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Effects of Piracetam on Indices of Cognitive Function in a Delayed Alternation Task in Young and Aged Rats

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ROUX, S., I. HUBERT, A. LENÈGRE, D. MILINKEVITCH AND R. D. PORSOLT. *Effects of piracetam on indices of cognitive function in a delayed alternation task in young and aged rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 683–688, 1994.—The effects of piracetam (64, 128, and 256 mg/kg PO) on the performance of a delayed alternation in a Skinner Box were investigated. Test sessions consisted of 36 trials during which animals were first presented with a single lever (left or right) followed 5, 10, or 20 s later by two levers. A press on the lever opposite to that presented previously (nonmatching to sample) was rewarded. The number of correct responses and the reaction times to the one- and two-lever presentations were recorded. All animals received all treatments in a balanced order. Aged animals showed clear deficits on all three parameters. Piracetam was without effect on the performance of young animals but dose-dependently decreased the choice reaction times (two levers) in aged animals without affecting the other two parameters. These results suggest that piracetam does not affect short-term memory but may facilitate choice behavior in aged animals.

Piracetam Aging Cognition Short-term memory Attention Animal model

ALTHOUGH there are no accepted behavioral models of Alzheimer's disease, it seems reasonable to propose that the best available animal model of human aging is the aging animal. It is nonetheless essential to demonstrate a parellelism between the cognitive deficits observed in aged animals and those observed in man (1,10). Furthermore, it is important to identify those changes observed in aging animals that can be ascribed to memory deficits and those related to other cognitive factors such as attention and information processing speed (9). Indeed, insufficient attention to the behavioral processes grouped under the label cognition in animal modeling has been blamed for the frequent failure of clinical studies of cognition enhancers to produce the clinical effects predicted for them on the basis of preclinical research (15).

One such compound is piracetam, where both positive and negative findings have been reported concerning its efficacy in improving different aspects of cognitive function in man and in animals (8,13,15). The experiments described below present findings with piracetam in an operant delayed alternation task performed in young and aged rats. In this task, young and aged animals are trained to retain spatial information—which of two retractable levers was previously presented—over short periods of time (5–20 s). A correct response is to press on the lever opposite to that which was previously presented (delayed nonmatching to sample). This task, therefore, represents a measure of short-term retention. In addition, however, the animals' reaction times to both the single and two-lever presentations are also measured. Intuitively, these two parameters would appear to measure other aspects of cognitive performance probably more related to attention processes and information processing speed (7). The measurement of multiple parameters in this situation could, therefore, represent a more discriminating technique for measuring drug effects on cognition.

The results obtained showed clear differences on all three parameters between young and aged rats, suggesting age-related cognitive impairment. Piracetam had no effect on correct responding or on simple reaction times in either young or aged animals. On the other hand, piracetam dose-dependently improved choice reaction times in aged animals without

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affecting the same parameter in young animals. These pharmacological findings indicate that the three parameters measured may, indeed, represent different aspects of cognitive function, and that the procedure may be useful for characterizing the cognition enhancing properties of drugs such as piracetam.

METHOD

Animals

Male rats of Wistar strain, supplied by the Centre d'Elevage Roger Janvier (France), were used. They were aged 3–5 months (young rats) and 21–23 months (aged rats) and weighed, respectively, 315–425 g and 480–620 g at the beginning of the experiments. They were housed two per cage in transparent macrolon cages (33 × 21 × 18 cm) on wood shavings. In addition to the 45 mg pellets gained during the experiments, they were maintained with restricted access to food (15 g per day of UAR 113 standard rodent diet, given after the last animal was tested each day) and had free access to water. The animal house was maintained at a temperature of 21 ± 1°C on a nonreversed light–dark cycle with illumination from 0800 to 2000 h.

Drug

Piracetam (U.C.B., Belgium) was dissolved in distilled water and administered orally 60 min before test sessions (see below). The control treatment was the vehicle (distilled water). The doses of piracetam (64, 128, and 256 mg/kg) are expressed as mg of powder. All administrations were given in a volume of 5 ml/kg body weight.

Apparatus

The apparatus consisted of standard sound attenuated Skinner Boxes (Model E10.10, Coulbourn Instruments, Le High Valley, PA) fitted with a house light, one or two retractable levers, and a food pellet dispenser (45 mg pellets). In the one-lever configuration, the lever was located centrally above the food receptacle (10 cm above the floor). In the two-lever configuration, the two levers were located at the same level but on either side of the food receptacle. The Skinner Boxes were connected to a MED.PC programming system (MED Associates, East Fairfield, VT), which controlled the experiments and collected the data automatically.

Experimental Procedure

The animals were subjected to a three phase training procedure consisting of the following steps:

Lever-pressing acquisition. The aim of this phase was to train animals, on the presentation of a single retractable lever, to press on it to receive a food pellet reward. During the week preceding this phase, all animals were placed on the partial food deprivation schedule described above (15 g per day) and were handled daily.

Training consisted of 10 acquisition sessions where all lever presses were reinforced with a food pellet (FR1). During the first seven sessions, the boxes were equipped with one fixed lever situated centrally above the food receptacle, to avoid spatial preference for the right or left side of the experimental panel. On sessions 1 and 2, a reinforcement was automatically delivered at regular 60-s intervals. If the animal pressed the lever, a reinforcement was also delivered and the next automatic delivery was delayed 10 s. From session 3 to 7, the animals were given a reinforcement only if they pressed the lever. After the seventh session, the boxes were fitted with two retractable levers located on either side of the food receptacle. During each session (sessions 8 to 10) the left or right lever was presented in a pseudorandom sequence every 5 s. A response on the lever resulted in the retraction of the lever and the delivery of a reinforcement. If the animal did not press the lever within 30 s, the lever was retracted without reinforcement and was represented 5 s later. Sessions terminated after the animal completed 50 responses or after 15 min had elapsed. All animals received an oral administration of distilled water 60 min before each session.

Animals that did not correctly press the lever at the end of this phase (less than 50 responses per session for young rats and less than 20 responses per session for aged rats) were discarded from the experiment. In fact, no young animals were discarded, whereas about 25% of aged animals were discarded.

Delayed alternation training (one delay). The animals were then submitted to 20 delayed alternation acquisition sessions over 4 consecutive weeks. The aim of this phase was to train the animals, presented first with a single lever and then 5 s later with two levers, to press on the lever opposite to that previously presented to gain a food reinforcement (delayed nonmatching to sample).

Each session consisted of 35 trials separated by a 10-s interval. A trial started with the presentation of a single lever (left or right). A response on the lever resulted in the retraction of the lever and the delivery of a reinforcement. Five seconds later, two levers were inserted into the chamber. A response on the lever not presented 5 s previously (correct response) resulted in the retraction of the two levers and the delivery of a reinforcement. A response on the same lever (incorrect response) resulted in the retraction of the two levers without reinforcement. A failure to lever press within 20 s during either the one-lever or two-lever presentations (response omission) resulted in the retraction of the lever(s) without reinforcement. Sessions terminated after the animal had completed 35 trials (i.e., 35 choice responses between the two levers) or after 30 min had elapsed. All animals received an oral administration of distilled water 60 min before each session.

Animals that did not adequately learn the delayed alternation (less than 60% correct responses per session during the last two sessions) were discarded from the experiment (about 15% of the young animals and 25% of the aged animals).

Delayed alternation training (three delays). The animals were then submitted to four three-delay training sessions. The aim of this phase was to establish stable delayed alternation responding during sessions in which the animals were exposed to three delays (5, 10, and 20 s) between the one-lever and two-lever presentations.

Each session consisted of 36 trials separated by a 20-s interval. The different delays were randomly presented and equally distributed throughout the session. Otherwise, the procedure was the same as that described above for a single delay. Sessions terminated after the animal had completed 36 trials or after 30 min had elapsed. All animals received an oral administration of distilled water 60 min before each session.

Fifteen animals in both the young and aged groups were retained for the following drug testing phase. They were chosen on the basis of the stability of their performance during the four three-delay training sessions.

Behavioral Parameters Measured

The following three behavioral parameters were measured for each animal:

Percent correct responses—the number of correct responses during a session was expressed as a percentage of the total completed choice responses at each delay; simple reaction time—the reaction times to each one-lever presentation were measured and expressed as the mean value per session; choice reaction time—the reaction times to each two-lever presentation (three delays combined) were measured and expressed as the mean value per session.

Drug Testing Procedure

During the drug testing phase, all animals were submitted to all the treatment conditions (64, 128, and 256 mg/kg piracetam and vehicle) with the treatment orders balanced between the different animals.

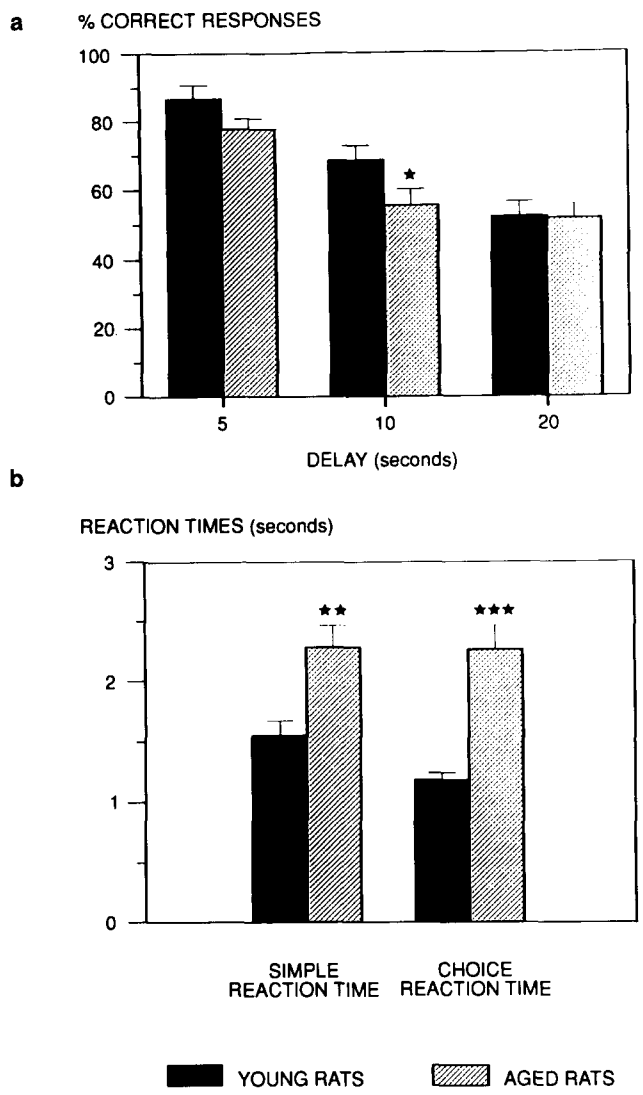

FIG. 1. Comparison of young and aged rats on three performance parameters in the delayed alternation task. (a) Percent correct responses at three delays. (b) Simple and choice reaction times. $n = 15$ per group. Data are presented as means (±SEM). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (two-tailed nonmatched Student's t-test).

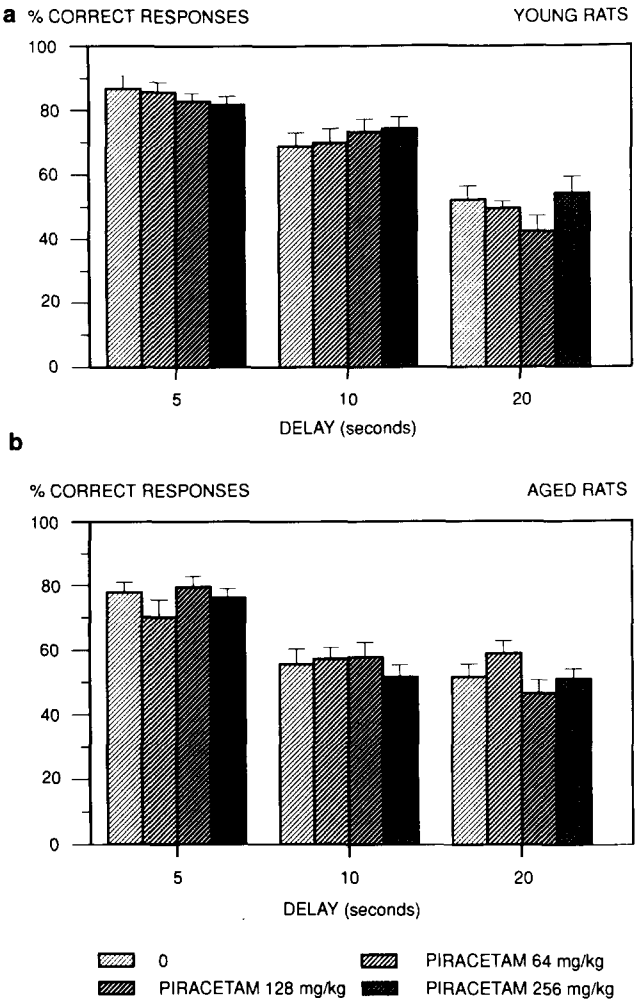

FIG. 2. Effects of piracetam on percent correct responses in young rats (a) and aged rats (b) at three delays during the delayed alternation task. $n = 15$ per group. Data are presented as means (±SEM).

The animals were given two treatment test sessions per week with at least one drug-free training session between each treatment test session. A treatment test session was performed as described immediately above with three delays. The drug-free training sessions consisted of 20 trials using a single delay (5 s) with an intertrial interval of 20 s.

All treatments were given PO 60 min before a test session. Drug testing was performed in blind conditions. For the drug-free training sessions, all animals continued to receive an oral administration of distilled water 60 min before each session.

Throughout training and testing, the experiments were always conducted at the same time of day (1000 and 1400 h) and individual animals were always tested in the same chronological order and in the same experimental chambers.

Statistical Analysis

All results were analyzed for statistical significance using analysis of variance (ANOVA) followed by individual t-tests

and linear trend analyses using the residual of the analyses of variance as the denominator (18).

RESULTS

Comparison of Young With Aged Animals

The data were first analyzed to compare the performance of young and aged animals on the three parameters measured in the delayed alternation task (Fig. 1).

Inspection of Fig. 1a suggests that aged control animals had generally lower correct response scores than young controls, which was confirmed by the presence of a significant age effect in the ANOVA [age, $F(1, 28) = 5.586, p < 0.05$]. Figure 1a also indicates that the percent of correct responses clearly declined with increasing delays, which was similarly confirmed by the presence of a highly significant delay effect in the ANOVA [delay, $F(2, 56) = 27.463, p < 0.001$]. The data suggest further that there was an age difference at the 5-s and particularly the 10-s delay, whereas there was little difference between the two age groups at the longest delay (20 s). This was not confirmed by the ANOVA where the age × delay interaction in the ANOVA was not significant [age × delay, $F(2, 56) = 1.222$, NS]. On the other hand, individual t-tests conducted at each delay suggested that the difference observed at the 10-s delay was statistically significant, $t(28) = 2.388, p < 0.05$.

There were, however, clear and highly significant differences between young and aged animals on the measures of both simple reaction time and choice reaction time (Fig. 1b) [ANOVA: simple reaction time, age $F(1, 28) = 13.729, p < 0.01$; choice reaction time, age $F(1, 28) = 27.378, p < 0.001$].

Taken together, these results suggest that aged animals made fewer correct responses at the intermediate delay and had longer simple and choice reaction times than young animals.

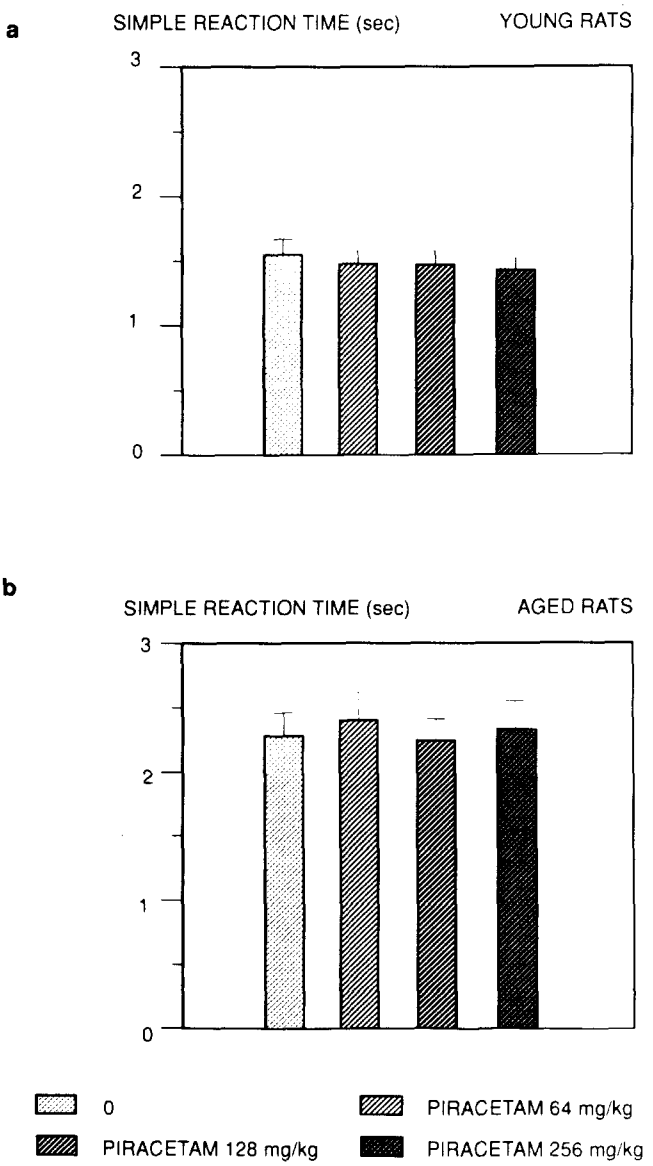

FIG. 3. Effects of piracetam on simple reaction times in young rats (a) and aged rats (b) during the delayed alternation task. $n = 15$ per group. Data are presented as means (± SEM).

Effects of Piracetam in Young Animals

The effects of piracetam on the performance of young animals are shown in the upper parts (a) of Figs. 2, 3, and 4 for percent correct responses, simple reaction times, and choice reaction times, respectively.

Inspection of these figures suggests that piracetam had no effects, at any dose tested, on any of the three parameters measured. This was generally confirmed by the results of the ANOVA, where the F values for drug treatment were less than unity for the first two parameters. With the choice reaction time measure there was a significant treatment effect [treatment, $F(3, 42) = 3.060, p < 0.05$], but the data do not suggest any clear relation between the dose administered and the score observed, nor do individual t-tests conducted between the drug treatments and the control.

Effects of Piracetam in Aged Animals

The effects of piracetam on the performance of aged animals are shown in the lower parts (b) of Figs. 2, 3, and 4 for percent correct responses, simple reaction times, and choice reaction times, respectively.

Inspection of these figures suggests that piracetam had no effects, at any dose tested, on the first two parameters measured (percent correct responses, simple reaction times). This was confirmed by the results of the ANOVA where the F values for drug treatment were less than unity for both parameters. In contrast, piracetam dose dependently reduced the choice reaction times in aged animals. This effect was confirmed by the ANOVA [treatment, $F(3, 42) = 3.65, p < 0.05$] and the presence of a dose-related linear trend [linear component, $F(1, 42) = 10.705, p < 0.01$]. Individual t-tests conducted between the drug treatments and the control also suggested a statistically significant effect, $t(42) = 3.248, p < 0.01$, at the highest dose tested (256 mg/kg).

DISCUSSION

The present results show that aged Wistar rats (21–23 months old) are capable of learning and performing a complex memory task in a Skinner Box. On the other hand, they show clear deficits as compared with young rats of the same strain. This is indicated first by the fact that aged rats were more frequently rejected during the training phases of the task than young animals. The reasons for these failures to learn cannot be clearly identified, but no doubt reflect a complex of memory, attentional, and motivational factors (14). On the other hand, clear deficits were still observed in aged animals that

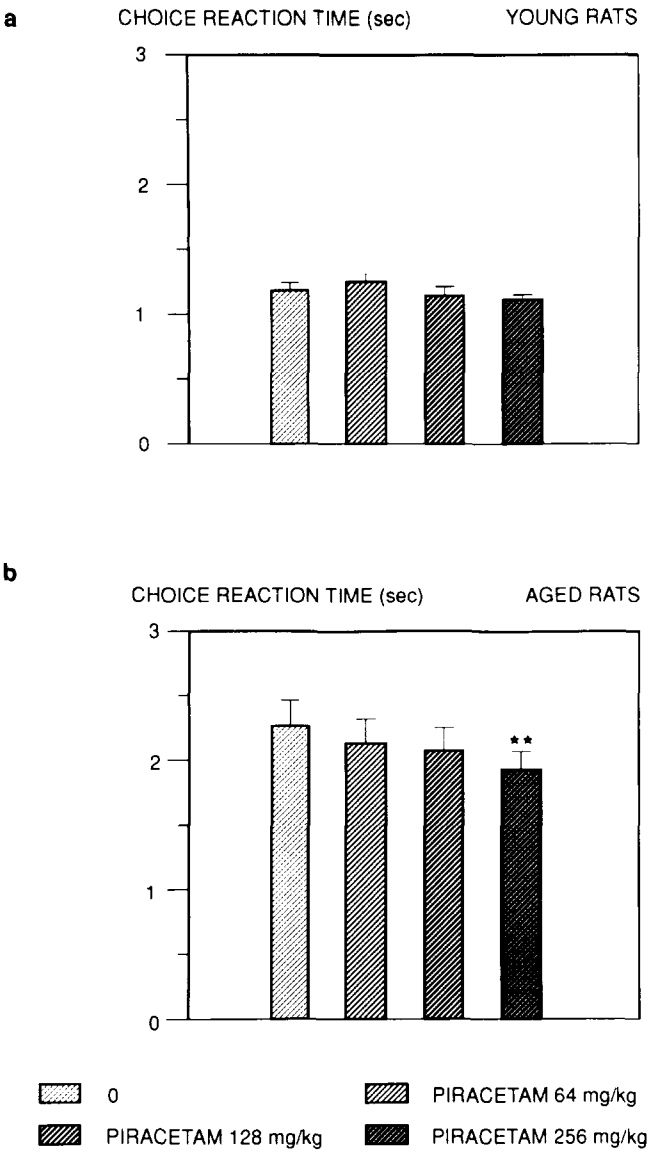

FIG. 4. Effects of piracetam on choice reaction times in young rats (a) and aged rats (b) during the delayed alternation task. $n = 15$ per group. Data are presented as means (±SEM). $^{**}p < 0.01$ (two-tailed matched Student's t-test).

had mastered the task, and these deficits lend themselves more readily to interpretations concerning the cognitive processes involved. For example, it seems reasonable to suppose that correct choice responding, when the animals are required to retain spatial information over an imposed period of time, represents a measure of short-term or working memory. This interpretation is reinforced by the fact that performance is superior when the delay is short and becomes less accurate as the delay is increased. In the present experiments, for example, performance even in young animals became virtually random (50% correct) when the imposed delay reached 20 s. In general, our findings with the correct responses parameter are very similar to those described by Dunnett et al., using a similar procedure (3). The two reaction time parameters, on the other hand, do not appear to measure memory, but may well measure other aspects of cognitive performance. Intuitively, the simple reaction time measure would appear to reflect the animal's capacity to react to an unpredictable spatial stimulus, an attentional process, whereas the choice reaction time measure would seem more to reflect decision-making processes or information processing speed (7). A motor component cannot, of course, be ruled out, but it should be emphasized that the motor requirement in a Skinner Box is considerably less than that, say, in a radial maze (17). In any case, behavioral measures can never be expected to represent pure measures of internal processes (4).

The data obtained with piracetam in the present experiments provide pharmacological evidence for the differentiation of the processes discussed above. Piracetam was without effect on any parameter measured in young animals. This finding would suggest that a drug such as piracetam could not facilitate cognition in animals where performance is already at an optimal level (11). This is particularly true for the reaction time measures, where the young animals were responding very rapidly. The present results suggest, however, that piracetam does not affect short-term memory at all. There was no effect of piracetam on the correct responses measure in conditions where memory in young animals was suboptimal (i.e., at long delays) nor did piracetam affect the same measure in aged animals, which generally made fewer correct responses, particularly at the intermediate delay. Furthermore, whereas aged animals showed clearly slower reaction times to both the one-lever and two-lever presentations, piracetam was completely without effect on simple reaction time but appeared to facilitate choice reaction time. These results, therefore, provide a pharmacological confirmation that the three parameters reflect different aspects of cognitive function. The difference between simple and choice reaction times suggests, furthermore, that the effects observed with piracetam do not simply represent some generalized psychomotor stimulation.

Although there is an extensive literature on the effects, often conflicting, of piracetam and derivatives in animal memory tasks (8,13,15), there appears to be a growing interest in drug effects on attentional as opposed to memory processes in aging (5,7). Studies in humans have consistently suggested that attentional processes decrease with age (2). It is, thus, of interest to note that, like in our own study, other authors have shown that piracetam and derivatives facilitate choice reaction times in aged rats (6). Furthermore, experimental studies in elderly motorists have shown that piracetam facilitates driving performance, an effect most probably related to attentional factors (16). An interesting finding was that piracetam was mainly effective in subjects with clearly deficient pretest performance. This kind of observation appears to correspond to the differential effects observed in aged and young rats in the present study, where piracetam was only active in animals showing a deficit (the aged group). Finally, clinical studies of drug therapy in Alzheimer's disease have suggested that drugs such as tacrine improve attentional function (simple and choice reaction time) rather than memory in patients with mild to moderate pathology (12).

Taken together, the findings reported in the present study suggest that piracetam has differential effects on cognitive function in aged animals. This finding could be of relevance for more accurately predicting the kind of clinical effects to be expected with piracetam-like drugs.

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REFERENCES

1. Bartus, R.; Dean, R. L. Developing and utilizing animal models in the search for an effective treatment for age-related memory disturbances. In: Gottfries, C. G., ed. Normal Alzheimer's disease and senile dementia: Aspects of etiology, pathogenesis, diagnosis and treatment. Brussels: EVB; 1985:231-267.
2. Davis, D. R.; Jones, D. M.; Taylor, A. Selective and sustained attention tasks individual and group differences. In: Parasurama, R.; Davis, D. R., eds. Varieties of attention. Orlando: Academic Press; 1984:395-447.
3. Dunnett, S. B.; Evenden, J. L.; Iversen, S. D. Delay-dependent short-term memory deficits in aged rats. Psychopharmacology (Berlin) 96:174-180; 1988.
4. Gage, F. H.; Dunnett, S. B.; Bjorklund, A. Age-related impairments in spatial memory are independent of those in sensorimotor skills. Neurobiol. Aging 10:347-352; 1989.
5. Jäkälä, P.; Sirvio, J.; Riekkinen, P.; Haapalinna, A.; Riekkinnen, P. Effects of atipamezole, an α2-adrenoceptor antagonist, on the performance of rats in a five-choice serial reaction time task. Pharmacol. Biochem. Behav. 42:903-907; 1992.
6. Kubota, A.; Kurasawa, M.; Furuya, I. Pharmacological study of aniracetam I: Choice reaction time, shortening action of aniracetam in aged rats. Jpn. Pharmacol. Ther. 14:47-54; 1986.
7. Moore, H.; Dudchenko, P.; Bruno, J. P.; Sarter, M. Toward modeling age-related changes of attentional abilities in rats: Simple and choice reaction time tasks and vigilance. Neurobiol. Aging 13:759-772; 1992.
8. Nicholson, C. D. Pharmacology of nootropics and metabolically active compounds in relation to their use in dementia. Psychopharmacology (Berlin) 101:147-159; 1990.
9. Olton, D. S.; Wenk, G. The development of behavioral tests to assess the effects of cognitive enhancers. Pharmacopsychiatry 23(Suppl. II):65-69; 1990.
10. Porsolt, R. D.; Roux, S.; Lenègre, A. Preclinical evaluation of potential cognition enhancers. In: Racagni, G.; Mendlewicz, J., eds. Treatment of age-related cognitive dysfunction: Pharmacological and clinical evaluation. Basel: Karger; 1992:50-62.
11. Rupniak, N. M. J. Profile of cholinomimetic drugs in primates: Status of screens for potential Alzheimer therapies. Drug Dev. Res. 27:77-88; 1992.
12. Sahakian, B. J.; Owen, A. M.; Morant, N. J.; Eagger, S. A.; Boddington, S.; Crayton, L.; Crockford, H. A.; Crooks, M.; Hill, K.; Levy, R. Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer's disease: Assessment of attentional and mnemonic function using CANTAB. Psychopharmacology (Berlin) 110:395-401; 1993.
13. Sanger, D. J.; Joly, D. Psychopharmacological strategies in the search for cognition enhancers. Pharmacopsychiatry 23(Suppl. II):70-74; 1990.
14. Sarter, M. Measurement of cognitive abilities in senescent animals. Int. J. Neurosci. 32:765-774; 1987.
15. Sarter, M.; Hagan, J.; Dudchenko, P. Behavioral screening for cognition enhancers: From indiscriminate to valid testing. Part I. Psychopharmacology (Berlin) 107:144-159; 1992.
16. Schmidt, U.; Brendemühl, D.; Engels, K.; Schenk, N.; Ludemann, E. Piracetam in elderly motorists. Pharmacopsychiatry 24: 121-126; 1991.
17. Willig, F.; Palacios, A.; Monmaur, P.; Mharzi, M.; Laurent, J.; Delacour, J. Short-term memory, exploration and locomotor activity in aged rats. Neurobiol. Aging 8:393-403; 1987.
18. Winer, B. J. Statistical principles in experimental design (2nd ed.). New York: McGraw-Hill; 1971:177-185.