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Oral Administration of Adrafinil Improves Discrimination Learning in Aged Beagle Dogs

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MILGRAM, N. W., C. T. SIWAK, P. GRUET, P. ATKINSON, F. WOEHRLÉ AND H. CALLAHAN. *Oral administration of adrafinil improves discrimination learning in aged beagle dogs.* PHARMACOL BIOCHEM BEHAV **66**(2) 301–305, 2000.—Aged beagle dogs were trained on either a size or intensity discrimination task 2 h following treatment with either 20 mg/kg of adrafinil or a placebo control. Training continued until the dogs reached a predetermined criterion level of performance, or failed to acquire the task after 40 sessions. The treatments and tasks were then reversed, with both the test order and treatment order counterbalanced. Thus, half of the animals were first tested on the intensity discrimination, and half of these were first tested under adrafinil. Treatment with adrafinil produced significant improvement in learning, as indicated by a decrease in both errors and trials to criterion. An effect of adrafinil on motivation may partially account for these findings; however, adrafinil did not significantly affect response latency. Adrafinil is believed to serve as an alpha-1 adrenoceptor agonist. The improved learning may also result from enhancement of vigilance due to facilitation of noradrenergic transmission in the central nervous system. © 2000 Elsevier Science Inc.

Adrafinil Modafinil Discrimination learning Cognition Canine Aging

(DIPHENYLMETHYL)SULFINYL-2 ACETOHYDROXAMIC ACID (adrafinil) is a newly developed pharmaceutical with a unique profile of producing behavioral stimulation in the absence of stereotypy. In animal studies, treatment with adrafinil has been reported to increase locomotor activity in mice (13), monkeys (11), and dogs (16,17). The locomotor facilitating effect of adrafinil differs from those produced by psychomotor stimulants, such as amphetamine. Adrafinil does not produce stereotypy; nor does it have peripheral sympathetic effects (13). Adrafinil also affects a more restricted set of central nervous system structures than amphetamine (4). Adrafinil is commonly believed to serve as an alpha-1 adrenergic receptor agonist, although evidence exists to suggest other mechanisms of action could also be involved (9).

These activating effects of adrafinil suggest an application as a vigilance-enhancing drug. This possibility has been confirmed in studies with human subjects with problems associated with arousal or vigilance (3,7,8). Possibly because of these effects on vigilance, adrafinil has also been found to produce improvement in aspects of cognitive function related to attention and vigilance (14).

Despite the evidence of these vigilance-promoting effects of adrafinil in humans, no studies have looked at the effects of adrafinil on cognition in animals. The present investigation addressed the possibility that cognitive functioning of aged dogs could be improved by treatment with adrafinil. A crossover design was used to test the effectiveness of adrafinil on discrimination learning ability in a group of aged beagle dogs. Each dog was tested on two separate learning tasks—a size discrimination task, and an intensity discrimination task. Using a counterbalanced procedure, adrafinil was administered during learning of one of the tasks, and a placebo was administered during learning of the other.

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METHOD

Subjects

Subjects were eight beagle dogs, five females and three males, ranging in age from 7.5 to 10 years of age at the start of the study. The dogs were purchased from Harlan–Sprague–Dawley Breeding, and had been in the test facility for approximately 6 months before the start of the study.

Test Apparatus

As described previously (10), the testing box consisted of a wooden box equipped with a sliding Plexiglas tray containing two laterally placed food wells. The front of the box consisted of movable stainless steel bars, which provided adjustable openings that enabled the animals to have access to the food wells. The experimenter was visually separated from the dog by a screen with a one-way mirror. The food tray was presented and withdrawn by opening a hinged door at the bottom of the screen. A lamp attached to the front of the box provided the only source of light, and assured that the dog could not see the experimenter when the screen was lowered.

Data acquisition was controlled by a dedicated program developed in the Asyst programming language. Timing, randomization procedures, and the location of the objects were determined by the program. Just prior to the start of each trial, a tone was presented as a cue to both the animal and experimenter

Training and Behavioral Tests

Before starting the study, every animal underwent a standard four-phase pretraining procedure as described previously (10). Behavioral test sessions consisted of 10 discrete trials with a 30-s intertrial interval, and were administered daily. The dogs were first trained to approach food presented to them on the tray. Next, they were trained to displace objects on the tray to obtain a reward, which consisted of either Hill's® Science Diet Treats® Canine Maintenance® or Derby Pet Food® or Vital® dog food.

After completing this initial pretraining phase, the animals were trained on a standard object discrimination learning and reversal task. This was done to familiarize the dogs with discrimination learning problems. Two objects were used—a yellow coffee jar lid, and a blue Lego block. Displacing the correct object led to a food reward. Prior to the start of training, each animal was given 10 trials with food located beneath both of the objects. This was done to establish object preferences. When a preference existed, on the subsequent object discrimination training phase, the animals' nonpreferred object was selected to be associated with the reward. A random procedure was used to select the positive object for animals that showed no object preferences.

During the discrimination learning tasks, the dog was presented with the two objects, one covering each of the lateral food wells. On each trial, an error occurred whenever the animal responded to its initially preferred objects. On each session, the animals were allowed a single correction trial, where they were allowed to correct their error to obtain the reward. The correct object was presented equally often to both sides (five times each) and the order of presentation was such that the same object was never presented on the same side for more than three successive trials.

Test sessions were repeated daily on consecutive days until the animal achieved a criterion level of performance that included: (a) obtaining either 9 correct responses out of 10 on a single test session or 8 correct out of 10 on two successive sessions, and (b) performing at an accuracy of at least 70% over three successive additional sessions following the achievement of the criterion level. An animal was assumed to fail the task when it had completed a total of 40 test sessions without achieving the criterion.

After completing the initial discrimination learning task, the animals were then trained on an object reversal task. The test procedures were identical except that the positive and negative discriminanda were switched. Thus, if an animal was initially rewarded for approaching the Lego block, during the reversal task the animal had to approach the coffee jar lid to obtain the reward.

No drugs or other interventions were administered during the pretraining phase. Adrafinil or placebo treatment commenced on the day following completion of the reversal learning task. Treatment was started when the dogs began either a size or an intensity discrimination task. The test procedures used for the size and intensity discrimination tasks were identical to those followed for the object discrimination learning task. The size discrimination task required dogs to respond selectively to one of two objects that differed only in size. The small object was a single red wooden block, and the large object was constructed from two of the small wooden blocks. For the intensity discrimination, the objects were two blocks that were identical except that one was white and the other black.

Experimental Design

Either adrafinil, at a dose of 20 mg/kg, or a placebo was given daily by oral administration of capsules placed in soft dog food. The placebo consisted of capsules identical in appearance containing lactose. At all times, the experimenter administering the capsules was blinded as to its contents.

A crossover design was used to evaluate the effect of adrafinil on discrimination learning. Every animal was tested on both the size and intensity discrimination tasks. Half of the animals were tested on the size discrimination task first; the other half was tested on the intensity discrimination task. Half of the animals in each of these groups, in turn, were administered adrafinil 2 h before the behavioral test session; the other half were given a placebo capsule containing only lactose 2 h prior to the behavioral test.

An 8-day washout period followed completion of the first task. Each animal was subsequently switched to the second task, and to the opposite treatment condition. Thus, animals tested on the size discrimination under adrafinil were now tested on the intensity discrimination task after administration of placebo. A counterbalanced procedure was used in assigning animals to test group. This resulted in the following four groups with two subjects per group: group 1 was tested first on size discrimination under adrafinil and second on intensity discrimination under placebo. Group 2 was tested first for size discrimination under placebo and second for intensity discrimination under adrafinil. Group 3 was tested first for intensity discrimination under adrafinil and second for size discrimination under the placebo. Group 4 was tested first for intensity discrimination under the placebo and second for size discrimination under adrafinil.

Statistical Analysis

Three performance measures were used in the statistical analysis; errors to criterion, trials to criterion, and response latency. The errors measure was the total number of incorrect

responses made up to and including the criterion session. The trials measure was the total number of trials taken to complete the criterion. Trials in which an animal made no response were not counted as errors. Response latencies were defined as the time interval between presentation of the tray and contact by the animal with one of the objects.

All statistical analysis used the Statistica software package. We first compared performance on the two tasks (Fig. 1). There was a marginally significant difference in errors to criterion ($t = 0.0503$ two-tailed t-test) and a statistically significant difference in total trials to criterion ($t = 0.0313$, two-tailed t-test). Because the tasks were not equivalent in difficulty, the distributions for the two tasks were normalized, and each animal was assigned a standard score, which was calculated by subtracting the animal's score from the mean of the distribution and then dividing by the standard deviation.

A three-way analysis of variance was used to test for the effects of adrafinil on acquisition. The three factors were treatment (adrafinil vs. placebo), a within-subject factor, treatment order (drug or placebo first), and task order (size discrimination or intensity discrimination first); both were between-subject factors.

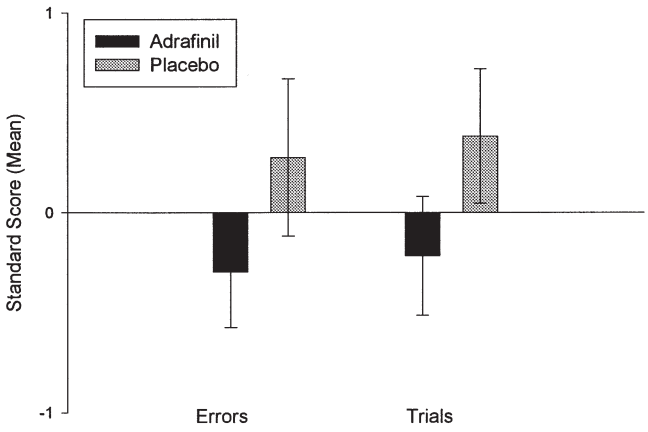

FIG. 2. The origins of the significant overall effect of adrafinil on learning is shown. Discrimination learning occurs more rapidly when animals are treated with adrafinil than under placebo control condition. Normalized scores were used to prevent the results being confounded by differences in task difficulty.

RESULTS

Learning

Analysis of both the errors to criterion and trials to criterion measures revealed statistically significant main effects of treatment, $F(1, 4) = 10.38$, $p = 0.0322$, for the errors measure, and, $F(1, 4) = 16.14701$, $p = 0.015886$, for the trials measure, and statistically significant interactions between task order and treatment for the errors and trials measures, respectively, $F(1, 4) = 11.64$, $p = 0.027$; $F(1, 4) = 7.791$, $p = 0.0493$. There was no effect of order of drug administration, while the effect of task order was marginally significant for both errors and trials, $F(1, 4) = 7.08$, $p = 0.0503$; $F(1, 4) = 5.874$, $p = 0.0725$. Figure 2 shows that these effects reflect more rapid learning after treatment with adrafinil than after treatment with the placebo. Figure 3 illustrates that the speed of learning depended on task order. Animals learned the size discrimination task more rapidly when the intensity task was first.

In one subject, the improved learning under adrafinil appeared to be linked to an effect on response motivation. Under the placebo control condition, this animal showed periods of depressed motivation, which were manifest by frequent failures to respond to either object. Figure 4 shows that this impairment was largely eliminated by treatment with adrafinil. Over the placebo session this subject failed to respond on 50 out of a possible 260 trials (19.2%). It responded on all but 2 out of 50 trials (4%) under adrafinil.

Latency Analysis

We also looked at the effect of adrafinil on response latencies. For each animal, the mean latency was calculated for

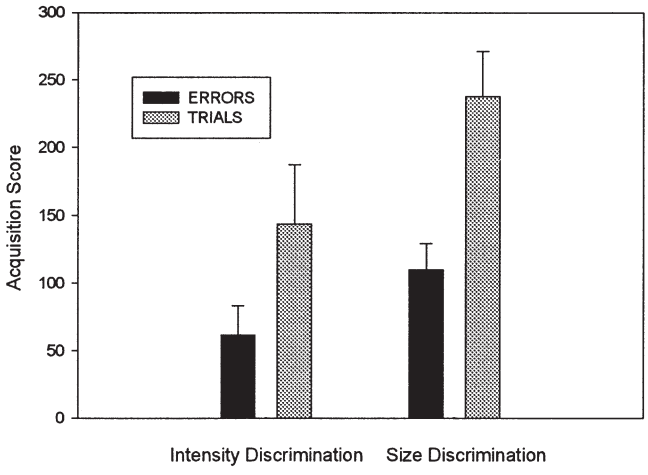

FIG. 1. Comparison of errors and trials to criterion measures on size and intensity discrimination learning tasks. The data from the two treatment conditions were combined for each of the tasks. The size discrimination task was found to be more difficult, using both measures of learning (errors and trials to criterion).

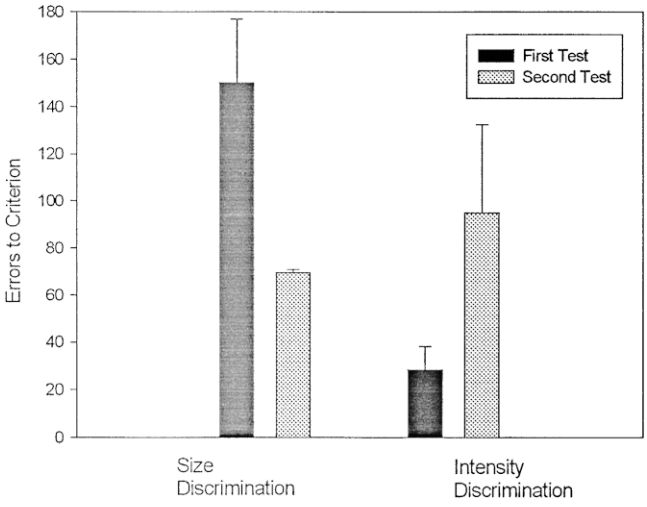

FIG. 3. The number of errors made to achieve criterion are plotted as a function of task order. Speed of learning depended upon the task order. Size discrimination learning was faster in animals tested on the intensity discrimination first. The reverse was not true. Learning was slower on the intensity task when it followed the size discrimination.

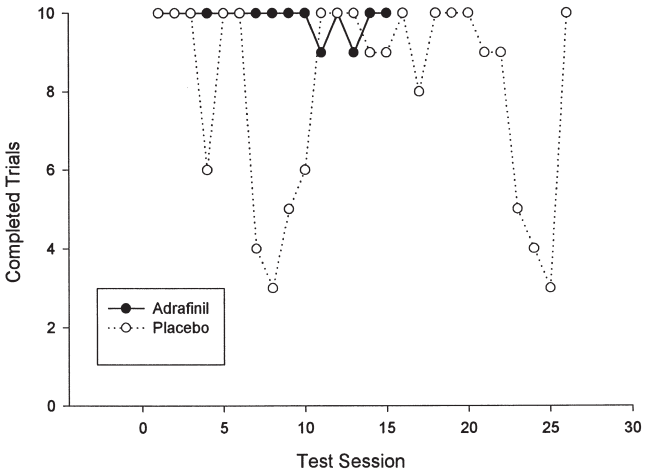

FIG. 4. Effect of adrafinil on motivation to respond in a single subject. This subject showed frequent response failures under the placebo control condition but not under the adrafinil condition.

each session. In doing these calculations, all responses that were greater than or equal to 5 s were first filtered out to prevent biasing effects from very long latency responses. For each animal, grand means were then calculated over the entire test period. This procedure did not reveal any statistically significant differences under the two treatment conditions. On the other hand, there were marked individual differences, and these tended to be consistent from session to session. Figure 5 shows data from two different subjects, one of which showed a decrease in latency under adrafinil and the other showed an increase in latency.

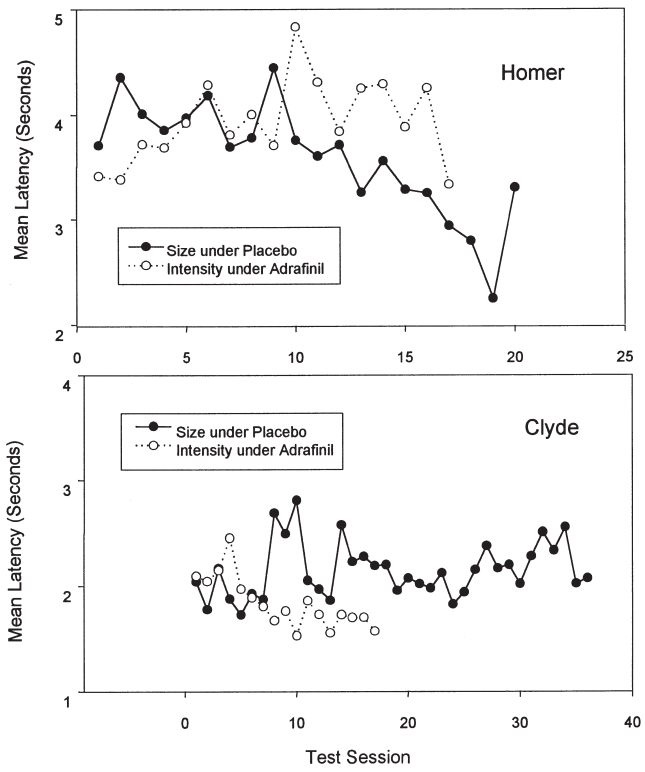

FIG. 5. Response latencies over successive training sessions are shown for two subjects that received the same treatments. One animal responded more rapidly, on the average, under adrafinil than under the control condition. The other animal showed an opposite effect, and responded more rapidly under the placebo control condition than it did under adrafinil.

DISCUSSION

The present results demonstrate that repeated daily administration of adrafinil can facilitate discrimination learning in canines. Discrimination learning under adrafinil was acquired with both fewer errors and fewer trials than under the control condition. Adrafinil has been found to enhance cognitive functioning of human subjects, but to the best of our knowledge, this is the first published evidence of adrafinil having cognitive enhancing properties in nonhuman subjects.

The effect of adrafinil depended in part on both order of testing and task. Adrafinil showed a greater cognitive enhancing effect on animals tested on the size discrimination learning problem first than it did on animals tested first on the intensity discrimination task. This probably reflects two factors: (a) the effectiveness of prior training on one task on subsequent training on a second, and (b) differences in task difficulty. Size and intensity discrimination learning problems can be solved in two ways: the first is by forming specific associations between the correct object and reward; the second involves learning the specific rule, namely that only one of the objects is associated a reward. To the extent that rule learning occurs, acquisition of one discrimination problem should facilitate the subsequent acquisition of another problem, which involves the same rule. The results of the present experiment provide partial support of this second alternative. Size discrimination learning was faster in the animals tested first on the intensity discrimination task than it was in animals tested on the size discrimination problem first. The converse, however, was not true. When the intensity discrimination task followed the size discrimination task, the learning occurred more slowly than when intensity discrimination learning was tested first.

Another factor that may have affected the outcome of this study is age; the subjects could all be categorized as either middle aged, or old. We have previously found that acquisition of a size discrimination task is generally more difficult in aged dogs than it is in young dogs (5). We also have preliminary evidence suggesting a much smaller age effect on intensity discrimination learning.

Aged dogs show deficits in a number of cognitive tasks other than size discrimination learning. These include discrimination reversal learning, acquisition of an object recognition memory task, acquisition of a visuospatial task, and performance at moderate to long delays on a spatial memory task (1,6,10). On the other hand, simple procedural learning tasks and object discrimination learning are not age sensitive (10). Clearly, it is important to determine whether the facilitatory effect of adrafinil is specific to discrimination learning or whether the effect is more general. Further studies should also include young dogs to establish if the improvement under adrafinil occurs exclusively in cognitively impaired animals.

The underlying mechanism of action of adrafinil has not been established with certainty. However, considerable evidence indicates that adrafinil serves as an alpha-1 adrenergic agonist (13). Thus, the behavioral response to adrafinil is similar to that produced by adrenergic agonists. Furthermore,

many of the behavioral effects of adrafinil can be blocked by prazosin, a selective alpha-1 adrenergic antagonist.

This agonistic action on brain adrenergic systems could account for the cognitive enhancing effects of adrafinil in aged subjects. Brain noradrenergic systems show a particular sensitivity to age-related degeneration (19). Furthermore, an extensive literature indicates a role of norepinephrine in learning and memory. Usher et al. (18) found that firing rate of noradrenergic neurons in the locus coeruleus correlated with performance on a discrimination learning task. Disruption of noradrenergic function can impair learning (15), while noradrenergic agonists can enhance both learning and working memory (2). Moreover, these effects are most marked in aged animals. Most of this literature has focussed on the alpha-2 adrenergic receptor. Puumala et al. (12), however, recently reported that St-58, a putative alpha-1 agonist, improved water-maze navigation of rats.

As discussed in the introduction, studies with human subjects indicate that adrafinil enhances vigilance. Thus, with psychometric testing adrafinil results in increased attention, as well as improved concentration and memory (14). All of these effects could account for the effectiveness of adrafinil on discrimination learning in dogs. In humans, adrafinil can also decrease reaction time. We had expected to see a corresponding effect of adrafinil in the dog on response latency, but this was not the case. There were no significant differences in response latency between the adrafinil and placebo conditions. There were, however, individual differences, and we cannot rule out the possibility of adrafinil affecting response latency in other testing paradigms.

Adrafinil also affects general arousal or motivation. Thus, adrafinil causes a robust increase in exploratory behavior (16,17). The improved discrimination learning appeared to be linked to a nonspecific increase in motivation in at least one subject. During the placebo tests, this animal showed frequent response failures in which the animal did not respond to either object. This was not the case, however, when the animal was administered adrafinil. This particular animal also showed frequent response failures during both the pretraining phase and during subsequent testing on another cognitive task. Thus, in this one case reliable behavioral responding occurred only when the animal was administered adrafinil.

To conclude, the present results provide the first evidence with nonhuman subjects that adrafinil can serve to enhance cognition. This effect could be caused by changes in attention, motivation, vigilance, or memory. Adrafinil is currently used as a vigilance-promoting agent for the elderly. Its effectiveness in improving learning in aged canines raises the possibility that it could also be effective in improving cognitive dysfunction in humans with either age-associated memory impairment or dementia.

REFERENCES

1. Adams, B.; Chan, A.; Callahan, H.; Siwak, C.; Tapp, D.; Ikeda-Douglas, C.; Atkinson, P.; Head, E.; Cotman, C.W.; Milgram, N. W.: Use of a delayed non-matching to position task to model age-dependent cognitive decline in the dog. Behav. Brain Res. 108:47–56; 2000.
2. Arnsten, A. F. T.: Catecholamine regulation of the prefrontal cortex. J. Psychopharmacol. 11:151–162; 1997.
3. Dewailly, P.; Durocher, A. M.; Durot, A.; Bukowski, J.V.; Frigard, B.; Herbin, H.; Lemaire, P.; Kohler, F.; Betrancourt, J. C.; Lubin, S.: Adrafinil et ralentissement du sujet âgé institutionnalisé: De la significativité statistique à la pertinence clinique (résultat d'une étude multicentrique en double aveugle versus placebo). Acta Med. Interpsychiatrie 6:1–8; 1989.
4. Engber, T. M.; Dennis, S. A.; Miller, M. S.; Contreras, P. C.: Brain regional substrates for the actions of the novel wake-promoting agent modafinil in the rat: Comparison with amphetamine. Soc. Neurosci. Abstr. 23:793; 1997.
5. Head, E.; Callahan, H.; Muggenburg, B. A.; Cotman, C. W.; Milgram, N. W.: Visual discrimination learning and beta amyloid accumulation in the dog. Neurobiol. Aging 19:415—425; 1998.
6. Head, E.; Mehta, R.; Hartley, J.; Kameka, M.; Cummings, B. J.; Cotman, C. W.; Ruehl, W. W.; Milgram, N. W.: Spatial learning and memory as a function of age in the dog. Behav. Neurosci. 109:851–858; 1995.
7. Israel, L.; Fondarai, J.; Lubin, S.; Salin, B.; Hugonot, R.: Olmifon(r) et patients àgés ambulatoires. Efficacité, versus placebo, de l'Adrafinil sur l'éveil dans les activités de la vie quotidienne. Psychol. Med. 21:1235–1255; 1989.
8. Kohler, F.; Lubin, S.: Étude en médecine générale de l'intérét thérapeutique d'Olmifon chez des malade présentant des symptomes précoces de vieillissement cérébral handicapant leur activité quotidienne. Étude ouverte pragmatique chez 304 patients. Vie Med. 2:335–344; 1990.
9. Milgram, N. W.; Callahan, H.; Siwak, C.: Adrafinil: A novel vigilance promoting agent. CNS Drug Rev. 5:193–212; 1999.
10. Milgram, N. W.; Head, E.; Weiner, E.; Thomas, E.: Cognitive functions and aging in the dog: Acquisition of non spatial visual tasks. Behav. Neurosci. 108:57–68; 1994.
11. Milhaud, C. L.; Klein, M. J.: Effets de l'Adrafinil sur l'activité nocturne du macaque rhésus (*Macaca mulatta*). J. Pharmacol. (Paris) 16:372–380; 1985.
12. Puumala, T.; Greijus, S.; Narinen, K.; Haapalinna, A.; Riekkinen, P.; Senior, Sirviö, J.: Stimulation of alpha-1 adrenergic receptors facilitates spatial learning in rats. Eur. Neuropsychopharmacol. 8:17–26; 1998.
13. Rambert, F. A.; Pessonnier, J.; De Sereville, J.-E.; Pointeau, A.-M.; Duteil, J.: Profil psychopharmacologique originil de l'adrafinil chez la souris. J. Pharmacol. (Paris) 17:37–52; 1986.
14. Saletu, B.; Grunberger, J.; Linzmayer, L.; Stohr, H.: Pharmaco-EEG, psychometric and plasma level studies with two novel alpha-adrenergic stimulants CRL 40476 and 40028 (Adrafinil) in elderlies. New Trends Exp. Clin. Psychiatry 2:5–31; 1986.
15. Sirviö, J., Jr.; Riekkinen, P.; Valjakka, A.; Jolkkonen, J.; Riekkinen, P. J.: The effects of noradrenergic neurotoxin, DSP-4, on the performance of young and aged rats in spatial navigation task. Brain Res. 563:297–302; 1991.
16. Siwak, C.; Callahan, H.; Gruet, P.; Takagi, N.; Milgram, N. W.: Adrafinil: A novel compound with both behavioral activating and cognitive enhancing effects in aged dogs. Soc. Neurosci. Abstr. 24:687; 1998.
17. Siwak, C.; Gruet, P.; Muggenburg, B. A.; Murphey, H. L.; Callahan, H.; Milgram, N. W.: Behavioral activating effects of adrafinil in aged canines. Pharmacol. Biochem. Behav. 66:293–300; 2000.
18. Usher, M.; Cohen, J. D.; Servan-Schreiber, D.; Rejkowski, J.; Aston-Jonmes, G.: The role of locus coeruleus in the regulation of cognitive performance. Science 283:549–554; 1999.
19. Vijayashankar, N.; Brody, H. J.: A quantitative study of the pigmented neurons in the nuclei locus coeruleus and subcoeruleus in man as related to aging. J. Neuropathol. Exp. Neurol. 38:490–497; 1979.