL structures interact when a task requires or encourages object and spatial information to be integrated. This integration has not only been demonstrated in the current study, but has also been shown in other previous studies using tasks that maintain a focus on object recognition, while incorporating a spatial component, as in the object-in-place task (Bussey et al., 2000b) or other place-object conditional tasks (Bussey et al., 2001). Disruption of either HPC or PRh function has been shown to produce deficits in performance on these types of tasks (Barker et al., 2007; Bussey et al., 2000b; Bussey et al., 2001; Ennaceur et al., 1997).

Previous studies have provided a significant amount of evidence supporting the functional dissociation between the PRh and HPC in recognition tasks. Neurotoxic lesioning of the PRh has been shown to impair performance on the SOR task, while performance on standard allocentric spatial memory tasks remains intact (Bussey et al., 2000; Bussey et al., 1999; Ennaceur et al., 1996). Additionally, lesions
of the fornix—the major output of the hippocampus—produce deficits in spatial memory, whereas object recognition memory is essentially unaffected (Ennaceur et al., 1996; Bussey et al., 2000). Finally, PRh plus postrhinal lesioned rats are impaired on the SOR task but not the radial arm maze, whereas HPC lesioned rats exhibit the opposite effects (Winters et al., 2004). However, some studies have demonstrated evidence to the contrary, with HPC lesion-induced impairments observed in object recognition memory tasks (Liu & Bilkey, 1998a, b, c, 1999, 2001; Wiig & Bilkey, 1994). This discrepancy could be accounted for by possibility that some “object” tasks could involve a “spatial” component, so even though the HPC is not necessary for object recognition, it could facilitate it by incorporating spatial and contextual information during object encoding (Bussey & Aggleton, 2002; Winters et al., 2004).

Thus, the way in which some experiments run the SOR task may include a spatial or contextual component where the HPC would be recruited during object encoding, which could account for the HPC-lesion induced deficits that are sometimes observed. In other studies, the influence of spatial and contextual cues on the SOR task may be minimal, which would tend to make it a very PRh-based task, and therefore damage to the HPC system would be less likely to affect performance (Winters et al., 2004).

However, under these circumstances, even though the HPC may not be necessary for object recognition, activation of this area could facilitate recognition memory processes. Such an effect can be observed in experiment 7 of the current study, where SOR performance appears to be facilitated by intra-HPC infusions of nicotine at 1.0 µg/µl. The same principle can also apply to the contributions of the
PRh to spatial tasks, in that certain “spatial” tasks could involve an “object” component; for example, the PRh becomes important when object discriminations are perceptually difficult (Buckley, Booth, Rolls, & Gaffan, 2001; Bussey, Saksida, & Murray, 2002b). Thus, if the spatial cues the animal must use to navigate or discriminate are perceptually similar or overlapping, then the spatial task may require the specialized functions of the PRh to help discriminate these cues for successful performance. In this case, damage to the PRh would be expected to have a detrimental effect on “spatial” task performance. Or conversely, activation of this brain area could facilitate performance on spatial tasks through the use of object identity information. Our findings seem to be consistent with the latter point, where a trend towards facilitation of object-location performance was observed following intra-PRh infusions of nicotine; this possibility has not yet been systematically assessed, therefore it would be beneficial to run further OL experiments that manipulate the perceptual discriminability of the spatial cues to delve deeper into the functions of the PRh. Indeed, it is possible that the recruitment of nAChR in both the HPC and PRh during object recognition and object-location tasks is encouraging the formation of more robust configural representations of the “object-in-space” during encoding, an effect which may ultimately increase the longevity of such representations. This more holistic representation may occur because the specialized information processing functions of these brain regions is being enhanced by nAChR activation. Consequently, even though both of these brain regions are not necessary for performance on the SOR and object-location tasks,
activation of nAChR in the HPC or PRh during these tasks can produce facilitative effects, as demonstrated in the set of results from the current study.

The results of our experiments, in conjunction with previous findings, suggest that even though these MTL lobe structures are functionally distinct, they can interact to enhance performance on tasks they are not entirely necessary for. Therefore, these findings support the view for functional heterogeneity of MTL structures, which can by definition account for the functional dissociations observed, as well as accommodate the finding that similar behavioural effects can be observed by damage to different structures (Bussey & Aggleton, 2002). It would be interesting to direct future studies toward determining which other memory tasks these structures contribute to, even though they are not necessary for the execution of such tasks.

*Possible mechanisms for nicotinic facilitation of object and location memory in the PRh and HPC*

nAChR have several subtypes that are composed of homologous subunits which can either be identical (homomeric receptors) or different (heteromeric receptors); therefore the facilitative action of nicotine observed in our object recognition and object-location tasks could be due to activation of heteromeric nAChR receptors, homomeric nAChR receptors, or both. A study using *in vitro* light microscope autoradiography determined the distribution of high affinity nicotinic heterometric receptors and low affinity nicotinic, α7 containing homomeric receptors in a plethora of brain areas, including the HPC and PRh (Tribollet et al.,
The results of this study showed that the levels of α-bungarotoxin – a compound used to label nAChR containing α7 subunits – were found to be moderate in the PRh; whereas, the levels of epibatidine – the compound used to label heteromeric nAChR – were found to be quite low. The moderate levels of α-bungarotoxin and low levels of epibatidine in the PRh suggest that the effects of nicotine on object recognition, and object-location memory tasks may be primarily mediated by action at the α7 nAChR. In contrast, epibatidine and α-bungarotoxin binding sites were both detected within the hippocampus; however, they appear to be differentially distributed throughout this structure (Tribollet et al., 2004). Thus, the detection of both heteromeric and homomeric nAChR subtypes in the hippocampus would indicate that both of these subtypes are involved in the facilitative effects observed in the OR and object-location tasks. Further research targeting nAChR subtypes specifically would provide further evidence for which subtypes are mediating these facilitative effects.

The results from previous research might provide insight into how activation of nAChR could enhance recognition memory. Administration of α7 or α4β2 agonists induce LTP in the rat dentate gyrus (Buccafusco, 2005). Additionally, a study measuring LTP in vivo in the mouse dentate gyrus found that both α7 and α4β2 nAChR are essential for full-sized LTP – induced by choline (selective α7 agonist) and epibatidine (potent α4β2 agonist), respectively. Intraperitoneal application of these drugs, prior to recording electrode stimulations (pre-application) found that epibatidine-induced LTP was increased by post-application of choline, and vice versa, reaching the same level of nicotine-induced LTP; nicotine being a non-
selective nAChR agonist. This evidence suggests that both nAChR subtypes play an important role in PRh LTP (Matsuyama and Matsumoto, 2003). Thus, activation of nAChR during encoding of object and spatial information might enhance LTP processes in the PRh and HPC. Enhanced LTP in HPC or PRh may possibly facilitate the formation of more complete representations by encouraging the formation of greater or better associations, perhaps between the PRh and HPC circuitry during and after memory acquisition. LTD could also play an important role in facilitating long-term object memory, with one form of PRh LTD mediated by mAChR activation. It has been demonstrated that in vitro application of a cholinergic receptor agonist caused a long-lasting depression of synaptic transmission, which was prevented by application of scopolamine, a mAChR antagonist (Massey et al., 2001). Facilitating LTD processes could produce greater decremental neuronal responses observed following exposure to familiar versus novel object (Brown & Bashir, 2002; Cho et al., 2000) or spatial (Etkin et al., 2006) stimuli, thus increasing subsequent recognition abilities. Therefore, it appears as though both LTP and LTD processes are likely involved in facilitating recognition memory within PRh and HPC. The involvement of nAChR in PRh or HPC LTD has not yet been examined to our knowledge; therefore it would be valuable to investigate the effects of nAChR activation on LTD processes in the future. Furthermore, conducting studies that vary the time at which drug administration occurs (pre-sample, post-sample, pre-choice) could help determine which stage(s) of memory (encoding, consolidation, retrieval) nAChR are exerting their effects. It would also be interesting to investigate whether activation of nAChR
in the PRh and HPC at the same time result in an even greater facilitation of object recognition and spatial memory.

It will also be important to eventually determine whether nAChR are normally involved in recognition memory or whether they are just capable of modulating object information acquisition processes. The LTP induced by epibatidine or choline in the mouse dentate gyrus was shown to be significantly, but not completely, suppressed by pre-application of mecamylamine, a non-selective nAChR antagonist (Matsuyama and Matsumoto, 2003). However, there were no depressive effects observed by application of mecamylamine after the establishment of LTP induced by epibatidine or choline. Thus, it is likely that the induction but not expression or maintenance of nicotinic LTP is mediated by the activation of α4β2 or α7 nAChRs. The results of this study suggest that nAChR may modulate object and spatial memory processes but are not necessary normally, since application of mecamylamine showed the reduction, but not prevention, of LTP. Thus, this nicotine-induced LTP may be a special form of LTP that is not necessarily required for learning and memory, but can have a neuromodulatory role to facilitate encoding and/or storage. However, these effects have only been demonstrated in the HPC, so in the future it will be necessary to determine whether similar events also occur in the PRh by conducting studies that will correlate the physiological effect of nAChR agonists and antagonists in the PRh with their behavioural effects.

Conclusion
Object recognition and object-location tasks are becoming increasingly prevalent in basic and preclinical research studies of the mechanisms of memory processes. The current set of experiments show that not only do nAChR facilitate performance on object recognition and object-location memory tasks, but these effects may be mediated by nAChR action in both the PRh and HPC. This result was somewhat surprising since these brain areas have been shown to be functionally distinct, with the PRh being necessary for object recognition but not spatial memory tasks, and the HPC displaying the opposite pattern of results. However, the tasks examined in the current study may require complex information processing under certain conditions, and it is quite reasonable to assume that even when these distinct brain regions are not necessary for a task, that their contributions can produce facilitative effects. Future research will further help to clarify the specific mechanisms by which nAChR produce these facilitative effects.
References


