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Chronic treatment with sulbutiamine improves memory in an object recognition task and reduces some amnesic effects of dizocilpine in a spatial delayed-non-match-to-sample task

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Abstract

The effect of a sulbutiamine chronic treatment on memory was studied in rats with a spatial delayed-non-match-to-sample (DNMTS) task in a radial maze and a two trial object recognition task. After completion of training in the DNMTS task, animals were subjected for 9 weeks to daily injections of either saline or sulbutiamine (12.5 or 25 mg/kg). Sulbutiamine did not modify memory in the DNMTS task but improved it in the object recognition task. Dizocilpine, impaired both acquisition and retention of the DNMTS task in the saline-treated group, but not in the two sulbutiamine-treated groups, suggesting that sulbutiamine may counteract the amnesia induced by a blockade of the *N*-methyl-D-aspartate glutamate receptors. Taken together, these results are in favor of a beneficial effect of sulbutiamine on working and episodic memory.

Keywords: DNMTS; Episodic memory; Object recognition; Radial maze; Rat; Sulbutiamine; Working memory

1. Introduction

Sulbutiamine is a drug currently used against somatic and psychic inhibition with apathy and decreased activity. For example, it shortens the duration of psycho-behavioral inhibition occurring during major depression (Loo et al., 2000) and improves the episodic memory in schizophrenic patients (Patris et al., personal communication). Past experiments showed in mouse that a chronic treatment with large doses of sulbutiamine (300 mg/kg) improved the acquisition of an operant task, an effect that could be mediated by an increase in hippocampal cholinergic activity (Micheau et al., 1985). More recent biochemical results showed that a chronic sulbutiamine treatment at doses close to the therapeutic dose (12.5 mg/kg) had positive modulatory effects on both dopaminergic and glutamatergic cortical transmission (Trovero et al., 2000). Numerous studies indicate that a facilitation of central glutamate transmission improves memory in various tasks including spatial tasks such as radial maze or non spatial tasks including object recognition (Parada-Turska and Turski, 1990; Puma et al., 1998; Pussinen and Sirvio, 1999; Staubli et al., 1994) and that peripheral injections or in situ infusion in the prefrontal cortex of dopamine agonists can improve memory in rodents (Bernaerts and Tirelli, 2003; Floresco and Phillips, 2001; Levin and Rose, 1995). Taking together, these results suggest that at therapeutic doses, sulbutiamine may improve memory in the rat.

Abbreviations: DNMTS, delayed-non-match-to-sample; N-F, difference of exploration time between the new object and the familiar object in the choice trial.

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This study aimed to evaluate in the rat effects of sulbutiamine on memory, given chronically and at therapeutic doses, using two tasks. The first is a delayed-non-match-to-sample (DNMTS) task in radial maze, considered as accounting for working and episodic spatial memories (Chiba et al., 2002; Chrobak and Napier, 1992; Dougherty et al., 1998). With this task we also assessed the capacity of sulbutiamine to reverse amnesia induced by dizocilpine (i.e. MK-801), an antagonist of *N*-methyl-D-aspartate (NMDA) glutamatergic receptors. Inhibiting glutamatergic transmission by dizocilpine induces amnesia, although precise action on memory is controversial. According to one study, dizocilpine disrupts retrieval but not acquisition (Harrod et al., 2001). In some studies reference memory was impaired when the drug was administered either before or immediately after acquisition (Ciamei et al., 2001; Hlinak and Krejci, 2002; Mele et al., 1996). Other workers have found that dizocilpine disrupts rather specifically working memory, while consolidation and retention are less affected (Brosnan-Watters et al., 1996; Heale and Harley, 1990; Keseberg and Schmidt, 1993; Parada-Turska and Turski, 1990; Shapiro and O'Connor, 1992). Then we tested the sulbutiamine effect on the potential effects of dizocilpine on acquisition, consolidation and retention.

The second task we used is an object recognition task, considered as evaluating non spatial working and/or episodic memory (Bartolini et al., 1996; Ennaceur and Delacour, 1988).

2. Methods

2.1. Subjects

The subjects were 30 experimentally naive Sprague–Dawley Han rats (Centre d'Elevage René Janvier, France), weighing 145 ± 2 g at the beginning of the experiment. They weighted 418 ± 6 g at the end of the study. They were housed in a regulated environment (temperature: 22 ± 1 °C) with a 12:12 h light/dark cycle (light on at 07:00 h). All tests were carried out between 09:00 h and 17:00 h. The rats were housed in individual cages (45 × 30 × 20 cm) on sawdust bedding, with tap water available ad-lib in the home cage. They were maintained to 85% of their free-feeding weight by daily feeding with measured rations.

All experiments were performed within the guidelines of the French Ministry of Agriculture for experiments with laboratory animal (law 87 848).

2.2. Apparatus

The radial maze consisted of a central octagonal platform (25 cm diameter), eight adjoining arms (50 cm L, 10 cm W) containing a cup at the distal end and side walls (25 cm H). Removable barriers (25 cm H, 10 cm W) were used to close selected arms during acquisition trials. The walls and barriers were made of transparent Plexiglas. The floor of the maze was made of white plastic-coated wood and was covered with sawdust. The maze stood 50 cm above the floor on a rotating base in a testing room, which contained many extra-maze visual cues. A video camera was fixed to the ceiling above the maze to monitor the animals' activity.

The apparatus of the object recognition task was an open box made of grey opaque Plexiglas (40 cm L, 40 cm W, 40 cm H). The objects to be discriminated (3.5 cm L, 6 cm H) differed in both color and shape. They were a frosted glass blue egg cup and an earthenware white egg cup. Apparently, they had no natural significance for rats and they had never been associated with a reinforcement. In order to rule out the possibility of scent traces left on the objects and therefore the dependency of the recognition capacity of rats on the olfactory cue, the objects and the ground of the box were washed with clear water and dried between each trial. A video camera was fixed to the ceiling above the box to monitor the animals' activity.

2.3. Radial maze training

Training and testing of animals in the maze occurred during the 4–5 day work week. The main steps of training are summarized in Table 1. Animals were habituated to the maze apparatus and rewards (45 mg food pellets, Phymep, France) and trained on a standard task for 6 daily sessions. The rat was placed in the centre of the maze and allowed to visit all 8 arms that were baited with a food pellet. The session continued until the rat had entered all 8 arms. Then,

Table 1
Timing and main steps of the study

Time	Main steps of the study	Inter-trial interval
Weeks of training	Training	
1–2	Habituation to the maze (6 sessions)	
2–10	Training to DNMTS (36 sessions)	30 s/5 min
Weeks of treatment	Experiment 1—Sulbutiamine in DNMTS	
1–2	Effect on memory (2 sessions) or retention (8 sessions)	5 min/1 h/4 h
3	Effect on retention (1 session)	24 h
	Effect on acquisition + consolidation (1 session)	24 h
4	Effect on consolidation (1 session)	24 h
	Effect on acquisition + consolidation + retention (1 session)	24 h
Experiment 2—Sulbutiamine on object recognition		
5	Effect on acquisition + consolidation + retention (1 session)	24 h
Experiment 3—Sulbutiamine on dizocilpine-induced amnesia		
6	Effect on memory (1 session)	5 min
8	Effect on retention (1 session)	4 h
	Effect on acquisition + consolidation (1 session)	4 h
9	Effect on consolidation (1 session)	4 h

animals were trained for 36 daily sessions in a delayed-non-match-to-sample (DNMTS) task. Each session consisted of an acquisition trial and a retention trial. On the acquisition trial, 4 arms chosen at random were baited with a food pellet and barriers precluded entry into the other 4 arms. The rat was placed in the centre of the maze and the trial continued until it had entered the 4 baited arms or until 5 min had elapsed. Then, the animal was removed from the apparatus and returned to its home cage. The barriers were removed and the maze was rotated (at random: 0°, ±45°, ±90°, ±135°, 180°) so as to preclude the use of intra-maze cues. On the retention trial, the 4 arms that were blocked during the acquisition trial (i.e. the spatial location of these four arms) were baited with a food pellet. The rat was placed in the centre of the maze for free exploration and the trial continued until it had entered the 4 baited arms or until 5 min had elapsed. The inter-trial interval was 30 s for the first 19 sessions and 5 min for 17 sessions.

2.4. Drug treatments

After completion of DNMTS acquisition, animals were randomly assigned to 3 groups ($n = 10$/group) and subjected for 9 weeks to the drug study. They received one daily injection 7 days per week of either saline or sulbutiamine (12.5 or 25 mg/kg). On the days when animals were not subjected to a behavioral task, treatment was given from 11:00 h and 12:00 h.

Sulbutiamine (Laboratoires Servier, France) was suspended with acacia gum in 0.9% NaCl (saline). Dizocilpine (MK-801; Tocris, France) was dissolved in saline. Dizocilpine was injected at doses from 0.1 to 0.3 mg/kg. All injections were given intraperitoneally in a volume of 1 ml/kg.

2.5. Experiment 1: effect of sulbutiamine on performance in the DNMTS task

The experiment took 4 weeks to complete (see Table 1). During the first week and during the first 3 days of the second week of treatment, the inter-trial interval was 5 min and treatment was injected 30 min before the acquisition trial. During the fourth day and the fifth day of the second week of treatment, the inter-trial interval was 1 h and 4 h, respectively, and treatment was injected 30 min before the retention trial. On the next 2 weeks of treatment, the inter-trial interval was 5 min and treatment was given 30 min before the acquisition trial on the first day of the week. On the other days, the inter-trial interval was 24 h, i.e. a session was conducted over 2 consecutive days with the acquisition trial made on the first day and the retention trial made on the second day. The first session with the 24 h inter-trial interval was designed to study the effect of sulbutiamine on retention; then the treatment was given 4 h after the acquisition trial and 30 min before the retention trial. The second session with the 24 h inter-trial interval was designed to study the effect of sulbutiamine on acquisition and consolidation; then the treatment was given 30 min before the acquisition trial and 4 h after the retention trial. The third session with the 24 h inter-trial interval was designed to study the effect of sulbutiamine on memory consolidation; then the treatment was given just after the acquisition trial and 4 h after the retention trial. Finally, the fourth session with the 24 h inter-trial interval was designed to study the effect of sulbutiamine on all the phases of memory (i.e. acquisition, consolidation and retention); then the treatment was given 30 min before the acquisition trial and 30 min before the retention trial.

2.6. Experiment 2: effect of sulbutiamine on dizocilpine-induced memory impairment in the DNMTS task

During the fifth week of treatment, animals were subjected to a test in an object recognition task. For greater convenience in the presentation of results, this experiment will be called experiment 3 (see below). Then, animals were re-trained in the DNMTS task with an inter-trial interval of 5 min (sixth week of treatment) and of 4 h (seventh to ninth week of treatment) (see Table 1). The experiment 2 consisted of five tests with at least 2-day interval between two consecutive tests. Each test was conducted according to a cross-over design over two consecutive daily sessions. Each group of treatment was subdivided in 2 subgroups. One subgroup was given dizocilpine at the first session (dizocilpine session) and saline at the second session (saline session); the other subgroup was subjected to saline session and dizocilpine session in the reverse order. The first test was designed to study the effect of sulbutiamine on dizocilpine-induced amnesia with an inter-trial interval of 5 min. The treatments – i.e. either saline or sulbutiamine (12.5 or 25 mg/kg), plus either saline or dizocilpine – were given 30 min before the acquisition trial; the dose of dizocilpine was 0.1 mg/kg. Afterwards, two tests were conducted with dizocilpine at doses of 0.1 and 0.2 mg/kg that did not produce any significant amnesia (data not presented). Therefore, subsequent tests were conducted with the dose of 0.3 mg/kg of dizocilpine. The second test was designed to study the effect of sulbutiamine on dizocilpine-induced disruption of retention; the duration of inter-trial interval was 4 h and the treatments were given 30 min before the retention trial. The third test was designed to study the effect of sulbutiamine on dizocilpine-induced disruption of acquisition and consolidation; the treatments were given 30 min before the acquisition trial. Finally, the fourth test was designed to study the effect of sulbutiamine on dizocilpine-induced disruption of memory consolidation; the treatments were given immediately after the acquisition trial.

2.7. Experiment 3: effect of sulbutiamine on object recognition

The experiment 3 was conducted on the fifth week of treatment (see Table 1). On the first day of the week, the rats

were allowed to explore the apparatus for 15 min, 30 min after administration of the treatment. Then, rats were subjected to the object recognition task on the second and on the third days. The test consisted of a 3-min sample trial and a 3-min choice trial separated by a 24-h inter-trial interval. On the sample trial, two identical objects were presented in two corners of the box. On the choice trial, one of the objects presented in sample trial (termed as familiar objects) was replaced by a new object. 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. In order to study the effect of sulbutiamine on all the phases of memory (i.e. acquisition, consolidation and retention), the treatment was given 30 min before the sample trial and 30 min before the choice trial.

2.8. Measurements and statistical treatments

DNMTS task performance was analyzed only on animals that entered the 4 baited arms within 5 min during the acquisition trial and during the retention trial. Performance indices included: the time taken to complete the acquisition trial and the time taken to complete the retention trial; the total number of errors made during the acquisition trial and the total number of errors made during the retention trial. Entries made during the retention trial in un-baited arms (i.e. in arms baited during the acquisition trial; range 0–4) are considered episodic memory errors, re-entries made during the retention trial in baited arms made may be considered working memory errors and re-entries made during the retention trial in un-baited arms working/episodic memory errors. However, since one feature of episodic memory is to be a long term memory (Tulving, 2002), the distinction between episodic memory errors and working memory errors appears to be relevant when the delay between the acquisition and the retention is long (i.e. when inter-trial interval is either 4 h or 24 h) while episodic memory errors may reveal working memory errors when the inter-trial interval is 5 min.

In the object recognition test, the basic measurement was the time spent by the rats in exploring the objects during the sample trial and during the choice trial. Exploration of an object was defined as follows: directing the nose to the object at a distance ≤ 2 cm and/or touching it with the nose; turning around or sitting on the object was not considered as exploratory behavior. Object recognition task indices included the following parameters: the total exploration time in the sample trial, the total exploration time in the choice trial, the difference of exploration time between the new object and the familiar object in the choice trial (N-F) and the discrimination index, that is $100 \times$ (N-F) divided by the total exploration time in the choice trial.

In experiments 1 and 3, the effect of sulbutiamine on performance was analyzed by a one-way analysis of variance (ANOVA). Significant ANOVAs were followed by a Dunnett's *t*-test. In experiment 3, "N-F" and the discrimination index were compared with 0 by a one-tailed paired Student's *t*-test. In experiment 2, the performance was compared between dizocilpine session and saline session for each treatment group, by a one-tailed Student's *t*-test.

Results are expressed as mean ± S.E. and the significant threshold was 0.05.

3. Results

3.1. Baseline performance in the DNMTS task

As can be seen in Tables 2 and 3, animals made few errors during the acquisition trial. The number of errors made during the retention trial increased according to the inter-trial interval duration (from 5 min to 24 h) and this resulted mainly from the increase of the number of episodic memory errors. After completion of the acquisition of the DNMTS task, saline-treated animals made one visit of an arm within 8 s by mean.

3.2. Experiment 1: effect of sulbutiamine on performance in the DNMTS task

Chronic treatment with sulbutiamine (12.5 and 25 mg/kg) induced an increase of the time taken to complete

Table 2
Effect of sulbutiamine on performance (mean ± S.E.) in the DNMTS task with 24 h inter-trial interval

Time of injection	Acq+4 h/Ret−30 min				Acq−30 min/Ret+4 h			
Groups	*n*	Acquisition errors	Retention errors		*n*	Acquisition errors	Retention errors	
			Total	Episodic			Total	Episodic
Saline	10	0.4±0.3	3.3±0.7	2.8±0.4	10	0.5±0.2	3.0±0.4	2.6±0.3
S 12.5	9	0.4±0.3	3.8±0.4	3.4±0.3	10	0.4±0.3	3.1±0.6	2.5±0.3
S 25	9	0.2±0.1	3.1±0.5	3.0±0.4	9	0.0±0.0	3.0±0.4	2.9±0.4

Performance indices, i.e. the number of errors made during the acquisition trial (Acquisition Errors), the total number of errors made during the retention trial (Retention Errors/Total) and the number of episodic memory errors (Retention Errors/Episodic), were recorded on the third week of treatment, at the first and second session with 24 h inter-trial interval. The effect of treatment was studied either on retention (time of injection 4 h after the acquisition trial and 30 min before the retention trial: Acq+4 h/Ret−30 min) or on both acquisition and consolidation (time of injection 30 min before the acquisition trial and 4 h after the retention trial: Acq−30 min/Ret+4 h); S 12.5 = sulbutiamine 12.5 mg/kg; S 25 = sulbutiamine 25 mg/kg.

Table 3
Effect of sulbutiamine on performance (mean±S.E.) in the DNMTS task with 24 h inter-trial interval

Time of injection	Acq+0 min/Ret+4 h				Acq−30 min/Ret−30 min			
Groups	*n*	Acquisition errors	Retention errors		*n*	Acquisition errors	Retention errors	
			Total	Episodic			Total	Episodic
Saline	10	0.3±0.2	3.2±0.5	2.6±0.4	10	0.4±0.2	2.7±0.6	2.3±0.4
S 12.5	9	0.2±0.1	3.3±0.5	2.9±0.5	9	0.2±0.1	4.0±0.2	3.6±0.2*
S 25	10	0.4±0.4	3.6±0.4	3.3±0.3	10	0.1±0.1	3.0±0.4	2.8±0.4

Performance indices, i.e. the number of errors made during the acquisition trial (Acquisition Errors), the total number of errors made during the retention trial (Retention Errors/Total) and the number of episodic memory errors (Retention Errors/Episodic), were recorded on the fourth week of treatment, at the third and fourth session with 24 h inter-trial interval. The effect of treatment was studied either on consolidation (time of injection immediately after the acquisition trial and 4 h after the retention trial: Acq+0 min/Ret+4 h) or on acquisition, consolidation and retention (time of injection 30 min before the acquisition trial and 30 min before the retention trial: Acq−30 min/Ret−30 min); S 12.5=sulbutiamine 12.5 mg/kg; S 25=sulbutiamine 25 mg/kg. Difference vs. saline group (Dunnett's *t*-test).
* $p<0.05$.

trials (data not shown). This effect appeared on the second day of treatment and attenuated after 2 weeks of treatment. However, in the majority of the sessions, one or two animals treated with sulbutiamine were excluded from the experiment because they did not complete the acquisition trial or the retention trial within 5 min. Except this slowing effect, sulbutiamine did not significantly modify the performance during both the first and the second week.

Sulbutiamine did not significantly modify DNMTS performance when treatment was given either before the retention trial (Table 2, left), before the acquisition trial (Table 2, right) or just following the acquisition (Table 3, left). When treatment was given both before acquisition and before retention (Table 3, right), sulbutiamine (12.5 mg/kg) increased the number of episodic memory errors ($p<0.05$). The other performance indices were not significantly modified by sulbutiamine.

3.3. Experiment 2: effect of sulbutiamine on dizocilpine-induced memory impairment in the DNMTS task

The results of the experiment 2 are presented in Table 4 (total number of errors made during the acquisition trial and during the retention trial) and in Fig. 1 (numbers of episodic memory errors, of working memory errors and of working/episodic memory errors). Sulbutiamine did not curve slowing in this experiment. On the other hand, in some animals, dizocilpine caused such a disruption of performance that they did not finish the trials within 5 min. These animals were excluded from the test. In the first test, administration of dizocilpine (0.1 mg/kg) 30 min before the

Table 4
Effect of sulbutiamine on dizocilpine-induced amnesia in the DNMTS task

Intertrial	Time of injection	Groups	*n*	Acquisition errors		Retention errors	
				Saline	Dizocilpine	Saline	Dizocilpine
5 min	Acq	Saline	10	0.6±0.2	0.6±0.3	1.0±0.4	3.1±0.9**
	–	S 12.5	10	0.5±0.2	0.1±0.1	1.8±0.8	1.6±0.6
	30 min	S 25	10	0.5±0.4	0.9±0.5	1.4±0.4	2.0±0.6
4 h	Ret	Saline	9	0.2±0.2	0.0±0.0	2.9±0.7	8.4±1.7*
	–	S 12.5	10	0.0±0.0	0.1±0.1	3.8±1.0	2.9±0.5
	30 min	S 25	9	0.0±0.0	0.3±0.2	2.3±0.6	2.9±0.6
4 h	Acq	Saline	9	0.2±0.2	1.7±0.4**	1.9±0.5	5.4±0.9**
	–	S 12.5	10	0.2±0.2	3.1±1.9	2.6±0.5	4.4±1.2
	30 min	S 25	10	0.3±0.3	4.5±2.5*	3.1±0.6	4.4±1.0
4 h	Acq	Saline	9	0.4±0.3	0.1±0.1	2.6±0.7	3.8±1.0
	+	S 12.5	9	1.1±0.7	0.3±0.2	2.8±1.0	2.2±0.3
	0 min	S 25	10	0.0±0.0	0.2±0.2	2.0±0.4	2.7±0.5

In the first test (with a 5-min inter-trial interval), the effect of treatment was studied on acquisition, consolidation and retention (time of injection 30 min before the acquisition trial: Acq−30 min). In the following tests (with a 4 h inter-trial interval), the effect of treatment was studied on (i) retention (second test: time of injection 30 min before the retention trial: Ret−30 min), (ii) acquisition and consolidation (third test: time of injection 30 min before the acquisition trial: Acq−30 min), (iii) consolidation (fourth test: time of injection immediately after the acquisition trial: Acq+min). Performance indices are the total number of errors (mean±S.E.) made during the acquisition trials and during the retention trials of saline sessions and of dizocilpine sessions; S 12.5=sulbutiamine 12.5 mg/kg; S 25=sulbutiamine 25 mg/kg. Difference "dizocilpine session" vs. "saline session" (one-tailed paired Student's *t*-test).
* $p<0.05$.
** $p<0.01$.

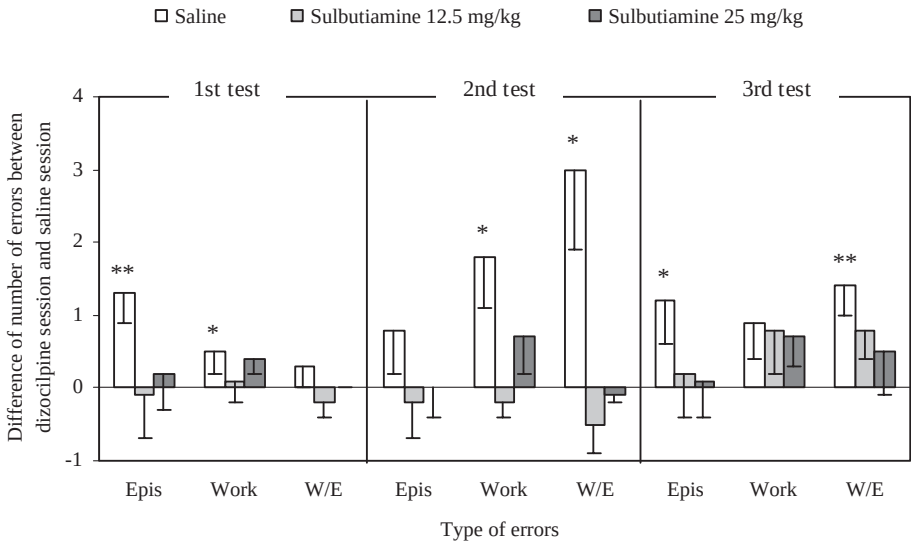

Fig. 1. Effect of sulbutiamine on dizocilpine-induced amnesia in the DNMTS task. Data (mean; S.E.) represents the differences, between "dizocilpine session" and "saline session", of the number of episodic memory errors (Epis), of the number of working memory errors (Work) and of the number of working/episodic memory errors (W/E). Left, first test: effect of treatment on acquisition, consolidation and retention: dose of dizocilpine = 0.1 mg/kg, inter-trial interval = 5 min, injections made 30 min before the acquisition trial; middle, second test: effect on retention: dose of dizocilpine = 0.3 mg/kg, inter-trial interval = 4 h, injections made 30 min before the retention trial; right, third test: effect on acquisition: dose of dizocilpine = 0.3 mg/kg, inter-trial interval = 4 h, injections made 30 min before the acquisition trial. Statistical analysis (one-tailed paired Student's *t*-test): $*p < 0.05$; $**p < 0.01$.

acquisition trial with a 5-min inter-trial interval did not significantly change the number of errors made by the three groups during the acquisition trial. Dizocilpine increased the total number of errors made on the retention trial, the number of episodic memory errors and the number of working memory errors–but did not significantly change the number of working/episodic memory errors–made by the saline group. On the other hand, dizocilpine did not produce any significant effect in the two sulbutiamine groups. In the second test, administration of dizocilpine (0.3 mg/kg) 30 min before the retention trial with a 4-h inter-trial interval significantly increased the total number of errors, the number of working memory errors and the number of working/episodic memory errors–but did not significantly increase the number of episodic memory errors–made during the retention trial by the saline group. In contrast, dizocilpine did not significantly change performance in the two sulbutiamine groups. In the third test, administration of dizocilpine (0.3 mg/kg) 30 min before the acquisition trial with a 4-h inter-trial interval increased the number errors made during the acquisition trial. This effect was statistically significant in the saline group and in the group treated with sulbutiamine (25 mg/kg), but not in the group treated with sulbutiamine (12.5 mg/kg). In the saline group, dizocilpine increased the total number of errors made during the retention trial, the number of episodic memory errors and of working/episodic memory errors, but not the number of working memory errors. On the other hand, performance indices of sulbutiamine groups recorded during the retention trial were not significantly modified by dizocilpine. In the fourth test, dizocilpine (0.3 mg/kg) administered just after the acquisition trial with a 4-h inter-trial interval did not significantly alter performance during the retention trial in both the saline and the sulbutiamine groups.

3.4. Experiment 3: effect of sulbutiamine on object recognition

Sulbutiamine did not significantly modify the total time spent to explore objects on both the sample trial and the choice trial (Fig. 2). The memory performance (Fig. 3) of the animals in the saline group was ambiguous. They did not spend more time exploring the new object than the familiar object (i.e. "N-F" not significantly > 0), suggesting that they

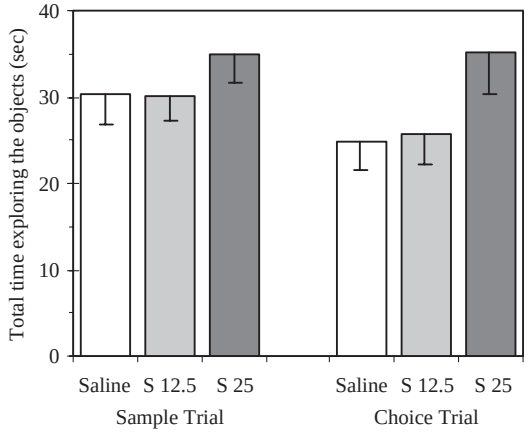

Fig. 2. Effect of sulbutiamine on the total time exploring the objects (mean; S.E.) on the sample trial and on the choice trial. Saline or sulbutiamine at a dose of 12; 5 mg/kg (S 12.5) or 25 mg/kg (S 25) ($N = 10$ animals/group) was administered 30 min before sample trial and 30 min before choice trial.

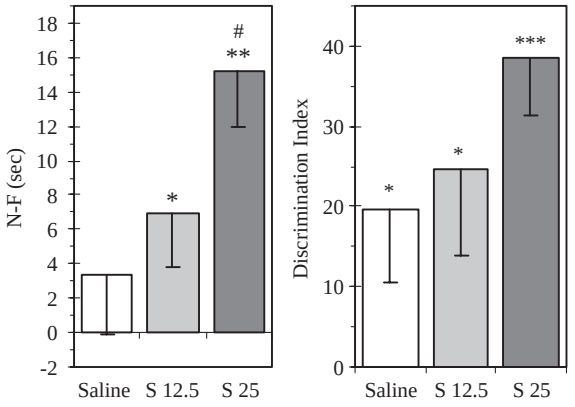

Fig. 3. Effect of sulbutiamine at doses of 12.5 mg/kg (S 12.5) and 25 mg/kg (S 25) on the memory performance (mean; S.E.) on the choice trial: difference of exploration time between the new object and the familiar object (N-F; left) and discrimination index (right). Difference vs. saline group (Dunnett's t-test): $^{\#}p<0.05$. Difference vs. 0 (one-tailed paired Student's t-test): $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

did not remember the familiar object. In another hand, they presented a discrimination index which was significantly >0, this suggested that they remembered the familiar object Conversely, both "N-F" and the discrimination index were significantly >0 for the two groups treated with sulbutiamine, indicating that these animals remembered the familiar object. The ANOVAs calculated on "N-F" and on the discrimination index did not reach statistical significance ($p=0.07$ and $p=0.34$, respectively). However, post-hoc comparison with the Dunnett's test revealed a difference significant at $p<0.05$ between the group sulbutiamine (25 mg/kg) and the saline group for "N-F".

4. Discussion

4.1. Lack of effect of sulbutiamine on performance in the DNMTS task

Sulbutiamine did not improve memory in the DNMTS task, whatever the delay between acquisition and retention and whatever the phase of memory examined (i.e. acquisition, consolidation or retention). It has been reported that rats can develop some serial searching strategies like visiting adjacent arms and thus can perform without using spatial memory (Dale and Innis, 1986). Therefore, the performance of an animal which exhibits such a stereotyped behavior cannot be improved by a drug that enhances memory. However, since in the present study not any animal did exhibit this strategy, this cannot explain the lack of effect of sulbutiamine on the performance in the DNMTS task. The animals were healthy, young and they achieved the task rapidly and efficiently, indicating that they were motivated and well trained in the DNMTS task. Therefore, they may have presented optimal performance that was quite impossible to improve with a pharmacological treatment.

4.2. Effect of dizocilpine on the performance in DNMTS

Dizocilpine induced a marked amnesia in saline-treated rats. In the first test, when inter-trial interval was 5 min, dizocilpine increased both episodic memory errors and working memory errors. Thus, because in the 5 min inter-trial interval condition, what we call episodic memory errors reflects rather working memory errors, the dizocilpine-induced amnesia may result from a disruption of working memory, an effect that has already been found in numerous studies (Ciamei et al., 2001; Levin et al., 1998; Maurice et al., 1994; Parada-Turska and Turski, 1990). In the third test, dizocilpine administered before the acquisition trial (i.e. acting during both the acquisition phase and the consolidation phase, but not during the retention phase) increased the number of episodic memory errors (and also the number of working/episodic memory errors) made during the retention trial. This result suggests that dizocilpine disrupted the acquisition and/or the consolidation of information. However a deleterious effect of dizocilpine on consolidation can be excluded since in the fourth test, dizocilpine did not induce amnesia when administered just after acquisition. Therefore, dizocilpine impaired the acquisition of the information during the acquisition trial, leading to an increase of the number of episodic memory errors made during the retention trial.

In the second test, dizocilpine administered before the retention trial (i.e. acting during the retention phase) increased the number of working memory errors and the number of working/episodic memory errors but did not significantly alter the number of episodic memory errors. In other words, dizocilpine clearly increased the number of working memory errors, while it did not disrupt the restitution of the information learned 4 h before. Taken together, these results suggest that dizocilpine disrupts both the working memory and the acquisition of information stored in episodic memory, but does not impair the retention or the consolidation of information stored in episodic memory.

4.3. Effect of sulbutiamine on dizocilpine-induced amnesia in the DNMTS task

Dizocilpine increased the number of errors made by rats treated with sulbutiamine (25 mg/kg) during the acquisition trial in the third test of experiment 2. Except for this case dizocilpine had no any significant effect on the DNMTS performance in the sulbutiamine treated rats. These results suggest that sulbutiamine can reduce the amnesia induced by dizocilpine. More precisely, sulbutiamine can reduce the disruption of working memory and the deficit of acquisition of information stored in episodic memory.

4.4. Effect of sulbutiamine on object recognition

In the third experiment sulbutiamine increased the difference of exploration time between the new object and

the familiar object and the discrimination index was higher in the group treated with the highest dose of sulbutiamine than in the control group. These results suggest an improvement of memory by sulbutiamine. Since in this experiment, treatment was active during both acquisition and retention, it cannot be determined on which phase of memory sulbutiamine exerted its effect.

5. Conclusion

In conclusion the results of this study show that in rats, sulbutiamine is able to reduce both the impairment of working memory and the impairment of acquisition in a spatial DNMTS task which are induced by dizocilpine. While sulbutiamine per se does not improve the animals' performance in this task, it may enhance memory in an object recognition task. These results suggest a beneficial effect of sulbutiamine on episodic/working memory.

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