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Effects of Aniracetam on Delayed Matching-to-Sample Performance of Monkeys and Pigeons[1]

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PONTECORVO, M. J. AND H. L. EVANS. *Effects of aniracetam on delayed matching-to-sample performance of monkeys and pigeons.* PHARMACOL BIOCHEM BEHAV 22(5) 745–752, 1985.—A 3-choice, variable-delay, matching-to-sample procedure was used to evaluate drugs in both pigeons and monkeys while tested under nearly-identical conditions. Aniracetam (Roche 13-5057) improved accuracy of matching at all retention intervals following oral administration (12.5, 25 and 50 mg/kg) to macaque monkeys, with a maximal effect at 25 mg/kg. Aniracetam also antagonized scopolamine-induced impairment of the monkey's performance. Intramuscular administration of these same doses of aniracetam produced a similar, but not significant trend toward improved matching accuracy in pigeons.

Aniracetam Short-term memory Cognition enhancement Scopolamine Pigeons Monkeys Behavior Matching-to-sample

A growing interest in drug-facilitated memory performance [4, 20, 23, 46] has emerged both from basic research on learning and memory and from the search for therapeutic alternatives for memory impairments due to aging, toxicant exposure or brain injury [7, 12, 37]. However, establishing the specificity of drug effects on memory processes has proven difficult [27,30]. Memory-enhancing effects can be confounded with changes in other aspects of performance such as arousal, motivation or response bias. Furthermore, many compounds that have been tested in well controlled studies have often produced small or inconsistent effects, or have proven effective only in cognitively impaired subjects [1, 2, 3, 14, 20, 44]. Thus, in spite of a decade of intensive research and improved test procedures, new compounds remain in great demand.

One new compound, Aniracetam (Ro 13-5057; 1-anisoyl-2-pyrrolidinone), has shown particular promise for enhancing memory and cognitive performance [12]. Aniracetam protected learning and retention of active and passive avoidance tasks by mice and rats against the disruptive effects of hypoxia, electro-convulsive shock, protein synthesis inhibition and the administration of scopolamine. The present study extended these findings in several important ways.

First, we examined the effects of aniracetam in an appetitively motivated task rather than the shock-motivated procedures previously employed [12]. If aniracetam selectively improves cognition, then it should prove efficacious in both appetitively and aversively motivated tasks.

Second, we examined both normal and scopolamine-impaired subjects. The demonstration that aniracetam's effects are not limited to subjects with experimentally induced cognitive impairments (e.g., [12]) would have implications both for the biochemical basis of normal memory, and for the potential therapeutic applications of the compound.

Third, we examined aniracetam's effects on short-term memory performance. The previous study of aniracetam [12] assessed aniracetam's effects on the ability to learn or retain a constant set of relationships among events; i.e., "long term memory," "reference memory" [28] or "constant memory" [45]. In constrast, our procedure emphasized memory for a unique event or relationship among events, relevant only for a brief period of time in a specific context; i.e., short-term memory, "working memory" [28], or "unique memory" [45]. Short-term memory is particularly important when evaluating cognition-enhancing drugs since short-term memory impairment is an important consequence of aging, brain injury and exposure to drugs and toxicants (e.g., [10, 11, 19, 32]) and relatively few drugs have been shown to facilitate short-term memory performance [20, 35, 43].

Finally, we extended the study of aniracetam's effects to two new species: pigeons (*Columba livia*) and macaque monkeys (*Macaca fascicularis*). Pigeons are useful experimental subjects because they are inexpensive and because a considerable literature exists concerning short-term memory [26,38] and the effects of drugs (e.g., [39,44]) in this species. Monkeys are an even more appropriate model because their phylogenetic position enhances extrapolations from experimental animals to humans.

Delayed matching-to-sample (DMS) performance was

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used to assess memory. On each trial a "sample" stimulus was presented, extinguished, and followed after some delay by a choice of three "comparison" stimuli. The subject was reinforced for choosing the stimulus which matched the hue of the sample. Short-term retention was quantified as the percentage of correct choices, as a function of the delay (retention) interval. Relative to other tests of animal's short-term memory (e.g., delayed alternation or radial arm maze performance), DMS has the advantages of precisely specifying the to-be-remembered stimulus, and of permitting the psychophysical manipulation of retention interval duration. Furthermore, since the subject must compare stimuli from before and after the retention interval (sample and comparison stimuli respectively), DMS tasks are relatively free from confounding effects due to overt postural mediation (e.g., [21]).

We now report the effects of 12.5, 25 and 50 mg/kg aniracetam on DMS performance in monkeys (Experiment 1) and in pigeons (Experiment 2). In Experiment 3 we report the ability of aniracetam pretreatment to overcome scopolamine-induced impairment of monkeys' DMS performance.

EXPERIMENT 1

Subjects

Six young adult (2.3–3.1 kg) feral female cynomolgus macaques (*Macaca fascicularis*) obtained from Hazelton Research Laboratories, Reston, VA, were housed individually in a room with an automatically timed cycle of 12 hr light and 12 hr darkness. Animal care conformed to NIH guidelines [33]. The monkeys were tested daily for 1 hr in the middle of the light period. All monkeys received free access to water during the last 3 hr of the light period and then were deprived of water for at least 16 hours prior to the next test session. The monkeys were fed 5 biscuits of Purina Monkey Chow (Ralston Purina Co., St. Louis, MO) in the morning at least one hour prior to the test session and 6–8 biscuits in the afternoon during the ad lib water period. Three monkeys, having inhaled behaviorally-inactive concentrations of toluene approximately one year prior to the start of the experiment, did not differ in control performance or response to aniracetam from previously unexposed monkeys.

Apparatus

Each monkey was placed in a 35.8×35.8×46.1 cm stainless steel transfer cage, which was secured inside one of 3 similar light-proof and sound attenuating enclosures (51.3×51.3×80.6 cm inside dimensions). White noise (Ralph Gerbrands Co., Arlington, MA, No. 64651 noise generator) was present continuously. A houselight (28 VDC×0.1 A) located above and behind the transfer cage provided diffuse illumination throughout the session, except for a 7 sec blackout following each incorrect response.

Three stimulus-response keys (2.6 cm diameter) were centered horizontally (7.7 cm apart center to center) at eye level on the wall facing the transfer cage. The keys could be illuminated from the rear with red, yellow or green light. A force of approximately 50 g was required to depress a key and register a response. A stainless steel spout located 2.6 cm below the center key delivered Welchade Grape Drink (Welch Foods Inc, Westfield, NY), mixed 1:1 with water, following each correct response. The apparatus was controlled and the data were recorded by a PDP-8A computer (Digital Equipment Corp., Maynard, MA) with SKED interface (State Systems, Inc., Kalamazoo, MI).

Procedure

A 3-choice, variable-delay matching-to-sample task was used. Each trial began with the presentation on the center key of a flashing (0.1 sec on, 0.1 sec off) red "observing" stimulus (this use of the red stimulus induced no bias toward or against the red stimulus during choice responding). Following a single response (depression of the center key), or the expiration of a 10 sec limited hold, the observing stimulus was replaced on the center key by the to-be-remembered sample stimulus (red, yellow or green). The sample remained on for 3.0 sec, and terminated in a variable retention interval during which no stimuli were presented. One of 3 retention intervals was selected at random for each trial: 0.2 sec, (hereafter called 0 sec), 6.0 sec or 12.0 sec. Following the retention interval all 3 keys were illuminated, each with one of the comparison stimuli (red, yellow and green). The position of the colors was randomized across trials. A response to the key illuminated with the sample color, terminated the stimuli and produced a 0.5 ml of juice. A response to a key that did not match the sample, or the expiration without a response of a 3.0 sec limited hold, produced a 7.0 sec timeout from reinforcement, during which the houselight and stimulus keys were extinguished. An incorrect choice, or a failure to respond was followed by a repetition of the same sample-delay-comparison sequence (correction trial). Only data from initial (not correction) trials were recorded for analysis in this study. The interval between the termination of the reinforcer, or timeout period and the presentation of the next observing stimulus (the inter-trial interval, ITI) was 1.0 sec. Responses during the ITI, delay and timeout periods had no programmed consequences. The session duration was 50 min. There was no restriction on the number of trials per session. The monkeys typically completed between 100 and 200 trials per session.

Drug regimen. Because aniracetam is not highly water soluble (soluability=0.13% in water; see [40]) and because doses of up to 150 mg/sugject were required, drug doses were prepared for individual monkeys by mixing appropriate amounts of aniracetam in a syringe with Welch's Grapeade. This mixture was then injected into the monkey's morning meal. The monkey was fed the biscuits one at a time and was observed to insure that all of the biscuits were eaten. The procedure took about 15 minutes and testing began approximately 1 hour later.

On the basis of Cummin *et al.*'s report [12] and our pilot data, we initially tested doses of 0, 25 and 50 mg/kg aniracetam. Drug tests were conducted twice weekly with a minimum of 3 days between successive tests. The order of dosing was counter-balanced across animals with a minimum of 2 determinations at each dose. The monkeys were subsequently tested with doses of 0 and 12.5 mg/kg. Because the control levels of accuracy for some monkeys increased during the interim period, the data from this follow-up study were analyzed separately from the earlier data.

Data analysis. For each monkey the accuracy of matching (percent correct) was averaged across replications of each drug dose. These means were then submitted to repeated measures analyses of variance [16] with dose and retention interval as factors. The percent of trials with observing responses and the mean choice reaction time were also recorded and analyses of variance were used, to com-

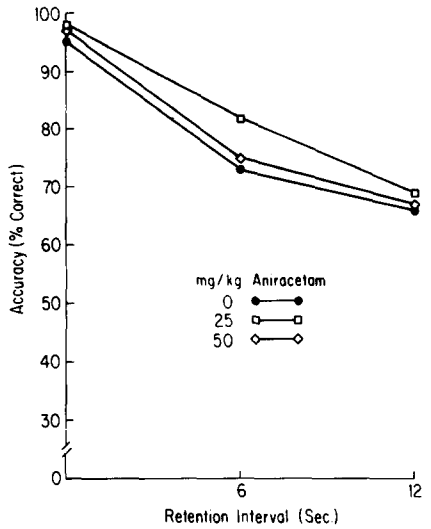

FIG. 1. Effects of 0, 25 and 50 mg/kg aniracetam on DMS accuracy in the monkey. Points represent means of 6 monkeys.

pare these values between vehicle and drug sessions. A criterion of $p<0.05$ was required for significance.

Results and Discussion

Accuracy of matching declined as a function of retention interval (Fig. 1). The mean levels of accuracy under control conditions were comparable to those obtained in other studies using similar DMS tasks (e.g., [36]). Aniracetam doses of 25 and 50 mg/kg significantly improved accuracy (overall dose effect: $F(2,10)=5.59$) but the dose by retention interval interaction was not significant. Individual monkeys differed in their sensitivity to aniracetam (Fig. 2). Monkeys 14, 10, 3 and 1 showed a fairly large and consistent facilitation of performance, whereas monkeys 12 and 8 showed only marginal effects.

In the follow-up study with 0 and 12.5 mg/kg aniracetam, there was no significant effect of aniracetam and no dose by retention interval interaction (mean accuracy at the 0, 6 and 12 sec retention intervals=99, 80 and 71% and 99, 79 and 75% for 0 and 12.5 mg/kg aniracetam respectively). Note that the control levels of accuracy in this follow-up study tended to be higher than those in the study with 25 and 50 mg/kg aniracetam. Although this trend was not statistically reliable, similar increases in retention with practice have previously been reported [13] and this trend may have partially obscured the effects of 12.5 mg/kg aniracetam.

Finally, no dose of aniracetam (12.5, 25 or 50 mg/kg) produced significant changes in the probability of an observing response or choice reaction time (mean p(obs)=.88, .86 and .90 for 0, 25 and 50 mg/kg aniracetam and .97 and .98 for 0 and 2 mg/kg aniracetam; mean reaction time=1.303, 1.318 and 1.388 sec for 0, 25 and 50 mg/kg aniracetam and 1.454 and 1.422 sec for 0 and 12 mg/kg aniracetam). Thus, aniracetam facilitated accuracy, but did not alter motor performance.

The route of drug administration may have limited the magnitude of the drug effect and increased between-subject variability in Experiment 1. Although aniracetam was effectively injected into the food biscuits and the monkeys ate with relatively little spillage, monkeys occasionally stored

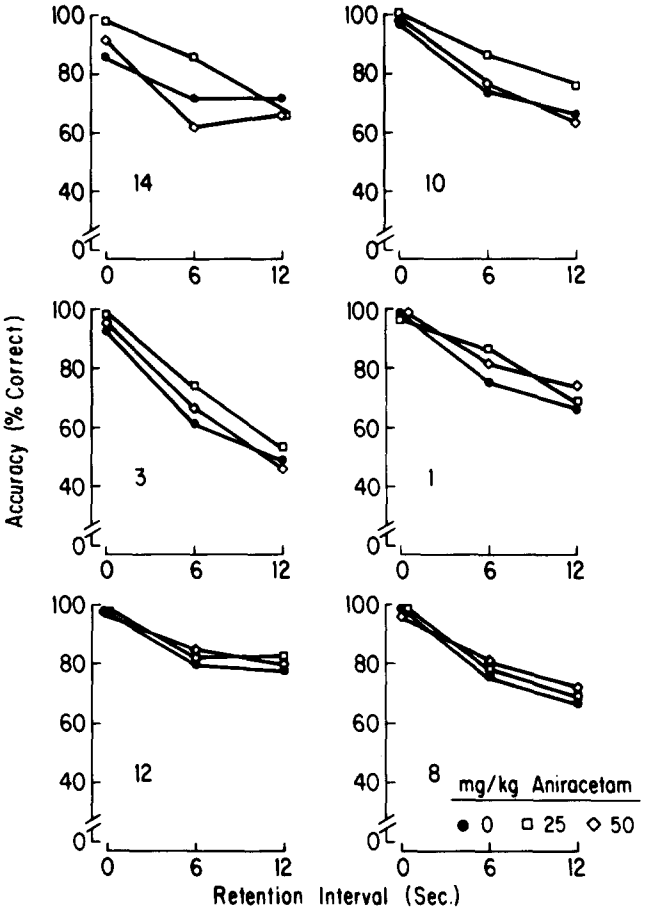

FIG. 2. Effects of 0, 25 and 50 mg/kg aniracetam on DMS accuracy by 6 individual monkeys.

food in their cheek pouches. Thus, both the administration-to-test interval, and the peak concentration of aniracetam in the blood, may have varied across animals or days, depending on a monkey's eating behavior.

Experiment 2 was, therefore, performed as a systematic replication of Experiment 1. Pigeons were used as subjects in Experiment 2 to test the between-species generality of aniracetam's effects. Aniracetam was administered IM in Experiment 2, thereby overcoming the potential problems related to oral administration of the drug.

EXPERIMENT 2

Subjects

Twelve experimentally naive adult male White Carneaux pigeons (Palmetto Pigeon Plant, Sumter, SC) were individually housed in a room with an automatically timed 12 hour light-dark cycle. Prior to the start of the experiment, the pigeons were food deprived to 80% of their ad lib weight. Thereafter, most birds obtained all of their daily food ration in the DMS experiment. Supplemental feeding (Purina Pigeon Checkers) was given as needed to maintain the 80% weight.

Apparatus

Four identical operant conditioning chambers (No. E10-

10, Coulbourn Instruments Inc., Lehigh Valley, PA) were enclosed in light-proof and sound-attenuating cubicles. White noise was presented through a speaker in the ceiling of the chamber. A houselight, located near the floor on the front wall of the chamber provided diffuse illumination throughout the session, except for a 2.5 sec period following an incorrect response.

Three 2.6 cm diameter stimulus response keys (Coulbourn No. 21-18) were centered horizontally 8.2 cm apart (center to center), 25.2 cm above the floor on the front wall of the chamber. A fourth key was centered 8.5 cm below the center key. All 4 keys could be illuminated from the rear with white, red, yellow or green light. A force of approximately 13 g was required to depress the keys and register a response. A food hopper, located 5.5 cm below the lower center key provided 2.5 sec access to mixed grain following each correct response. The computer and interface were the same as in Experiment 1.

Procedure

The pigeons were trained to perform a 3-choice, DMS task similar to that employed with the monkeys. Each trial began with the presentation of an observing stimulus (white light, maximum 10 sec duration) on the lower center key. Following a response, or expiration of the limited hold, the observing stimulus was replaced on the lower key by the sample stimulus (red, yellow or green). The sample (3.0 sec in duration) terminated in a variable retention interval: 0.1 sec (hereafter referred to as 0 sec), 4.0 sec or 8.0 sec. At the conclusion of the retention interval the comparison stimuli (red, yellow and green) appeared on the three top keys with position randomized across trials. A response to the key illuminated with the sample color produced 2.5 sec access to mixed grain. An incorrect choice, or a failure to respond within 3.0 sec, produced a 2.5 sec timeout from reinforcement. In all other respects the DMS procedure was identical to that in Experiment 1.

After achieving stable DMS performance (approximately 3 months after the start of training) all birds received IM injections of 0, 12.5, 25 and 50 mg/kg aniracetam in a vehicle of isotonic saline and 10% (v/v) Tween 80 (J. T. Baker Chemical Co., Phillipsburg, NJ). Injections were given in the pectoral muscle in a volume of 1.0 ml/kg, approximately 50 min prior to the test session. The order of drug doses was counter-balanced across birds. Drug tests occurred twice weekly, on the day following vehicle testing.

Results and Discussion

As in Experiment 1, matching accuracy decreased as retention interval duration increased. The control levels of matching accuracy were lower than those we have previously reported for a two choice DMS task [44] but were still considerably above chance even at the longest delays.

Aniracetam increased matching accuracy. However, the magnitude of the effect was somewhat smaller than that in Experiment 1. Accuracy at the 0, 4 and 8 sec retention intervals increased from means of 94, 67 and 65% correct following 0 mg/kg aniracetam (vehicle) to 94, 72 and 66% following 12.5 mg/kg, 94, 72 and 69% following 25 mg/kg and 94, 71 and 69% following 50 mg/kg aniracetam. Both the overall effect of aniracetam, and the dose by retention interval interaction approached, but did not achieve statistical significance, $F(3,33)=2.33$, $p<0.1$; $F(6,66)=1.92$, $p<0.1$ respectively. As in Experiment 1, aniracetam did not change the probability

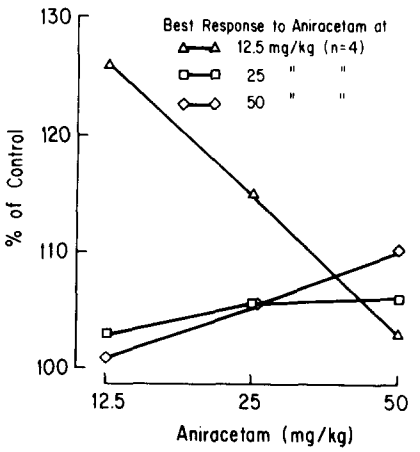

FIG. 3. Different sensitivity to aniracetam in 3 subgroups of pigeons. Effect of aniracetam is shown as percentage of control (vehicle) accuracy at 4-sec retention interval. Note that some pigeons (△) were sensitive to 12.5 but not 50 mg/kg aniracetam, whereas others (□) were sensitive at 50 but not 12.5 mg/kg. Similar results occurred at the 8 sec retention interval. At the 0 sec retention interval, all doses of aniracetam had minimal effects for all subgroups. Mean control accuracies at 0, 4 and 8 sec were 94, 57 and 56%, 94, 78 and 74% and 93, 66 and 65% in the groups that showed maximal facilitation at 12.5, 25 and 50 mg/kg aniracetam respectively.

of an observing response or choice reaction time, $p(\text{obs})=.98$ for 0, 12, 25 and 50 mg/kg aniracetam; mean reaction time=0.846, 0.836, 0.874 and 0.872 sec.

Several factors may have contributed to the failure to achieve significant results in Experiment 2. First, aniracetam's duration of action may have been short, relative to the 50-min absorption time. However, there were no differences in aniracetam's effects between the first and second 25-min halves of each test session. Furthermore, Schwam, Kuehn, Rumennik and Sepinwall [40] have recently reported that IM administration of aniracetam 60 min prior to the test session facilitates DMS performance by monkeys. Thus, it is unlikely that a shorter absorption time would have increased the magnitude of aniracetam's effect in Experiment 2.

Alternatively, variation in the maximally effective dose among individual pigeons may have precluded the demonstration of overall significant effects of any aniracetam dose. To illustrate this point the data from individual pigeons were grouped according to the dose of aniracetam that produced the greatest percentage change from the vehicle scores, and plotted for the 4-sec retention interval, where the effects of aniracetam, and these individual differences, were most apparent (Fig. 3). Note that one sub-group of pigeons showed a maximal facilitation of accuracy following 50 mg/kg of aniracetam and little facilitation at 12.5 mg/kg, whereas a different sub-group showed maximal facilitation at the low dose with little improvement at the high dose. Although there was a trend toward lower baseline accuracies in the sub-group showing maximum facilitation at 12.5 mg/kg (Fig. 3), there were individual pigeons in each of the other sub-groups with accuracy scores below the means for this group. Thus, there was no conclusive evidence for a relationship between baseline accuracy and sensitivity to aniracetam.

A final possibility is that cognition-enhancing effects of aniracetam are minimal in normal animals, but are more vis-

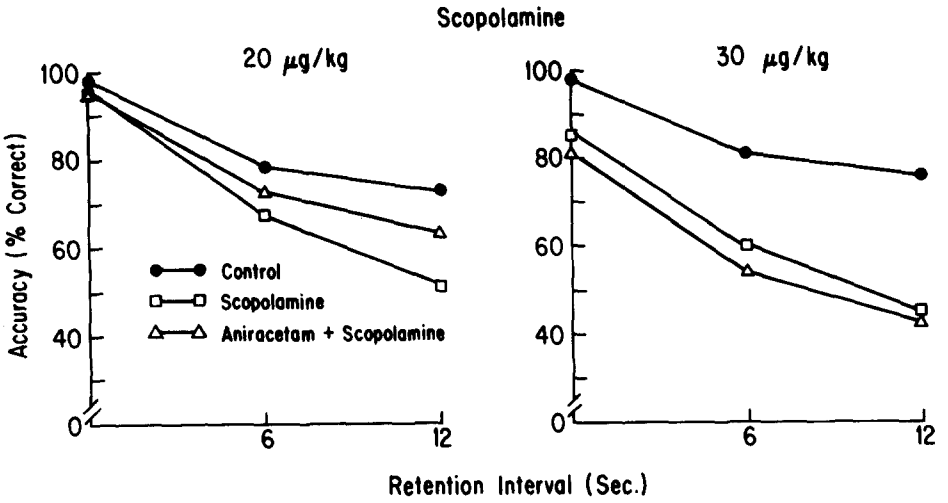

FIG. 4. Effects of pretreatment with 25 mg/kg aniracetam on DMS performance by scopolamine-treated monkeys (N=5). Note that aniracetam antagonized the effects of 20 μg/kg (left) but not 30 μg/kg scopolamine (right).

ible in cognitively-impaired animals [7,12]. Experiment 3 determined whether pretreatment with aniracetam could counteract scopolamine-induced impairment of DMS performance.

EXPERIMENT 3

The rationale for studying aniracetam's effects in scopolamine treated animals are three: First, since cholinergic neurotransmission has been implicated in cognitive processes [8, 15, 17, 42], scopolamine-impaired performance may provide a more appropriate and specific baseline for assessing aniracetam's cognitive enhancing effects than could be achieved with more global challenges such as electroconvulsive shock or hypoxia. Second, dysfunction of cholinergic neurotransmission has been implicated in neuropsychiatric disorders for which treatment with aniracetam might be appropriate (e.g., [5,37]). Finally, Cumin *et al.* [12] reported that aniracetam counteracts scopolamine-induced deficits in passive avoidance retention by rats and mice. Thus, the present experiment further tests and extends their findings.

Subjects

Five of the 6 monkeys from Experiment 1 were used. Monkey 3 was excluded because it showed an atypically-steep dose-response function for scopolamine. Apparatus and method for DMS was identical to that of Experiment 1.

Procedure

One hour before each twice-weekly drug session, each monkey received either 0 or 25 mg/kg aniracetam in food biscuits as in Experiment 1. Twenty minutes before the session the monkey was injected IM with either normal saline, or 20 or 30 μg/kg scopolamine HBr (Sigma Chemical Co., St. Louis, MO) dissolved in saline (dose expressed as the salt; injection volume=0.2 ml/kg). Thus, there were three test conditions: Vehicle Control (juice in morning meal, then saline injection), Scopolamine Alone, (either 20 or 30 μg/kg) and Aniracetam-plus-Scopolamine. All tests with 20 μg/kg scopolamine were conducted prior to those with 30 μg/kg scopolamine and the results from the two doses were analyzed separately.

Results

Figure 4 shows the accuracy of matching as a function of aniracetam pretreatment and retention interval for the two doses of scopolamine. There was a significant overall effect of drug treatment (Control vs. Scopolamine vs. Aniracetam-plus-Scopolamine) for both doses of scopolamine, $F(2,8)=15.3$ and 23.4, for 20 and 30 μg/kg scopolamine, respectively. Accuracy fell below control values after both 20 μg/kg, $F(1,4)=27.9$, and 30 μg/kg scopolamine, $F(1,4)=34.4$. At 20 μg/kg, scopolamine impaired accuracy primarily at the 6 and 12 sec retention intervals (treatment by retention interval interaction: $F(2,8)=19.2$). However, at 30 μg/kg, the impairment was evident across all retention intervals and did not increase significantly with retention interval duration (treatment by retention interval: $F(2,8)=3.31$, $p<0.1$). Pretreatment with aniracetam partially antagonized the effects of 20 μg/kg scopolamine, producing accuracy scores intermediate between the control and scopolamine-only values. There was a significant difference in accuracy between the scopolamine and aniracetam-plus-20 μg/kg scopolamine conditions, $F(1,4)=10.4$, and a significant treatment by retention interval interaction, $F(2,8)=4.99$. Pretreatment with aniracetam did not, however, antagonize the effects of 30 μg/kg scopolamine. There were no significant differences between the 30 μg/kg scopolamine and the aniracetam-plus-30 μg/kg scopolamine conditions.

There was also a significant difference in choice response time among the Control, Scopolamine and Aniracetam-plus-Scopolamine conditions for both doses of scopolamine, $F(2,8)=9.20$, and 18.3 for the 20 and 30 μg/kg conditions respectively. Scopolamine increased response time at both doses (means=1.419 and 1.312 sec on the two control conditions and 1.768 and 1.888 sec following 20 and 30 μg/kg scopolamine, $t(4)=3.94$ and 4.21). Aniracetam partially antagonized the effects of 20 μg/kg but not 30 μg/kg scopolamine (means=1.670 and 1.848 sec, $t(4)=3.01$ and 0.52

respectively). There were no significant differences in the probability of an observing response among the control, scopolamine and scopolamine-plus-aniracetam conditions.

DISCUSSION

Administration of aniracetam improved the accuracy of DMS in both monkeys and pigeons, although the effect reached statistical significance only in the monkeys (Fig. 1). Both pigeons and monkeys exhibited individual differences in sensitivity to aniracetam (Figs. 2 and 3) as has been reported for other purported cognition-enhancing compounds [2, 3, 4]. Finally, aniracetam partially antagonized the impairment of DMS performance produced by 20 μg/kg scopolamine, but did not antagonize the more severe impairment produced by 30 μg/kg scopolamine (Fig. 4).

Thus, aniracetam moderately enhanced accuracy in two animal species not previously studied, using two different routes of administration and (in monkeys) with both normal and scopolamine-treated subjects. Schwam *et al.* [40] have recently reported similar small improvements in DMS performance of squirrel monkeys following aniracetam. Although the magnitude of aniracetam's effects in both the present and the Schwam *et al.* study was modest, this 10–15% average facilitation compares favorably to the negligible effects we have previously obtained in similar studies with compounds such as arginine vasopressin (unpublished data), 1-desamino-8-D-argine vasopressin [44] and d-amphetamine [44]. The present facilitation of matching accuracy also compares favorably to the absence of facilitation for normal monkeys following physostigmine administration [36]. Bartus and his colleagues [2, 4, 7] have reported somewhat greater facilitation of short-term memory performance (25–35%) for individual monkeys following physostigmine and arecoline, with smaller (10–20% at best) and less consistent effects following administration of vasopressin analogues, nootropics and CNS stimulants. However, only arecoline produced consistent, significant effects similar to those reported here. Furthermore it should be noted that Bartus's studies employed aged monkeys and a delayed response task and, thus, do not duplicate the present results. Additional systematic studies will be necessary to determine the relative efficacy of aniracetam and other purported cognition enhancing compounds.

The present results, together with those of Cumin *et al.* [12], clearly show that aniracetam can enhance cognitive performance in both appetitively and aversively motivated tasks. Thus, aniracetam's effects are not limited to a specific motivational system. That aniracetam improved response accuracy but did not alter the probability of an observing response or choice reaction time (except following 20 μg/kg scopolamine) further suggests that aniracetam selectively improves cognitive processes at doses which do not affect motor performance.

The present results also extend the findings of Cumin *et al.* to include performance of short-term memory tasks. However, the results suggest that aniracetam's effects are not specific to short-term memory. Aniracetam facilitated DMS performance across all retention intervals, and the magnitude of facilitation did not increase monotonically with retention interval duration (Fig. 1). This result suggests that aniracetam facilitates processes such as attention, encoding or retrieval that occur independent of retention interval duration, and thus, independent of short-term memory processing. That aniracetam's effects are not limited to short-term memory is further supported by the demonstration that aniracetam can improve passive avoidance performance even when administered prior to a retention trial, 48 hours after the initial learning [12].

On the other hand, it is premature to conclude that aniracetam has no effect on short-term retention, since aniracetam did partially reverse the impaired retention following 20 μg/kg scopolamine (Experiment 3). This result is doubly interesting because the effects of scopolamine on short-term retention have also been the subject of some controversy, with some studies [8,36] reporting a specific decrement in retention following anticholinergic administration (as reported here for 20 μg/kg scopolamine) and others [24, 42, 44] reporting deficits in performance across all delay intervals (as reported here for 30 μg/kg). Further research is needed to elucidate the factors that determine whether drugs like scopolamine and aniracetam will alter the time course of retention. We suggest that factors such as the baseline level of stimulus control, which has been shown to affect the magnitude of drug response in a discrimination task [18] may also influence the magnitude and nature of effects seen in short-term memory tasks.

That aniracetam can antagonize the effects of scopolamine suggests that aniracetam may act to facilitate cholinergic neurotransmission. However, biochemical studies indicate that aniracetam does not bind to cholinergic receptors and thus is not likely to serve as a direct cholinergic agonist (Keller, Burkard and Mohler, cited in Cumin *et al.* [12]. Alternatively, aniracetam may partially reverse the effects of scopolamine by increasing the synthesis or release of acetylcholine (ACh). Although appropriate studies with aniracetam have only recently appeared, certain data do suggest such mechanisms of action for related nootropic compounds: specifically, ACh synthesis has been demonstrated to be sensitive to oxidative metabolism [9,22], and both behavioral [6,12] and neurochemical [25, 29, 34] experiments suggest that nootropic compounds such as piracetam may increase oxidative metabolism. Furthermore, piracetam and the related compound oxiracetam have been shown to increase the levels of free choline and decrease the levels of ACh in rat hippocampus and cortex [6, 35, 47], which may indicate that the compounds also increase ACh release. Finally, aniracetam can reverse scopolamine-induced deficits in glucose utilization throughout the brain [31], and aniracetam and related nootropic compounds can increase high affinity choline uptake in the hippocampus [41], suggesting a role for these compounds in energy utilization and ACh synthesis.

Thus, the present demonstration that aniracetam can partially antagonize the effects of scopolamine on short-term retention is consistent both with the antiamnestic effects [12] and with the neurochemical mechanisms of nootropic drugs reviewed above. Never-the-less it would be premature to conclude that aniracetam's effects are limited to the facilitation of cholinergic neurotransmission. Further investigations of the behavioral and neurochemical mechanisms of aniracetam and related compounds are necessary, and may lead to the development of still more efficacious compounds for therapeutic use with human populations.

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