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Bicuculline, AP-7 and behavioral activity in rats

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Short communication

Abstract. The effects of bicuculline (0.25 mg/kg i.p.) and AP-7 (5 nmols icv) on the processes of retrieval, consolidation of conditioned reflexes, object recognition and locomotor activity were tested in rats. Neither AP-7, nor bicuculline nor AP-7 with bicuculline changed locomotor and exploratory activity in the open field test. Coadministration of AP-7 with bicuculline however, facilitated retrieval of passive avoidance in rats, but was without effect on consolidation in this test. Also neither AP-7 nor bicuculline when were given alone had an effect on influenced consolidation. We found no differences in effects of either AP-7 or bicuculline on object recognition regardless whether administered alone or in combination.

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Neuronal excitability, and synaptic plasticity, cognitive performance, learning and memory are frequently the function of the balance of GABAergic inhibitory and glutaminergic excitatory inputs (Stephens 1995). Manipulations leading to increased inhibition can be expected, to have similar consequences to those reducing excitation (glutaminergic antagonists). On the other hand, several lines of evidence suggest a clear metabolic interaction between GABA and glutamate (Simantov 1990). The $GABA_A$ receptor belongs to the group of ligand-gated receptors such as the glycine receptor, and glutamate receptor (Betz 1990).

The N-metyl-D-aspartate (NMDA) receptor subtype of excitatory the amino acids (EAA) receptor system has been proposed to play a role in synaptic plasticity, cognitive performance (Morris 1989), and learning and memory (Collingridge and Singer 1990). First suggestions that glutamate was involved in learning were made over 30 years ago (Vogel et al. 1966). The evolution of the working hypothesis has paralleled the induction of selective glutamate receptor antagonists (Watkins and Evans 1981), and the development of our knowledge of long-term potentiation (LTP), which is believed to resemble some elementary features of memory formation at the neuronal level (Collingridge and Singer 1990).

GABA is released in different brain areas during learning of different tasks and after the induction of LTP. $GABA_A$ antagonist facilitates LTP (Brioni 1993).

The purpose of the present study was to investigate the effect of the antagonist of the $GABA_A$ receptor and the antagonist of the NMDA receptor on behavioral activity in rats. Electrophysiological and biochemical studies demonstrated that bicuculline is a specific $GABA_A$ receptor antagonist (Kardos et al. 1984), and 2 amino-7-phosphonoheptanoic acid (AP-7) is a competitive antagonist of the NMDA receptor.

These antagonists were used to estimate the role of NMDA and $GABA_A$ receptors in behavioral tests.

Subjects were white, male Wistar rats weighing 160-180 g ($n = 264$). The animals were fed on a standard diet and housed in group cages in an air-conditioned room with a 12 h light/ 12 h dark cycle beginning at 7 a.m.

Bicuculline was administered intraperitonelly (i.p.) in the dose of 0.25 mg/kg (Car et al. 1996).

AP-7 was administered into the lateral ventricle of the brain (icv) (Herman 1970) in a dose of 5 nmols icv (Car et al. 1996). Two days before behavioral tests, under ether anesthesia, a burr hole of 0.5 mm in diameter was drilled in the rats skull, 2.5 mm laterally and 1 mm caudally from the point of intersection of bregma and the superior sagittal suture on the right side of the head. Icv injection was made to a depth of 4.5 mm with a Hamilton microsyringe (Herman 1970). After termination of each experiment, all animals were killed by decapitation, their brains were removed and the site of injection was verified macroscopically. Animals with inappropriate injection sites were deleted from the calculations.

The open field test was used for estimation of locomotor and exploratory activity of rats. The apparatus consisted of a square (100 cm x 100 cm) of white floor which was divided by 8 lines into 25 equal squares, and surrounded by white walls, 47 cm high. Four plastic bars, 20 cm high, were located at 4 different line crossings in the central area of the floor. A single rat was placed inside the apparatus for 1 min of adaptation. Subsequently, crossings, rearings and bar approaches were counted manually during 5 min. AP-7 and bicuculline were administered 30 min before the test.

The passive avoidance response was induced using the one trial learning method of Ader et al. (1972). The apparatus consisted of a 6 x 25 cm platform illuminated with a 25 W electric bulb connected through a 6 x 6 cm opening with a dark compartment (40 x 40 x 40 cm). The floor of the cage was made of metal rods of 3 mm in diameter, spaced at 1 cm. The investigation took advantage of the natural preference of rats to stay in dark compartments. The test lasted for 3 days. On the 1st day, after 2 min of habituation in the dark compartment, they were immediately removed. Two similar trials, at an interval of 2 min, were carried out on the 2nd day. After the first trial the rats were allowed to stay in the dark compartment for 10-15 s. In the second trial when the rat entered the dark compartment it received a foot shock (0.25 mA, 3 s) delivered through the metal rods. The presence of the passive avoidance was checked 24 h later. The rats were placed on the illuminated platform once more and the latency to enter the dark compartment was measured, with the cut time of 300 s. To determine the effect of AP-7 and bicuculline on consolidation according to the protocol proposed by Matthies (1980), the drugs were administered on the 2nd day immediately after the completion of induction of passive avoidance and on the 3rd day time to enter the dark compartment was measured as consolidation. To determine the effect of AP-7 and bicuculline on retrieval these substances were administered on the 3rd day, 30 min before the test for preservation of the passive avoidance response.

The apparatus used for object recognition test was a plastic box 62 cm long, 38 cm wide and 20 cm high covered with a wire mesh lid. Objects for discrimination test were made of glass or porcelain and existed in duplicate. Clearly they had no natural significance for rats and they had never been associated with reinforcement. Their weight was such that they could not be displaced by rats.

The behavioral procedure was similar to that described by Ennaceur and Delacour (1987) and Cavoy and Delacour (1993) with some modifications by Braszko et al. (1995).

It may be summarized as follows: all rats were submitted to two habituation sessions at an interval of 60 min, in which they were allowed 3 min to explore the apparatus. Twenty four hours later testing began. Each session consisted of two trials each lasting 3 min and separated by 60 min period. In the first trial (T1) rats were exposed to two identical objects which constituted samples "a" and "b". The objects were placed in the two back corners of the box. The time of each object exploration by the rats was recorded. Immediately after T1 the rat was removed, and received one of the following: saline (n = 13), AP-7 (n = 15), bicuculline (n = 15) or a combination: AP-7 + bicuculline (n = 13) as an icv or ip injection. After 60 min rats were returned to the apparatus. In the second trial (T2) rats were exposed to two objects which were introduced into the apparatus: the familiar object "a" and a new object "b" placed in the same positions as during T1. The object a presented during T2 was duplicate of the sample presented in T1 in order to avoid olfactory trails. From rat to rat, the role (familiar or new object) as well as the relative position of the two objects were counterbalanced and randomly permuted.

The time of each object exploration by rats was recorded. Exploration of an object was defined as touching it with the nose. Turning around or sitting on the object was not considered as exploratory behavior. From this measure the following variables were defined: A - the time spent on exploring object "a" and "b" in T1; A' and B - the time spent in exploring the familiar and the new object in T2 respectively.

Object recognition was measured by the variable B-A' (discrimination beetwen the new object "b" and the familiar one "a"). As B-A' time may be biased by differences in overall levels of exploration, the total exploration in T2 expressed as B +A' and B - A'/B + A' were also computed.

Behavioral tests was conducted on different groups of rats. This allows to exclude commulative effects of drugs. In each test we used control group of rats which received only saline.

The experiments were conducted in the same room as passive avoidance testing as well locomotor and exploratory activity tests.

The statistical significance of the results was computed by analysis of variance (ANOVA) followed by modified t statistics and Benferroni's procedure (Wallenstein et al. 1980) when multiple means were to be compared.

The effect of AP-7, bicuculline and AP-7 with bicuculline on locomotor and exploratory activity of rats in the open field test is shown in Fig. 1.

Neither AP-7, nor bicuculline, nor AP-7 together with bicuculline changed the mobility in rats, estimated on the basis of the number of fields crossed, rearings and bar approaches. $F(3.54) = 2.84$, F (crossings) = 1.993854, df = 42, F (rearings) = 0.84966211, df = 42, F (bar approaches) = 1.918677, df = 42.

The effect of AP-7, bicuculline and AP-7 with bicuculline on retrieval of passive avoidance in rats is shown in Fig. 2A.

Neither AP-7 in a dose of 5 nmols (icv) nor bicuculline (O.25 mg/kg i.p.) changed the latency in rats. Coadministration AP-7 and bicuculline significantly prolonged the latency to enter the dark compartment. $P < 0.001$, F (3.52) = 2.76, F = 22.07406, df = 51.

The effect of AP-7, bicuculline and AP-7 with bicuculline on consolidation of passive avoidance in rats is shown in Fig. 2B.

Neither AP-7, nor bicuculline, nor AP-7 with bicuculline changed the latency in rats, i.e. they did not change consolidation of passive avoidance. F (3.51) = 2.75, F = 0.28889109, df = 51.

The effect of AP-7, bicuculline and AP-7 with bicuculline on object recognition in rats is shown in Table I.

The time spent in exploring object "a" in T1 (variable A) was comparable in all groups. Object recognition memory measure B - A' was not significantly different between the groups. The total time spent on exploring object "b" and "a" in T2 (B + A') was similar in all groups. The variable B - A' / B + A' in different groups were at the same level. This shows that object recognition scores were not biased by differences in overall levels of exploration. F(B-A') = 0.2116453, F (3.51) = 2.76, df = 50; F(B-A'/B+A') = 0.78129519, F(3.41) = 2.37, df = 78; F(B+A') = 0.5735284, F(3.41) = 2.37, df = 78.

In the present experiments we did not observe significant changes in the locomotor activity after inhibition of GABA-A receptor by administration of bicuculline or

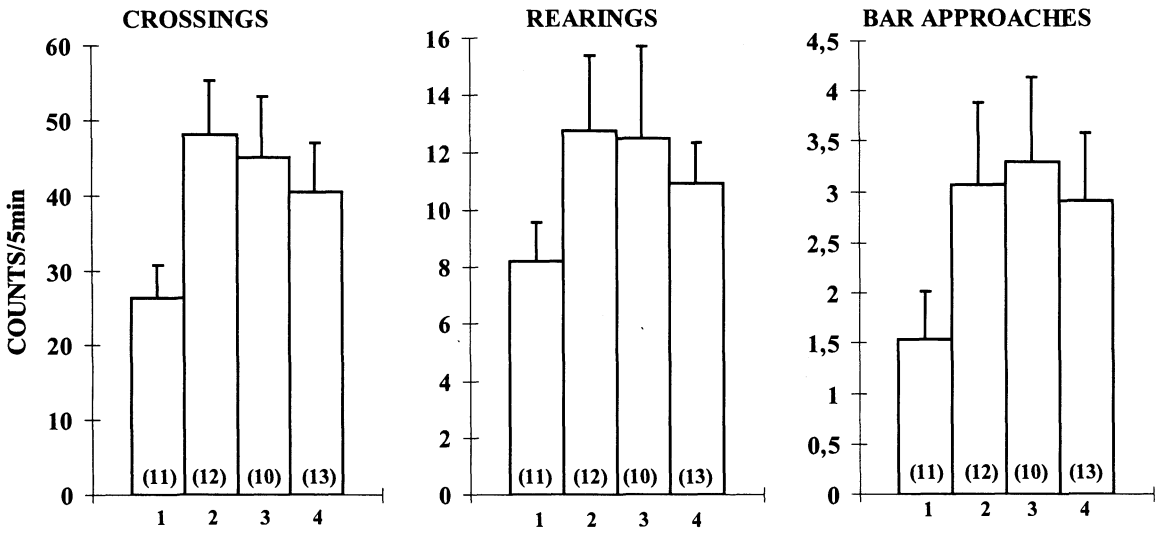
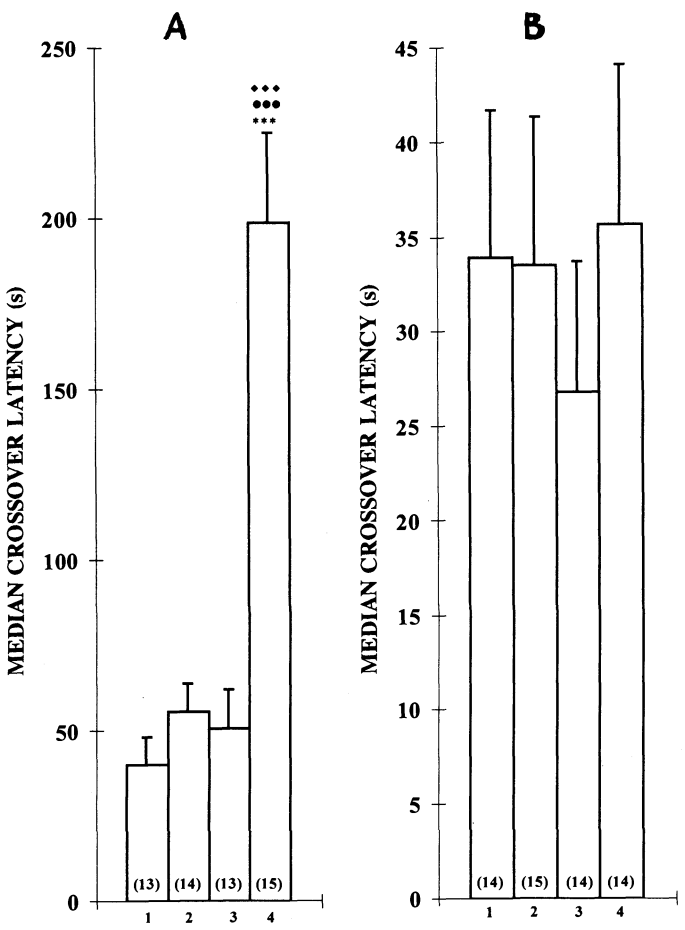


Fig. 1. The effect of AP-7, bicuculline and AP-7 with bicuculline on the number of crossings, rearings and bar approaches in the open field. Columns represent means ± SEM of the number of animals indicated in the columns. 1. saline 5 µl icv and saline 0.1 ml/100g i.p. 2. AP-7 5 nmols icv and saline i.p. 3. saline icv and bicuculline 0.25 mg/kg i.p. 4. AP-7 icv and bicuculline i.p. $F(3.54) = 2.84$ F(crossings) = 1.9938354, $df = 42$; F(rearings) = 0.84966211, $df = 42$; F(bar approaches) = 1.918677, $df = 42$.



after inhibition of NMDA receptor by administration of AP-7, alone or in combination.

The competitive NMDA antagonist weakly or inneffectively produce an increase in dopamine turnover in limbic structures and can enhance locomotor activity (Hirammatsu et al. 1989, Danysz et al. 1994).

It is an interesting fact, that inhibition of both the excitatory and the inhibitory receptor types enhance the retrieval process. We suggest some interpretation of this effect. The role of nonspecific (non-associative) effects of the NMDA receptor antagonists on learning is difficult to eliminate. It is possible that anxiolytic effects that are also seen with NMDA receptor antagonists (Stephens

Fig. 2. A, the effect of AP-7, bicuculline and AP-7 with bicuculline on retrieval in the passive avoidance situation in rats. Columns represent means ± SEM of the number of animals indicated in the columns. 1. saline 5µl icv and saline 0.1 ml/100g i.p. 2. AP-7 5 nmols icv and saline i.p. 3. saline icv and bicuculline 0.25 mg/kg i.p. 4. AP-7 icv and bicuculline i.p. $F(3.52) = 2.76$, $F = 22.07406$, $df = 51$ *** $P(1\text{-}4)<0.001$, ◆◆◆ $P(2\text{-}4)<0.001$ (filled squares), ●●● $P(3\text{-}4)<0.001$ (filled circules). B, the effect of AP-7, bicuculline and AP-7 with bicuculline on consolidation in the passive avoidance situation in rats. Columns represent means ± SEM of the number of animals indicated in the columns. 1. saline 5µl icv and saline 0.1 ml/100 g i.p. 2. AP-7 5 nmols icv and saline i.p. 3. saline icv and bicuculline 0.25 mg/kg i.p. 4. AP-7 icv and bicuculline i.p. F (3.51) = 2.75, $F = 0.28889109$, $df = 51$.

TABLE I

The rats were treated icv with 5 nmols of AP-7, and i.p. 0.25 mg/kg of bicuculline. For further details see text. Variables (in seconds) describing object recognition (see text). Values are means from 13-15 subjects and ± SEM (in parentheses). F(B-A) = 0.2116453, F(3.51) = 2.76, df = 50; F(B-A/B+A) = 0.78129519, F(3.41) = 2.37, df = 78; F(B+A) = 0.5735284, F(3.41) = 2.37, df = 78

Object recognition				
Variables	Treatment			
	Saline	AP7	Bic	AP7+Bic
B-A'	0.85 (1.02)	0.8 (0.4)	0.38 (0.54)	0.23 (0.57)
A	13.92 (1.26)	16.33 (1.88)	14.76 (2.16)	17.46 (1.9)
B+A'	8.54 (1.05)	8.0 (1.23)	9.31 (1.46)	6.46 (1.17)
B-A'/B+A'	-0.018 (0.1)	0.25 (0.086)	0.034 (0.096)	-0.028 (0.071)

et al. 1986) are responsible for observed retention deficits. Also $GABA_A$ receptor antagonists has the anxiogenic properties (Brioni 1993). The inhibition of the $GABA_A$ receptors by bicuculline can enhance this anxiolytic effects evoked by AP-7. On the other hand, modulatory compounds of NMDA receptors, like antagonists, may be more beneficial as one could modulate the effect of high glutamate concentrations in brain. Palmer et al. (1989) showed that chemical stimulation of the neocortex of anaesthetized rats with NMDA, in combination with a various GABA antagonists, increased the extracellular concentration of glutamate and aspartate in the striatum. The antagonist of $GABA_A$ receptor used in these study probably promote the effect of AP-7 on retrieval in the passive avoidance situation.

In our study AP-7 and bicuculline, when given alone or in combination, did not influence memory consolidation. Results obtained from the majority of studies show no effect of post-training injection of NMDA receptor antagonists and they suggest that there is no clear indication of NMDA receptor involvement in memory consolidation (Danysz et al. 1995). It is possible that NMDA receptors are involved in the early stages of memory consolidation that already take place during acquisition (Danysz et al. 1995). The effect of bicuculline on consolidation processes is dose-dependent (Brioni and McGough 1988) and the dose used in our study was lower than that inducing memory consolidation. We suggest independent influence of AP-7 and bicuculline on the consolidation of memory storage.

The object recognition test was used to estimate the working memory (Ennaceur and Delacour 1987). In the present study we observed no changes in object recognition in rats after administration of AP-7 and bicuculline neither alone nor in combination. Pharmacological studies of the influence of NMDA receptor antagonist on working memory are unequivocal. In most studies, specific impairing effect of NMDA receptor antagonists on this kind of memory is restricted to a narrow dose range and is easily confounded by non-associative factors (Danysz et al. 1995). To our knowledge there is no data on the effects of the $GABA_A$ receptor antagonists on working memory. However, further studies are needed to determine the precise modulatory effects of $GABA_A$ and NMDA receptors on memory and learning processes.

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