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Pharmacology, Biochemistry and Behavior 76 (2003) 161–168
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Adrafinil disrupts performance on a delayed nonmatching-to-position task in aged beagle dogs

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Received 2 April 2003; received in revised form 5 July 2003; accepted 19 July 2003

Abstract

Previous studies in humans and dogs have reported beneficial effects of adrafinil on specific cognitive functions. The effects in dogs are limited to a single study examining discrimination learning. We wanted to further explore the cognitive effects of adrafinil in dogs. The purpose of the present study was to determine the effect of oral administration of adrafinil on visuospatial function in dogs. Eighteen aged beagle dogs were tested on a delayed nonmatching-to-position (DNMP) task 2 h following one of three possible treatments; 20 mg/kg of adrafinil, 10 mg/kg of adrafinil or a placebo control. All dogs were tested under each treatment for eight test sessions. A 2-day washout period was given between treatments and the order of treatments was varied. Treatment with 20 mg/kg of adrafinil produced a significant impairment in working memory as indicated by an increase in the number of errors over the 8-day test period. The disturbance of memory functions from adrafinil could be a result of increased noradrenergic transmission in the prefrontal cortex.

Keywords: Adrafinil; Modafinil; Delayed nonmatching-to-position; Dog; Alpha-1 noradrenergic

1. Introduction

Adrafinil is a stimulant that has behavioral activating effects without the adverse side effects common to other stimulants, which include stereotypy (Rambert et al., 1986; Milhaud and Klein, 1985; Lyons and French, 1991; Saletu et al., 1986) and anxiogenesis (Hascoët and Bourin, 1998). Administration of adrafinil produces increases in locomotor activity in mice (Hascoët and Bourin, 1998; Hascoët et al., 1995; Rambert et al., 1986; Duteil et al., 1979), rats (Delini-Stula and Hunn, 1990), monkeys (Milhaud and Klein, 1985), and dogs (Siwak et al., 2000a,b). The increase in activity is dose dependent (Delini-Stula and Hunn, 1990; Milhaud and Klein, 1985; Duteil et al., 1979). Adrafinil is metabolized to an active form called modafinil. Dose-dependent increases in locomotor activity also occurred in mice, rats, and monkeys after treatment with modafinil (Ferraro et al., 1997; Simon et al., 1994, 1996; Duteil et al., 1990; Lagard and Anton, 1990).

Adrafinil and modafinil are both believed to serve as alpha-1 adrenergic agonists. Intact postsynaptic alpha-1 receptors seem to be required for the development of adrafinil-induced hyperactivity (Duteil et al., 1979), and alpha-adrenergic antagonists, including phenoxybenzamine, prazosin, and yohimbine, block the increase in motor activity induced by adrafinil. There is, however, evidence that adrafinil and modafinil inhibit GABA release (Tanganelli et al., 1992, 1995; Ferraro et al., 1996a,b, 1998, 1999), cause changes in brain metabolism (Touret et al., 1994; Piérard et al., 1995), and act on hypocretin-producing neurons (Chemelli et al., 1999; Scammell et al., 2000).

In clinical trials with human subjects, adrafinil was beneficial in treating problems of vigilance, attention concentration, learning, memory, affective troubles, and depressive manifestations (Boyer, 1994; Defrance et al., 1991; Fontan et al., 1990; Kohler and Lubin, 1990; Dewailly et al., 1989; Israel et al., 1989; Saletu et al., 1986). These beneficial effects are proposed to result from the effects of adrafinil on adrenergic transmission (Jouvet et al., 1991; Fontan et al., 1990; Guyotat, 1987; Oeuvray et al., 1986).

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0091-3057/$ – see front matter © 2003 Published by Elsevier Inc.
doi:10.1016/S0091-3057(03)00211-9

The present study used the aged beagle dog as a model of aging. Dogs exhibit similar patterns of cognitive decline, behavioral changes, and neuropathology as humans. Aged beagles are not impaired on a simple object discrimination task or the reversal (Head et al., 1998; Milgram et al., 1994). Aged dogs show deficits on a variety of cognitive tests compared to young animals including landmark discrimination (Milgram et al., 2002a), oddity discrimination (Milgram et al., 2002b), size discrimination and reversal learning (Tapp et al., 2003a), spatial list learning (Tapp et al., 2003b), recognition memory (Callahan et al., 2000), and spatial working memory (Chan et al., 2002; Head et al., 1995). Locomotor activity, exploratory, and social behaviors also exhibit changes with age in dogs, equivalent to those observed in humans (Siwak et al., 2001, 2002, 2003). Aged dogs also develop similar age-related neuropathology as humans. β-amyloid deposition, in the form of diffuse senile plaques, is a prominent feature of the aged dog brain (Head et al., 2000).

We previously reported that adrafinil improved the performance of aged beagle dogs on a visual discrimination task (Milgram et al., 2000). The purpose of the present study was to further explore the effect of adrafinil on cognitive function in aged beagle dogs. We specifically looked at the performance of aged beagle dogs on a visuospatial working memory task (Chan et al., 2002). We have previously reported age-dependent deterioration in visuospatial working memory function (Adams et al., 2000a; Chan et al., 2002) but with increased variability in performance among aged dogs. Some aged animals show impaired performance while others do not differ from young dogs (Adams et al., 2000b). We therefore divided the aged animals into two groups, poor and good performers, based on their baseline performance on the visuospatial task.

2. Materials and methods

This placebo-controlled, fully blinded study was performed in accordance with the National Institutes of Health guidelines for the care and use of research animals. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed and the study was approved by the Institutional Animal Care Committee at the study site, University of Toronto.

2.1. Subjects

Subjects were 18 beagle dogs, 8 females and 10 males, ranging in age from 9 to 12 years of age from the University of Toronto. The dogs were individually housed in 1.07 × 1.22 m pens and maintained on a 12:12-h light/dark cycle. Pens were washed daily between 8:00 and 10:00 a.m., during which time the animals were exercised in groups in a separate room for 15 min. Water was available ad libitum. Dogs were fed approximately 300 g of Purina Dog Chow daily. All dogs were in good health at the time of behavioral testing.

2.2. Test apparatus

The test apparatus, as described previously (Milgram et al., 1994), consisted of a wooden box 0.609 × 1.15 × 1.08 m, with vertical aluminum bars at the front, a moveable Plexiglas tray, with three food wells, a small overhead incandescent light, and a wooden partition containing a one-way mirror and hinged door to separate the investigator from the animal. The heights of the vertical bars can be adjusted for each dog to allow access to the food placed in the tray wells. A dedicated computer program was used for controlling all timing procedures, for specifying the location of the correct choice, and for capturing data. The test sessions were once daily.

2.3. Experimental design

2.3.1. Pretraining

The dogs were previously trained on a delayed nonmatching-to-position (DNMP) task (Adams et al., 2000a,b; Chan et al., 2002). Each trial of the task involves two components. The first is the sample phase in which the dog was presented with a sample object in one of three wells on the tray. The sample object had a food reward placed beneath it. The tray was then removed for a delay period of 10 s. After the delay, the tray was presented a second time with the sample object covering the same well and a second identical object covering the second well. The dog was required to go to the object in the new location to receive the food reward. The dogs were considered to have made an incorrect choice if they come into contact with the sample object that was previously presented. They were allowed to correct on their first error only. Once the subjects passed at 10 s, the delay was increased to 20 s and then to 30 s. The longer delays make the task more difficult. The dogs were given 12 trials/day with a 60-s interval between trials.

2.3.2. Test procedures

A variable delay DNMP procedure was used to assess the effects of adrafinil. The delay was either 20 or 70 s, the order of which varied randomly within a session such that half of the trials were 20 and half were 70. The dogs were given 12 trials/day for 8 days on each dose, with a 2-day wash out period between the doses. Three treatment levels were used, 0, 10, and 20 mg/kg of adrafinil. The order of treatments was randomly assigned to each dog such that six dogs started with 0 mg/kg, six dogs with 10, and six dogs with the 20 mg/kg dose. All dogs received each dose. Adrafinil was administered 2 h prior to cognitive testing. Capsules were placed in a wet dog food and administered orally. The experimenters were blinded to the treatment. The placebo consisted of identical capsules containing lactose.

The memory measure used was errors made over the 8-day period of each treatment. Response latency was measured by the experimenter using key presses on a computer from the time the tray began moving toward the dog until the animal made contact with the object on the tray.

The dogs were also divided into two groups based on their baseline level of performance. Dogs whose performance during baseline testing was less than 70% accurate were grouped as poor performers. Dogs who maintained an average accuracy of 70% or greater were considered good performers. This grouping produced nine poor performers and nine good performers.

2.4. Statistical analysis

All statistical analyses were performed using SPSS v.10.0 for windows with the alpha level at .05. The memory measure assessed was errors, the total number of incorrect responses, made over each 8-day period. A three-way analysis of variance was used to test for the effects of adrafinil on memory. Dose and delay intervals were within-subject factors and baseline performance was a between-subject factor. A simple main effects analysis was used to interpret a significant interaction. Boneferroni's test was used for pairwise comparisons.

Response latency measures were also assessed. The Shapiro–Wilk statistic indicated nonnormality of the latency data. The Wilcoxon signed ranks test for related samples was used to compare latencies between each dose and delay. The Mann–Whitney test for independent samples was used to compare the group categories created using the baseline performance.

3. Results

3.1. Memory measure

The ANOVA revealed a significant main effect of dose [$F(2,32) = 6.12$, $P = .011$]. The dogs made more errors when tested under the 20 mg/kg dose compared to the 10 mg/kg dose ($P = .002$) and the placebo dose ($P = .031$; Fig. 1). The 10 and 0 mg/kg doses did not differ ($P = .99$). A significant main effect of delay was also obtained [$F(1,16) = 66.01$, $P < .0001$]. Errors were higher at the 70-s than at the 20-s delay (Fig. 2). The main effect of baseline performance was also significant [$F(1,16) = 32.49$, $P < .0001$]. Dogs whose baseline performance was poorer performed more poorly under adrafinil as well.

The interaction between dose and baseline performance was significant [$F(2,32) = 3.60$, $P = .050$]. The simple main effects analysis revealed that the interaction is due to a significant difference in errors between the 10 and 20 mg/kg doses for the poor performers only (Fig. 3).

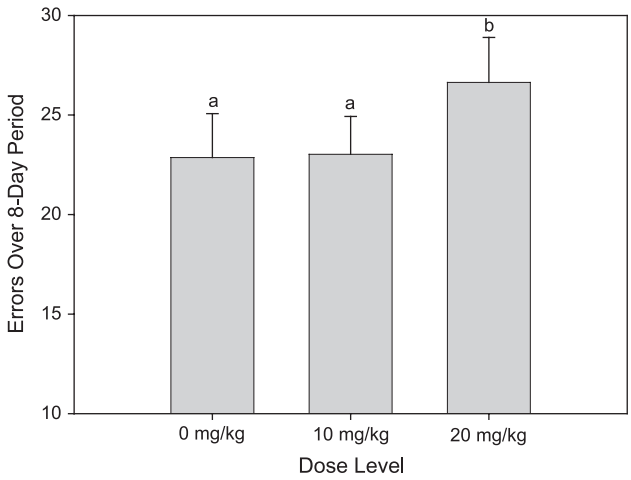

Fig. 1. The graph shows the total number of errors made during the 8-day period of each treatment ($N = 18$). The dogs made more errors when tested under the 20 mg/kg dose compared to the 10 mg/kg and placebo doses; a is significantly different than b.

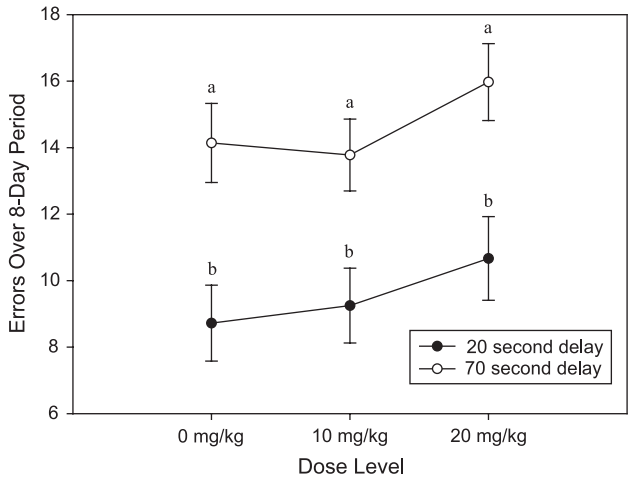

Fig. 2. The total number of errors at each delay interval is plotted. Significantly more errors were made at the 70-s delay interval compared to the 20-s delay interval. This effect was not affected by adrafinil; a is significantly different than b.

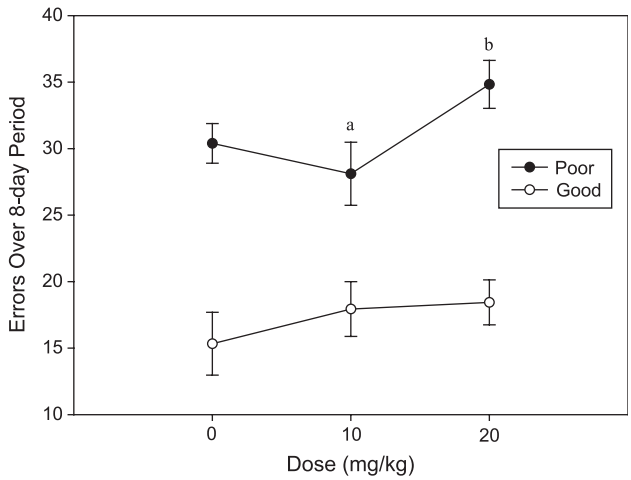

Fig. 3. The graph plots the total number of errors over each 8-day testing period for the dogs divided into good and poor performers. Both groups were impaired by adrafinil at the 20 mg/kg dose. The poor performers made significantly more errors under the 20 mg/kg dose than the 10 mg/kg dose; a is significantly different than b.

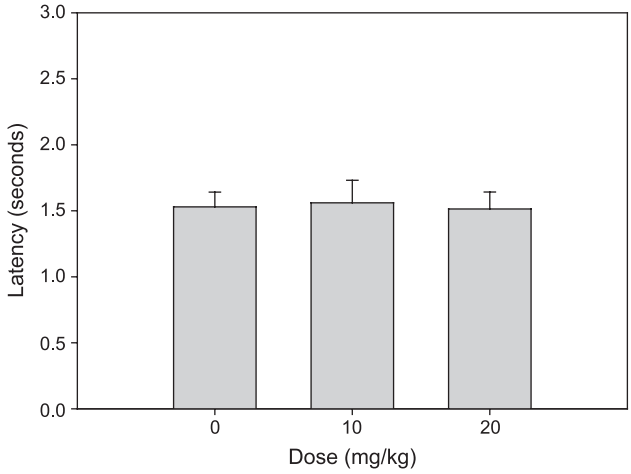

Fig. 4. The mean latency (seconds) for all dogs ($N=18$) at each dose is plotted. Adrafinil did not alter the latency to respond to the stimuli at any dose level.

3.2. Response latency

Adrafinil did not have an effect on response latency. The latency under 20 mg/kg did not differ from the 10 mg/kg ($P=.98$) or placebo ($P=.44$) doses (Fig. 4). The latency under the 10 mg/kg and placebo doses also did not differ ($P=.69$). Latencies at 20 s did not differ from latencies at 70 s at 0 mg/kg ($P=.56$), 10 mg/kg ($P=.81$), or 20 mg/kg ($P=.95$). The latencies of the poor and successful performers did not differ at the 0 mg/kg ($U=39.00$, $P=.89$), 10 mg/kg ($U=40.00$, $P=.97$), or 20 mg/kg ($U=33.00$, $P=.51$) doses.

4. Discussion

Adrafinil, at a dose of 20 mg/kg, disrupted performance on the DNMP task, a test of visuospatial memory. Dogs performed worse under the 20 mg/kg dose compared to the placebo and 10 mg/kg conditions. We previously reported an improvement in discrimination learning in aged dogs treated with adrafinil (Milgram et al., 2000), which could be related to effects on attention or motivation. We found no effect of adrafinil on performance of a delayed nonmatching-to-sample (DNMS) task (Siwak et al., 2000c). The present study indicates that adrafinil impairs working memory on a DNMP test, which could be linked to disruptive effects on noradrenergic function in the prefrontal cortex. The DNMP task is a working memory test that relies on the integrity of the prefrontal cortex (Braver et al., 2001; D'Esposito et al., 1999; Owen et al., 1999; Petrides et al., 1993, 2000). The effects of adrafinil on cognitive performance in dogs are consistent with an alpha-1 adrenergic mechanism of action.

The underlying mechanism of action of adrafinil has not been established with certainty but seems to require an intact central noradrenergic system (Duteil et al., 1979; Delini-Stula and Hunn, 1990; Simon et al., 1983; Chermat et al., 1981; Rambert et al., 1986). Studies of cognitive function involving noradrenergic transmission have focused on the effects of alpha-2 adrenergic agents. Alpha-2 agonists lead to improvements in performance on spatial delayed response tests (Franowicz and Arnsten, 1998; Li et al., 1999; Franowicz et al., 1999), delayed matching-to-sample tests (Jackson and Buccafusco, 1991), and DNMS tests (Arnsten and Goldman-Rakic, 1990) in both young and aged monkeys. Reversal performance in aged monkeys is also improved with alpha-2 agonists (Steere and Arnsten, 1997). Alpha-2 antagonists lead to declines in performance (Li et al., 1999). The prefrontal cortex may be the site of action of the alpha-2 agonists (Li et al., 1999; Avery et al., 2000).

The effects of alpha-1 agonists on cognitive performance are less consistent. Alpha-1 agonists may facilitate the acquisition or encoding of new information (Puumala et al., 1996, 1998) but do not participate in spatial working memory as assessed with the DNMP task in rats (Puumala and Sirviö, 1997). Puumala and Sirviö (1997) suggest that modulation of alpha-1 adrenoceptors may affect motor activity and motivation rather than cognitive ability directly. Arnsten et al. (1999), using the delayed alternation task in rats, reported impairments in prefrontal cortex functions. Delayed response tests in aged and young monkeys also indicate that alpha-1 stimulation impairs spatial working memory performance (Arnsten and Jentsch, 1997; Mao et al., 1999).

Arnsten (1998) suggests that noradrenaline impairs prefrontal cortex function through its actions at alpha-1 adrenergic receptors. Noradrenaline has a higher affinity for alpha-2A than alpha-1 receptors; thus, low levels of noradrenaline in the prefrontal cortex may engage alpha-2 receptors while higher levels engage alpha-1 (Arnsten, 1998). Alpha-1 stimulation can increase excitatory currents in apical dendrites thereby increasing background noise, which can interfere with the signal transfer to prefrontal cortex cell bodies. The prefrontal cortex can no longer inhibit processing of irrelevant information and working memory functions are impaired.

The stimulation of alpha-1 receptors can affect behavioral activity in addition to cognitive function. Arnsten (1998) suggests that alpha-1 receptor mechanisms might contribute to the occurrence of behavioral problems in demented people. Siwak et al. (2000a) found that 20 mg/kg of adrafinil produced increases in locomotor activity in aged dogs. This increase in activity could disrupt performance on cognitive tasks by interfering with sustained attention. In an earlier study, we reported that 20 mg/kg of adrafinil enhanced visual discrimination learning in aged dogs (Milgram et al., 2000). The present study, using a visuospatial memory task, however, found impairment rather than improvement. The neuropsychological tests are administered with the dogs placed inside a wooden box, where they must wait for the duration of the intertrial intervals and the delay

intervals for objects to be presented. The discrimination learning task used in our previous report is a quick task to administer. The intertrial intervals were only 30 s and there was only a single presentation for each of the 10 trials. The DNMP task used 60 s intertrial intervals and a delay interval for two presentations per each of 12 trials, making the task much longer to complete and requiring sustained attention. Hyperactivity would be more disruptive in a test where the dog has to wait for longer periods of time.

If hyperactivity contributed to the decline in performance under adrafinil, however, we would expect to see a decrease in response latency, the time it took the dog to move toward the tray and select an object. In humans, adrafinil and modafinil administration leads to decreases in reaction time in elderly people (Saletu et al., 1986). Adrafinil did not affect response latency in dogs suggesting that hyperactivity may not have accounted for the disruptive effects on performance. We previously reported increased locomotor activity in aged dogs at a minimum dose of 20 mg/kg in a novel environment (Siwak et al., 2000a,b) but not in a familiar situation (Siwak et al., 2000b). The test box in this study is a highly familiar situation for the dogs since they are tested in it daily. Hyperactivity may not have been induced in this situation. Additionally, the method of measuring the response latency may not have been sensitive enough to detect effects of adrafinil since latencies were not recorded automatically.

Other mechanisms may account for the impairment in working memory observed following adrafinil administration. Recent studies, using modafinil, indicate that hypocretin-producing neurons may be another potential site of action for modafinil and adrafinil (Chemelli et al., 1999; Scammell et al., 2000). Hypocretins (also called orexins) are newly discovered neuropeptides that function as neurotransmitters with excitatory activity (Kilduff and Peyron, 2000; De Lecea et al., 1998; Sakurai et al., 1998). These peptides appear to play a role in feeding, blood pressure regulation, neuroendocrine regulation, thermoregulation, metabolism, and sleep regulation (Kilduff and Peyron, 2000; Peyron et al., 1998). Hypocretin-producing neurons are found in the hypothalamus and have axonal projections to areas within the hypothalamus and to several other brain areas, including the locus coeruleus and raphe nuclei (Kilduff and Peyron, 2000; De Lecea et al., 1998; Peyron et al., 1998). Actions of adrafinil on the hypocretin-producing neurons could contribute to the working memory impairment, but the role of this system in working memory has yet to be explored.

Future research should explore the alpha-1 and hypocretin mechanisms of action. Use of an alpha-1 antagonist (i.e., prazosin) or a hypocretin antagonist to determine if the working memory impairment is reversed would provide valuable clues to the mechanism through which adrafinil is affecting working memory.

The effects of adrafinil vary as a function of both task and dose. We have found that a 20 mg/kg dose of adrafinil improves discrimination learning (Milgram et al., 2000), does not affect object recognition memory in a DNMS task (Siwak et al., 2000c), and impairs spatial working memory. All of these studies used the 20 mg/kg dose of adrafinil because we found that 20 mg/kg was the minimum effective dose required to produce a reliable increase in locomotor activity in the dog (Siwak et al., 2000a). The induced hyperactivity might have served as a distracter to disrupt working memory, even though the latency measure was not sensitive to it. The facilitation of discrimination learning may reflect effects of adrafinil on memory consolidation since the effects of adrafinil may persist for up to 10 h (Siwak et al., 2000a).

This is our first study to examine the effect of 10 mg/kg of adrafinil on cognition. We found that 10 mg/kg of adrafinil did not reliably affect locomotor activity (Siwak et al., 2000a). Although 10 mg/kg did not lead to hyperactivity, it did produce an EEG arousal effect. Adrafinil induced an arousal state in cortical EEG in dogs, and the effects of 10 mg/kg did not differ from the 20, 30, or 40 mg/kg doses (Siwak et al., 2000c). Our earlier research also indicates that some dogs do not respond to adrafinil administration (nonresponders or negative responders) and metabolic differences exist between dogs (Siwak et al., 2000a). Thus, the data from the present study show that the 20 mg/kg dose is disruptive to spatial working memory, but more research needs to be conducted to further examine the effects of lower doses on cognition.

Central nervous system stimulants frequently interfere with appetite in animals, which could affect performance variables. The test procedures used in this study used food as a reward and a change in appetite could have affected the outcome. Evidence from our previous discrimination learning study (Milgram et al., 2000) indicates that the beneficial effects of adrafinil may be partly related to performance motivation. One dog in that study frequently stopped responding during baseline and control conditions. When given adrafinil, the total number of responses increased dramatically. We observed this effect again in a second dog exhibiting similar response failures on a DNMP test (Siwak et al., 2000c). In addition, Nicolaidis and De Saint Hilaire (1993) examined the effect of modafinil (the main metabolite of adrafinil) on feeding behavior in rats. The results showed that although the frequency of meals taken by the rat was significantly reduced in comparison to controls, there was no change in the size or duration of the meals taken, using 20 and 40 mg/kg. The reduction of feeding observed was due to an increase in the meal to meal interval. Thus, modafinil reduced feeding not by diminishing the size of meals but by lengthening the meal-to-meal interval. This suggests that an alteration in appetite did not drive the results of this study.

4.1. Baseline performance

Dogs that were classified as poor performers during baseline testing continued to perform at low accuracy during

the placebo condition. Several reports have identified two subgroups of aged animals; one whose performance on a variety of behavioral tests does not differ from that of young animals, and a second group whose performance is dramatically worse compared to young animals (Gallagher and Burwell, 1989; Rowe et al., 1998; Adams et al., 2000b). Adams et al. (2000b) identified various groups of aged dogs based on their performance, compared to young dogs, on a variety of cognitive tests. Some aged dogs are impaired on some tests while others, successful agers, perform at levels equivalent to young dogs on some tests. The present study divided the aged dogs into two groups based on their baseline performance of the DNMP task. The poor performers continued to perform worse than the good performers under adrafinil treatment. Both groups, the poor performers and good performers, showed impaired performance when given the 20 mg/kg dose of adrafinil.

4.2. Delay

Lengthening of the delay interval on the DNMP task increases the demands on working memory and leads to a decline in performance (Tapp et al., 2003b; Chan et al., 2002). The information obtained on the sample presentation must be retained for a greater period of time rendering the task more difficult. Both poor and good performers were affected the same way by the longer delay interval, performance declined. This effect was not affected by treatment with adrafinil.

4.3. Conclusions

Adrafinil, at a dose of 20 mg/kg, impaired performance of aged dogs on a visuospatial working memory task. Previous work found improvement in discrimination learning at this dose. This suggests that adrafinil selectively improves encoding, the acquisition of new information, while disrupting working memory possibly through attentional mechanisms. Evidence from the present and our previous studies suggests that the effects of adrafinil seem to depend on the task used. Also, lower doses may produce different effects on cognition. Further research should explore these possibilities.

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