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Review article

Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging

Joseph A. Araujo[a], Christa M. Studzinski[a], Norton W. Milgram[a,b,*]

[a]*Department of Pharmacology, University of Toronto, Ontario, Canada*
[b]*Division of Life Sciences, University of Toronto at Scarborough, Scarborough, Ontario, Canada*

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Abstract

Memory decline in human aging and dementia is linked to dysfunction of the cholinergic system. Aging dogs demonstrate cognitive impairments and neuropathology that models human aging and dementia. This paper reviews recent evidence suggesting cholinergic involvement in canine cognitive aging based on studies with the anti-cholinergic drug, scopolamine, and a novel acetylcholinesterase inhibitor, phenserine. In particular, we examine: (1) the cognitive specificity of scopolamine's impairment in dogs, (2) the effect of age on scopolamine impairment and (3) the effect of phenserine on cognitive performance in dogs. Our findings indicate that working memory performance is disrupted by scopolamine at doses that do not disrupt non-cognitive behavior or long-term, semantic-like, memory, as indicated by performance of previously learned discriminations. This pattern of deficits is also seen in human and canine aging. We demonstrate that aged dogs are more sensitive to the impairing effects of scopolamine than young dogs, suggesting a decrease in cholinergic tone with increasing age. Dogs receiving phenserine demonstrate improved learning and memory compared to placebo controls. Our findings suggest that cholinergic decline could result in memory impairment, but that the memory impairment may be secondary to deficits in attention and/or encoding of new information. Together, these results suggest that the canine cholinergic system declines with age and that the aged dog is a unique model for screening therapeutics and for examining the relationship between amyloid pathology and cholinergic dysfunction in age-dependent cognitive decline.

Keywords: Acetylcholinesterase inhibitor; Aging; Alzheimer's disease; Anti-cholinergic; Attention; Cholinergic; Cognition; Dog; Encoding; Memory; Phenserine; Scopolamine

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Abbreviations: Aβ, amyloid-β; AChEI, acetylcholinesterase inhibitor; AD, Alzheimer's disease; CAT, choline acetyltransferase; DNMP, delayed-non-matching-to-position; DNMS, delayed-non-matching-to-sample; SAL, saline; SCP, scopolamine; SL, spatial list task.

* Corresponding author. Division of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, Ontario, Canada M1C 1A4. Tel.: +1 416 287 7402; fax: +1 416 287 7642.
E-mail address: milgram@utsc.utoronto.ca (N.W. Milgram).

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1. Introduction

Over the past 14 years, our laboratory has characterized several parallels between age-dependent cognitive decline in dogs and cognitive deficits observed in human aging and dementia (Adams et al., 2000a,b). In particular, dogs show impairments on tests of visuospatial working memory (Chan et al., 2002; Head et al., 1995) and executive function (Tapp et al., 2003a,b). Simple procedural and discrimination learning, by contrast, show less age sensitivity (Milgram et al., 1994). Dogs also show a pattern of brain aging that parallels aged and demented humans in many respects, including the deposition of amyloid-beta (Aβ; Head et al., 2000), increases in oxidative stress (Head et al., 2002), reduction of cerebral volume (Tapp et al., 2002) and alterations of the cerebral vasculature (Su et al., 1998). Collectively, these cognitive and neuropathological findings support the use of the aged dog as a model of aging and Alzheimer's disease (AD).

In human aging and dementia, multiple neurotransmitter systems appear to be compromised including the glutaminergic, histaminergic, dopaminergic and cholinergic systems (Gsell et al., 2004; Perry et al., 1998; Francis, 2003). Changes in cholinergic function have been characterized to the greatest extent and arguably show the strongest correlation with cognitive decline. Furthermore, the cholinergic hypothesis led to the development of the initial clinically effective therapeutics for AD (Blount et al., 2002; Ibach and Haen, 2004; DeLaGarza, 2003). To further validate the canine model, we investigated the role of the cholinergic system in canine cognition and aging. This review presents an overview of our findings and discusses the implications for studies of human aging and AD.

2. The cholinergic hypothesis of human aging and Alzheimer's disease

The cholinergic hypothesis proposes that dysfunction of the cholinergic system contributes to the memory decline seen in aging and dementia (Bartus et al., 1982). The groundwork and support for this hypothesis is based on pharmacological research and on histological analysis of brain pathology in AD patients.

2.1. Pharmacological evidence

Drachman and Leavitt (1974) were the first to demonstrate a link between cholinergic dysfunction and age-associated memory impairment. Scopolamine, an anticholinergic drug, caused memory impairments in healthy young humans that paralleled the memory impairments seen in non-demented drug-free elderly. More recent studies indicate that scopolamine is increasingly disruptive with increasing age and declining cognitive status (Flicker et al., 1992; Molchan et al., 1992; Ray et al., 1992; Sunderland et al., 1987; Tariot et al., 1996). This sensitivity pattern is consistent with the hypothesis that cholinergic tone decreases with increasing age and dementia.

Subsequent work showed that mild doses of scopolamine produced memory impairments in non-human primates that were similar to memory deficits observed in aged monkeys and humans. Specifically, scopolamine produced impairment in recent memory (Bartus and Johnson, 1976; Bartus, 2000), which according to Bartus (2000) is characterized by the following: (1) the information to be remembered is a discrete and brief event; (2) the information is trial-specific; (3) the ability to remember the event decays rapidly; and (4) it is analogous to episodic recent memory in humans (e.g. where one left their keys). In rodents, scopolamine caused impairments not only on measures of recent memory (Andrews et al., 1994; Dodart et al., 1997; Kirkby et al., 1995; Pilcher et al., 1997; Ravel et al., 1992), but also reference memory (Biggan et al., 1996), conditioned learning (Flood and Cherkin, 1986) and conditioned memory (Sessions et al., 1998). Similarly, scopolamine-induced deficits were also found in primate tests of visual discrimination learning (Bartus and Johnson, 1976) and attention shifting (Davidson et al., 1999). This absence of

specificity has led researchers to debate the contribution of the cholinergic system in cognition, aging and AD, as well as the usefulness of scopolamine models (e.g. Blokland, 1995). Unfortunately, many published studies do not use incremental doses of scopolamine to dissociate specific from non-specific effects, or dose-dependent effects.

Further support of the cholinergic hypothesis extends from studies showing memory-enhancing effects of cholinesterase inhibitors. Cholinomimetic drugs enhanced memory in animal models of aging and AD (Bartus, 1979; Muir et al., 1995). In fact, augmentation of cholinergic transmission with acetylcholinesterase inhibitors (AChEIs) presently is the standard AD therapy, demonstrating clinically significant improvements on cognitive function in mild to moderate AD (Blount et al., 2002; Ibach and Haen, 2004; DeLaGarza, 2003). Collectively, pharmacological evidence indicates that decline of the cholinergic system contributes to the cognitive decline in aging and AD, but the specific role of the cholinergic system remains controversial.

2.2. Cholinergic dysfunction in Alzheimer's disease

Cholinergic deficits are consistently reported in AD patients. Initially, reduced choline acetyltransferase (CAT) activity was detected in the cortex, hippocampus and amygdala of AD patients (Davies and Maloney, 1976; Bowen et al., 1976; Perry et al., 1977). Further, these changes in CAT activity were correlated with level of premorbid cognitive impairment (Perry et al., 1978; Wilcock et al., 1982). Subsequently, a selective loss of cholinergic cells were found in the cholinergic basal forebrain, particularly in the nucleus basalis of Meynert (Whitehouse et al., 1981, 1982), establishing an anatomical correlate with the biochemical evidence of cholinergic dysfunction in AD.

Basal forebrain cholinergic cell loss also was reported in aged animal models (Haba et al., 1988; Michalek et al., 1989; Rosene, 1993; Thal et al., 1992; Luine et al., 1986; Mesulam et al., 1987; Strong et al., 1980; Mundy et al., 1991; Voytko et al., 1995; Armstrong et al., 1993; Fischer et al., 1992; Riekkinen et al., 1992), but losses in cholinergic cells were not observed consistently and were not always correlated with cognitive decline (reviewed by Sarter and Bruno, 1998). Furthermore, selective lesions of the basal forebrain cholinergic system caused impairments in non-mnemonic processes such as attention (Muir et al., 1995; Baxter et al., 1995; Voytko et al., 1994), although memory deficits were also reported (Torres et al., 1994). Thus, cholinergic cell loss in animal models is not consistently linked to memory dysfunction.

3. Cognitive specificity of scopolamine impairment in dogs

As previously mentioned, recent memory is particularly susceptible to cholinergic blockade using scopolamine but performance on other tasks may also be disrupted. In many instances, the specificity of the effect cannot be established because of an absence of incremental dose paradigms and a limited selection of measures. To investigate the specificity of scopolamine's effect in dogs, we tested aged dogs on tests of recent memory, discrimination performance and non-cognitive behaviors (Araujo et al., 2004).

3.1. The effect of scopolamine on recent memory in the dog

We tested 12 aged dogs on a delayed-non-matching-to-position task (DNMP) to examine the effects of scopolamine on visuospatial working memory. In the DNMP, a subject is initially presented with an object at a specific sample location. Following a delay, the subject is then presented with two identical objects, one at the sample location and one at a novel location (Chan et al., 2002; Adams et al., 2000b). The subject is required to choose the object at the novel location; thus it must remember the location of the sample object over a delay interval. Each trial constitutes a discrete event and the information is trial-specific since the locations differ between trials. Furthermore, we have established that performance on the task declines as the delay interval increases (Adams et al., 2000b), thereby establishing a rapid decline in the ability to remember. Thus, the DNMP meets the criterion for a measure of recent memory as defined by Bartus (2000) (see Section 2.2).

In our initial experiment, we used aged beagles to examine the effect of three scopolamine doses on DNMP performance using delays of 20, 70 and 110 s. The dogs were impaired by 15 μg/kg (s.c.), but not at dose levels of 5 or 10 μg/kg (s.c.) (Fig. 1). Furthermore, the dogs were impaired at all delays, which questioned whether memory was impaired, or whether the deficit was related to a deficiency in encoding or attention. The ability of detecting a memory deficit, however, was confounded by a potential floor effect because performance under scopolamine approached chance at the 70- and 110-s delays and the 20-s delay may have been too long to approximate a condition with low memory demands. We also examined the effect of scopolamine on latency to respond on the DNMP, which provided a measure of reward-based motivation (Kirkby et al., 1995). No effect of any dose on mean latency to respond was found indicating that scopolamine did not affect motivation (Fig. 2).

3.2. The effect of scopolamine on discrimination performance in the dog

To determine whether scopolamine is disruptive of previously acquired memories (long-term memory), we trained dogs on a discrimination-learning problem until they performed at a minimum of 70% accuracy over 3 days. Subsequently, the dogs were tested following no treatment (control), 1-h following a subcutaneous saline injection (vehicle), and 1-h following a subcutaneous scopolamine injection.

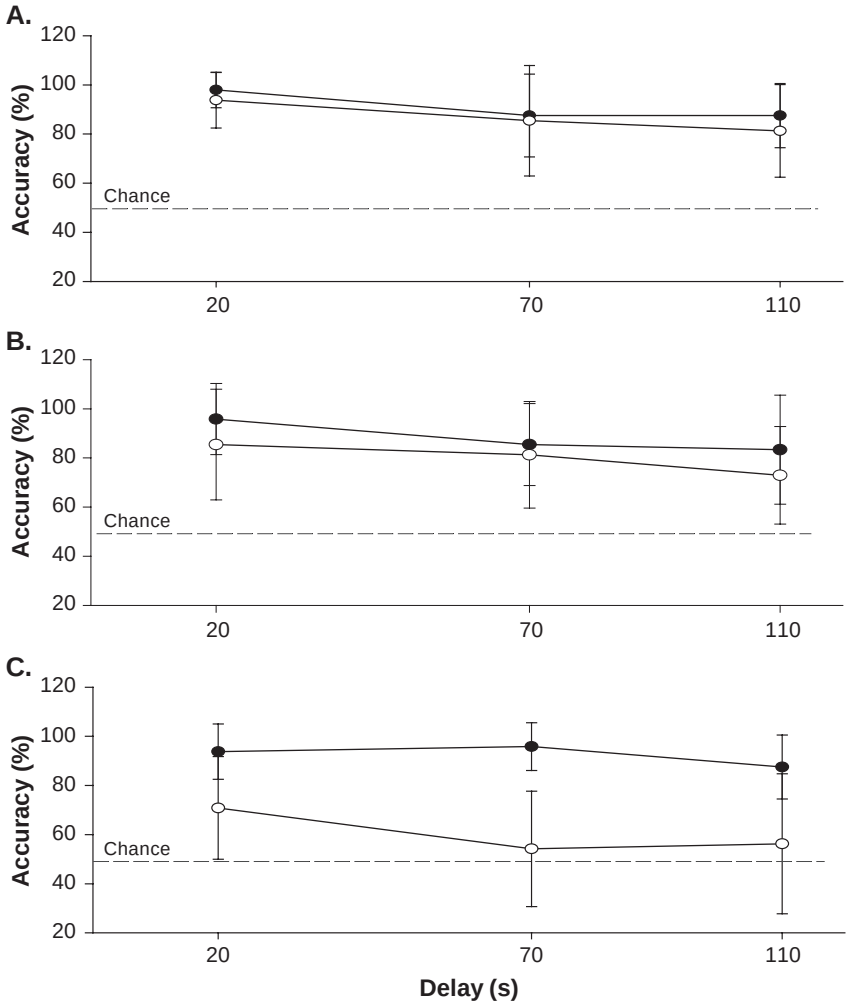

Fig. 1. Performance of aged dogs on the DNMP following three doses of scopolamine (white circles) compared to control (black circles). Scopolamine doses of 5 (1A), or 10 (1B) μg/kg did not significantly affect performance. By contrast, a 15-μg/kg dose (C) impaired performance at all delays. Performance at the 70- and 110-s delays approached chance (indicated by the dashed strait line).

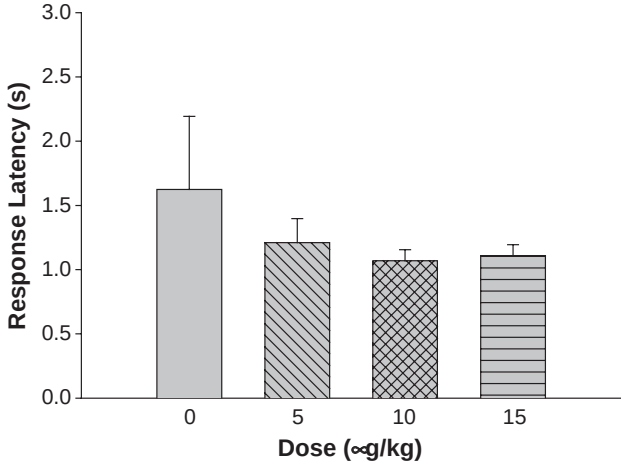

Fig. 2. Mean response latencies of aged dogs on a DNMP task following three doses of scopolamine. Although a dose-dependent DNMP performance decrement was observed (Fig. 1), latencies were not affected by any scopolamine dose compared to control. This indicates that the recent memory impairment is independent of effects on motivation or of the motor performance decrement observed on the skilled motor task (Fig. 5).

We first tested scopolamine on a variable-distance landmark discrimination task (Milgram et al., 1999, 2002a). The correct response consisted of displacing one of two identical coasters closest to an external landmark, a wooden yellow peg. An important feature of this task is that increasing the distance between the landmark and the rewarded coaster increases the level of difficulty. On any given trial in the landmark experiment, the landmark was placed at one of three locations: either at distances of 1 or 4 cm from the rewarded coaster, or directly on the coaster, which served as a control condition. To directly compare the results in the present study with that in the DNMP study, we administered a scopolamine dose of 15 μg/kg to four aged dogs that were able to perform at 70% accuracy when the landmark was placed at a distance of at least 1 cm from the rewarded coaster. As expected, performance declined with increasing distance; however, scopolamine had no effect regardless of the level of difficulty (Fig. 3). These results indicate that scopolamine does not disrupt visuospatial function or memory for previously acquired rules. To

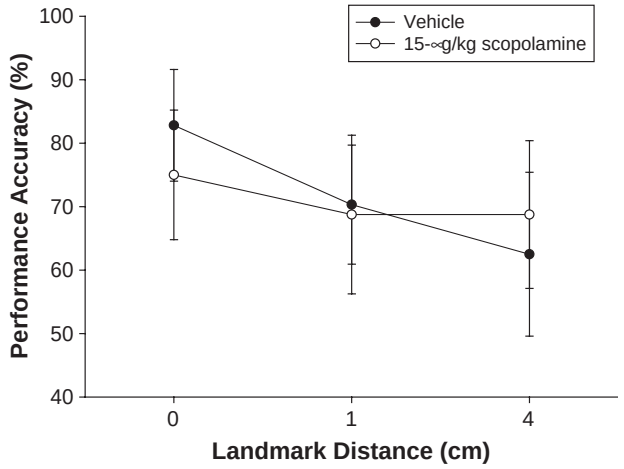

Fig. 3. Performance of aged dogs on a landmark discrimination task. As the distance between the rewarded coaster and the landmark increased, performance accuracy declined. Scopolamine, however, did not impair performance compared to the saline control.

confirm this finding, we tested five young dogs on the same task and obtained identical results. We then conducted similar experiments using a black–white discrimination task and an oddity discrimination task in aged animals.

The black–white discrimination task required subjects to respond to one of two identical blocks that differed only in color (white vs. black) (Milgram et al., 2005). Five dogs were tested using a scopolamine dose of 15 μg/kg. Performance under scopolamine did not differ from control or vehicle conditions. Subsequently, we tested nine dogs on an oddity discrimination task (Milgram et al., 2002b). Subjects were presented with three objects; two identical objects and one odd object. Subjects were required to respond to the odd object of the three. After completing training, we tested all subjects following two doses of scopolamine (15 and 30 μg/kg). We found a highly significant impairment following the higher dose, but not the lower dose; however, a greater, albeit non-significant, effect was found on the oddity (two identical non-rewarded stimuli) compared to the black–white (one non-rewarded stimulus) discrimination, which may suggest a scopolamine-induced sensitivity to increased distracters.

3.3. The effect of scopolamine on non-cognitive behavior in the dog

To further examine the specificity of the impairing effects of scopolamine in dogs, we examined two measures of exploratory behavior: the curiosity test and open field activity (Siwak et al., 2001). For the curiosity test, seven objects were placed in an open field arena and the dogs were permitted to explore them for a 10-min period. Eight dogs were tested following injections of saline and scopolamine (15 μg/kg; s.c.); however, no differences were found between the two conditions. On the open-field activity test, we examined doses of 15 and 30 μg/kg (s.c.). The higher dose resulted in a significant decrease in groom frequency, a significant increase in fall frequency, and a non-significant trends towards increased activity with increasing dose (Fig. 4). Collectively, these results indicated a generalized behavioral disruption following 30 μg/kg (s.c.) of scopolamine, but not following 15 μg/kg (s.c.).

We subsequently confirmed this hypothesis using a skilled motor task in which dogs were required to use their paw to retrieve a food reward on a sliding coaster (manuscript in preparation). Initially, the maximal distance at which each animal could successfully retrieve the food reward was determined. Then animals were tested on a variable-distance paradigm whereby they were required to retrieve the food reward from their maximal distance, half their maximal distance and at a control distance of 0 cm. This allowed us to differentially tax motor function within a session: the longer distances taxed motor function to a greater extent. Aged dogs were impaired following 30 μg/kg (s.c.) at all distances; including the control condition. By contrast, the 15 μg/kg (s.c.) only impaired performance at the subjects' maximal distance (Fig. 5), indicating that scopolamine could impair skilled motor performance when the task was sufficiently difficult. The motor skill required in this motor task contrasts with the motor requirements used in the cognitive tasks mentioned previously; the cognitive tasks required dogs to displace objects with their nose and not their paw. Thus, the motor skill involved was less than that required for the motor task and, therefore, should not have been impaired by scopolamine. Consistent

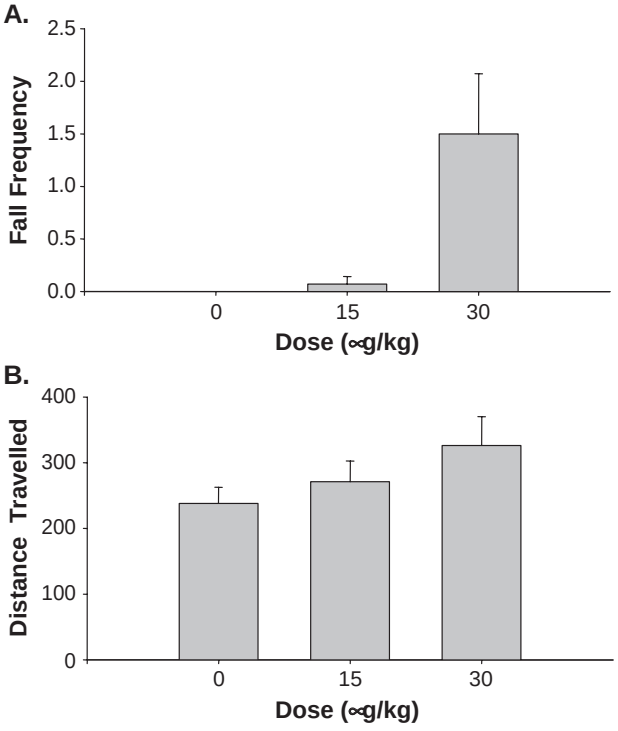

Fig. 4. Performance of aged dogs on an open-field activity test. With increasing dose, a significant increase in fall frequency (A) and a non-significant increase in activity (B) were observed.

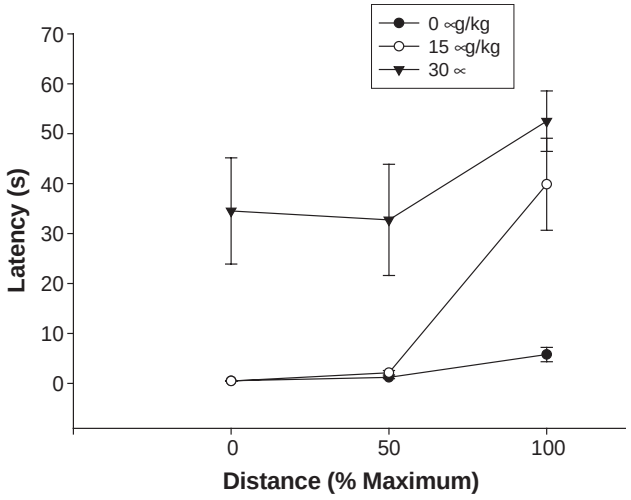

Fig. 5. Performance of aged dogs on a skilled motor task. The 30-μg/kg-scopolamine dose impaired performance at all distances tested, including the control condition (0 distance). By contrast, performance following a 15-μg/kg dose only impaired performance at the most difficult level (maximal distance).

with this interpretation, mean response latency on the DNMP following a 15-μg/kg-scopolamine dose did not differ from mean control latency (Fig. 2) indicating that the recent memory impairments were not secondary to motor skill deficits.

3.4. The implications of specific scopolamine-induced recent memory deficits in the dog

These studies indicate that DNMP performance, a measure of recent memory, is more susceptible to scopolamine-induced impairment than performance of previously learned discriminations, which may represent long-term semantic-like memory performance since the rules and information to be remembered are invariant between trials. Furthermore, recent memory disruption by scopolamine can occur independently of disruption of non-cognitive behaviors. In this respect, recent memory in the dog, like non-human primates, appears to be highly dependent on the cholinergic system. The pattern of impairment produced by scopolamine also parallels the progression of deficits occurring in aging and AD; deterioration in recent memory precedes impairments in semantic memory (Bartus, 2000). This suggests that a 15-μg/kg-scopolamine dose provides a model of aging and AD in aged dogs. Additionally, normal aged dogs also show deficits in recent memory prior to semantic-like memory, which suggests that the canine cholinergic system is compromised with age.

The response to scopolamine in the canine studies was dose dependent; treatment with a 30-μg/kg dose of scopolamine produced global impairment but only recent memory performance was impaired following 15 μg/kg of scopolamine. These results suggest that the diversity of scopolamine-induced impairments reported in rodents reflects differences in dose selection and possibly also scopolamine effects on secondary processes such as motivation or motor ability (Bartus, 2000). This suggestion is consistent with evidence of non-specific performance deficits following administration of scopolamine (Godding et al., 1982) and of a dose-dependent dissociation of working and reference memory deficits (Biggan et al., 1996) Alternately, the deficits observed at some scopolamine doses might model a more compromised state of cholinergic disruption; both semantic memory decline and changes in activity are reported in advanced AD.

Finally, we also should note that we did not find clear evidence of a delay-dependent scopolamine effect. This suggests that the affected process(es) may be non-mnemonic, which was supported by the findings of greater scopolamine effects with increased number of distracters in the oddity task compared to the black-white discrimination task.

4. Interaction between age effects and scopolamine

In humans, a pattern of age sensitivity to scopolamine impairment is observed that presumably occurs due to decreased cholinergic tone (Flicker et al., 1992; Molchan et al., 1992; Ray et al., 1992; Tariot et al., 1996; Sunderland et al., 1987). Consequently, we examined the effect of age on scopolamine impairment on various tests of recent memory. In all of the experiments, we used a scopolamine dose of 15 μg/kg (s.c.), which we previously found caused specific recent memory impairment in aged dogs (Araujo et al., 2004). The following studies were intended to test the hypothesis that aged dogs would be more sensitive to scopolamine impairment than young dogs.

4.1. Effects of age on DNMP performance

We first tested 12 aged and 6 young dogs on a fixed-delay DNMP, using only two possible locations and a delay of 30 s, following administration of scopolamine. We confirmed our previous findings that DNMP performance was impaired in the aged group, however, no impairment was found in the young group. The variability between age groups did not provide conclusive evidence of age differences. Consequently, we trained seven aged and seven young dogs to an equivalent level of control performance on the DNMP using delays of 5, 55 and 110 s and three possible locations. We included the 5-s delay to further examine the delay dependency of the scopolamine effect. Once again, we found that aged dogs, but not young dogs, were significantly impaired by scopolamine (Fig. 6). We also failed to find an interaction between delay and scopolamine impairment suggesting that the memory deficit was secondary to impaired attention or encoding. The increased sensitivity to scopolamine in aged dogs supports the hypothesis that cholinergic tone is decreased in aged dogs.

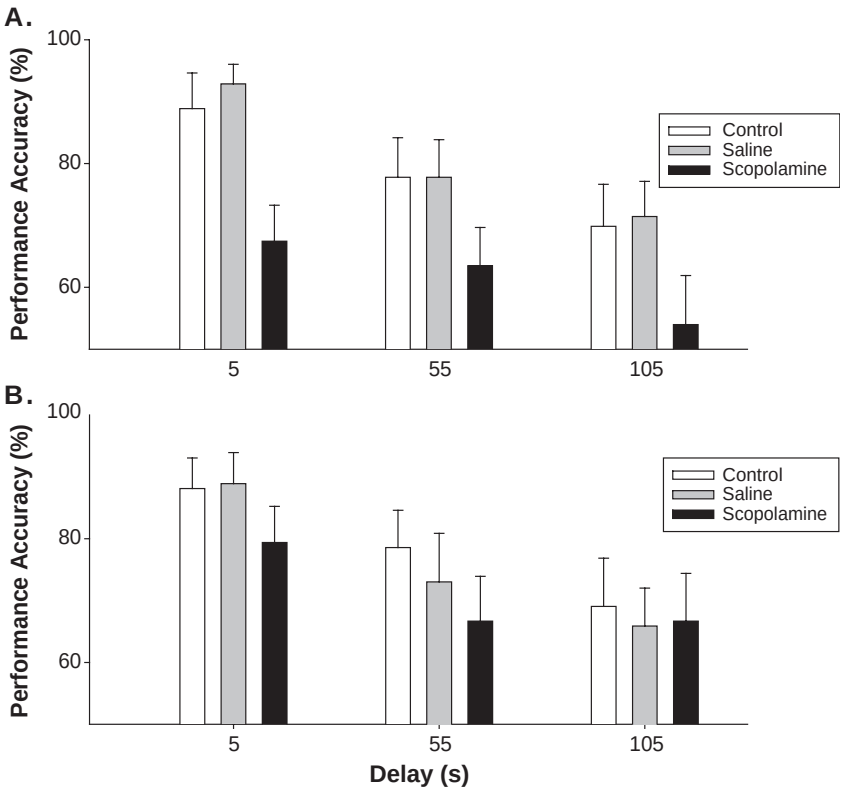

Fig. 6. Age sensitivity to the impairing effects of scopolamine (15 μg/kg) on DNMP performance. Aged dogs (A) demonstrated impairment following scopolamine at all delays tested compared to control and saline conditions. By contrast, young dogs (B) were not impaired by the same dose of scopolamine.

4.2. Effects of scopolamine on object recognition memory in young dogs

We also used another task to assess recent memory, a delayed-non-matching-to-sample task (DNMS), which provides an index of object recognition memory (Callahan et al., 2000). In the DNMS, a dog was presented initially with a sample object. Subsequent to a delay, two objects were presented: the sample object and a novel object. The dog was rewarded for responding to the novel object. For inclusion in the scopolamine study, six young beagle dogs were trained to a performance level of at least 70% at a 30-s delay. We attempted to train aged dogs on the same task, but were unsuccessful. Following administration of scopolamine, young dogs were significantly impaired compared to vehicle injection, which indicated two important points. First, young dogs were susceptible to scopolamine-induced recent memory impairments. Second, that the absence of an effect on the DNMP likely was not due to increased metabolism in young dogs compared to aged. We hypothesized that the difference in sensitivity between the DNMP and DNMS was due to task difficulty, although the DNMP and DNMS are not directly comparable (Adams et al., 2000a).

4.3. Effects of scopolamine on spatial list memory

To examine the hypothesis that scopolamine-induced recent memory deficit varied as a function of task difficulty, we examined the effects of scopolamine on four young and six aged dogs on a spatial list task (SL). The SL is a visuospatial recent memory task that is identical in principle to the DNMP, but has increased memory demands because the subject must remember two locations as opposed to one (Tapp et al., 2003b). In the SL, dogs are initially presented with an object over one of three food wells and allowed to respond to the object and retrieve the hidden food reward beneath. Following a delay, the dog is presented with an identical object over one of the two food wells not used in the previous presentation; once again, the dog is permitted to respond to the object and retrieve the food reward beneath it. Following a second delay, three identical objects are presented over the three food wells. The subject is rewarded for responding to the object over the food well not rewarded in the previous two presentations. All subjects were trained to a consistent performance using delays of both 5 and 10 s. Following scopolamine, there was an overall impairment. There was no significant difference between age groups, albeit aged dogs showed lower performance accuracy under scopolamine than young dogs. Once again, we found no interaction between the drug effect and delay. We also did not find that primacy errors (i.e., errors to the location in the first presentation) were increased by scopolamine, which we would expect if scopolamine impaired memory because this location is stored in memory for a longer period of time (i.e., for two delays) than the location in the second presentation (i.e., for one delay).

To further characterize the scopolamine impairment, we examined the number of errors that occurred over blocks of three trials (i.e., trials 1–3, trials 4–6, trials 7–9 and trials 10–12). We found that errors towards the end of the test session were increased by scopolamine compared to control and vehicle, which suggested that scopolamine increased susceptibility to proactive interference. Thus, scopolamine may impair inhibitory control, or the ability to disregard irrelevant information from previous trials (Tapp et al., 2003b).

4.4. Implications of age and task sensitivity

Overall, we found that aged dogs are more sensitive to the effects of scopolamine than young dogs, which also occurs in humans and suggests that cholinergic tone decreases with age in both dogs and people. This age-dependency further validates the canine model as a model for human aging and Alzheimer's disease. We currently are investigating the possibility that cholinergic markers are altered with age in the dog, which would provide further insight into correlations between cholinergic alterations and Aβ pathology.

The task-specific sensitivity to scopolamine-induced impairment in young dogs supports the hypothesis that particular functions are more susceptible to cholinergic disruption than others (Bartus, 2000). Furthermore, we did not see an interaction between drug effect and delay, which suggests that encoding or attention, and not memory primarily, is impaired by cholinergic blockade. Additional support for an attentional hypothesis was provided by the findings of a scopolamine-induced increase in errors towards the end of the SL session. This pattern of errors may model working memory deficits among the elderly due to an increasing inability to inhibit attention to interfering or irrelevant stimuli (Connelly and Hasher, 1993; May et al., 1999; McDowd and Filion, 1992; Moscovitch and Winocur, 1995). This hypothesis is also consistent with the findings of greater scopolamine effects on the oddity discrimination compared to the black-white discrimination task.

5. The effects of cholinomimetic therapy on canine cognition

The results of the previous experiments suggested that the cholinergic system is diminished with age in dogs and likely contributes to the cognitive decline, particularly in recent memory. We, therefore, examined the effects of augmenting cholinergic transmission with the specific AChEI, phenserine, on canine cognition (manuscript in preparation). Phenserine is a novel AChEI currently in development for AD and demonstrates a favorable safety profile, likely due to its short plasma half-life (Greig et al., 2000).

5.1. The effects of phenserine on object recognition memory

We conducted a pilot study in six beagle dogs (between ages 3 and 7) intended to provide initial safety and efficacy data on phenserine using the DNMS. The six dogs previously were unable to acquire the DNMS after 80 test sessions (800 trials); thus all had experience with the task, but were unable to achieve an accuracy level of 80%. The animals were divided into two groups matched for prior DNMS performance accuracy and were then tested once daily over 20 days on the DNMS, 1 h following phenserine administration. No animal acquired the task over the 20 days (i.e., reached the 80% learning criterion), however a marginally significant trend ($p<0.1$) for higher performance accuracy was seen in the phenserine group over the last 5 days compared to the first 5 days. By contrast, the performance of the control group did not differ over the 20 days.

5.2. The effects of phenserine on visuospatial function and memory

Seven senior beagle dogs (age >12 years), with extensive practice on the DNMP, were tested on DNMP relearning. Two groups, matched for their prior performance of the DNMP, were tested for DNMP relearning on a 5-s delay following either phenserine or placebo administration 1 h prior to testing. The phenserine group committed fewer errors to relearn the DNMP than the placebo group. Furthermore, when compared to their most previous performance, the phenserine group committed fewer errors and the placebo group committed more errors to relearn. Neither of these results, however, was significant.

After relearning, the dogs were tested on a variable-delay DNMP using delays of 20 and 80 s, following both saline and scopolamine (15 μg/kg, s.c.) injections 45-min prior to testing. As expected, the placebo group demonstrated decreased performance accuracy at the longer delay than the shorter delay following saline, and was impaired at both delays following scopolamine. By contrast, the phenserine group did not show a delay-dependent effect and was not impaired at the 20-s delay following scopolamine treatment (Fig. 7). These results suggest that, first, phenserine improves working memory, and, second, that phenserine partially attenuates scopolamine-induced deficits. The animals were subsequently retested on the variable-delay DNMP following saline and scopolamine injection approximately 1 week after phenserine and placebo treatment was discontinued. Discontinuation of placebo did not result in any differences compared to the previous performance; however, dogs discontinued from phenserine now demonstrated a delay-dependent decrease in performance accuracy under saline (Fig. 7). No difference was observed in either group under scopolamine compared to their previous performance. This finding supports the interpretation that

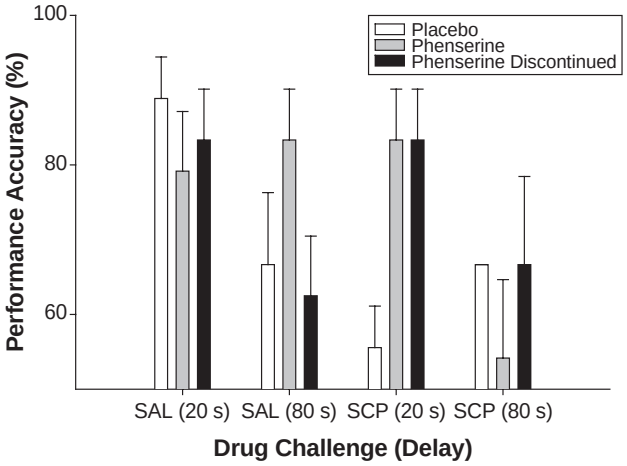

Fig. 7. The effects of saline and scopolamine on placebo and phenserine treated dogs tested on the DNMP. Dogs receiving placebo demonstrated the expected delay-dependent impairment under saline (SAL) and impairment at 20 and 80 s under scopolamine (SCP). By contrast, phenserine-treated dogs (phenserine) did not demonstrate a delay-dependent impairment following saline (SAL) administration and did not demonstrate impairment at the 20-s delay following scopolamine (SCP) administration. After phenserine was discontinued (phenserine discontinued), the same dogs performed less accurately at the 80-s delay compared to the 20-s delay, which suggested that phenserine improved memory. A continued protection from scopolamine-induced memory impairment was also seen at the 20-s delay after phenserine was discontinued, suggesting a long-term (i.e., 1 week) protective effect of phenserine.

phenserine improves memory and also suggests that phenserine may provide long-term (i.e., approximately 1 week) protection from cholinergic inhibition after treatment cessation.

5.3. The effects of phenserine on complex discrimination learning

Dogs do not exhibit consistent impairments in simple discrimination learning with increasing age (Milgram et al., 1994); however, age-dependent learning impairment is seen when the complexity of the discrimination task is increased (Tapp et al., 2003a; Milgram et al., 2002b). We tested 11 aged beagle dogs (between ages 7 and 10) on a complex oddity discrimination task that had two levels of difficulty, based on object similarity. The dogs were divided into two groups matched for previous discrimination learning. Both groups were tested first on the less difficult discrimination and then on the more difficult discrimination. Phenserine was administered to one of the groups during the easier discrimination and to the other group for the more difficult discrimination; thus, both groups were tested following phenserine treatment and both groups were tested on both difficulty levels. The dogs treated with phenserine acquired the more difficult discrimination with fewer errors than the control group, but no differences between groups was found on the less difficult problem (see Fig. 6 in Studzinski et al., in press). We also found that dogs in the placebo group committed significantly more errors on the second discrimination, which confirmed that it was more difficult than the first discrimination.

5.4. The implications of phenserine-induced cognitive improvement in the dog

These studies provide further evidence of cholinergic involvement in canine cognition by demonstrating that phenserine improves measures of learning (oddity discrimination task), and memory (DNMP). The results also suggest that administration of phenserine may provide protection against cholinergic decline and that performance of young animals also may benefit from cholinomimetic therapy. The present experiments not only support the hypothesis that cholinergic augmentation improves memory, as seen with the absence of a delay-dependent deficit following saline on the DNMP, but that cholinergic augmentation also improves learning. The improvement seen on both tasks is consistent with an attentional hypothesis of cholinergic function; however, we cannot exclude the alternative possibility that cholinergic enhancement affects more than one cognitive process.

6. Discussion

The following sections briefly summarize our collective findings and their implications to the cholinergic hypothesis.

6.1. Extending the canine model of aging and AD

Our results further extend the canine model of aging and AD by providing evidence that the canine cholinergic system diminishes with age. Not only did the pattern of scopolamine impairment parallel the decline observed in aged dogs and early AD, but aged dogs were also more sensitive to the impairing effects of scopolamine than young dogs. The cognitive enhancement following phenserine administration seen on the DNMP and oddity task, which are both sensitive to age-dependent cognitive decline in dogs (Adams et al., 2000b; Chan et al., 2002; Milgram et al., 2002b), further suggests that the cholinergic system is diminished in aged dogs. We are currently investigating the possibility that cholinergic markers, such as muscarinic receptors, are modified with age in the dog.

The finding of naturally occurring cholinergic deficits in the aging dog extends the aging and AD-like pathology previously described in the dog (Cummings et al., 1996a,b; Head et al., 2000, 2002; Su et al., 1998; Torp et al., 2000), which should provide a useful tool for examining hypotheses related to aging and AD. The dog is likely to be particularly useful for examining in vivo links between cholinergic deficits, Aβ pathology and cognitive decline, all of which are consistently seen in AD and, to a lesser extent, in normal aging. In vitro experiments suggest that cholinergic neurotransmission via M1 and M3 muscarinic

receptors augments alpha-secretase cleavage of amyloid precursor peptide, thereby reducing the formation of Aβ1-42, the Aβ isoform believed to be most neurotoxic (reviewed by Roberson and Harrell, 1997). This suggests that early treatment with AChEIs may provide protection from Aβ pathology. On the other hand, basal forebrain cholinergic neurons are particularly sensitive to Aβ toxicity. It is unclear, at present, which pathology occurs earliest and to what extent they contribute to the ensuing cognitive decline.

Finally, the finding that phenserine, an AChEI, improves learning and memory in aged dogs indicates that the canine model can serve as a pre-clinical screening model for the cognitive enhancing effects of therapeutics intended for aging and AD. While cholinergic enhancement of cognition is observed in many animal models (Bartus, 1979; Muir et al., 1995), the canine model accurately predicts ineffective compounds as well (e.g. early ampakine compounds), which suggests that the canine model demonstrates pharmacological validity (see Studzinski et al., in press for a review). Since the dog exhibits neuropathology and cognitive decline that parallels human aging and AD, various endpoints for providing evidence of efficacy and/or mechanism of action can be included in pre-clinical screening with the dog.

6.2. The role of cholinergic dysfunction in cognition

These experiments indicate that cholinergic decline can contribute to the memory deficits observed in aging and AD. We found that recent memory is impaired specifically at low scopolamine doses, but when larger doses are used the impairment is more generalized including impairments in semantic-like memory and non-cognitive behaviors. Furthermore, increasing recent memory demands exacerbated the scopolamine-induced disruption of recent memory. Together, this suggests that many of the reported scopolamine impairments in animal models may be due to the dose or task chosen (Bartus, 2000; Blokland, 1995; Roberson and Harrell, 1997). One implication is that some reported impairments in animal models might not accurately reflect specific cholinergic effects. Like others (Bartus, 2000; Biggan et al., 1996), we found that the use of low scopolamine doses cause cognitive impairments that parallel those seen in aging and Alzheimer's disease, indicating that cholinergic dysfunction can contribute the deficits seen in aging and AD.

Many studies, by contrast, indicate that attentional functions, and not necessarily memory, are particularly susceptible to cholinergic dysfunction (Holley et al., 1995; Muir et al., 1995; Robbins et al., 1989; Sarter and Bruno, 1997; Sarter, 1994; Voytko et al., 1994). In our studies, the absence of scopolamine impairment by delay effects, the increase in errors towards the end of an SL session following scopolamine, and the findings of improved learning and memory following the administration of an AchEI support an attentional hypothesis of cholinergic function. Thus, our findings do not contradict an attentional hypothesis, but they do suggest that disruption of attentional processes can impair performance on measures of recent memory. These findings also support a previously indicated link between the cholinergic system and encoding (Rosier et al., 1998), which also could be attributed to attentional disruption.

7. Conclusions

The present review further extends the canine model of aging and AD by demonstrating that cholinergic function in dogs likely diminishes with age. This highlights the utility of the aging dog in examining the links between cholinergic and Aβ pathology and in screening therapies for aging and AD. Our work also supports the hypothesis that cholinergic dysfunction contributes to the early deficits in memory observed in aging and AD. Finally, the present results support the hypothesis that memory deficits are secondary to deficits in attention and/or encoding, but does not exclude other explanations.

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