Nicotinic Cholinergic Modulation of Sensorimotor Gating and Working Memory in Two Strains of Inbred Mice

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Abstract

Nicotinic acetylcholine receptor (nAChR) stimulation has been found to enhance sensorimotor gating and the trial-specific store of mutable information known as working memory. Working memory function is dependent upon effective stimulus filtering, which leads to the question as to whether nAChR agonists augment working memory function directly, or whether this effect is mediated indirectly through a strengthening of sensorimotor gating. In the present studies, the C57BL/6J and 129X1/SvJ strains of inbred mice were tested at 2 and 8 months of age in the pre-pulse inhibition paradigm (PPI) and the working memory version of the Morris Water Maze task (MWM). The prototypic and non-selective nAChR agonist, nicotine (0.5-2.0 mg/kg), and the $\alpha 4\beta 2$ nAChR subtype specific agonist, RJR-2403 (0.06-0.54 mg/kg), were employed for cholinergic stimulation. Nicotine administration, but not RJR-2403, tended to enhance PPI in 2 month, but not 8 month-old animals. Both nicotine and RJR-2403 were ineffective in modulating working memory function. Hence, with these strains, at these ages, in these paradigms, and at these particular drug dosages, sensorimotor gating and working memory do not appear to be correlated processes nor does nAChR stimulation appear to modulate either process within these experimental parameters. This research demonstrates the importance of fully characterizing the dosage spectrum of drugs in animals commonly used in behavioural testing and, thus, further research into this area is warranted.

Résumé

L'activation des récepteurs nicotiniques pour l'acétylcholine (nAChR) accroît la filtration des stimuli sensori-moteurs ainsi que la mémoire de travail. Le fait que la mémoire de travail soit dépendante de la filtration des stimuli soulève la question à savoir si les agonistes des nAChR joue un rôle direct ou indirect via le renforcement de la filtration des stimuli.

Nous avons utilisé deux espèces de souris (C57BL/6J et 129X1/SvJ) consanguines âgées de 2 et 8 mois dans les tests de pré pulse inhibition (PPI) ainsi que dans une version du Morris water maze (MWM) pour la mémoire de travail. La nicotine (agoniste non sélectif), ainsi que le RJR-2403 (agoniste spécifique) furent utilisés pour la stimulation cholinergique.

Les résultats révèlent que la nicotine et non le RJR-2403 a produit une tendance à l'augmentation des performances dans le PPI chez les souris âgées de 2 mois, exclusivement. De plus, la nicotine et le RJR-2403 furent inefficace dans la modulation des fonctions de la mémoire de travail.

En résumé, il semble que la filtration de stimuli sensori-moteurs ainsi que la mémoire de travail ne seraient pas des processus en corrélation et que l'activation des nAChR ne module pas ces processus, du moins, avec les espèces de souris, l'âge, les tests comportementaux ainsi que les drogues utilisés. Davantage de recherche est nécessaire pour clarifier ces incertitudes.

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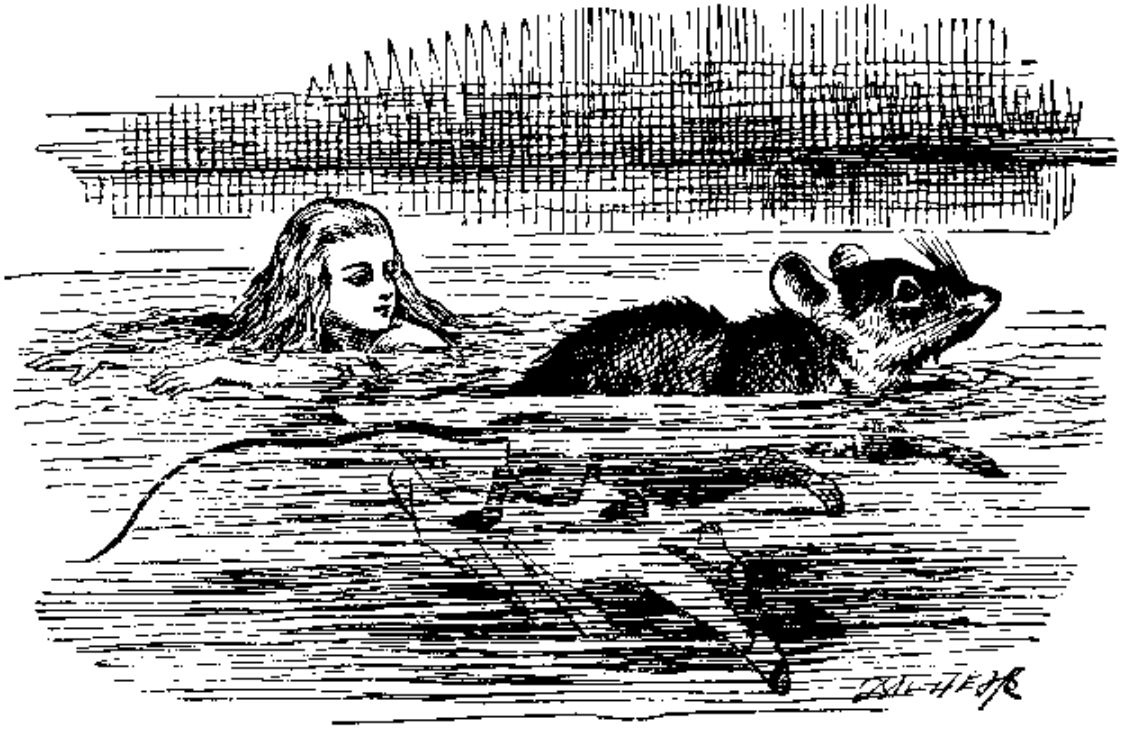
I am very privileged to have been given the opportunity to study science at a graduate level. These last two years have given me incredible insight into both academics at this level and myself. As such, I must first thank my advisor, Joseph Rochford, for having enough faith in my abilities to take me on as a research assistant despite the lack of experience I originally had in this field. I must also thank the members of my lab and the staff at the Douglas Hospital Research Centre for all of their help. Next, I thank all my friends, both here and abroad, who worked so hard to keep me sane during these two years and the Pagé family for their warm hospitality. Most importantly, I thank the people who have provided me with endless love and support throughout my life, my family.



"O Mouse, do you know the way out of this pool?"

- Alice's Adventures in Wonderland by Lewis Carroll

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I. INTRODUCTION:

Effective cognitive processing is, essentially, effective information processing. As an organism interacts with the environment, tremendous amounts of information are continuously being presented. There is no way to effectively process all of the presented stimuli due to the finite amount of information that can be managed at any given time. Therefore, there must be certain mechanisms which confer the ability to identify salient information, hold that information for a short period of time if it is only required for a particular task, and then save information deemed important enough in a more permanent long-term store (Ellis, 1983; Ericsson and Kintsch, 1995). These stages are known as attention, working memory, and reference memory, respectively.

Predominantly, organisms process external stimuli in a sequential and ordered fashion (Mesulam, 1998). Each unit of any given sensory experience may then be seen as a 'snap-shot' of the environment and the organism's relationship to it. In order for higher processing levels to extract useful information and to prevent cognitive overload, there needs to be a mechanism in place to combine multiple 'snap-shots' into a cohesive picture of the environment (Ericsson and Kintsch, 1995). Working memory fills this role by retaining 'trial specific' information, information that is relevant until the task at hand has been completed, before replacing it with an updated view of the environment (Granon *et al*., 1995; Hyde *et al*., 1998). For example, before crossing a street an individual looks to the left and sees an oncoming vehicle, she then looks to the right and sees no oncoming traffic. In this case, her working memory allows her to remember that there is still a car coming to her left even though she is no longer focusing her attention in that direction and so she does not cross the street until the vehicle has passed. Upon crossing, there is

no need for her to remember the car and, thus, that information is forgotten as her view of the world is updated. In this manner, working memory acts to combine all of the immediately relevant information together to create a working model of the surrounding environment (Mesulam, 1998). The important feature to note here is that working memory is continually being updated and is, thus, finite in its capacity. As such, certain mechanisms need to protect the salient information residing in working memory from being 'bumped out' by irrelevant stimuli before the task at hand has been completed. This role is filled by attentional and more reflexive pre-attentional processes which act as a gateway into the working memory stores (Downing, 2000; Hoffman and Ison, 1980).

Reference memory is thought to be an organism's immutable stockpile of information (Levin *et al*., 1996). Hence, this store may be defined by its long life span and large capacity (Hyde *et al*., 1998). In actuality, 'reference memory' is a broad umbrella term that encompasses the multiple types of long-term storage. These areas include declarative, or conscious, memory involved in recall, and non-declarative, or unconscious, memory which seems to be involved in the nuancing of memories with emotions, conditioning, and/or priming; learned skills which have become automatic would also seem to fall under non-declarative memory (Squire and Zola, 1996). What is important to note here is that reference memory is an organism's store of rules and templates upon which any incoming information will be compared to and built on.

Because incoming information is processed in a sequential fashion and because of the inherently limited processing capacity of the cognitive system, selectively focusing attention on relevant information while avoiding extraneous 'noise' will enhance the function of working memory (Downing, 2000; Johnston *et al*., 1970). Until recently, the

interaction between the processes of selective attention and working memory was held to be a 'bottom-up' process (Mesulam, 1998, refs. therein). That is, selection of relevant stimuli was a solely attentional function and those selected stimuli were then passed along to working memory. This hypothesis has since been altered, however, due in large part to the work of Desimone and Duncan (1995; Desimone, 1996) which demonstrates that, at least in visual attention and memory, there is also a 'top-down' mechanism in play. In this readapted model, not only does attention select relevant stimuli to be processed, but memories act directly on attention to determine which stimuli are the most salient thus leading to 'biased-competition' between the internal and external forces (Miller et al., 1996). Because of the complex interactions between these two processes, it may be difficult in behavioural testing to determine which process is influencing the other. There are, however, pre-attentional stimulus selection mechanisms in place which act at a reflexive level and so may be seen as purely independent mechanisms, distinct from mnesic control, and operating independently of learning (Hoffman and Ison, 1980).

Sensorimotor gating is a stimulus filtering process that acts to prevent two closely presented stimuli from competing with each other (Della Casa *et al*., 1998; Schauz and Koch, 1999). That is, the individual is able to focus on a relevant stimulus without being distracted, or minimally so, by a second stimulus presented within 30-500 milliseconds of the first (Della Casa *et al*., 1998). Defects in sensorimotor gating are seen in mental illnesses such as schizophrenia where the inability to filter out competing stimuli leads to sensory overload and eventual cognitive fragmentation (Braff and Geyer, 1990). Though there has been debate as to whether or not sensorimotor gating is in fact a pre-attentional mechanism rather than a particularly specialized attentional process still subject to

attentional modulation, the work of Gewirtz and Davis (1995) demonstrated that, because behavioural indicators of sensorimotor gating fail to habituate (i.e. modulate) except in a very specific and artificial set of experimental parameters, sensorimotor gating is likely exempt from attentional influences. Thus, the current model of cognitive processing presented here begins with pre-attentional filtering of rapidly presented information (*sensorimotor gating*), selection of appropriate stimuli to attend to (*selective attention*, which may or may not be influenced by memory depending on the situation), limited retention of 'trial-specific' information which will be purged and updated when the task has been completed (*working memory*), and, finally, the long-term storage of important information (*reference memory*).

Not surprisingly, all of the processes described above are thought to occur in similar areas of the brain. For instance, several brain areas have been implicated in playing a role in sensorimotor gating including the hippocampus, nucleus accumbens, amygdala, and medial prefrontal cortex (Paylor and Crawley, 1997). The hippocampus and prefrontal cortical areas have also been implicated in playing a role in both working and reference memory, as well (Bernstein *et al*., 1985; MacPherson *et al*., 2002; Miller *et al*., 1996; Wall and Messier, 2001). In particular, as with sensorimotor gating, the medial prefrontal cortex has been demonstrated to be of particular importance in working memory while the orbitomedial prefrontal cortex and the neocortex of the medial temporal lobe are held to be the important cortical structures governing reference memory (Squire and Zola, 1996; Wall and Messier, 2001). What may be more interesting, however, is that, while all of the above processes involve the same neurotransmitters (Levin and Simon, 1998; Paylor and Crawley, 1997), it seems that

sensorimotor gating and working memory but not reference memory are under the modulatory influence of nicotinic acetylcholine receptors and both processes may be enhanced by the administration of nicotinic acetylcholinergic agonists (see next section) (Levin *et al*., 2002; Rezvani and Levin, 2001; Schreiber *et al*., 2002). Taken together, this information has sparked a debate as to how these agonists exert their influences. That is, do the agonists facilitate working memory directly by increasing its capacity and duration or do they act at the pre-attentional level of sensorimotor gating, enhancing the stimuli filtering mechanism and, thus, increasing the efficacy of working memory indirectly (Park *et al*., 1999; Warburton and Rusted, 1993)? Despite this controversy and repeated testing on the modulation of one of the processes or the other, little or no attempt has been made to test cholinergic modulation of both sensorimotor gating and working memory in the same group of individuals. Additionally, given the recent evidence demonstrating the existence of multiple nicotinic receptor subtypes (see below for review), it remains to be demonstrated whether the same subtype or set of subtypes might mediate both sensorimotor gating and working memory.

I.1. Acetylcholine:

I.1.1. Biosynthesis:

Originally discovered in 1921 by Otto Loewi (Perry *et al*., 1999), the neurotransmitter acetylcholine (ACh) is found throughout the body, playing an important role in both the peripheral and central nervous systems (PNS and CNS, respectively) (Kandel, 1991). ACh is synthesized from two precursors, acetyl coenzyme A (CoA) and choline. Acetyl CoA is synthesized within nervous tissue, formed within the mitochondria from pyruvic acid and CoA via a four step enzymatic process (Feldman and

Quenzer, 1984). Choline, on the other hand, is gathered through the diet and arrives at the nervous tissue via the circulatory system (Graham, 1990; Kandel, 2000). Once both components are present, the enzyme choline acetyltransferase (ChAT) catalyzes the reaction to form ACh (Schmidt and Rylett, 1993).

Following synthesis, ACh is stored in vesicles within the nerve terminals until its release into the synaptic cleft where it binds to specific cholinergic receptors (Graham, 1990). Finally, ACh within the synaptic cleft is broken down by acetylcholinesterase (AchE) into choline, which is taken back up into the nerve terminal for recycling, and acetic acid (Graham, 1990; Feldman and Quenzer, 1984).

I.1.2. Cholinergic Neuroanatomy:

Within the rat CNS, cholinergic innervation arises primarily from six cell clusters located in the basal forebrain and upper brainstem (Kobayashi and Isa, 2002). Labeled Ch1 to Ch6, these areas correspond to the following nuclei: Ch1, the medial septal nucleus. Ch2, the vertical limb nucleus of the diagonal band. Ch3, the lateral area of the horizontal limb nucleus of the diagonal band. Ch4, the nucleus basalis magnocellularis (NBM). Ch5, the nucleus pedunculopontinus. Ch6, the laterodorsal tegmental nucleus. These areas subsequently give rise to the afferents that comprise the major source of ACh innervation for the hippocampus (Ch1 and Ch2), thalamus (Ch5 and Ch6), olfactory bulb (Ch3), amygdala and the cortex (Ch4) (Figure 1) (Mesulam *et al*., 1983).

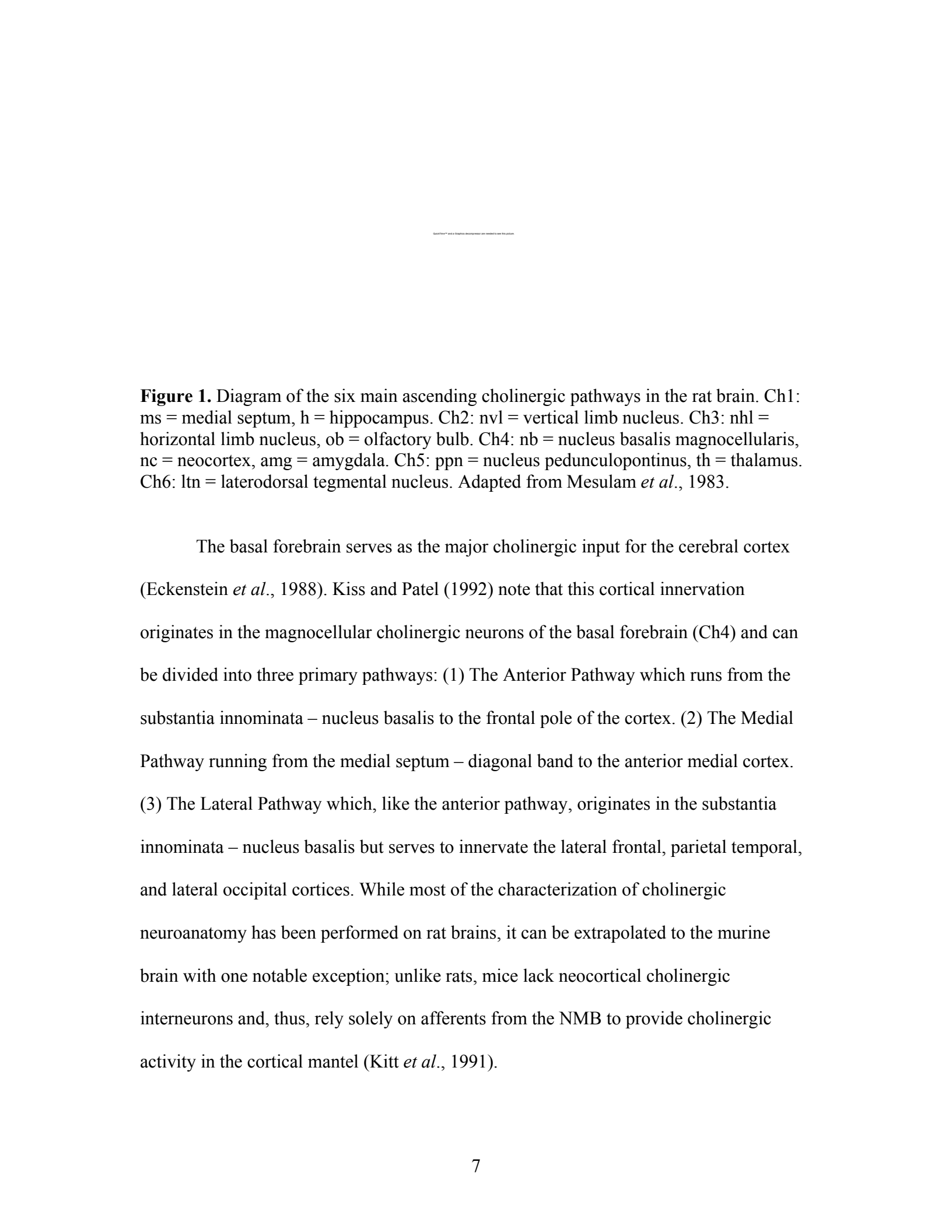


Figure 1. Diagram of the six main ascending cholinergic pathways in the rat brain. Ch1: ms = medial septum, h = hippocampus. Ch2: nvl = vertical limb nucleus. Ch3: nhl = horizontal limb nucleus, ob = olfactory bulb. Ch4: nb = nucleus basalis magnocellularis, nc = neocortex, amg = amygdala. Ch5: ppn = nucleus pedunculopontinus, th = thalamus. Ch6: ltn = laterodorsal tegmental nucleus. Adapted from Mesulam *et al*., 1983.

The basal forebrain serves as the major cholinergic input for the cerebral cortex (Eckenstein *et al*., 1988). Kiss and Patel (1992) note that this cortical innervation originates in the magnocellular cholinergic neurons of the basal forebrain (Ch4) and can be divided into three primary pathways: (1) The Anterior Pathway which runs from the substantia innominata – nucleus basalis to the frontal pole of the cortex. (2) The Medial Pathway running from the medial septum – diagonal band to the anterior medial cortex. (3) The Lateral Pathway which, like the anterior pathway, originates in the substantia innominata – nucleus basalis but serves to innervate the lateral frontal, parietal temporal, and lateral occipital cortices. While most of the characterization of cholinergic neuroanatomy has been performed on rat brains, it can be extrapolated to the murine brain with one notable exception; unlike rats, mice lack neocortical cholinergic interneurons and, thus, rely solely on afferents from the NMB to provide cholinergic activity in the cortical mantel (Kitt *et al*., 1991).

I.1.3. Nicotinic Acetylcholine Receptors:

Acetylcholine receptors can be divided into two broad categories; the metabotrophic muscarinic receptors and the ionotrophic nicotinic receptors (Brioni *et al*., 1997). Though most research has been focused on the muscarinic receptor, there has recently been a major upswing in nicotinic acetylcholine receptor (nAChR) research due to its possible role in the modulation of attention and memory (Kim and Levin, 1996). nAChRs are found both pre and post-synaptically and are activated by ACh binding to specific sites on the receptor (Stahl, 2000b). ACh binding causes a reconformation of the nAChR ion channel allowing cations, like calcium, to flow freely through the channel and causing the ionic charge gradient across the membrane to shift thus bringing about a local depolarization and signal modulation/potentiation before the channel closes (Dani, 2001). The receptors themselves are comprised of five subunits, each of which has four transmembrane regions (Stahl, 2000a). The subunits are classified as α, β, γ, δ, ε, the α and β types of which are most common within the mammalian brain (Levin and Simon, 1998; Stahl, 2000a). To date, eight α subunits (α2–α9) and three β subunits (β2–β4) have been characterized (McGehee, 1999).

nAChRs have been found to consist of single subunits, called homooligomers (eg. the α7 nAChR), or they may consist of two or more differing subunits called heterooligomers (eg. the α4β2 nAChR) (Mihailescu and Drucker-Colin, 2000).

Based on sequence homology, it has been postulated that the homooligomeric nAChRs, specifically the α7, are, evolutionarily, the oldest nicotinic receptors (Clementi *et al*., 2000). The α7 nAChR is located pre-synaptically and contains five ACh binding sites which modulate the release of ACh, glutamate (Glu), serotonin (5-HT), and GABA

(Court *et al*., 2000; Stahl, 2000b). These receptors are found to be highly concentrated within the hippocampus, particularly in the CA3 and dentate granule cells, and are noted for their high calcium permeability and relatively low affinity for ACh (Levin *et al*., 2002). The α4β2 receptor subtype, on the other hand, while only having two ACh binding sites, is located both pre and post-synaptically and, thus, not only modulates neurotransmitter release pre-synaptically, as does the α7 nAChR, but is also involved in the propagation of the excitatory signal post-synaptically (Stahl, 2000b). Moreover, the α4β2 receptor has been found to bind ACh and the prototypic nAChR agonist, nicotine, with an extremely high affinity, and is the most common nAChR in the mammalian CNS (Court *et al*., 2000; Levin and Simon, 1998; Mihailescu and Drucker-Colin, 2000). Both the α4β2 and α7 receptors have recently become the focus of several lines of research as they, specifically, have been implicated in the modulation of cognitive functioning (Jones *et al*., 1999).

I.1.4. Acetylcholine and Cognitive Functioning:

Since the mid-twentieth century, the evidence supporting a role for ACh in cognitive functioning has continued to build (Drachman, 1977). For example, blockade of the α4β2 and α7nAChRs in the ventral hippocampus using the nicotinic antagonists dihydro-β-erythrodine (DHβE) and methyllycanconitine (MLA), respectively, has been demonstrated to significantly impair working memory performance while chronic nicotine administration ameliorated the DHβE induced deficits (Bettany and Levin, 2001). Moreover, significant working memory impairment has been reported after DHβE and MLA administration into both the ventral tegmental area (VTA) and the substantia nigra (Kim and Levin, 1996; Levin *et al*., 2002). Destruction of the basal forebrain

cholinergic neurons using selective cholinergic neurotoxins such as 192 IgG-saporin, a ribosome inactivating toxin coupled to an antibody for rat p75[1], demonstrates a significant working memory deficit in subjects following lesioning of 75% of the entire basal forebrain cholinergic system (Wrenn *et al*., 1999). It should be noted, however, that several studies using 129 IgG-saporin have failed to reproduce working memory deficits (Chappell *et al*., 1998). For example, Lehmann *et al*. (2000) found that 192 IgG-saporin administration produced profound motor defects but no cognitive deficits in their subjects. In this experiment, however, the concentration of ACh was reduced by approximately 40% and it may be that much more significant lesioning is required to produce cognitive impairment. This finding points to a functional redundancy of the basal forebrain cholinergic system much like that of the dopaminergic system which seems to necessitate lesioning of more than 90% before Parkinsonian motor deficits are seen in experimental primate subjects (Wrenn *et al*., 1999).

On the other hand, facilitation of neurotransmission using ACh agonists has been found to increase attentional and mnemonic functioning in several different tasks. For instance, the α4β2 agonist SIB 1765F has been shown to increase visual attention (Grottick and Higgins, 2000) and administration of the α7agonist GTS-21 appears to improve pre-attentional stimuli filtering mechanisms in mice known to exhibit sensory gating deficits (Stevens *et al*., 1998). There is also a strong case to be made for cholinergic agonists increasing performance in working memory related tasks (see Levin and Simon, 1998 for review). Interestingly, despite the enhancement of early stages of stimulus selection and mnemonic processing, reference memory appears to remain

[1] A neurotrophin receptor expressed only by cholinergic neurons.

relatively unaffected by nicotinic agonist administration (Picciotto, 1997; Rezvani and Levin, 2001).

I.1.5. Acetylcholine and Aging:

Given the aging human population, coupled with increased longevity, the need to understand the physiological and behavioural changes that occur during the aging process has become of particular importance to our society. As such, there appears to be a continual growth in the research and literature directed towards determining the neurological basis for the cognitive decline that often occurs with age. As noted above, ACh is widely held to play a role in cognitive functioning; thus, it follows that changes in the cholinergic system may be partially responsible for age related cognitive deficits (Ikegami *et al*., 1992).

Of the age related cholinergic changes, the most pronounced may be the atrophy of the basal forebrain cholinergic neurons (Muir, 1997); the most severely affected parts of which appear to be the neurons of the intermediate and posterior basal nucleus (De Lacalle *et al*., 1996). The reason as to why this degeneration occurs is still unknown. However, Turrini *et al*. (2001) argue that the resultant loss in cholinergic function may be due to cholinergic neuron shrinkage and, thus, a diminution in the number of synapses formed as well as a decline in the area for synaptic transmission. Changes in the biosynthesis of ACh are also apparent with aging. Comparisons of acetylcholinergic change between young and old rats (3 and 21 months, respectively) revealed a significant decrease in the function of both ChAT and AchE in the cerebral tissue of the old rats (Sastry *et al*., 1983). As well, several lines of research indicate a decline in choline uptake into ACh synthesizing neurons in senescent individuals (Gibson *et al*., 1981;

Wurtman, 1992). Together, these changes in cholinergic functioning have been highly correlated to age related deficits in the attentional and memory processes (Bartus *et al*., 1982).

A particularly elegant experiment suggesting the importance of changes in ACh function to age-related working memory deficits was carried out by Ikegami (1994) using inbred mice. In this experiment, mice from the extremely long-lived BDF_1 strain were tested at 28 months of age in the radial arm maze (a test of working spatial memory function) and contrasted against 5 month old "young" animals before biochemical analysis of brain ACh levels. The results revealed that the aged animals could be divided into two sub-groups: those who demonstrated "good" performance, Group A, and those who performed "badly", Group B. While both groups of old animals exhibited both working and reference memory impairments compared to the young, Group B required significantly more trials to obtain the level of acquisition seen in by Group A. Neurochemically, both aged groups demonstrated lower levels of ACh in the hippocampus and striatum compared to the young. However, Group B also demonstrated lower levels of ACh in the cortex compared to the young, strengthening the correlation between ACh decline and memory dysfunction. Adding weight to this correlation are findings that acute injection of nicotine and grafting of cholinergic-rich brain tissue into cognitively deficient individuals have both been demonstrated to significantly improve age-related working memory deficits in rats tested in several working memory paradigms (Levin, 1992; Levin and Torry, 1996; Muir, 1997).

Aging does not always result in cognitive impairment, however, and some "old" individuals often perform as well as their young counterparts (Colombo and Gallagher,

1998). This divide between age-impaired (AI) and age-unimpaired (AU) individuals has been exploited by several labs to determine the functional neurochemical and neuroanatomical differences between the two groups. For example, neurochemical analysis performed on AI and AU individuals, 24 25 month old Long-Evans rats classified as such based on their performance in a working memory paradigm, revealed that the AI group demonstrated a lower ACh release capacity *in vivo* than did their AU counterparts (Quirion *et al*., 1995). This finding was positively correlated to an increase in the expression of muscarinic M_2 auto-receptors in the AI subjects which was believed to be impairing normal ACh release. These data would seem to support the role of ACh in proper mnemonic functioning. However, correlation does not imply causation and one must also consider that the behavioural changes seen with age are occurring against a background of extremely complex neurochemical and neuroanatomical alterations (Muir, 1997). For instance, Smith *et al*. (1995) failed to observe any deficits in spatial memory in 24-25 month old Long-Evans rats compared to their 6-7 month old counterparts despite finding age-related reductions in both nicotinic and muscarinic binding sites in the striatum, thalamus, and basal forebrain. Though in conflict with the Quirion *et al*. findings, these data do not nullify the hypothesis that changes in cholinergic functioning results in impaired cognitive functioning, however, as Smith *et al*. found their aged population to be heterogeneous in their performance. That is, there was no distinction of AI and AU individuals and, thus, correlations like those in the Quirion *et al*. experiment could not be performed.

Further evidence implicating cholinergic dysfunction in age-related memory impairment stems from the observations of cholinergic deficits in such age-related

pathologies as Alzheimer's Disease (AD). The hallmark of AD is severe memory loss coupled with the appearance of classic senile plaques consisting primarily of the amyloid β protein (Aβ) (Aubert *et al*., 1995; Verbeek *et al*., 1997). Recent ligand binding studies have determined that, within the cholinergic system, the α4 nAChR subunit seems to be most affected by the pathology with reductions in related heterooligomerous receptors of 30-50% when compared to age matched controls (Court *et al*., 2001). Interestingly, α4 mRNA levels remain unchanged in AD patients, compared to age matched controls, suggesting that the receptor loss is occurring at a post-transcriptional level (Nordberg, 2001). The α7 receptor has also been shown to be involved in AD pathology in that it forms a complex with the $A\beta_{1\text{-}42}$ protein which may result in inhibiting the release of ACh and upsetting the homeostatic calcium equilibrium leading to neuronal stress and eventual cell death (Wang *et al*., 2000). To date, several lines of cholinergic therapy have been followed in the search for a treatment for AD including ACh precursor therapy, administration of cholinergic agonists, and attempts to halt ACh degradation (Winkler *et al*., 1998). Though most of these attempts have met with little success, hope for an effective therapy remains bright as both acute and chronic administration of nicotine has been repeatedly shown to improve the attentional processes of individuals suffering from AD (Sahakin *et al*., 1989; White and Levin, 1999). There are drawbacks to the use of nicotine, however; primarily its noxious gastrointestinal and cardiovascular side effects as wells as its addiction risk (Bencherif *et al*., 1996). As such, several lines of research have focused on developing new pharmaceuticals which will stimulate centrally located nAChRs while having little or no activity at the periphery. Additionally, extensive research has been devoted to identifying the nicotinic receptor subtype(s) involved in

addiction (Mihailescu and Drucker-Colin, 2000); the hope here is that the receptors mediating the addictive properties of nicotine will be distinct from those involved in cognitive processing. As such, development of agonists specific for those nicotinic receptor involved in cognitive processes could represent a potentially effective novel form of therapy.

I.2. Pharmacology:

I.2.1. Nicotine:

Nicotine is the prototypic nAChR agonist and, as such, has long been associated with increasing acetylcholinergic transmission within both the PNS and CNS (DeSarno and Giacobini, 1989; Felix and Levin, 1997). Though generally classified as a "non-selective" nAChR agonist, that is, having roughly the same functional efficacy at each nAChR subtype tested (Lloyd *et al*., 1998), nicotine has been found to have a particularly high affinity for the $\alpha 4\beta 2$ receptor subtype (Felix and Levin, 1997) where its efficacy is equipotent to that of endogenous ACh (Brioni *et al*., 1997). Specifically, systemic administration of nicotine has been found in microdialysis studies to increase ACh release by 70% (presumably through an action at presynaptic nicotinic receptors), norepinephrine (NE) by 155%, and dopamine (DA) by 130%, while having no effect on 5-HT release (Summers *et al*., 1996).

Behaviourally, nicotine has been repeatedly demonstrated to increase the attentional faculties of both humans and rodents (Stolerman *et al*., 2000; Gould and Wehner, 1999; Heishman, 1998). The resultant attentional increase following nicotinic administration is thought to occur, in part, due to the rise in the available transmitter and transmitter mimetic resources, thus increasing the relative power and sustainability of the

brain's attentional processing mode (Mancuso *et al*., 1999). Nicotine has also been repeatedly found to enhance the working memory process, as well (Attaway *et al*., 1999; Levin *et al*., 1999; Levin *et al*., 1995). The underlying mechanisms for working enhancement, however, are a subject of much debate. Though nicotine has been shown to induce long-term potentiation (LTP), a process thought to underscore the neurological changes that occur during mnemonic plasticity (Matsuyama *et al*., 2000), it may be that the working memory enhancements are actually the result of the nicotinic enhancement of selective attention (Park *et al*., 1999).

I.2.2. RJR-2403:

One cannot rule out the effects of nicotine on the PNS as a potential confound in behavioural testing. Thus, to truly elucidate the central effects of nAChR stimulation in cognitive paradigms, one needs to employ a nAChR agonist with little or no effect in the periphery. RJR-2403, (E)-N-methyl-4-(3-pyridinyl)-3-butene-1-amine, is a potent agonist of the $\alpha 4\beta 2$ nAChR subtype which shows very poor affinity for the $\alpha 7$ nAChR as well as poor competition for non-nicotinic receptors (Bencherif *et al*., 1996; Papke *et al*., 2000). Specifically, RJR-2403, acting in a manner very similar to nicotine, has been found in microdialysis studies to increase ACh levels by 90%, NE by 125%, and DA by 130% above baseline levels while having no effect on 5-HT release (Summers *et al*., 1996). Unlike nicotine, however, RJR-2403 does not interact with PNS nAChRs (Lippiello *et al*., 1996). Thus, any behavioural modification recorded after administration may be taken as being almost entirely due to CNS modulation. Moreover, RJR-2403 has been safely tested *in vivo* at doses from 0.015 to 5.9 mg/kg and has been found to enhance working memory (Brown-Proctor *et al*., 2000; Lippiello *et al*., 1996). Taken together,

these findings reveal RJR-2403 to be an extremely effect tool in elucidating the effects of central $\alpha 4\beta 2$ stimulation on cognitive functioning.

I.3. Behavioural Characterization:

I.3.1. Pre-pulse Inhibition of the Acoustic Startle Response:

An acoustic startle response (ASR) is evoked when a loud and unpredictable auditory stimulus is presented to an organism, resulting in the activation of the cochlear nucleus - ventrolateral pons - reticular formation - spinal motor neuron pathway and the subsequent contraction of various antagonistic muscle groups (Joober *et al*., 2002). The rapidity with which the stimulus is presented is of particular importance in eliciting the ASR as the reflex will not occur in the presence of a startle-inducing stimulus if the rise-time is sufficiently long (Hoffman and Ison, 1980). For example, one is less likely to startle to an ambulance siren that is sounded a block away and is continued until it passes than by the same siren sounded when the ambulance is parked beside the individual. The ASR is not absolute, however, and may be modulated by the presentation of a non-startling stimulus, or "pre-pulse", presented 30-500ms before the startling noise (Kumari *et al*., 1996).

Pre-pulse inhibition (PPI) of the ASR refers to the phenomenon that occurs when presentation of a pre-pulse before the startle stimulus reduces the startle amplitude of an organism (Lehmann *et al*., 1999). This reflexive behaviour is highly conserved across much of the mammalian spectrum and is held to be an accurate reflection of sensorimotor gating (Bullock *et al*., 1997). Despite the vast amount of literature devoted to this subject, the neurological mechanisms underlying PPI are still an issue of hot debate and conflicting findings. For instance, Schauz and Koch (1999) determined that selectively

lesioning the NBM had no effect on the PPI phenomenon while Ballmair *et al*. (2001) argued that the NBM is critical for PPI as they found significant impairment of PPI following lesioning. What is clear, however, is that the cholinergic system plays some sort of modulatory role in this phenomenon, given that acute nicotine administration has been demonstrated to enhance PPI in 70 day old rats (Wistar, Sprague-Dawley, and Long-Evans strains) (Acri *et al*., 1995) and in human smokers compared to their nicotine deprived counterparts (Kumari *et al*., 1996) while chronic exposure to nicotine has been demonstrated to cause deficits in PPI in both adult Lister hooded and prenatally exposed Sprague-Dawley rats (Mirza *et al*., 2000; Popke *et al*., 1997). One potential confound in employing the PPI of an ASR is that it is dependent on an acoustic stimulus and, thus, hearing. Hence, subjects suffering from hearing loss may not display an accurate response to the paradigm (Carlson and Willott, 1996). An accurate measure of sensorimotor gating may still be obtained from these individuals, however, by employing an alternate PPI paradigm called pre-pulse inhibition of a tactile startle response (TSR) in which a light flash is substituted for the acoustic pre-pulse and the acoustic startle stimulus is replaced by a short but intense air puff (Joober *et al*., 2002).

I.3.2. Morris Water Maze:

Originally developed in 1981 by Richard Morris (1981) to determine the basis for spatial learning and working memory, the Morris Water Maze (MWM) has since become a mainstay in the realm of mnemonic testing. Briefly, the MWM consists of a large pool in which a submerged platform is typically placed and obfuscated by the addition of a substance that renders the water opaque. Animals are then released into the pool and, because water is deemed to be an aversive stimulus, the subject's time to find the

platform, dubbed “escape latency”, is measured (Morris, 1984). The MWM is particularly appealing because of the ease with which it can be altered to assess varying aspects of cognition. The reference memory of a subject may be recorded by keeping the hidden platform in a constant location within the maze but altering the point at which the animal is released into the pool (D’Hooge and De Deyn, 2001). For example, the animal must remember that the platform is in the northwest quadrant of the pool. Because the rules dictate that the platform should be there, the animal, once the task has been learned, should be able to orient itself, given the spatial map it has stored in reference memory over the learning process, and find the platform with relative ease. The number of trials in which it takes for the subject to learn this task may also be a relative measure of spatial learning (D’Hooge and De Deyn, 2001). Working memory can be assessed by moving the platform on each day of testing and by allowing the subjects to randomly acquire the platform in a first trial and then, after a given inter-trial interval, measure the escape latency of a second trial (Hodges, 1996). That is, the subject must remember a trial-specific piece of information, built on a reference memory based template, that will enable it to solve the maze on that particular day but will not be useful in subsequent tests and, thus, must be forgotten after completion of the task. Increasing the inter-trial interval should then put more strain on working memory by increasing the number of factors and events that may cause loss of the relevant information need to solve the maze. Because of this flexibility, the MWM has been used to elucidate the effects of many factors on memory including pharmacological administration, age, genetic background, and selective brain lesioning (D’Hooge and De Deyn, 2001; Grigoryan *et al*., 1996; Owen *et al*., 1997). Of particular interest are the findings that administration of cholinergic

agonists and antagonists significantly modulate MWM performance. (D'Hooge and De Deyn, 2001). For example, nicotine administration has been demonstrated to significantly improve spatial search strategy in animals, Sprague-Dawley rats, with lesions to both the nucleus accumbens and forebrain cholinergic projection system, indicating a role for ACh in visuo-spatial mnemonic tasks (Grigoryan *et al*., 1996).

I.4. Strain:

In recent years, the scientific community has witnessed huge leaps forward in the field of molecular genetics and its ability to define the gene(s) underlying various aspects of behaviour. With these advances, the creation of transgenic and knockout mice has become increasingly commonplace. However, given that the targeted genes are still based on and interacting with the subject's unaltered genetic background, it has become increasingly important to characterize the genetics and behaviour of the parent strain(s) (Banbury Conference, 1997; Crawley *et al*., 1997). Two parent strains of particular importance to characterize are the 129 strain, commonly used to create murine embryonic stem cell lines, and the C57BL/6 strain which is often used to parent new lines of mice (Homanics *et al*., 1999).

The C57BL/6J strain is one of the most frequently tested inbred mouse strains and has been investigated in paradigms ranging from operant learning and avoidance tasks to spatial learning and memory tests (Baron and Meltzer, 2001; Gould and Wehner, 1999; McCaughran *et al*., 2000; Bernstein *et al*., 1985). In particular, the C57BL/6J strain has been shown to perform extremely well in paradigms that provide contextual environmental stimuli such as the MWM (Owen *et al*., 1997; Restivo *et al*., 2002; Stavnezer *et al*., 2002). Conversely, the strain has also been demonstrated to perform

extremely poorly in tasks that reflect sensorimotor gating such as PPI of an ASR and TSR (Paylor and Crawley, 1997). The 129X1/SvJ strain[2], on the other hand, demonstrates excellent PPI of both startle responses (Paylor and Crawley, 1997) while exhibiting relatively poor performance in spatial discrimination and memory tasks (Owen *et al*., 1997). Taken together, this juxtaposition of performance levels in the two strains make them excellent candidates for determining the effects of nAChR agonist administration on sensorimotor gating and visuo-spatial working memory.

I.5. Summary:

Given the above considerations, the present series of experiments were designed to address several issues. The first, and principal, aim was to determine whether administration of nicotine and RJR-2403 would exert similar effects on both sensorimotor gating and spatial working memory function as assessed by the PPI of an ASR/TSR and MWM, respectively. PPI was chosen as the sensorimotor gating task because it has become a standard in the field for measuring this pre-attentional process (Paylor and Crawley, 1997). The MWM was selected based on its flexibility to both measure and tax working memory. Second, we were interested in determining whether the effects of nAChR agonists would differ depending on differing basal levels of either PPI or MWM performance. Accordingly, the C57BL/6J and 129X1/SvJ inbred strains of mice were chosen for their different performance levels in both the PPI and MWM paradigms. Finally, because a case has been made for cholinergic loss/dysfunction with age, two age groups of both mouse strains, 2 and 8 months, were chosen for testing.

[2] The strain formerly known as 129/SvJ. The change in nomenclature arose when genetic testing revealed genomic regions introduced into the 129/SvJ strain from an unknown donor strain(s), now indicated by the "X1" (Threadgill *et al*., 1997).

Given the neuroanatomical and biochemical overlaps in the processes of sensorimotor gating and working memory, it seems logical that they may overlap behaviourally as well. That is, modulation of the up-stream process of sensorimotor gating may indirectly affect an individual's working memory. Thus, it is important to test these two processes in the same individual to determine if a link is present. Though only correlations may be drawn from this kind of experiment it is, nevertheless, an important step in elucidating the order and flow of mammalian cognitive processing.

II. 2 MONTH OLD MICE

Experiment 1 – Pre-pulse Inhibition of an Acoustic Startle Response

II.1. Methods

II.1.1. Subjects:

Two separate cohorts (one to assess the effects of nicotine, one to assess the effects of RJR-2403) consisting of thirty-six male C57BL/6J and thirty-six male 129X1/SvJ mice were obtained at 8 weeks of age from Jackson Laboratories, Bar Harbor, Maine. Mice from the same strain were housed 9-12 animals per cage in clear polycarbonate cages measuring 48 x 26 x 15 cm. The mice were kept on a 12:12 hour light:dark cycle (lights on at 8:00 AM), fed ad libitum (rodent chow 5001 from Purina Mills), and given ad libitum access to water. The colony room was maintained at 20-22^{o} C and 35-45% humidity. Cages were changed on a weekly basis and bedding consisted of Beta Chip hardwood bedding from Charles River Co, St. Constant, Quebec. Following arrival, the mice were given 8 days to acclimate to their new surroundings before behavioral testing. All experimental procedures were conducted during the light portion of the cycle.

II.1.2. Apparatus:

Two SR-LAB startle stabilimeters (San Diego Instruments, California) were employed. Each stabilimeter consisted of a Plexiglas tube 2.5cm in diameter that rested on a 12.5 x 12.5 cm Plexiglas frame within a ventilated, sound-attenuating chamber (39 x 37 x 58 cm). A piezoelectric device mounted below the Plexiglas frame detected and

transduced motion within the tube. The delivery of acoustic stimuli was controlled by a PC microcomputer and SR-LAB interface assembly, which also digitized (0-4095), rectified, and recorded stabilimeter readings. Background noise (70 dB) and acoustic stimuli were delivered in each chamber through a Radio Shack Supertweeter (frequency response predominantly between 5 and 16 kHz) located 24 cm above the animal.

II.1.3. Drugs:

(-)-Nicotine [(-)-1-methyl-2-(3-pyridyl)pyrrolidine hydrogen tartrate salt], and RJR-2403 [E)-N-methyl-4-(3-pyridinyl)-3-butene-1-amine] were obtained from Sigma Labs (St. Louis MO). Both compounds were dissolved in distilled water.

II.1.4. Behavioural Characterization:

II.1.4.1. Pre-pulse Inhibition

On the first day of testing, all mice from both strains from both cohorts were tested for basal startle reactivity. No injections were administered on this day. Animals were placed in the startle apparatus, one C57BL/6J and one 129X1/SvJ per session, and given 5 minutes to acclimate, after which they were exposed to a total of 25 stimulus presentations in the range of 100, 105, 110, 115, and 120 dB. Each animal received 5 presentations of each stimulus intensity. Stimuli were randomly presented, with the restriction that there were no more than 2 successive presentations of the same intensity. Stimulus duration was 50 msec. Stimuli were presented on a variable time schedule that averaged 15 sec (range = 5 - 30 sec).

Once the data had been recorded for each cohort (i.e., the nicotine and the RJR-2403 cohorts), the average startle amplitude at 120 dB was calculated for each animal and these data were used to distribute (match) the subjects into four treatment groups for each strain within each cohort (n=9 per group). The startle amplitude at 120 dB was selected for matching because, as described below, this intensity was the intensity that would be used to assess PPI.

PPI was assessed on day 2. Following the 5 min acclimation period, the animals were subjected to 42 trials separated by a 5 – 30 sec inter-trial interval. The trials consisted of three types: (1) Null trials in which no stimulus was presented in order to measure basal levels of movement within the restrainer, (2) Pulse (or startle) trials, in which a 50 msec 120 dB stimulus was presented alone in order to measure startle reactivity in the absence of a prepulse stimulus, and (3) PPI trials, in which a 30 msec pre-pulse preceded the 120 dB startle stimulus by 70 msec. The pre-pulse consisted of a non-startling noise burst that was 3, 6, 9, 12, or 15 dB above the background noise level of 70 dB, depending on the trial. Testing commenced with two startle trials that were discarded from data analysis, previous experiments in our laboratory have indicated that the startle amplitude on these first two trials are elevated relative to subsequent startle trials, and inclusion of these trials in the data analysis therefore elevates estimates of basal startle reactivity. The subsequent 40 trials consisted of 5 null trials, 10 startle trials, and 5 trials at each of the pre-pulse intensities. The trials were presented in a random pattern with the same trial never occurring more than twice in succession.

To assess the effects of nicotine on PPI separate groups of animals within each strain from the nicotine cohort were administered i.p. either vehicle, 0.5 mg/kg, 1.0

mg/kg or 2.0 mg/kg 15 min prior to being placed in the startle chambers. Separate groups of mice within the RJR-2403 cohort were administered either vehicle, 0.06 mg/kg, 0.18 mg/kg, or 0.54 mg/kg. The injection volume in each case was 0.10 ml/20 g body weight. The nicotine dose range was selected on the basis of previous literature assessing the effects of this compound in mice (cf. Acri *et al*., 1994). The RJR-2403 doses reflect molar equivalents to each nicotine dose.

IIb. Experiment 1b – The Morris Water Maze

IIb.1. Methods

IIb.1.1. Subjects

Thirty-five male mice, both C57BL/6J and 129X1/SvJ, aged 10 weeks, from the nicotine cohort were tested in the Morris Water Maze. One animal from each strain within this cohort died between testing in the PPI task and commencement of the MWM. From the RJR-2403 cohort, thirty-one C57BL/6J and thirty-four 129X1/SvJ mice, aged 10 weeks, survived to MWM testing. Five C57BL/6J mice were killed and eaten by their cage mates while the two 129X1/SvJ mice died of natural causes. The housing and environmental parameters remained the same as in Experiment 1 (see section II.1.1.).

IIb.1.2. Apparatus

The Morris Water Maze consisted of a circular pool 160 cm in diameter and 45 cm deep. A circular platform, 10 cm in diameter, was placed in one quadrant of the pool. The pool was then filled with 22^{oC} water until the platform was approximately 0.5 cm

under the surface. Following submersion, the platform was obfuscated by the addition of powdered skim milk. A large visual cue was taped to the outside edge of the apparatus at each of the four compass points.

IIb.1.3. Drugs

(-)-Nicotine and RJR-2403, both obtained from Sigma Labs, were prepared in the same fashion and at the same dosage (0.5mg/kg, 1.0mg/kg, and 2.0mg/kg for nicotine, 0.06mg/kg, 0.18mg/kg, and 0.54mg/kg for RJR-2403) as in Experiment 1 (see section II.1.3.).

IIb.1.4. Behavioural Characterization

IIb.1.4.1. Training

For the first six days of training, each animal was given four trials per day. For each trial, the mouse was released into the maze at one of four randomly selected starting points (north, south, east, west). Throughout this phase the platform remained in a constant position within the pool. Animals were given 60 sec to find the platform and, once it had been acquired, had to remain sitting on the platform for 20 sec before escape latency was recorded. Following acquisition, the animal was removed from the maze and placed in a dry cage. If an animal was unsuccessful in finding the platform, the experimenter guided it there after the trial time had elapsed. No injections were administered during this phase.

IIb.1.4.2. Working Memory Training

Following the six days of primary training, the animals were tested in the working memory paradigm of the MWM. During the remainder of this experiment, the platform was moved everyday and placed in a random quadrant, however, platform location remained constant within each day. In this task, the animals received only two trials a day separated by a 1-minute inter-trial interval. The point at which the animals were placed in the pool was also randomized across days but remained constant for both trials within each day. During the first trial, escape latency was recorded but was not analyzed, as this was the trial in which the animal was required to locate the new position of the platform. Once the new location was found, the escape latency during the second trial was deemed to be a direct measure of the animals' working memory. Again, no injections were administered during this phase.

IIb.1.4.3. Working Memory Experiment

Once fully trained, the animals began receiving injections 15 minutes before their first trial. Individual animals received the same drug at the same dose as was administered them during the PPI testing phase described above. The trial regime remained the same as in the working memory training (see above) for the first two days of testing. On the third day, the ITI was lengthened to 2 minutes and continued to lengthen every two days thereafter to ITI s of 5, 10, and 20 minutes, respectively.

III. 8 MONTH OLD MICE

Experiment 2 – PPI of an Acoustic Startle Response

III.1. Methods

III.1.1. Subjects

Thirty male mice, retired breeders, from both the C57BL/6J and the 129X1/SvJ were obtained at 32 weeks (8 months) of age from Jackson Laboratories, Bar Harbor, Maine. The animals were given 8 days to acclimate to their new surroundings before testing commenced. The housing and environmental parameters remained the same as in Experiment 1 (see section II.1.1.).

III.1.2. Apparatus:

The same apparatus used in Experiment 1 was employed here (see section II.1.2.)

III.1.3. Drugs:

(-)-Nicotine, (-)-1-methyl-2-(3-pyridyl)pyrrolidine hydrogen tartrate salt, was obtained from Sigma Labs. The drug was mixed with a vehicle of distilled water to produce two different dosage levels; 1.0mg/kg and 2.0mg/kg, respectively (n = 10 per dosage group). Animals were weighed the day prior to injection. The average weight of each strain was then used to determine the appropriate volume of solution for individual injection (see section II.1.4.1.). No RJR-2403 was used with this group of animals.

III. 1.4. Behavioural Characterization:

Prepulse Inhibition: The same experimental paradigm used in Experiment 1 was employed here (see section II.1.4.).

IIIb. Experiment 2b – Morris Water Maze

IIIb.1. Methods

IIIb.1.1. Subjects

Following PPI testing, 28 C57BL/6J and 30 129X1/SvJ mice, were tested in the MWM. One C57BL/6J mouse died of natural causes while the other suffered massive spinal trauma and was deemed unfit to be tested upon between completion of the PPI paradigm and MWM commencement. The housing and environmental parameters remained the same as in Experiment 1 (see section II.1.1.).

IIIb.1.2. Apparatus:

The same apparatus used in Experiment 1b was employed here (see section IIb.1.2.)

IIIb.1.3. Behavioural Characterization:

The same experimental paradigm used in Experiment 1b was employed here (see section IIb.1.4.) with the notable exception that only the 1 and 10 min ITI times were used.

Experiment 2c – Pre-pulse Inhibition of a Tactile Startle Response

IIIc.1.1. Subjects

The same subjects used in section III b.1.1. were used in this paradigm. The animals were kept under the same environmental and housing parameters as noted in section II.1.1.

IIIc.1.2. Apparatus

The same apparatus used in section II.1.2. was partially modified for this experiment. Specifically, an air puff delivery device (San Diego Intruments, San Diego, CA) was affixed to the startle restrainers such that a 20 psi puff of air (which served as the startle stimulus) could be applied directly to the facial area of the animal. Second, a 25 W light bulb was affixed to the floor of the sound attenuating chambers, light in this experiment served as the pre-pulse stimulus.

IIIc.1.3. Drugs

Drug preparation and administration followed the procedure outlined in section III b.1.3.

IIIc.1.4. Behavioural Characterization

Due to an age-dependent reversal of expected PPI of an ASR magnitude between the strains (see results section), PPI of a tactile startle response (TSR) was employed to elucidate whether the age-related shift may be stimulus specific (eg. auditory) and, thus, may be occurring due to an age-related hearing deficit.

Subjects received intraperitoneal (i.p.) injection of drug (same dosage as was administered in previous tests) or vehicle 15 minutes before testing. Following the 5 min period of acclimation, the animals were subjected to 20 trials consisting of 5 air puff only startle trials at the beginning of the session, 10 pseudorandomly presented light + air puff PPI trials or air puff startle trials in the middle, and 5 air puff only trials at the end of the session. The air puff startle trials consisted of a 40 ms, 20 psi burst of air while PPI trials consisted of a 20 ms light pre-pulse 100 ms after by the air puff startle stimulus at the aforementioned intensity. The inter-trial interval ranged between 12-30 sec (mean of 15 sec) and, like the PPI of an ASR task, a background noise level of 70 dB was maintained throughout the experiment.

IV. RESULTS

IV.1. Two Month Old Mice

IV.1.1. Nicotine PPI

Figure 2 shows the mean startle amplitude for the C57BL/6J and 129X1/SvJ strains across the 5 stimulus intensities employed in this experiment. A strain x intensity ANOVA yielded significant main effects for Strain, $F_{1,70} = 100.26$, and for Intensity, $F_{4,280} = 15.48$, both ps < .0001, but no significant interaction. The strain effect indicates that C57BL/6J mice startled significantly more than 129X1/SvJ mice; the intensity effect indicates increased startle amplitude with increasing stimulus intensities.

Following the matching procedure based on the results from the 120 dB intensity trials, the means ± SEMs for the four C57BL/6J groups ranged between 214.50 ± 8.91 and 228.18 ± 25.22, while those for the four 129X1/SvJ groups ranged between 50.37 ± 8.91 and 55.07 ± 11.90.

During the PPI test, startle reactivity was calculated by averaging each individual's startle amplitude during the last 10 startle trials. A two-way ANOVA (strain x dose) revealed a significant main effect of strain, $F_{1, 62} = 229.26$, $p < 0.0001$ with the C57BL/6Js startling significant more (197.94 ± 9.78 versus 38.53 ± 2.51). The main effect of dose and the strain x dose interaction were not significant.

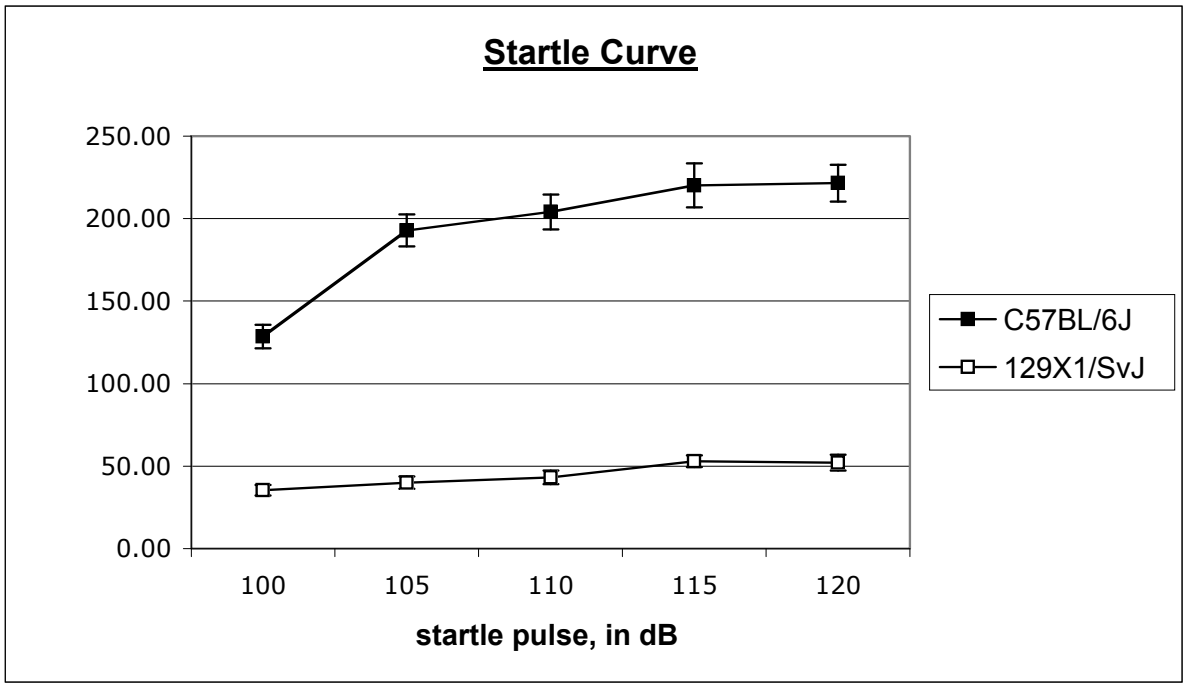
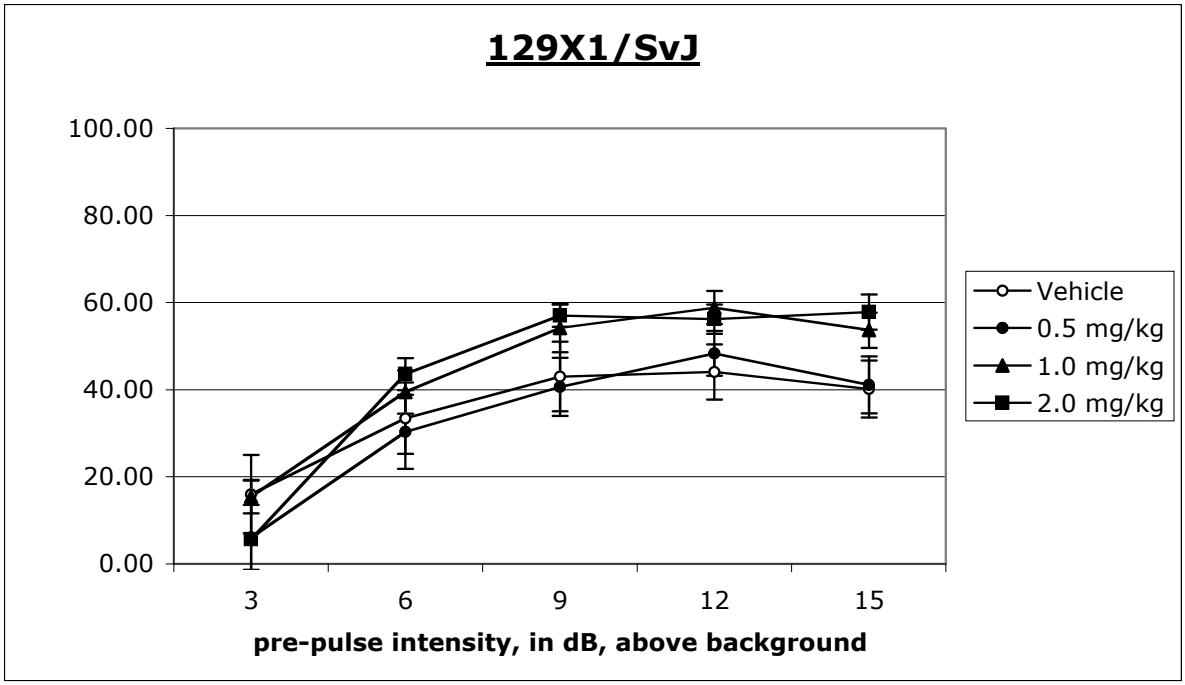


Figure 2. Mean startle amplitude (± SEM) of the C57BL/6J and 129X1/SvJ strains of mice, aged 2 months, over a range of acoustic startle stimuli, 100-120 dB in 5 dB increments.

Percent PPI was calculated at each pre-pulse level and was determined using the following equation: [(startle – PPI)/startle] x 100. The five trials at each pre-pulse level were then averaged giving the individual a percent inhibition score at each pre-pulse intensity. These data are presented in Figure 3. The three-way ANOVA (strain x pre-pulse intensity x dose) revealed no interaction between the three factors and no interaction between strain x dose and pre-pulse intensity x dose. There was a significant interaction between the strain and pre-pulse intensity, however, $F_{4, 252} = 10.46$, $p < 0.0001$. Simple main effects tests on the stain x pre-pulse intensity interaction and Tukey's post hoc tests revealed that both strains were inhibiting similarly at PPI3 and PPI15 but that the 129X1/SvJs showed significantly better inhibition at PPI6, 9, and 12. There was a significant main effect of dose, $F_{3, 63} = 3.03$, $p < 0.05$. However, subsequent Tukey's post hoc tests were only able to detect trends suggesting that administration of both 1.0 mg/kg ($p = 0.065$) and 2.0 mg/kg ($p = 0.074$) elevated PPI relative to vehicle administration.



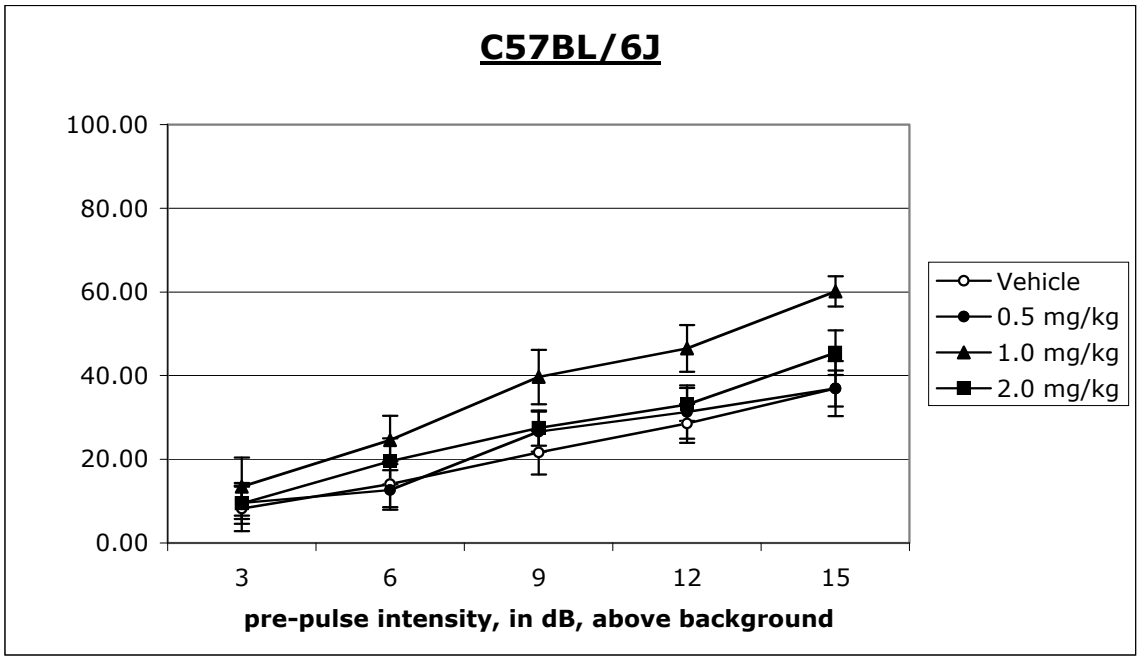


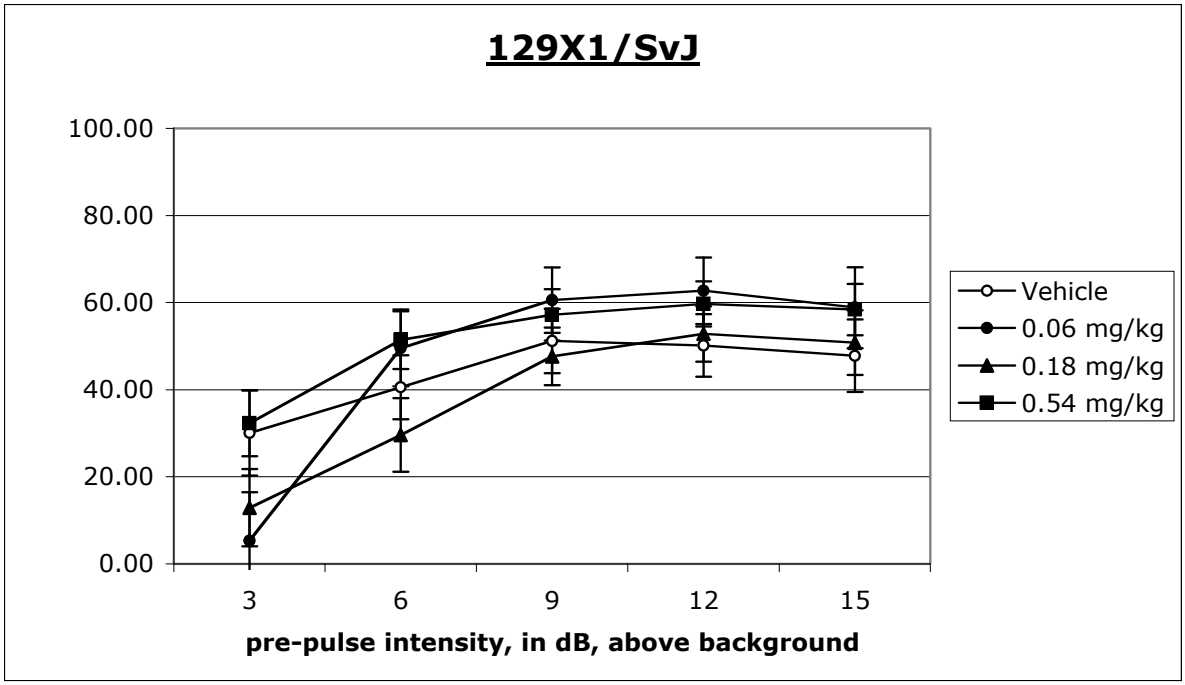
Figure 3. (Top) A comparison of percent inhibition (± SEM) in the PPI of an ASR paradigm between four groups of 2 month old 129X1/SvJ mice injected i.p. with nicotine or distilled water vehicle. (Bottom) A comparison of percent inhibition (± SEM) in the PPI of an ASR paradigm between four groups of 2 month old C57BL/6J inbred mice injected i.p. with nicotine or distilled water vehicle.

IV.1.2 RJR-2403 PPI

The results from the preliminary test assessing startle reactivity across the 100-120 dB range in the absence of drug were similar to those found for the nicotine cohort (i.e., C57BL/6J mice startled significantly more than 129X1/SvJ mice at all intensities, data not shown). With respect to the treatment groups following matching, startle amplitudes ranged from 201.80 ± 24.06 to 219.36 ± 25.80 for the C57BL/6Js and 54.80 ± 10.13 to 58.09 ± 12.57 for the 129X1/SvJs.

For the PPI test, startle amplitudes and percent inhibition were calculated as in section IV.1.1. A two-way ANOVA (strain x dose) calculated on the startle trials revealed a significant main effect of strain, the C57BL/6Js startling more (193.08 ± 8.04) than the 129X1/SvJs (53.74 ± 4.91), $F_{1, 64} = 232.59$, $p < 0.0001$, but no dose effect or interaction.

Figure 4 displays the percent pre-pulse inhibition for the four groups within each of the two strains. A three-way ANOVA (strain x pre-pulse intensity x dose) conducted on percent inhibition revealed a significant three way interaction, $F_{12, 256} = 3.68$, $p < 0.0001$, Simple main effects tests and subsequent Tukey's post hoc analysis revealed that the 129X1/SvJ strain had a higher percent inhibition than the C57BL/6Js at each of the pre-pulse intensities except PPI3 and 15 where both strains inhibited equally well. There was also significant difference between the 129X1/SvJ dosage groups at PPI3 with the 0.54mg/kg dosage group inhibiting significantly better than the 0.06mg/kg group. This trend was not seen in the C57BL/6J strain.



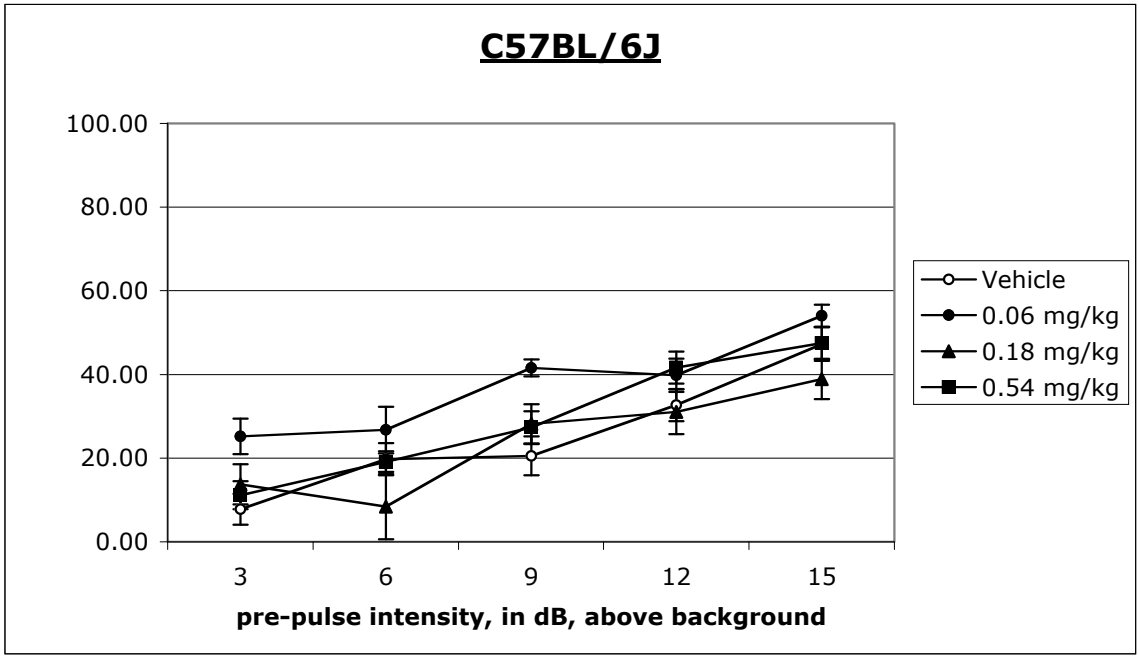


Figure 4. (Top) A comparison of percent inhibition (± SEM) in the PPI of an ASR paradigm between four groups of 2 month old 129X1/SvJ mice injected i.p. with RJR-2403 or distilled water vehicle. (Bottom) A comparison of percent inhibition (± SEM) in the PPI of an ASR paradigm between four groups of 2 month old C57BL/6J inbred mice injected i.p. with RJR-2403 or distilled water vehicle.

IV.1.3. Nicotine MWM

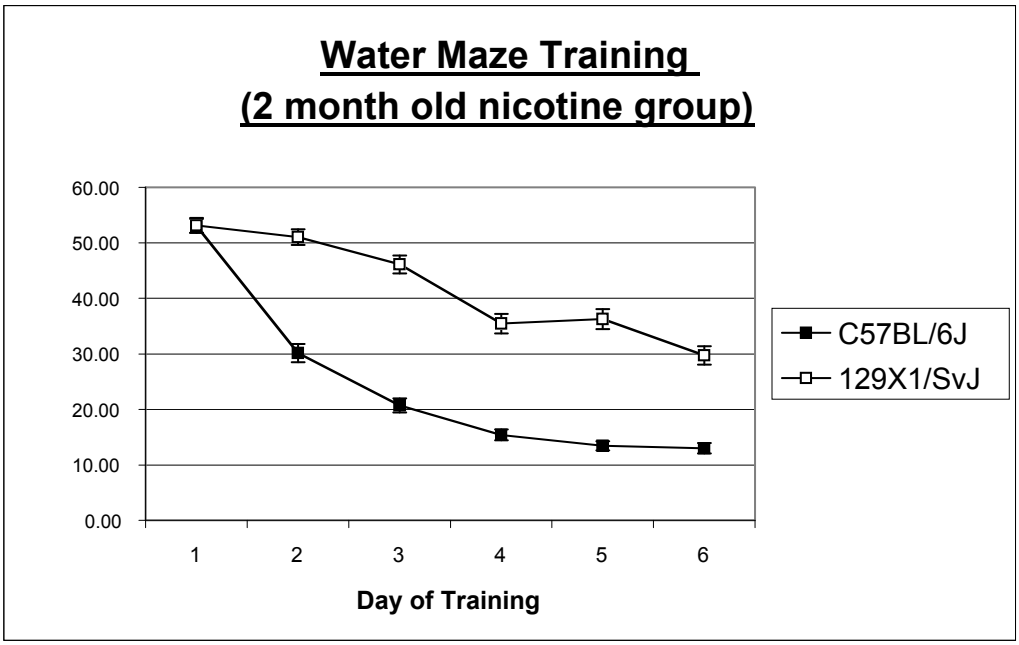
IV.1.3.1. MWM Training

An individual mouse's escape latency measure during the first 6 days of training was calculated by taking the mean of the 4 trials completed on the given day. The mean escape latencies for the two strains across the 6 training days are shown in Figure 5a. A two-way ANOVA (strain x day) conducted on the data revealed a significant interaction, $F_{5, 1410} = 23.53$, $p < 0.0001$. Simple main effects tests and Tukey's post hoc analysis revealed that the 129X1/SvJ strain had a longer escape latency than the C57BL/6J strain on all but the first day, where there was no significant difference between the groups.

IV.1.3.2. MWM Working Memory Training

Beginning on day 7 and continuing until the end of the experiment, escape latency was measured as the animal's time to find the platform on its second trial of each day. Figure 5b displays the trial 2 escape latencies for the three days of working memory training for the two strains. A two-way ANOVA (strain x day) revealed a significant main effect of day, $F_{2, 136} = 3.60$, $p < 0.05$, and strain, $F_{1, 68} = 8.14$, $p < 0.01$, but no interaction between the two. Subsequent Tukey's post hoc analysis showed that the C57BL/6Js found the platform more rapidly than the 129X1/SvJs and that the strains performed better on day 1 than on day 2 of working memory training due primarily to the poor performance of the 129X1/SvJs on day 2.

A.



B.

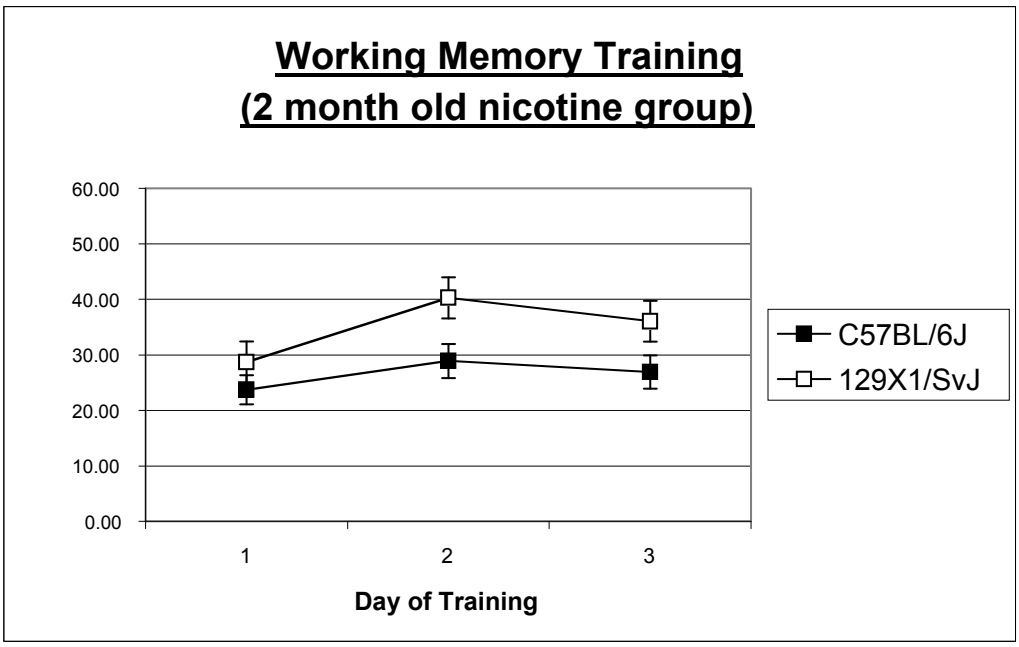


Figure 5a. Average escape latency (± SEM) of 2 month old C57BL/6Js and 129X1/SvJs over a 6 day training period in the Morris Water Maze. The platform remained in a fixed position over all 6 days. No drug was administered over this time period.
b. Average escape latency (± SEM) of the same individuals over 3 days of working memory training. During this period, the platform was moved to a different location each day. No drug was administered over this time period.

IV.1.3.3. MWM Working Memory Testing

The trial 2 latencies for both days at each inter-trial-interval (ITI) were averaged for each animal, and these data were used to calculate the means for each group. These data are displayed in Figure 6. A three-way ANOVA (strain x dose x ITI) revealed significant main effects for strain, $F_{1,61} = 25.79$, $p < .0001$, indicating that C57BL/6J mice found the platform more rapidly than did 129X1/SvJ mice, and for ITI, $F_{4,244} = 2.76$, $p < .05$. Subsequent Tukey's tests, however, failed to yield any significant differences between any pair of ITIs. The dose main effect, as well as all interaction effects, were not significant.

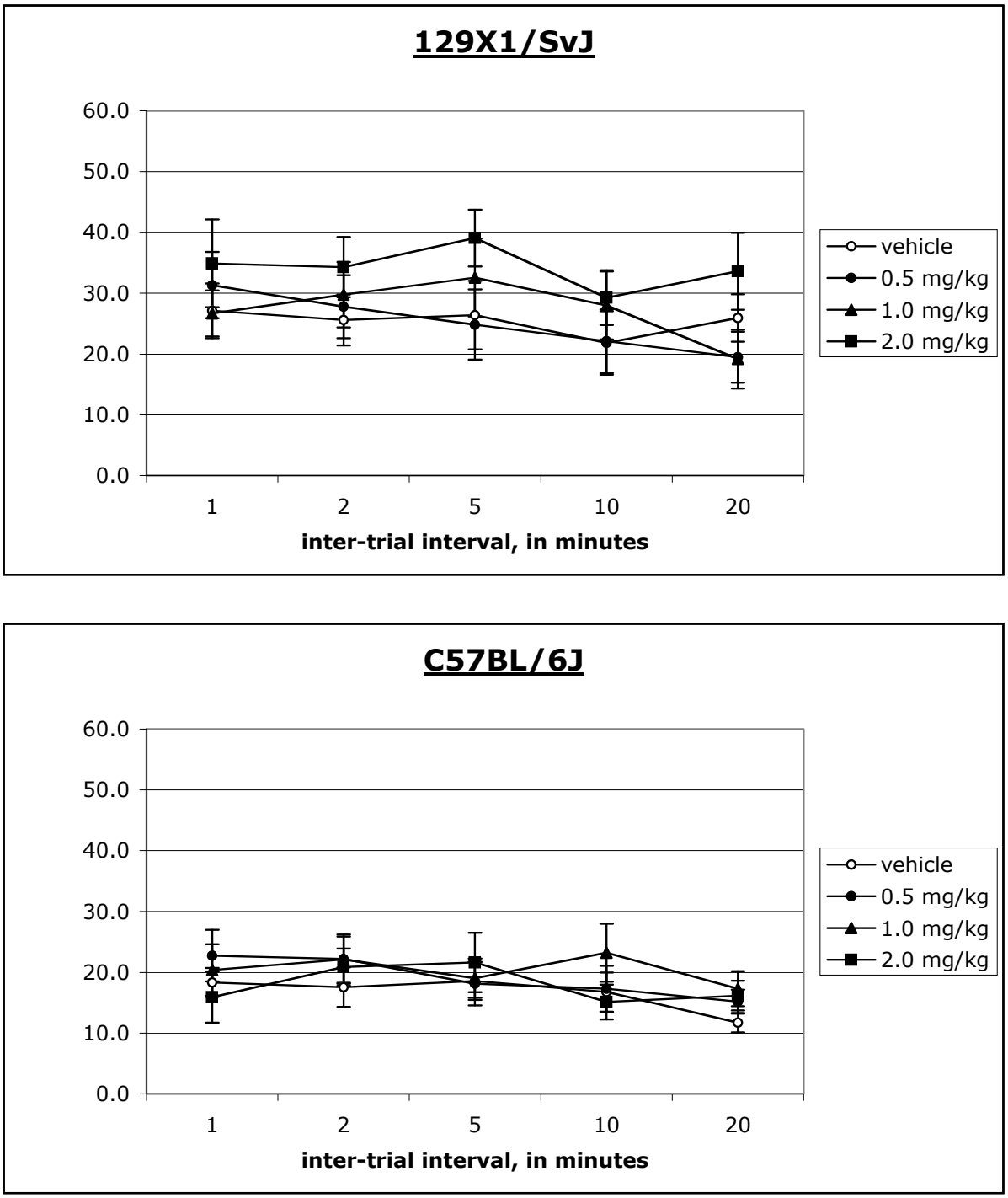


Figure 6. (Top) Mean escape latency (± SEM) of 2 month old 129X1/SvJ inbred mice injected i.p. with nicotine or vehicle in a working memory version of the Morris Water Maze. (Bottom) Mean escape latency (± SEM) of 2 month old C57BL/6J inbred mice injected i.p. with nicotine or vehicle in the same paradigm. The platform was moved following each day of training.

IV.1.4. RJR MWM

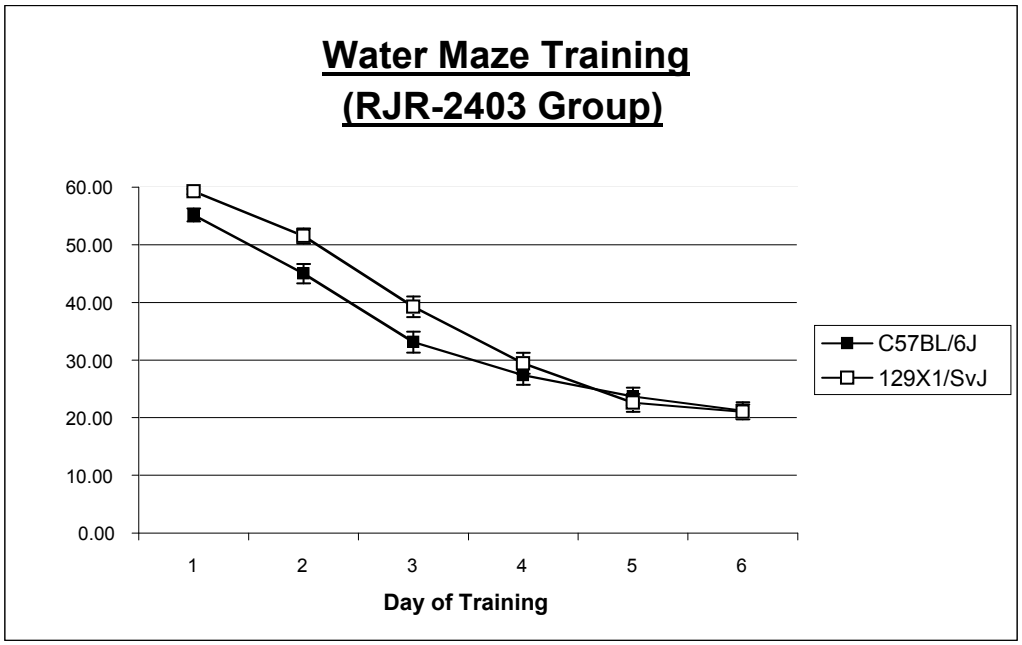
IV.1.4.1. MWM Training

Escape latencies were calculated as previously described and are displayed in Figure 7a. A two-way ANOVA (strain x day) conducted on the data gathered from the 6 days of training revealed a significant interaction effect, $F_{5, 1290} = 2.38$, $p < 0.05$. Subsequent simple main effects tests and Tukey's post hoc analysis revealed that the C57BL/6J strain performed better than the 129X1/SvJ strain on day 2 and 3 of training but, by day 4, the 129X1/SvJs were performing at a level equal to the C57BL/6Js.

IV.1.4.2. Working Memory Training

A two-way ANOVA (strain x day) conducted on the 3 days of working memory training (see figure 7b), revealed a significant main effect of day, $F_{2, 126} = 3.93$, $p < 0.05$. Ensuing Tukey's post hoc analysis showed that both strains performed significantly better on day 2 than on day 3. The strain main effect and the strain x day interaction were not significant.

A.



B.

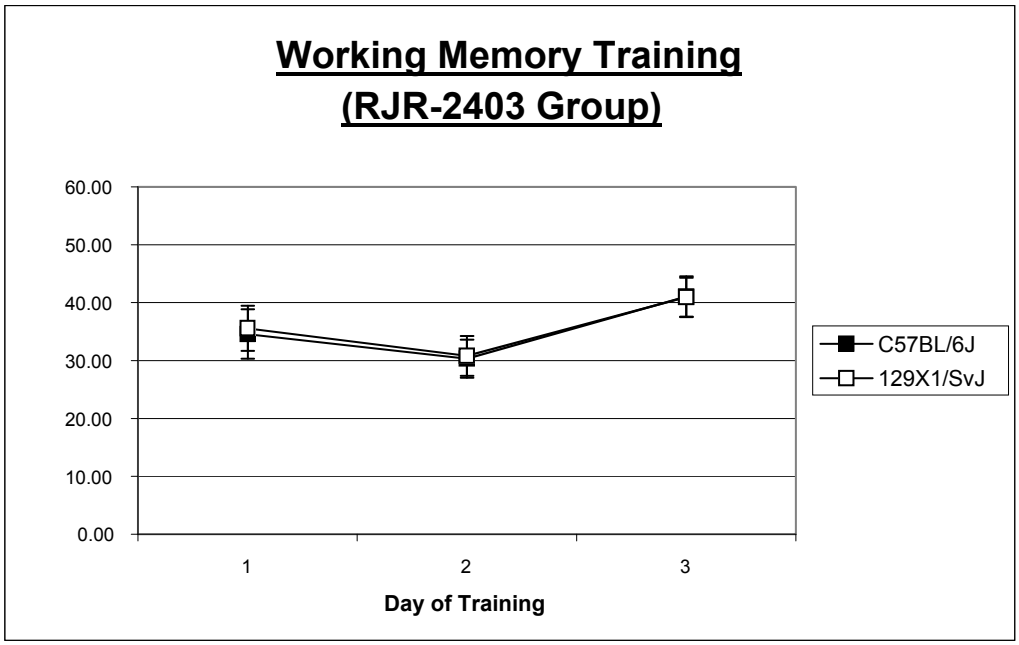
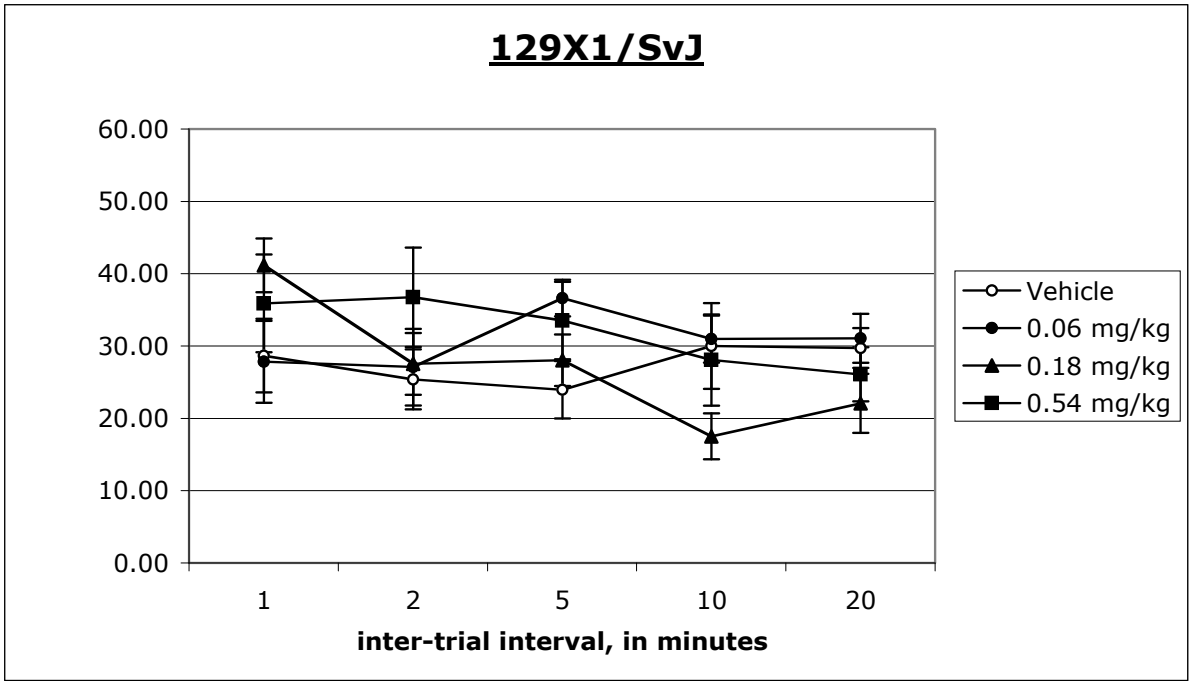


Figure 7a. Average escape latency (± SEM) of 2 month old C57BL/6Js and 129X1/SvJs over a 6 day training period in the Morris Water Maze. The platform remained in a fixed position over all 6 days. No drug was administered over this time period.
b. Average escape latency (± SEM) of the same individuals over 3 days of working memory training. During this period, the platform was moved to a different location each day. No drug was administered over this time period.

IV.1.4.3. MWM Working Memory Testing

Figure 8 displays the mean escape latencies, calculated as above in section IV.1.3.3., of the two strains over the ITIs following RJR-2403 or vehicle injection. The three-way ANOVA (strain x ITI x dose) performed on these data revealed a significant main effect of strain, $F_{1, 57} = 15.03$, $p < 0.001$. Tukey's post hoc analysis showed that the C57BL/6J strain consistently completed the maze in less time than the 129X1/SvJ strain (22.44 sec ± 1.04 versus 29.37 ± 1.06).



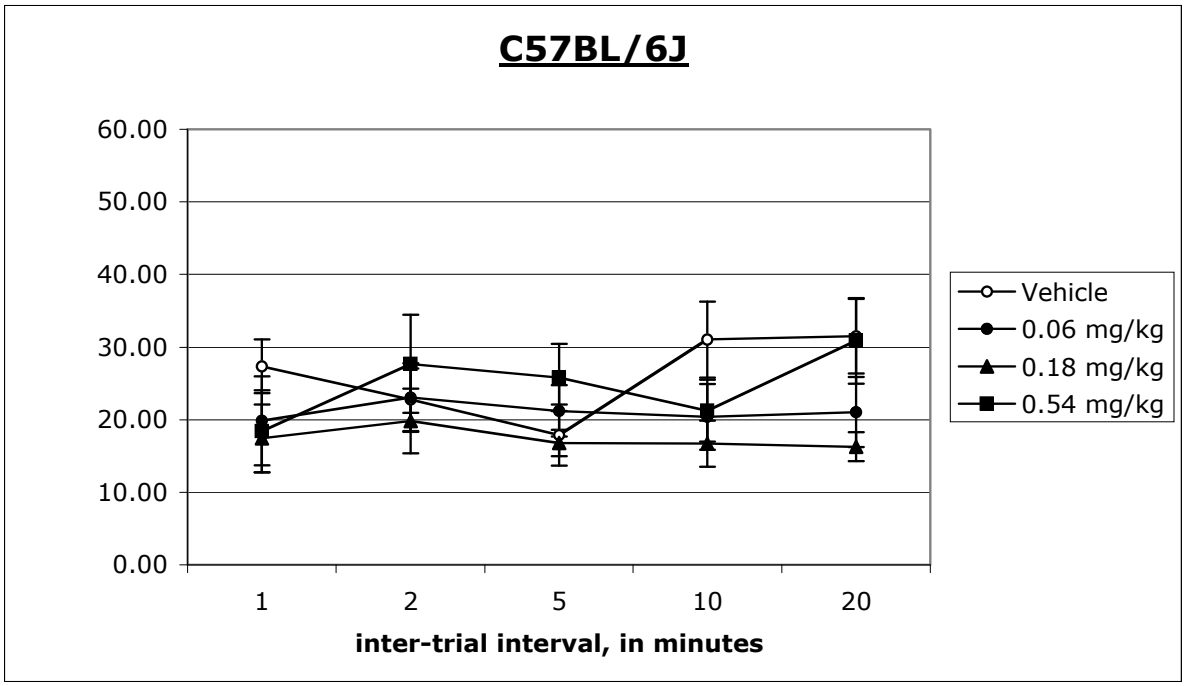


Figure 8. (Top) Mean escape latency (± SEM) of 2 month old 129X1/SvJ inbred mice injected i.p. with RJR-2403 or vehicle in a working memory version of the Morris Water Maze. (Bottom) Mean escape latency (± SEM) of 2 month old C57BL/6J inbred mice injected i.p. with RJR-2403 or vehicle in the same paradigm. The platform was moved following each day of training.

IV.2. Eight Month Old Mice

IV.2.1. PPI of an Acoustic Startle Response

Startle amplitudes and percent inhibition were calculated as in section IV.1.1. Figure 9 displays the startle curve seen in the 8 month old animals. Like the 2 month olds' startle curves, a two-way ANOVA (strain x intensity) yielded a significant main effect of both strain, $F_{1, 58} = 68.45$, $p < 0.0001$, and intensity, $F_{4, 232} = 30.00$, $p < 0.0001$. These results indicate that the C57BL/6J strain startled significantly more than the 129X1/SvJ strain and that both strains exhibited a higher startle amplitude with increased stimulus intensity. With respect to the treatment groups, C57BL/6J startle ranged from 106.18 ± 13.27 to 110.66 ± 14.26 while 129X1/SvJ startle amplitude ranged between 41.60 ± 8.43 and 43.36 ± 8.61.

A two-way ANOVA (strain x dose) calculated on the 10 startle trials during the PPI test revealed a significant main effect of strain, the C57BL/6Js startling more than the 129X1/SvJs, 89.43 ± 8.01 versus 36.93 ± 4.57, $F_{1, 54} = 34.73$, $p < 0.0001$, but no dose effect or interaction.

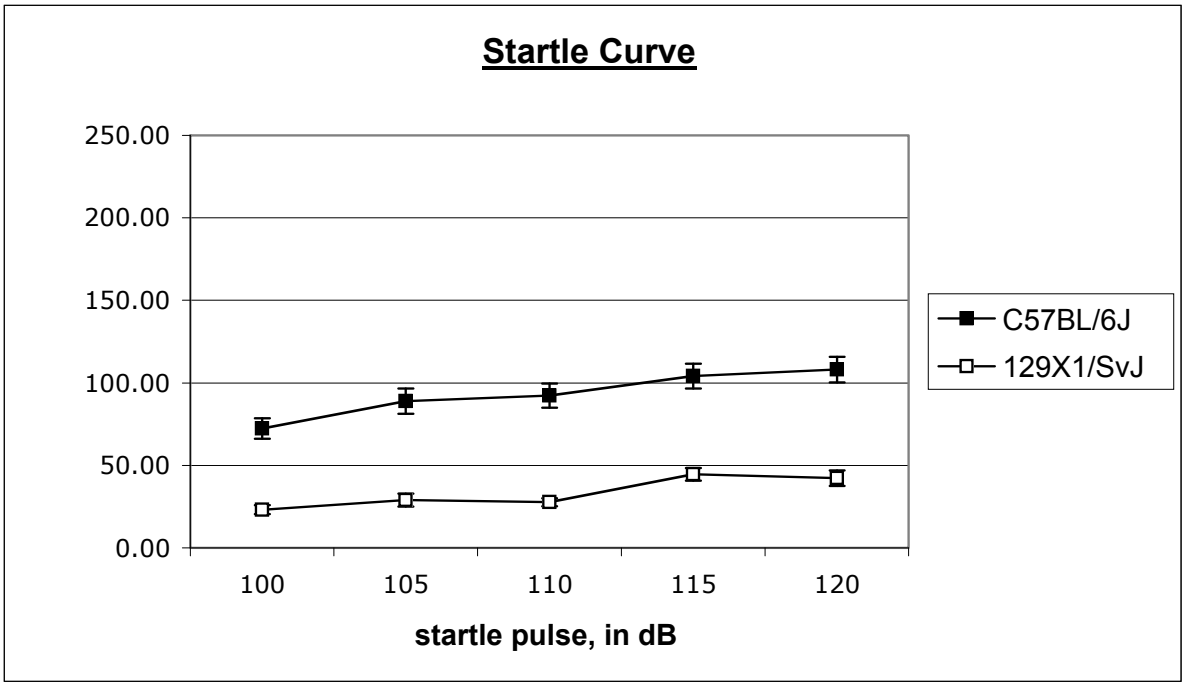


Figure 9. Mean startle amplitude (± SEM) of the C57BL/6J and 129X1/SvJ strains of mice, aged 8 months, over a range of acoustic startle stimuli, 100-120 dB in 5 dB increments.

A three-way ANOVA (strain x pre-pulse intensity x dose) on the percent pre-pulse inhibition measure (Figure 10) revealed a significant interaction between pre-pulse intensity x dose, $F_{8, 216} = 2.42$, $p < 0.05$, and an interaction between strain x pre-pulse intensity, $F_{4, 216} = 3.75$, $p < 0.01$. Post hoc analysis on the strain x pre-pulse intensity interaction using simple main effects tests and Tukey's post hoc analysis revealed that the primary difference was that the C57BL/6J strain were inhibiting significantly better than their 129X1/SvJ counterparts at each pre-pulse intensity. Subsequent simple main effects tests and Tukey's post hoc analysis performed on the pre-pulse intensity x dose interaction revealed no significant findings.

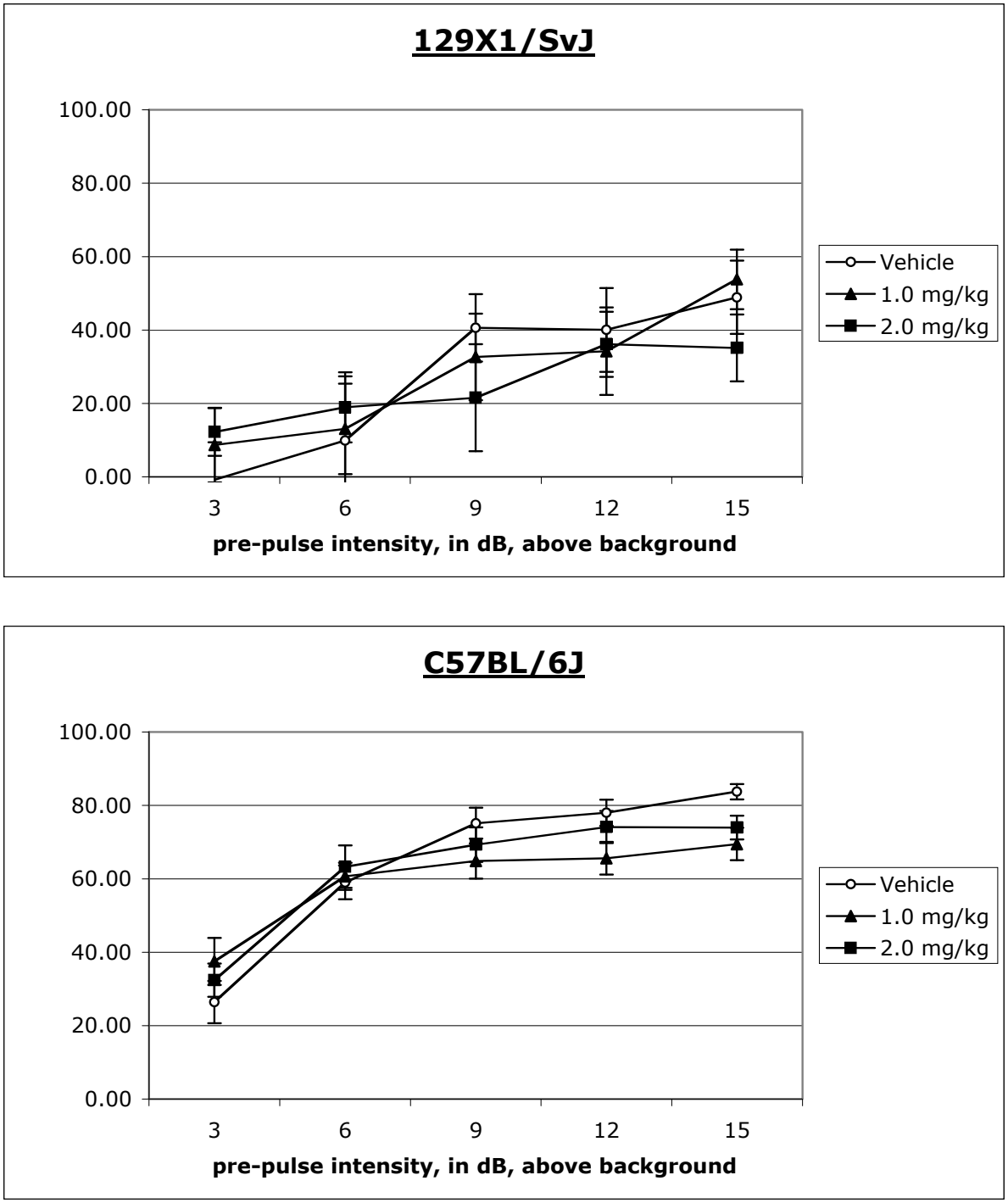


Figure 10. (Top) A comparison of percent inhibition (± SEM) in the PPI of an ASR paradigm between four groups of 8 month old 129X1/SvJ mice injected i.p. with nicotine or distilled water vehicle. (Bottom) A comparison of percent inhibition (± SEM) in the PPI of an ASR paradigm between four groups of 8 month old C57BL/6J inbred mice injected i.p. with nicotine or distilled water vehicle.

IV.2.1.1. Comparison between the age groups

In 2 month-old mice, the 129X1/SvJ mice displayed stronger PPI in relation to C57BL/6J mice, whereas in the older mice this pattern was reversed, in that the C57BL/6J mice showed stronger PPI. To further assess the nature of this reversal (i.e., to determine whether the C57BL/6J strain improved with age or whether the 129X1/SvJ strain deteriorated with age), two-way ANOVAs (age group x pre-pulse intensity) were conducted on the vehicle-treated groups (including both the nicotine and the RJR-2403 cohorts) within each strain. Analysis on the data from the 129X1/SvJ strain revealed a significant main effect between the pre-pulse intensities, $F_{4, 68} = 12.05$, $p < 0.0001$, but no age main effect or age x intensity interaction (Figure 11 top). However, the ANOVA conducted on the C57BL/6J data revealed a significant interaction effect, $F_{4, 68} = 14.59$, $p < 0.0001$. Subsequent analysis using simple main effects tests and Tukey's post hoc analysis revealed that the 8 month old group displayed a percent inhibition significantly higher than that of the 2 month old group (Figure 11 bottom). Analysis of the startle amplitude between the age groups using the Student-t test revealed further that, though there was no significant difference between the 129X1/SvJ groups, the C57BL/6J 8 month old group startled significantly less than their 2 month old counterparts, $t_{63} = 8.404$, $p < 0.00001$ (108.1 ± 7.80 for the 8 month olds versus 221.5 ± 11.11 for the 2 month old group).

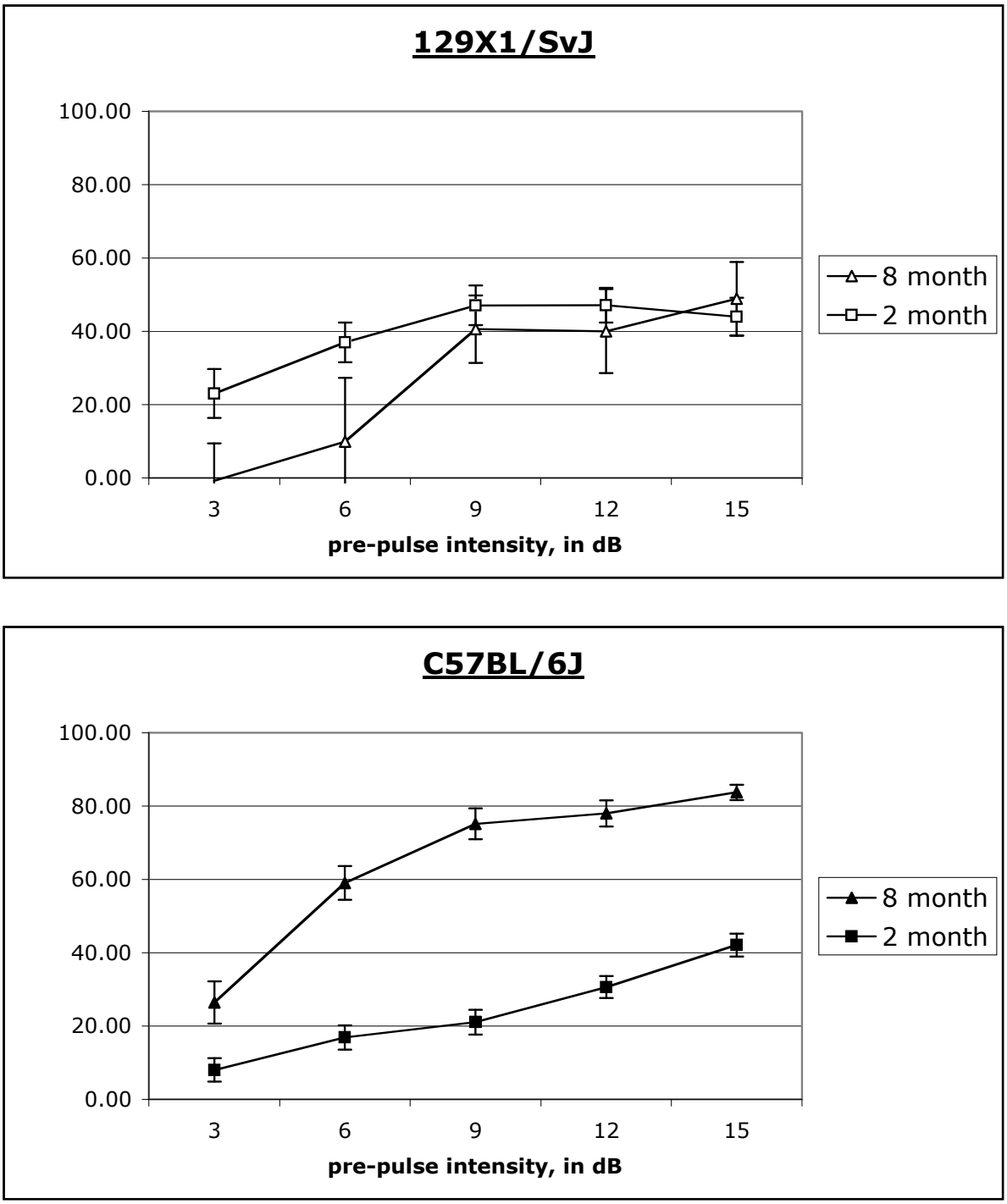


Figure 11. (Top) A comparison between percent inhibition (± SEM) of the 129X1/SvJ vehicle groups at the two ages, 2 and 8 months respectively, in the PPI of an ASR paradigm. (Bottom) A comparison between the percent inhibition (± SEM) of the C57BL/6J vehicle groups of the two ages, 2 and 8 months respectively, in the same paradigm.

IV.2.2. MWM

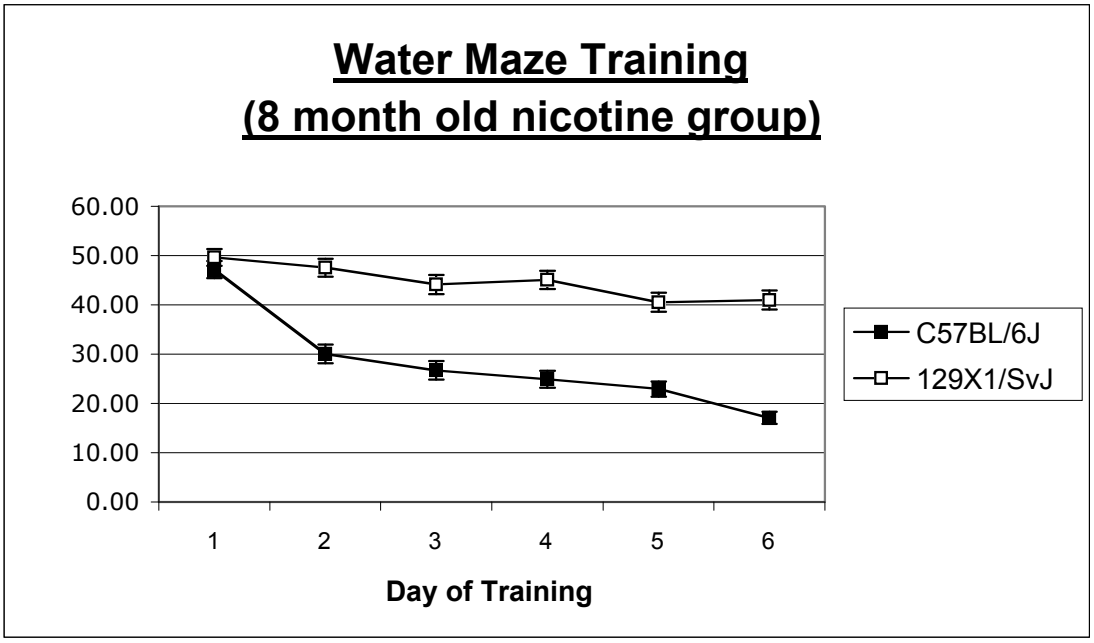
IV.2.2.1. MWM Training

A two-way ANOVA (strain x day) conducted on the data gathered from the first 6 days of training revealed a significant interaction effect, $F_{5, 1190} = 10.18$, $p < 0.0001$. Ensuing simple main effects tests and Tukey's post hoc analysis revealed that the C57BL/6Js were faster to find the platform than the 129X1/SvJs on all but the first day (Figure 12a).

IV.2.2.2. Working memory training

Analysis of the 3 days of working memory training (figure 12b) using a two-way ANOVA (strain x day) showed a significant interaction effect, $F_{2, 116} = 6.65$, $p < 0.01$. Simple main effects and Tukey's post hoc tests used to further analyze the data revealed that the C57BL/6Js performed better than the 129X1/SvJs on day 2 and that both strains performed better on day 1 than on day 2.

A.



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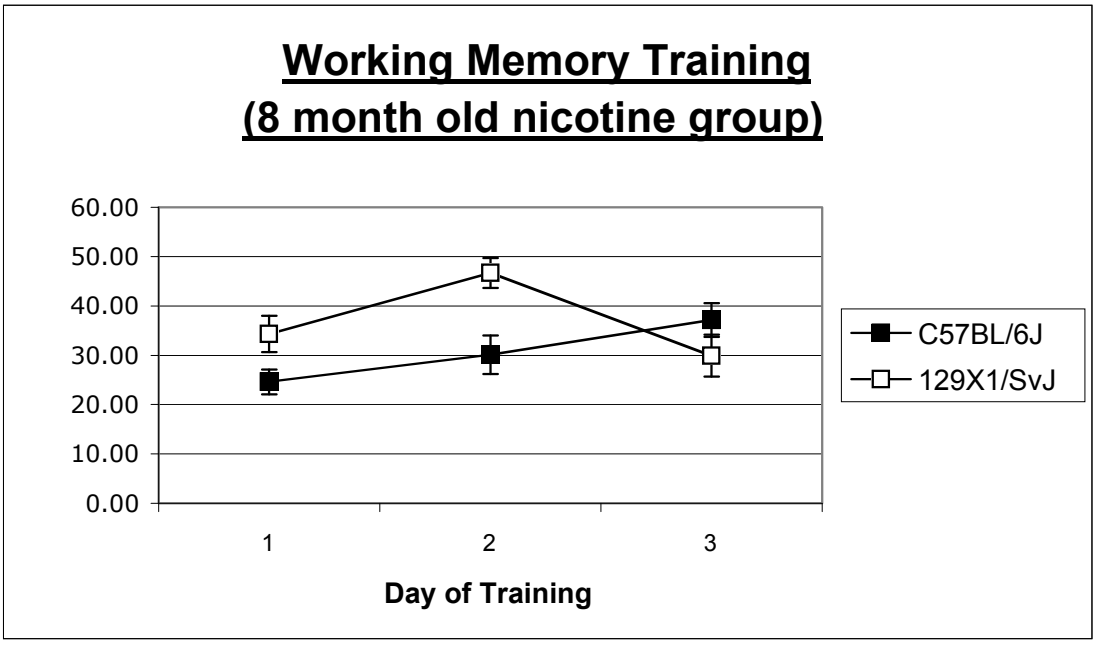


Figure 12a. Average escape latency (± SEM) of 8 month old C57BL/6Js and 129X1/SvJs over a 6 day training period in the Morris Water Maze. The platform remained in a fixed position over all 6 days. No drug was administered over this time period.
b. Average escape latency (± SEM) of the same individuals over 3 days of working memory training. During this period, the platform was moved to a different location each day. No drug was administered over this time period.

IV.2.2.3. Working Memory Testing

A three-way ANOVA (strain x ITI x dose) used to analyze the mean escape latencies (calculated as in section IV.1.3.3.) revealed a significant main effect of strain, $F_{1, 52} = 13.74$, $p < 0.001$, and ITI, $F_{1, 52} = 9.31$, $p < 0.01$. Ensuing Tukey's post hoc analysis revealed that the animals had shorter escape latencies at the 1 minute ITI (26.32 sec ± 2.01 versus 34.82 sec ± 2.17) and that the C57BL/6Js were solving the maze faster than the 129X1/SvJs (25.17 sec ± 1.91 versus 35.61 sec ± 2.17) (Figure 13).

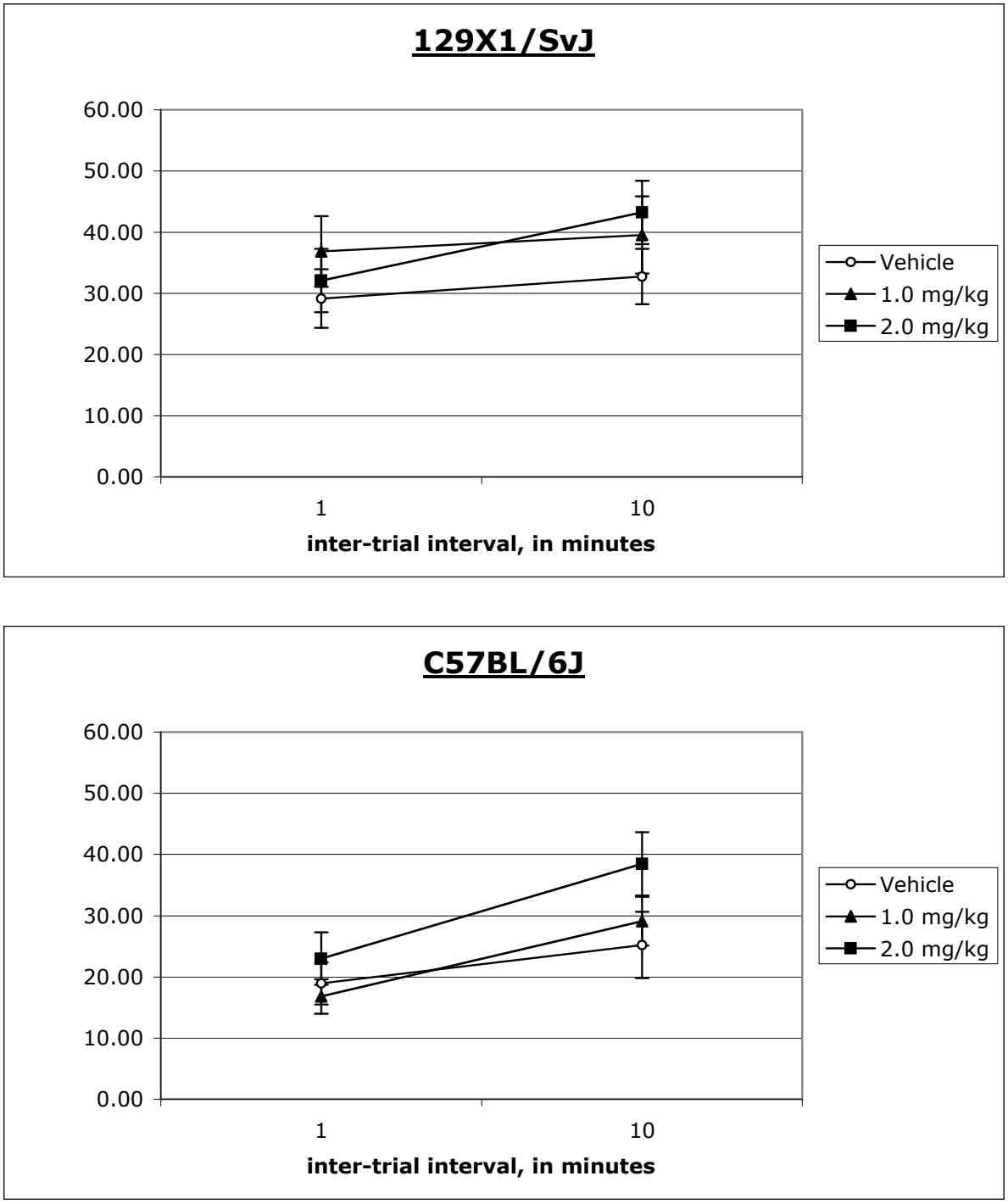


Figure 13. (Top) Mean escape latency (± SEM) of 8 month old 129X1/SvJ inbred mice injected i.p. with nicotine or vehicle in a working memory version of the Morris Water Maze. (Bottom) Mean escape latency (± SEM) of 8 month old C57BL/6J inbred mice injected i.p. with nicotine or vehicle in the same paradigm. The platform was moved following each day of training.

IV.2.2.4. Comparison between the age groups

To assess whether there were any direct effects of age in the various phases of the MWM test, we again collapsed the vehicle groups from the 2 month old cohorts and compared it to the 8 month old vehicle group for each strain. A two-way ANOVA (age group x day) performed on the C57BL/6Js during the first 6 days of training revealed a significant interaction effect, $F_{5, 1930} = 8.62$, $p < 0.0001$. The simple main effects tests and Tukey's post hoc analysis performed on these data revealed that the 8 month group had a shorter escape latency on days 1 and 2 (38.61 sec ± 1.40 versus 45.56 sec ± 0.84) while the 2 month old group exhibited a faster escape latency on days 4 and 5 (19.55 sec ± 0.67 versus 23.91 sec ± 1.16). The same analysis performed on the 129X1/SvJs also revealed a significant interaction effect, $F_{5, 1970} = 22.64$, $p < 0.0001$. Simple main effects tests and Tukey's post hoc analysis conducted on these data showed that, overall, the 2 month old group performed better than their 8 month old counterparts (39.60 sec ± 0.52 versus 44.66 sec ± 0.77).

A two-way ANOVA (age group x day) conducted on the C57BL/6J data gathered from the 3 days of working memory training showed a significant main effect of day, $F_{2, 188} = 4.45$, $p < 0.05$, but no age-dependent effect. The same analysis performed on the 129X1/SvJ data revealed a significant interaction effect, $F_{2, 194} = 4.13$, $p < 0.01$. The simple main effects tests conducted on these data showed that, while the groups are not different overall, the 2 month old group demonstrated a shorter escape latency on day 2 of the training (35.61 sec ± 2.58 versus 46.67 sec ± 3.06).

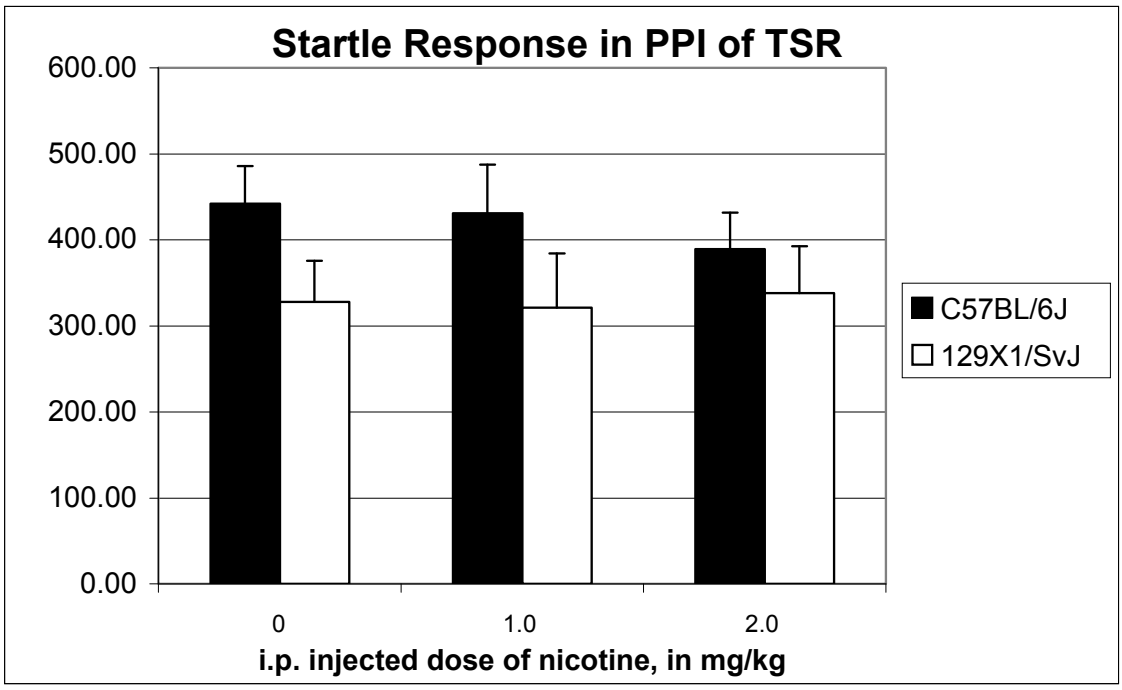
Comparison of both the C57BL/6J and 129X1/SvJ control groups' escape latencies from the experiment proper using a two-way ANOVA (age group x ITI), revealed no significant differences between the groups.

IV.2.3. Experiment 2c – PPI of a Tactile Startle Response

Despite the methodological differences, startle amplitudes and percent inhibition were calculated here as in section IV.1.1. A two-way ANOVA (strain x dose) conducted to determine differences in startle amplitude revealed a significant main effect of strain, $F_{1,52} = 4.55$, $p < 0.05$. Subsequent Tukey's post hoc analysis demonstrated that the C57BL/6Js startled with a greater amplitude than did the 129X1/SvJs (figure 14a)

A two-way ANOVA (strain x dose) conducted on the percent inhibition data demonstrated no significant differences (figure 14b).

A.



B.

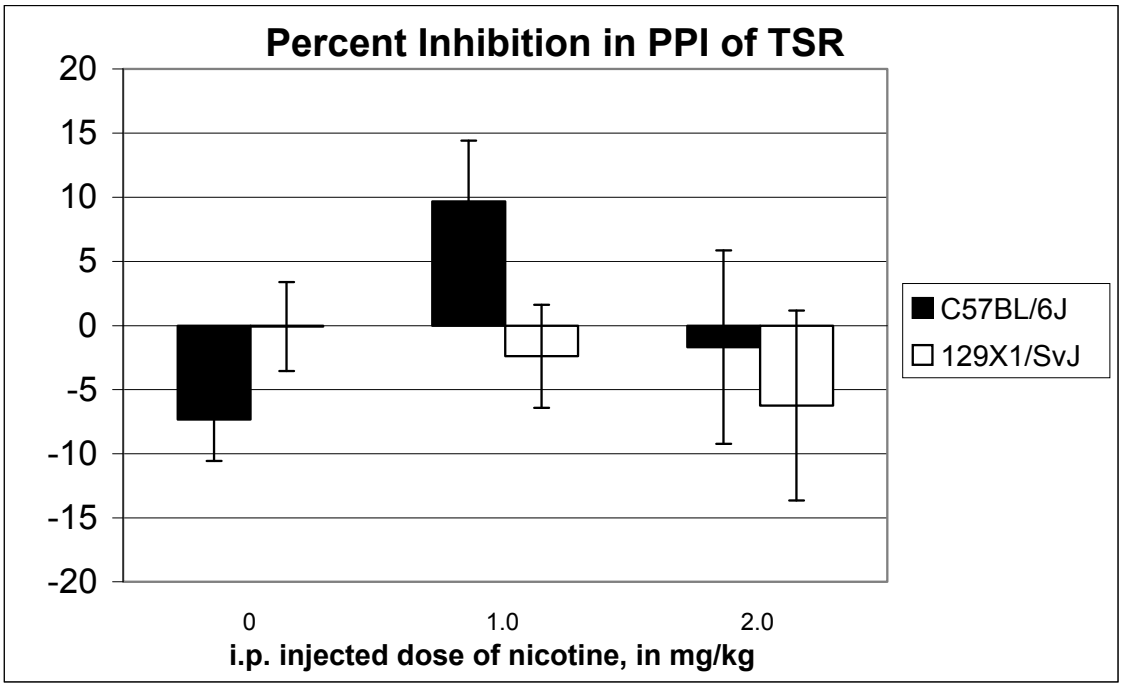


Figure 14a. Average startle amplitude (± SEM) of the C57BL/6J and 129X1/SvJ strains of inbred mice, aged 8 months, injected i.p. with nicotine or vehicle in the pre-pulse inhibition of a tactile startle response task.
b. Percent inhibition (± SEM) of 8 month old C57BL/6J and 129X1/SvJ mice injected i.p. with nicotine or vehicle in the PPI of a TSR paradigm.

V. DISCUSSION

In the present series of experiments, the effects of nicotine and the selective α4β2 agonist RJR-2403 were examined in both a sensorimotor gating and working memory paradigm in two strains of inbred mice of two different age groups. The intent of these studies was to determine if the enhancement of working memory that has been reported with nAChR agonist administration (see Levin and Simon, 1998 for review) is due to an increase in working memory capacity or if it is due to an increase in the power of an earlier sensory filtering mechanism. These experiments yielded the following principle findings:(1) Administration of 1.0 and 2.0 mg/kg of nicotine (but not of any RJR-2403 dose) produced a trend towards an increase in PPI in 2 month-old mice from both strains. However, this trend was not observed in 8 month-old animals in either strain. (2) An age-dependent reversal in the magnitude of PPI between the two strains in that, in comparison to the 129X1/SvJ strain, C57BL/6J mice displayed a lower level of inhibition at 2 months of age and a higher level of inhibition at 8 months of age. (3) The 129X1/SvJ mice tended to perform more poorly in all components of the MWM task (i.e., the training, working memory training and working memory testing phases) in comparison to C57BL/6J mice. Additionally, there were no marked effects of drug administration or age in the MWM working memory testing phase. Each of these three principle findings will be discussed in detail below.

V.1. Age-related drug effects in the PPI of an ASR

As noted above, one of the principle findings of this experiment was that the 2 month old mice injected with 1.0 mg/kg or 2.0 mg/kg of nicotine (but not RJR-2403)

tended to display a higher percent inhibition than vehicle-treated mice. This trend would seem to indicate that nicotinic administration does, in fact, enhance sensorimotor gating capacity in C57BL/6J and 129X1/SvJ mice. This conclusion is supported by the results from previous studies demonstrating that nicotine administration enhances PPI in other animal models (Schreiber *et al*., 2002; Acri *et al*., 1994). Obviously, one objection to this conclusion stems from the fact that the augmenting effect of nicotine administration on PPI was not statistically significant but, rather, a statistical trend (i.e., probability values were in the range $0.05 < p < 0.10$). The reasons for this outcome are unclear but may reflect a lack of statistical power. Although the group sizes employed in this experiment were sufficiently large to generate a significant main effect for dose, it is important to realize that subsequent Tukey's analysis across the range of the means for the 4 doses employed in this experiment assessed each pair-wise comparison not at the 0.05 level, but at the 0.0103 level (as determined using the statistical package *Datasim*, Bradley, 1988). Using the statistical package G*Power (Erdfelder *et al*., 1996), we calculated that the power to detect the maximum between-group difference observed in this experiment (mean = 40.59 for the 1.0 mg/kg nicotine group vs. 28.36 for the vehicle-treated group) with a group size of 9 was 85%. Additionally, it was determined that a group size of 12 would be required to achieve 95% power. Thus, in retrospect, it would have been optimal to add three subjects per group in this experiment to increase the ability to detect a significant dose effect.

Additionally, it is possible that the dose range of nicotine employed in this experiment was not optimal to evoke a more robust enhancement of PPI. For instance, Schreiber *et al*. (2002) reported that optimal enhancement of PPI in mice was observed at

a dose of 3mg/kg. The results observed in the present experiment, namely that 0.5 mg/kg was without effect but that 1.0 and 2.0 mg/kg were able to evoke a trend towards augmentation, are consistent with the suggestion that a higher dose of nicotine, such as 3.0 mg/kg, would have evoked a statistically significant augmentation of PPI.

As has been previously reported (Paylor *et al*., 1996), vehicle-treated 2 month old 129X1/SvJ mice displayed higher levels of PPI in comparison to mice from the C57BL/6J strain. Vehicle-treated 129X1/SvJ mice displayed lower levels of startle reactivity both in the startle test assessing reactivity across the 100-120 dB range and on the pure startle (i.e., pulse) trials that were presented during the PPI test session. This raises the possibility that the enhanced PPI observed in vehicle-treated mice may have resulted from the reduced startle reactivity. The suggestion here is that it may be easier to inhibit a weak startle response. This hypothesis in turn raises the possibility that the enhanced PPI evoked by nicotine administration might have occurred indirectly, through a modification of the startle response itself. There are, however, two difficulties with this latter hypothesis. First, nicotine administration did not alter startle reactivity to the 120 dB pulse trials in 129X1/SvJ (or, for that matter, in C57BL/6J) mice, as evidenced by the non-significant main effect of dose and strain x dose interaction. Second, nicotine was able to enhance PPI in both strains. As mentioned above, however, 129X1/SvJ and C57BL/6J mice exhibited marked differences in their startle reactivity. Therefore, considered collectively, these results suggest that nicotine enhanced PPI directly through an action on mechanism(s) involved in sensorimotor gating, as opposed to an indirect effect mediated through changes in startle reactivity. Moreover, the fact that nicotine was able to enhance PPI in both strains suggests that this compound is able to enhance

sensorimotor gating function independent of the basal level of function. That is, it can enhance PPI in animals that normally display relatively poor (eg. 129X1/SvJ) and relatively more robust (eg. C57BL/6J) sensorimotor gating.

Whereas nicotine administration resulted in a trend towards enhanced PPI, no such tendency was observed following administration of the centrally acting and selective α4β2 receptor agonist RJR-2403. To our knowledge, this is the first time that this compound has been assessed in a sensorimotor gating task. The inability of RJR-2403 to influence PPI therefore fails to support the hypothesis that centrally located α4β2 nAChRs are involved in PPI. It is, of course, possible that the dosages tested in the present experiment were not within the effective dose range. It is worth reiterating that the doses of RJR-2403 employed were molar equivalents to the nicotine doses that were administered. However, it is also possible that differences in pharmacokinetics (i.e., binding affinities) could account for the differential effects of nicotine and RJR-2403. Additionally, there is evidence to suggest that the ability of a variety of nAChR agonists to influence processes involved in stimulus filtering is restricted to a relatively narrow window within the dose response curve (see, for example, Rochford *et al*., 1996).

Despite the reservations listed above, the question remains as to why, within the dose ranges used, nicotine, but not RJR-2403, was able to exert the trend towards an augmentation of PPI. A difference in pharmacokinetics is one possibility but there are others. First, given that RJR-2403 is centrally acting, whereas nicotine can have both central and peripheral effects, it is possible that the peripheral actions of nicotine may have contributed to the PPI augmentation. This suggestion may appear puzzling at first, given that cognitive function is generally thought to be centrally mediated; however,

there are a number of ways through which nicotine's actions on the peripheral nervous system could influence cognitive function. To illustrate but one example, catecholamine release from the adrenal gland is mediated primarily through nicotinic receptors (McGaugh, 1983). Enhanced adrenal catecholamine release could enhance cognitive function either by modulating activity of central transmitters systems (McGaugh, 1983) or by increasing glucose availability and utilization in the brain (Wenk, 1989). We hasten to add that we are not committed to this particular mechanism; we mention it only to indicate how it would be feasible to suggest that a peripheral action of nicotine could translate into improved cognitive function. One way in which the hypothesis of a peripheral site of nicotine's actions could be tested is by examining the ability of the peripherally acting nAChR antagonist hexamethonium to reverse the augmenting effect of nicotine on PPI.

An alternative hypothesis is that nicotine may have an action at a receptor other than the $\alpha4\beta2$ subtype and that RJR-2403 is inactive at this receptor subtype. This suggestion, of course, raises the issue of the identity of this potentially important alternative receptor. As alluded to in the introduction, in addition to the $\alpha4\beta2$ receptor, the $\alpha7$ nAChR has been strongly implicated in the control of cognitive function. Because of the specificity of RJR-2403, it may be that its lack of effect was due to lack of $\alpha7$ stimulation. There are, however, two problems with this hypothesis. First, $\alpha7$ knockout mice display normal levels of PPI when compared to wild-type controls (Paylor *et al*., 1998). Second, two separate studies have shown that administration of the selective $\alpha7$ receptor agonists GTS-21 and AR-R17779 do not influence PPI in a variety of mouse and rat strains (Schreiber *et al*., 2002; Olivier *et al*., 2001). Given this evidence, it seems

unlikely that nicotine may have been acting through α7 nAChRs. Though it is possible that nicotine exerted its effects through other nAChRs (eg. α3β2 subtype), insufficient research has been conducted on these other subtypes to make any firm conclusions.

A final explanation for the trend and lack of RJR-2403 effect is one that involves the strain of the animals tested. Nicotine has been found not only to having varying effect in the PPI paradigm across species (Schreiber *et al*., 2002) but also to vary in its effectiveness across strains (Acri *et al*., 1995). It may be then that the doses of each drug used were not appropriate for the strains tested.

As mentioned above, the nicotine trend was only apparent in the 2 month old mice and not their 8 month old counterparts. This outcome contrasts with the results reported by Acri *et al*. (1995), who found that in three strains of rats (Wistar, Long-Evans, and Sprague-Dawley) nicotine improved PPI in 70-day old animals but was without effect in 40-day old animals. Two marked procedural differences between the present study and that of Acri *et al*. could potentially explain this discrepancy. First, it is possible that species differences (mouse versus rat) could explain this difference, although exactly how and why this factor would be important is difficult to identify. Second, note that the age of the rats that were found to be sensitive in the Acri *et al*. study (70 days) was very similar to the age of the mice (approximately 60-70 days) that were found to respond to nicotine in the present experiment. Assessing nicotine's effects at either an earlier (eg., 40 days as in the Acri *et al*. study) or a much later (8 month as in the present study) would indicate a lack of effect in both rats and mice. Put more specifically, there may be a relatively restricted "age-window" within which nicotine may be active in the PPI paradigm and this window is species-independent. Clearly additional studies will

be required in order to assess the validity of this hypothesis and, if it is proven to be the case, the neurobiological mechanism that would be involved. For example, it may be that 8 month old animals do not respond to nicotine because of the age-related loss of presynaptic nicotinic receptors (see Introduction) which would result in a reduction of nicotine-stimulated ACh release. The reason why younger animals would be insensitive to nicotine is, at present, equally unclear, although Acri *et al*. advanced the hypothesis that 40-day old male rodents are sexually immature as evidenced, in part, by lower androgen levels. Lower androgen levels could influence the rate of nicotine metabolism, which in turn effectively alter nicotine availability.

V.2. Age-dependent enhancement of PPI in C57BL/6J mice

With respect to the PPI of an ASR data, the most striking finding was that of the age related change in percent inhibition between the 2 month and 8 month old C57BL/6J mice. The most plausible explanation for this variation involves the phenomenon of hearing-loss induced plasticity. Specifically, the C57BL/6J strain is known to suffer from high frequency (> 20 kHz) hearing loss by 5 months of age due to outer hair cell degeneration and loss in the cochlea (Willott *et al*., 1994). Paradoxically, animals exhibiting this hearing loss also demonstrate an enhanced response to mid-range (eg. 12 kHz – 16 kHz) frequencies (Falls *et al*., 1997). The loss of some hearing ranges but increase in the salience of others has been dubbed "hearing-loss induced plasticity" (HLIP) and is a result of distinct neurological changes within the hearing pathway itself (Willott *et al*., 1994). The major neurological alteration in HLIP occurs within the inferior colliculus and is evidenced by a change in the recruitment of frequency

processing neurons in that, as high frequency processing areas are lost, the area for processing mid-range frequencies is seen to expand (Carlson and Willott, 1996). If hearing loss is indeed the underlying cause for the difference in percent inhibition exhibited by the C57BL/6Js, an observation which seems to be supported by the age related decrement in startle amplitude, the present study adds further credence to the hypothesis that certain kinds of hearing loss may actually increase the reaction, on a behavioural level, to areas of the individual's remaining auditory spectrum.

In order to derive a measure of sensorimotor gating that was not affected by hearing loss, the PPI of a TSR paradigm was used in conjunction with the 8 month old animals. Regrettably, no pre-pulse inhibition seemed to occur in either strain regardless of nicotine dosage. The reasons for this finding are difficult to ascertain especially because pre-pulse inhibition was readily evident in the PPI of an ASR task. However, this lack of PPI in the TSR task has been reported by other labs and the underlying mechanism(s) involved is currently under investigation (Torkamanzehi *et al*., 2003).

V.3. No effects of ITI, age, and drug administration in the MWM

In the MWM, both 2 month and 8 month 129X1/SvJ mice were impaired relatively to similarly-aged C57BL/6J mice. This finding replicates previous evidence (Stavnezer *et al*., 2002; Owen *et al*., 1997), demonstrating that this mouse strain performs poorly in the MWM. One potential reason for the poorer performance of the 129X1/SvJ in the MWM is that they are an albino strain and, thus, may suffer from visual impairments which would hinder acquisition of the hidden platform in this task. Age-related visual pathology has been studied in the 129X1/SvJ strain, however (Hengemihle

et al., 1999), and while 52% of mice over 17 months old had overt visual pathologies, 5 month old animals displayed a very low incidence of visual-related problems. Given that there was no statistical difference between the 129X1/SvJ age groups in MWM performance in the present study, we believe the impairments seen in these mice likely resulted from a cognitive, as opposed to a visual dysfunction.

Although there were some exceptions (discussed below), the 129X1/SvJ mice generally performed more poorly on all three variants of the MWM task (i.e., the training, working memory training and working memory testing phases). This makes it difficult to determine whether the 129X1/SvJ mice suffer from impaired working or reference memory function (or both).

Of the three-phases, the training phase would most likely be the phase in which reference memory function is most taxed. This is so because the animal must learn the general rules and effective search strategies needed to solve the task (i.e., that there is a platform located somewhere in the pool and that the animal must use the extra-maze spatial cues in order to identify its location). Assuming the animal has learned these rules as best as they can be acquired, the subsequent phases, namely, working memory training and working memory testing, would more or less exclusively tax working memory. This is so because the animal is now forced to retain the new location of the platform on each day. If, however, the animal has not fully accommodated the information needed for optimal reference memory function during training, then a deficit during the working memory training and testing phases could be due to sub-optimal reference memory function. In the 2-month old nicotine cohort and the 8-month old mice, performance of 129X1/SvJ mice was impaired on days 2-6 of the training phase relative to the C57BL/6J

mice. Thus, given the arguments outlined above, the continued poor performance of these mice in the working memory training and testing phases could have resulted from poor reference memory function rather than working memory function.

Moreover, it is interesting to note that (for reasons that are unclear) the 129X1/SvJ mice in the RJR-2403 cohort displayed a more modest impairment during the training phase. Specifically, these mice displayed longer escape latencies than did the C57BL/6J mice only on days 2-3 of training, from days 4-6 these animals performed as well (at least statistically) as did the C57BL/6J mice. This would suggest that these mice acquired reference memory function as effectively as the C57BL/6J mice. To complete the argument, it is worth noting that the performance of these same 129X1/SvJ animals in the working memory training and testing phases was not impaired. Thus, it would appear that animals displaying deficits during training also show deficits during working memory training and testing, whereas those that do not display significant reference memory deficits during training do not show working memory training or testing deficits. This finding would appear to be most parsimoniously interpreted as reflecting primarily a reference memory deficit in 129X1/SvJ mice.

This conclusion could also, in principle, explain the lack of effect of both nicotine and RJR-2403. Nicotine and other nAChR agonists have been repeatedly demonstrated to enhance working memory function without affecting reference memory function (see Levin and Simon, 1998 for review). Assuming that the impairment observed in 129X1/SvJ mice during the working memory training and working memory testing phases resulted more from a deficit in reference memory, as opposed to working memory function, it is not surprising that both nicotine and RJR-2403 were unable to rectify the

impairment. One drawback to this conclusion is that these agonists were also without effect in C57BL/6J mice. However, the facilitative effects of nAChR agonists are most evident in animals with working memory deficits; these agents evoke more modest, if any, effects in animals with normal working memory function presumably because working memory is already functioning at near-optimum levels. Thus, the lack of effect of nicotine and RJR-2403 in C57BL/6J mice may reflect a ceiling effect.

The working memory testing phase was designed to tax working memory function by assessing performance over increasing ITIs. The ability of working memory to hold task-specific information is presumed to be reduced with the passage of time. Therefore, one puzzling result from the working memory testing phase was that there were no marked or consistent differences between the ITIs. That is, in general, performance at the 20 min ITI was equivalent to that observed following the 1 min ITI. This implies one of two conclusions. First, during the working memory testing phase, animals were fully able to retain, over at least a 20 min period, the new platform location. This would be demonstrating a particularly long-lived working memory. To the best of our knowledge, the precise relationship between ITI-length and performance in the working memory task has not been extensively examined. Indeed, our decision to use the ITI durations that were employed was based on an examination of studies that have employed but a single ITI in each experiment and, generally, these ITIs were no longer than 10 minutes. The relationship between ITI and working memory function has been more precisely defined in the radial arm maze test and it has been shown that rodents can retain spatial information in working memory for as long as 2 hours (Maki *et al*., 1984).

Accordingly, a more pronounced effect of ITI might have been obtained had longer ITIs been used.

The second explanation for the lack of ITI differences could be that the animals were not trained long enough in the working memory paradigm. Whereas they learned the specific rules needed for reference memory function during the 6 days of the training phase, they may not have been given sufficient training (only 3 days) to accommodate the new contingencies imposed by the working memory paradigm (that is, that the platform changes location daily). Hence, there was no shift in acquisition time over the ITIs because the animals were randomly acquiring the platform on each trial.

In contrast to the results obtained in the PPI paradigm, there was no effect of age in the MWM. There are two possible reasons for this finding. First, it may be that our animals were too young to exhibit age-related deficits. Indeed, we appreciate that our "old" group of animals is misclassified in that 8 month old mice are more accurately categorized as "middle-aged". It would have been preferable to have tested older animals (eg. 27-28 months old) however all animal suppliers approached did not provide animal in this age range and time constraints imposed by the completion of this thesis made this option unfeasible. Furthermore, Bernstein *et al*. (1985) found no age-related differences in a working memory variant of the radial arm maze task between 8 month old C57BL/6J mice (classified as "young" by Bernstein *et al*.), and 27-28 month old animals (we are unaware of any evidence that has examined working memory performance in 129X1/SvJ mice within this extended age range). Thus, the C57BL/6J strain may be immune to age-related working memory impairments. Second, although age-related cognitive dysfunction, as demonstrated by poor performance in the MWM, has in some cases been

found to correlate with atrophy of cholinergic neurons in the NBM (Winkler *et al*., 1998), the correlation between cholinergic and spatial learning dysfunction has not been consistently shown and this may be due to differences in the strains or species tested (Sirvio, 1999; Ikegami, 1994). Hence, it may be that the C57BL/6J and 129X1/SvJ strains simply do not suffer from age-related cholinergic dysfunction. Along these lines it is also interesting to note that Bernstein *et al*. (1985) reported no age-related differences in ChAT activity in the 8 month and 28 month old C57BL/6J mice that were tested in the radial arm maze. It is, of course, possible that the use of other markers of cholinergic activity (eg., muscarinic or nicotinic receptor status) may have revealed age-related deficits; however, experiments assessing this possibility have as yet to be conducted in C57BL/6J or 129X1/SvJ mice.

Unlike in the PPI paradigm, where nicotine was found to produce a trend towards improved sensorimotor gating, both nicotine and RJR-2403 did not influence performance in the working memory testing phase of the MWM task in both strains of mice at both ages. As discussed previously, one explanation for the lack of drug effect may have to do with the doses employed. Because of the variability due to individual differences and/or strain on the sensitivity to nicotine (and possibly RJR-2403) and the narrow effective dose range for the drug, the doses tested in this experiment may have been too far above or below the behaviourally salient dose (Gould and Wehner, 1999b; Levin, 1992).

One additional issue that needs to be addressed at this point concerns that of repeated drug exposure. Specifically, the effects of nicotine and RJR-2403 on PPI were assessed following a single (and first) administration of the drugs. However, the effects

of these same compounds in the working memory testing phase of the MWM task were examined over multiple injections (10 in the case of 2 month old animals, 4 in the case of the 8 month old animals, see methods section). It has been clearly demonstrated that chronic exposure to nicotine up-regulates high affinity nAChRs, like the α4β2 receptor, but also results in the subsequent desensitization of these receptors (Buisson and Bertrand, 2001; Kem, 2000; Park *et al*., 2000). Chronic RJR-2403 administration has also been demonstrated to up-regulate [3H]-nicotine binding in the frontal cortex of Sprague-Dawley rats (Abdulla *et al*., 1996). Thus, the lack of effect of nicotine and RJR-2403 in the present experiments may have resulted from some change in the function of nAChRs consequent to repeated drug administration. The problem with this hypothesis is that, if anything, the cognitive enhancement evoked by nicotine appears to become more robust over repeated injections (Levin and Simon, 1998). In fact, one study found that the greatest enhancement in working memory, as measured in the radial arm maze, was found during the third and fourth weeks of chronic nicotine exposure (Levin *et al*., 1999). Thus, it is unlikely that the lack of effect of nicotine in the MWM found in these experiments can be attributed to repeated drug injection.

V.4. Conclusions

The principle aim of the present series of experiments was to determine whether the augmentation of working memory function that is sometimes observed following administration of nAChR agonists might be mediated through an effect on mechanisms involved in stimulus selection. Because we did not observe any augmentation of working memory function following either nicotine or RJR-2403 administration, it is difficult to

make a strong conclusion on this issue. We did, however, observe a trend towards enhanced sensorimotor gating following nicotine administration in 2 month old C57BL/6J and 129X1/SvJ mice. Thus, it would appear that nicotine can enhance sensorimotor gating without a concomitant increase in working memory function. This pattern of results could be taken as at least indirect support for the supposition that sensorimotor gating and working memory function act independently of each other.

Sensorimotor gating is but one mechanism that is involved in stimulus selection. Accordingly, the suggestion that sensorimotor gating and working memory are uncorrelated should not be taken to imply that other forms of stimulus selection can influence working memory. Nicotine and other nAChR agonists have been shown to influence animal tests purported to measure both selective attention (Rochford *et al*., 1996), or sustained attention (Grottick *et al*., 2002; Hahn *et al*., 2003; Mirza and Stolerman, 2000). These latter forms of stimulus selection processes are thought to be considerably more "effortful" (i.e., requiring more cognitive processing capacity) than sensorimotor gating, which is considered by some investigators to be a more primitive stimulus selection mechanism that does not impose significant processing demands (Schauz and Koch, 1999). It may be then that the more effortful forms of stimulus selection are more closely associated with working memory function.

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