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Aniracetam Restores Object Recognition Impaired by Age, Scopolamine, and Nucleus Basalis Lesions

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BARTOLINI, L., F. CASAMENTI AND G. PEPEU. *Aniracetam restores object recognition impaired by age, scopolamine, and nucleus basalis lesions.* PHARMACOL BIOCHEM BEHAV **53**(2) 277–283, 1996.—Object recognition was investigated in adult and aging male rats in a two-trials, unrewarded, test that assessed a form of working-episodic memory. Exploration time in the first trial, in which two copies of the same object were presented, was recorded. In the second trial, in which one of the familiar objects and a new object were presented, the time spent exploring the two objects was separately recorded and a discrimination index was calculated. Adult rats explored the new object longer than the familiar object when the intertrial time ranged from 1 to 60 min. Rats older than 20 months of age did not discriminate between familiar and new objects. Object discrimination was lost in adult rats after scopolamine (0.2 mg/kg SC) administration and with lesions of the nucleus basalis, resulting in a 40% decrease in cortical ChAT activity. Both aniracetam (25, 50, 100 mg/kg os) and oxiracetam (50 mg/kg os) restored object recognition in aging rats, in rats treated with scopolamine, and with lesions of the nucleus basalis. In the rat, object discrimination appears to depend on the integrity of the cholinergic system, and nootropic drugs can correct its disruption.

Aniracetam Nucleus basalis Scopolamine Working memory Object recognition

ANIRACETAM, and its analog oxiracetam, belong to a group of cognition enhancers, called nootropic drugs. In animals, these agents combine the ability to facilitate information acquisition with protection against learning and memory impairing drugs. They show a lack of stimulant or sedative effects on gross behavior, and have low toxicity (20,41). Their activity on learning and memory has been evaluated in different behavioral tests including one-trial passive avoidance, radial-arm maze, water maze, operant behavior (38,39), and T-maze with a stem left/right discrimination (1). Performance in the radial arm maze involves working and spatial memory (33), and spatial memory in the Morris water maze (29). None of these tests explore the episodic memory that is primarily affected in senile dementia and age-associated memory impairment (16). An object recognition test has been developed (14) that measures in the rat nonspatial working memory with the characteristics of the episodic memory assessed in nonhuman primates by visual recognition tests. Episodic memory here is meant as the ability of the rat to recognize an object previously seen only once. Recognition of the object does not depend on a reward but only on the innate exploratory behavior. In the present experiments, we investigated to what extent object recognition is impaired by age and cholinergic hypofunction and whether the impairment can be corrected by aniracetam and oxiracetam.

METHOD

Subjects

Male Wistar rats (Charles River), 3, 16–18, and 20–25 months old, were used. The rats were individually housed in macrolon cages with ad lib. food and water and maintained on 16 L : 8 D cycle, with light at 0700 h. The room temperature was 23 ± 1°C.

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Object Recognition Test

Apparatus. The apparatus (14) was formed by a white colored polyvinyl chloride box (70 × 60 × 30 cm) with a grid floor that could be easily cleaned. The apparatus was illuminated by a 75 Watt lamp suspended 50 cm above the box. The objects to be discriminated were also made of polyvinyl chloride, gray colored, and were in three different shapes: cubes of 8 cm side, pyramids, and cylinders of 8 cm height.

Procedure. The day before testing the rats were allowed to explore the box for 2 min. On the day of the test, a session of two trials was given. The intertrial interval was usually 60 min, but in some experiments different intertrial intervals were tested, as shown in the results. In the first trial (T1), two identical objects were presented in two opposite corners of the box, and the amount of time taken by each rat to complete 20 s of object exploration was recorded. Exploration was considered directing the nose at a distance <2 cm to the object and/or touching it with the nose. During the second trial (T2), one of the objects presented in T1 was replaced by a new object and rats were left in the box for 5 min. The times spent for the exploration of the familiar (F) and the new object (N) were recorded separately and a discrimination index D was calculated (N − F/N + F). Care was taken to avoid place preference and olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T2, and cleaning them carefully.

Motor Behavior

Motor behavior of some of the rats was investigated by means of the elevated platform test (4). Each rat was positioned at the beginning of a 5-cm wide, 60-cm long wood bridge suspended between two platforms. The rats were tested for their ability to remain on and traverse the bridge. The number of cm covered and of animals who fell from the bridge in 3 min was recorded.

Surgery

Under ketamine anaesthesia (100 mg/kg IP) bilateral lesions of the nucleus basalis of Meynert (nbM) were made by stereotaxic injection of 0.5 μl of 0.12 M quisqualic acid dissolved in saline with a 10 μl Hamilton syringe. The injection lasted 5 min and the syringe was left in place for 5 min after completion of the infusion. The coordinates used were 0.2 posterior to bregma, 2.8 mm lateral, and 6.8 mm below the dura (32). In the sham-operated rats, the needle was lowered into the cortex and no quisqualic acid was injected.

Choline Acetyltransferase Activity Determination

Cortical choline acetyltransferase (ChAT) activity was measured in the rats with the bilateral lesion of the nucleus basalis to assess cholinergic hypofunction. After completing the object recognition test, the rats were killed by decapitation, the brain quickly removed, and the cortical areas dissected out. The samples were homogenized in 20 vol of 10 mM ethylene-diaminetetra-acetic acid (EDTA) buffer (pH 7.4) and 0.2% (v/v) Triton X-100. ChAT activity was calculated by measuring the conversion of 1 · [^{14}C] acetyl-coenzyme A (Radiochemical Centre, Amersham, specific activity 60 mCi/mmol) to [C^{14}] acetylcholine (17). Incubation time was 15 min at 37°C. ChAT activity was expressed as μl/h/100 mg protein. Protein content of the homogenates was determined by the method of Bradford (3).

Drugs and Treatments

The following drugs were used: Ketalar, Park Davis; scopolamine HBr (Sigma Chemical Co., St. Louis, MO); N-butylscopolamine HBr (Boehringer, Ingelheim), quisqualic acid (Sigma), aniracetam (a gift of Roche, Basel, Switzerland), oxiracetam (a gift of Smith Kline Beecham, Baranzate, Milano). Scopolamine HBr and N-butyl scopolamine HBr were dissolved in saline and injected SC, in a volume of 0.3 ml, 60 min before the first session; aniracetam and oxiracetam were suspended in carboxymethylcellulose 0.5% and administered orally, in a volume of 0.3 ml, 90 min before the session. Control rats received saline and carboxymethylcellulose, SC and orally, respectively.

Statistical Analysis

Statistical analysis was carried out using the NCSS 5.0 program. Two-tailed Student's *t*-test was applied for variables within each group; for multiple comparisons between-groups ANOVA and subsequent Duncan's post hoc analysis were applied. Student's *t*-test was used to compare ChAT values.

RESULTS

The purpose of the first group of experiments was to ascertain for how long the rats were able to discriminate between the familiar object seen in the first trial and the novel object presented in the second trial. Table 1 shows that the time spent by 3-month-old rats exploring the familiar and novel objects was significantly different up to 60 min intertrial time. The discrimination index gradually decreased from 1 to 60 min, and discrimination between the two objects was lost when the intertrial times were 4 and 24 h. The mean duration of the T1 session was 224.5 ± 8.0 s, with no statistically significant differences between the groups of rats.

The effect of age on object discrimination was investigated in two groups of rats 16–18 and 20–25 months of age, respectively. To evaluate the general condition of the old rats, their motor behavior was assessed by the elevated platform test. The centimeters of bridge covered were 45.7 ± 5.4 with 35.7% falls (n = 51) for the 20- to 25-month-old rats, and 57.3 ± 1.5 with 13.3% falls (n = 50) for the 3-month-old

TABLE 1

EFFECT OF DIFFERENT INTERTRIAL TIMES ON OBJECT RECOGNITION IN 3-MONTH-OLD RATS

Intertrial Time		T2 Exploration Time in s (Mean ± SEM) F	T2 Exploration Time in s (Mean ± SEM) N	Discrimination Index D
1 min	(10)	3.3 ± 1.4	11.6 ± 2.5*	0.65
20 min	(8)	4.7 ± 1.0	14.8 ± 2.0*	0.51
60 min	(20)	7.7 ± 1.2	17.1 ± 1.2*	0.49
4 h	(9)	7.9 ± 0.9	10.2 ± 1.4	0.10†
24 h	(11)	8.1 ± 0.9	10.8 ± 1.2	0.14†

Number of rats in parenthesis. F = exploration time of familiar object; N = exploration time of new object; D = discrimination index (N − F/N + F).

*$p < 0.001$ N vs. F (two-tailed Student's *t*-test).

†$p < 0.05$, $F(4, 7) = 10.39$ vs. 1, 20, and 60 min intervals (one-way ANOVA and Duncan's post hoc test).

TABLE 2

AGE-ASSOCIATED IMPAIRMENT OF OBJECT RECOGNITION IN THE RAT

Age (Months)	Rat Number	Intertrial Time (min)	T2 Exploration Time in s (Mean ± SEM) F	T2 Exploration Time in s (Mean ± SEM) N	Discrimination Index D
3	15	60	6.7 ± 0.5	18.1 ± 1.1*	0.46
16–18	6	60	4.7 ± 1.3	9.7 ± 2.5*	0.35
20–22	9	60	6.1 ± 1.4	8.6 ± 1.7	0.19†
23–25	11	60	8.6 ± 0.9	9.8 ± 1.3	0.05†
23–25	10	1	7.8 ± 1.6	9.1 ± 1.9	0.07†

F = familiar object; N = novel object.

*$p < 0.001$ N vs. F (two-tailed Student's t-test)

†$p < 0.05$, $F(4, 49) = 7.50$ vs. 3 and 16–18 months (one-way ANOVA and Duncan's post hoc test). The group of rats tested after 1 min intertrial time received CMC and is the same group included for comparison in Table 3.

rats. The differences are not statistically significant. Old rats that were unable to walk on the bridge were discarded.

Table 2 shows that 16- to 18-month-old rats were able to discriminate between the familiar and novel objects 60 min after the first trial, but object discrimination was lost in rats of 20 to 22 and 23 to 25 months of age; even when the intertrial time was shortened to 1 min, 23- to 25-month-old rats were unable to discriminate between familiar and novel objects. T1 exploration time in 23- to 25-month-old rats was 264.5 ± 9.7 s ($n = 11$), with no statistically significant difference from 3-month-old rats. Similarly, there was no difference in exploration time of the familiar object between the young and old rats.

Table 3 shows that a single administration of aniracetam and oxiracetam at the dose of 50 mg/kg PO was able to restore object recognition in the aging rats tested with intertrial times of 1 and 60 min. Doses of aniracetam of 25 and 100 mg/kg tested with an intertrial of 60 min did not restore object recognition. None of the drugs at the doses significantly affected the duration of the T1 session.

The effect of cholinergic hypofunction on object recognition was investigated by scopolamine administration (0.2 mg/kg SC). Table 4 shows that the blockade of muscarinic receptors was followed by a shortening of the T1 session, the loss of object discrimination, and a marked increase in the time spent exploring the two objects during the second 5-min session. After administration of the same dose of N-butylscopolamine, a peripheral muscarinic receptor antagonist, object recognition was still present and the duration of the T1 session was unmodified. When aniracetam was administered at the

TABLE 3

NOOTROPIC DRUGS RESTORE OBJECT RECOGNITION IN AGING RATS

Treatment mg/kg os	Rats Number	T2 Exploration Time in s Mean ± SEM F	T2 Exploration Time in s Mean ± SEM N	Discrimination Index D
1 min intertrial time				
CMC	10	7.8 ± 1.6	9.1 ± 1.9	0.07
Aniracetam 50	6	6.4 ± 1.3	12.4 ± 2.2*	0.32†
Oxiracetam 50	5	2.5 ± 0.8	8.9 ± 2.7*	0.50†
60 min intertrial time				
CMC	8	7.7 ± 1.5	8.5 ± 1.4	0.04
Aniracetam 25	6	8.4 ± 1.3	10.2 ± 1.3	0.09
Aniracetam 50	15	6.3 ± 1.2	12.8 ± 1.3*	0.34‡
Aniracetam 100	5	6.9 ± 0.9	9.0 ± 0.9	0.13
Oxiracetam 50	5	3.9 ± 2.0	8.1 ± 1.8§	0.37‡

F = Familiar object; N = Novel object; T1 = exploration session; CMC = carboxymethylcellulose.

The drugs were administered 90 min before T1.

*$p < 0.05$ N vs. F (two tailed t-test).

†$p < 0.05$, $F(2, 20) = 6.53$, vs. CMC (one-way ANOVA and Duncan's post hoc test).

‡$p < 0.05$, $F(4, 38) = 2.99$, vs. CMC (one-way ANOVA and Duncan's post hoc test).

§$p < 0.001$ N vs. F (two tailed t-test).

TABLE 4

EFFECT OF SCOPOLAMINE AND NOOTROPIC DRUGS ON OBJECT RECOGNITION

Treatments	Rats Number	T1 Session Length in s Mean ± SEM	T2 Exploration Time in s Mean ± SEM F	T2 Exploration Time in s Mean ± SEM N	D
Saline	20	219.7 ± 9.1	5.1 ± 1.6	12.0. ± 1.2**	0.40
Scopolamine 0.2 mg/kg	10	110.7 ± 7.3†	21.4 ± 2.2‡	22.6 ± 2.7§	0.03¶
N-scopolamine 0.2 mg/kg	8	186.8 ± 20.0	4.5 ± 1.6	9.3 ± 1.6#	0.36
Oxiracetam 50 mg/kg	5	192.4 ± 19.4	4.6 ± 0.2	11.7 ± 1.5*	0.42
Aniracetam 50 mg/kg	5	207.3 ± 15.7	7.2 ± 1.9	14.7 ± 2.5#	0.33
Scopolamine 0.2 mg/kg + Oxiracetam 50 mg/kg	7	128.0 ± 14.3†	4.9 ± 1.0	11.7 ± 1.5*	0.40
Scopolamine 0.2 mg/kg + Aniracetam 25 mg/kg	5	145.2 ± 14.3†	17.9 ± 3.2‡	18.1 ± 2.2	0.02¶
Scopolamine 0.2 mg/kg + Aniracetam 50 mg/kg	6	192.4 ± 19.4	12.9 ± 1.3‡	25.8 ± 4.1*§	0.30
Scopolamine 0.2 mg/kg + Aniracetam 100 mg/kg	5	120.3 ± 13.1†	14.8 ± 2.9‡	23.3 ± 5.1§	0.20

F = Familiar object; N = Novel object; T1 = exploration session; D = Discrimination index. Scopolamine and N-buthyl-scopolamine were injected 60 min before T1.

*$p < 0.01$ N vs. F (two-tailed paired t-test).

†$p < 0.05$, $F(4,46) = 11.68$, vs. saline (one-way ANOVA and post hoc Duncan's test).

‡$p < 0.05$, $F(5,53) = 21.46$, vs. saline and N-scopolamine (one-way ANOVA and post hoc Duncan's test).

§$p < 0.05$, $F(4,48) = 26.85$, vs. saline and N-scopolamine (one-way ANOVA and post hoc Duncan's test).

¶$p < 0.05$, $F(8,70) = 6.70$, vs. saline and all other treatments (one-way ANOVA and post hoc Duncan's test).

#$p < 0.05$ N vs. F (two-tailed paired t-test).

dose of 50 mg/kg PO before scopolamine, T1 shortening was prevented, a decrease in the exploration time of the familiar object, and a significant difference in the times spent exploring the familiar and novel objects were found, indicating that discrimination between the two objects was restored. At the doses of 25 and 100 mg/kg PO aniracetam was inactive. Oxiracetam (50 mg/kg PO), administered before scopolamine, restored object recognition but did not prevent the decrease in T1 exploration time.

Aniracetam and oxiracetam given alone affected neither the duration of T1 session nor object discrimination. However, rats receiving aniracetam or oxiracetam (50 mg/kg PO) 90 min before the first session were still able to discriminate between novel and familiar objects after an intertrial time of 4 h, as indicated by a discrimination index of 0.25 ($n = 7$) and 0.22, ($n = 10$), respectively, while the discrimination index of the saline treated rats was 0.02 ($n = 9$). The difference between drug-treated and saline-treated groups was statistically significant ($p < 0.05$).

A cortical cholinergic hypofunction was also induced by placing bilateral lesions in the nbM. ChAT activity in the frontal and parietal cortex in both hemispheres of the lesioned rats was 42 and 40% lower, respectively, than in sham-operated rats (Table 5), a decrease demonstrating substantial destruction of the cholinergic cortical network. As shown in Table 6, in the lesioned rats the duration of T1 was significantly shorter than in sham-operated rats, and the ability to discriminate between familiar and novel objects was lost. Administration of aniracetam and oxiracetam (50 mg/kg PO) 90 min before the first session did not affect the duration of T1 session but restored object discrimination. On the contrary, amphetamine increased the length of the T1 session but did not restore object discrimination.

DISCUSSION

Object recognition is a form of nonspatial memory, usually investigated in monkeys and rats by rewarded matching- or nonmatching-to-sample-tasks. In the present work, object recognition memory was investigated by a nonrewarded one-trial test based on the spontaneous exploratory behavior of rats toward objects that have no special meaning for them (14). Under these conditions object recognition may be considered a form of episodic memory that lasts for at least 60 min. The duration was prolonged to at least 4 h by both aniracetam and oxiracetam, while persistence of object discrimination for

TABLE 5

CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN THE CEREBRAL CORTEX OF RATS WITH BILATERAL LESIONS OF THE NUCLEUS BASALIS

Conditions	Number of Rats	ChAT Activity (μmol/h/100 mg protein) Frontal Cortex	ChAT Activity (μmol/h/100 mg protein) Parietal Cortex
Sham operated	10	5.5 ± 0.3	4.9 ± 0.1
Lesioned	10	3.2 ± 0.2*	2.8 ± 0.2*
		−42.4%	−40.8%

The samples from both hemispheres were pooled.

*$p < 0.01$ vs. sham operated (two-tailed Student's t-test).

TABLE 6

EFFECT OF NUCLEUS BASALIS LESIONS AND NOOTROPIC DRUGS ON OBJECT RECOGNITION

Conditions and Treatments mg/kg os	Rats Number	T1 Session Length in s Mean ± SEM	T2 Exploration Time in s Mean ± SEM		Discrimination Index D
			F	N	
Sham operated	20	231.8 ± 9.0	5.1 ± 1.6	12.0. ± 1.2*	0.40
nbM lesion + CMC	14	196.0 ± 7.0†	5.4 ± 0.5	7.5 ± 0.8	0.15†
nbM lesion + Aniracetam 50	14	169.5 ± 12.3†	5.8 ± 0.7	11.5 ± 1.1*	0.33
nbM lesion + Oxiracetam 50	9	201.4 ± 15.6†	3.7 ± 0.7	7.9 ± 1.2§	0.33
nbM lesion + Amphetamine 0.5 s.c	5	255.0 ± 15.6	5.9 ± 2.0	7.1 ± 3.4	0.13‡

F = Familiar object; *N* = Novel object; T1 = exploration session; CMC = carboxymethylcellulose.
†$p < 0.05$, $F(4,61) = 3.40$, vs. sham operated and nbM + amphetamine (one-way ANOVA and post hoc Duncan's test).
*$p < 0.001$ N vs. F (two-tailed *t*-test).
§$p < 0.05$ N vs. F (two-tailed *t*-test).
‡$p < 0.05$, $F(4,61) = 3.73$, vs. sham-operated, aniracetam, and oxiracetam (one-way ANOVA and post hoc Duncan's test).

24 h was found after piracetam and pramiracetam administration (13).

Object recognition did not occur in rats older than 18 months, in rats treated with scopolamine, and those with bilateral nbM lesions. These three different experimental conditions, which have in common a cholinergic hypofunction, may impair object recognition at different steps of the cognitive process, namely attention, acquisition, and storage. The cholinergic hypofunction brought about by scopolamine results in a cognitive impairment including spatial and working memory (7). In aged rats, the extensive changes in brain neurotransmitter systems (21) include a diffuse cholinergic hypofunction (34) and are associated with the impairment of working and spatial memory (18,23,26,43), and acquisition of delayed matching- and nonmatching-to-sample responses (10). However, object recognition is not always lost in aging rats (5), presumably due to strain differences. On the other hand, because in our experiments object recognition in old rats was lost even after a 1 min intertrial interval, acquisition more than memory processes appear to be affected. The lack of difference between young and aged rats in the time spent in T1, and in exploring the familiar object shows that in healthy aged rats there is no impairment of motor and exploratory activity, as previously reported (45).

nbM lesions induced by quisqualic acid bring about a cholinergic hypofunction confined to the cortex, with relatively small impairment of other neuronal systems (12). However, the extent to which learning and memory impairment observed in rats with nbM lesions depends on cholinergic hypofunction is a matter of debate (11,15).

Although object discrimination was impaired by all three experimental conditions, a decrease in the time spent in T1 was observed only in rats with nbM lesions, and in those treated with scopolamine, and it may depend on the increase in spontaneous motility brought about by both scopolamine (2,31) and nbM lesions (27). The increase in motility reduces the time needed by the rats to explore the objects for 20 s. Conversely, only scopolamine prolonged also the time spent in exploring both the familiar and new objects, presumably as a consequence of the impairment of exploration abituation caused by this drug (2).

The administration of aniracetam and oxiracetam restored object recognition impaired by aging, scopolamine, and nbM lesions. When different doses of aniracetam were used, the inverse U dose-effect relationship typical of these drugs (35) was found. Oxiracetam, aniracetam, and piracetam have been shown to restore passive avoidance response (9,42), radial maze performance (28), and delayed matching-to-sample response (36) impaired by scopolamine. In normal rats, exploratory behavior was neither affected by aniracetam and oxiracetam in the present experiments, nor by piracetam and pramiracetam (13).

There are differences in the recovery of object recognition induced by the nootropics in the three experimental conditions. In the aged and nbM-lesioned rats the increase in the discrimination index following aniracetam administration results from an increase in the time spent exploring the novel object, with little effect on exploration time of the familiar object. The drug appears to restore the ability to recognize novelty. In the scopolamine-treated rats, aniracetam restores object discrimination by reducing the time spent in exploring the familiar object, and oxiracetam reduces the time spent in exploring both the familiar and novel object. In this case, the drugs appear to antagonize the effect of scopolamine on exploration habituation.

Because, in the present experiments, object recognition impairment is associated with a cholinergic hypofunction, and both aniracetam and oxiracetam activate the forebrain cholinergic system (19,35), it is tempting to attribute the improvement in object discrimination to this effect. This hypothesis is supported by the finding that in old rats both object recognition and extracellular ACh levels are restored by intracerebroventricular perfusion of NGF (40). However, other mechanisms for the action of the nootropic drugs can not be excluded because it has been shown that oxiracetam facilitates synaptic transmission in the hippocampus (37), aniracetam is a positive modulator of AMPA-sensitive ionotropic glutamate receptors (8,24), and the effect of nootropics on memory appears to be steroid sensitive (30).

Finally, the question of whether the demonstration of the efficacy of nootropic drugs in animal models is matched by a therapeutic activity in human memory disorders is still unresolved, because both negative (6,22) and positive clinical results have been reported (25,44).

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