Stimuli delivered to these electrodes evoked a compound muscle action potential in the ipsilateral limb which was used to monitor effects on peripheral neuromuscular transmission. Small discharges were also evoked in the contralateral limb which monitored transmission across polysynaptic pathways in the spinal cord. Effects were monitored for 1 h or longer depending on activity. Farnesylacetone epoxide at 300 mg/kg i.p. completely blocked the discharge in the contralateral limb but had no effect on the compound action potential in the ipsilateral limb (figure 2). This effect of farnesylacetone epoxide was observable 10 min after i.p. injection and was still evident 1 h later. Phenytoin at 400 mg/kg i.p. had a similar effect in blocking polysynaptic reflexes without any effect on peripheral neuromuscular transmission.

These results suggest that high doses of farnesylacetone epoxide and phenytoin do not effect transmission at the peripheral neuromuscular junction but do block polysynaptic transmission in the spinal cord.

Farnesylacetone epoxide initially caused hyperactivity at doses higher than 300 mg/kg i.p. followed by ataxia and finally a loss of the righting reflex in mice. These and other effects, such as hypothermia and ataxia, appeared within 5 min after injection and lasted longer than 1 h but usually were absent 2 h after injection. In contrast, the observable effects of phenytoin in mice, although similar, were longer in onset and duration. Phenytoin was more toxic than farnesylacetone epoxide after i.p. administration. Farnesylacetone epoxide at doses greater than 300 mg/kg i.p. but not p.o. did potentiate barbiturate sedation and also prevented the tremor and cholinergic symptoms after oxotremorine in mice.

A 50% inhibition of rat liver mitochondrial respiration in vitro was caused by farnesylacetone epoxide at a concentration of 5 μM as compared to the standard, rotenone, which caused a 50% inhibition at 0.5 μM. Farnesylacetone epoxide also caused a nonspecific block to agonists on the isolated guinea-pig ileum. Thus an organ bath concentration of 1×10^{-4} M reduced the contractile response to acetylcholine, nicotine and histamine by 50%. This concentration also inhibited the spontaneous beating of the isolated guinea-pig atria.

The pharmacological activity of farnesylacetone epoxide is in many ways similar to the polyhalogenated monoterpenes isolated from red alga[2]. The anticonvulsant activity is observed after high parenteral doses and not observed after oral administration. There is a nonspecific block of the response of isolated organs such as the ileum and atria to agonists. Mitochondrial respiration is inhibited which may relate to the sedative and anticonvulsant properties.

The pharmacological properties of farnesylacetone epoxide are not grossly altered in structural analogues which may suggest a nonspecific mode of action, however, the lack of knowledge of the precise mechanism of action of known anti-convulsants makes comparative evaluation of the mechanism of farnesylacetone epoxide very difficult.

1 R. Kazlauskas, P.T. Murphy and R.J. Wells, Experientia *34*, 156 (1978).
2 K.M. Taylor, D.D. Jamieson and I. Spence, in preparation.

A comparative study of the effects of muscimol and diazepam on the recall of noxious events[1]

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Summary. Muscimol induced in rats a recall deficit which possibly results from a dissociation of learning. In one out of the 2 experimental conditions studied, a cross dissociation of learning was found between diazepam and muscimol.

Several lines of research suggest a GABA involvement in memory processes. Blockade of GABA transaminase with amino-oxyacetic acid (AOAA) leads to a lack of consolidation across sessions[2]. Benzodiazepines, drugs acknowledged to facilitate GABA transmission[3], have been found - perhaps through a retrieval impairment - to exhibit amnesic-like effects[4].

In order to test further the hypothesis of a GABA control on memory, the effects of a direct GABA agonist, muscimol[5], were investigated in 2 experimental situations in which benzodiazepines were found to exert amnesic-like activity. Furthermore, an attempt was made to examine the relationship between the GABA agonist activity of benzodiazepines and their amnesic-like activity.

Material and methods. The experiments were carried out on male Wistar A.F. rats (180–220 g) housed 8 per cage with free access to food and water unless otherwise noted, and maintained in a 12 h/12 h light-dark cycle.

Situation A. The situation used has been described previously[6]. Briefly, the test situation was a ($36 \times 36 \times 30$ cm) translucent box with, in a corner, a drinking bottle the drinking tube of which terminated at a height of 3 cm above an electrifiable grid floor. The rats were deprived of water (but not of food) during the 16 h preceding their introduction into the test box.

Situation B. The test-apparatus were 3 operant chambers (housed in ventilated sound insulating cubicles) with an automatic magazine delivering 45 mg Noyes pellets. The boxes were equipped with a lever (5.5 cm above the grid floor) which the rat had to press to obtain reward. Electric current could be passed through the grid floor. The rats, maintained at 80–85% of their normal b. wt, were trained (16 daily sessions of 15 min) to press the lever of the Skinner box according to a continuously reinforced schedule.

Separate groups of rats were tested in the situation A or in the situation B. In the situation A, as well as in the situation B, the rats were subjected to 2 distinct experimental sessions: a session of learning with electric shocks and, 4 days later, a session of retest without shock.

'Shock-session'. Rats were individually placed in their appropriate test-apparatus, and, 30 sec later, were subjected to a contingent electric shock (situation A: 2 mA when drinking; situation B: 1 mA when pressing for food). After the electric shock, the animals remained 1 min in the apparatus and each time they drank or pressed the lever, were shocked again. Control rats with no shock, were given a 3-min placement in the apparatus without any shock.

'Test-session'. Each rat was placed for 5 min in the appropriate test-apparatus without any shock, and the time spent

Effects of muscimol (Mus) or diazepam (DZP) on the inhibition of drinking or pressing for food induced by previous contingent electric shocks

Treatment Learning-session	Test-session	Test-session Situation A Time spent drinking during 5 min (mean ± SEM)	Situation B Number of lever presses during 5 min (mean ± SEM)
No shock + H_2O	H_2O	175.7 ± 7.8	61.7 ± 5.3
Shock + H_2O	H_2O	8.5 ± 8.5	15.8 ± 9.6
Shock + Mus 0.5 mg kg^{-1}	H_2O	47.4 ± 24.6	-
Shock + Mus 1 mg kg^{-1}	H_2O	75.6 ± 13.1[c]	41.8 ± 4.7[a]
Shock + Mus 1 mg kg^{-1}	Mus 0.5 mg kg^{-1}	30.5 ± 10.0[d]	15.8 ± 8.7[d]
Shock + DZP 4 mg kg^{-1}	H_2O	96.1 ± 22.7[c]	45.0 ± 3.2[b]
Shock + DZP 8 mg kg^{-1}	H_2O	106.7 ± 17.3[c]	-
Shock + DZP 4 mg kg^{-1}	Mus 0.5 mg kg^{-1}	81.6 ± 20.3 NS	28.6 ± 3.5[e]
Shock + DZP 8 mg kg^{-1}	Mus 0.5 mg kg^{-1}	87.4 ± 31.4 NS	-

Drugs were given i.p. 30 min before the 'shock-session' or the 'test-session'. a,b,c indicate that animals treated before the 'shock-session' differ from the appropriate control rats with shocks at: [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$. d,e indicate that, in animals given muscimol or diazepam prior to the 'shock-session', rats given muscimol before the 'test-session' differ from appropriate control rats at: [d] $p < 0.05$; [e] $p < 0.01$; NS: not statistically significant.

drinking (situation A) or the number of lever presses (situation B) were recorded during this period.

Drugs were given i.p. (0.5 ml/100 g of b.wt) 30 min before the 'shock-session' and/or the 'test-session'. Diazepam was injected as a suspension in acacia gum.

The statistical comparisons between groups (8–10 rats per group) were done using Student's t-test or Darmois' t-test.

Results. As compared to control rats without shock, control rats with shocks exhibit in the situation A an intense inhibition of the drinking behavior ($t = 12.87$; $p < 0.001$), or in the situation B, a marked reduction of the number of lever presses for food ($t = 4.57$; $p < 0.001$). In rats treated with muscimol (1 mg kg^{-1}) or diazepam (4, 8 mg kg^{-1}), 30 min before the 'shock-session' only, the behavioral inhibition induced in both situations, was statistically reduced. Muscimol (0.5 mg kg^{-1}) injected 30 min before the 'test-session', totally abolish muscimol-induced reduction of the inhibition of the drinking behavior and of the lever presses. Given before the 'test-session', muscimol (0.5 mg kg^{-1}) statistically reduced the number of presses emitted by the rats treated with diazepam (4 mg kg^{-1}) before the 'shock-session' (at this dose, muscimol did not modify responding for food in rats without shock). In contrast, muscimol failed to reduce the drinking time of rats treated with diazepam (4, 8 mg kg^{-1}) before the 'shock-session'.

Discussion. The results reported here show that muscimol, a direct GABA agonist[5], interfers with the recall of noxious events. This is in agreement with the memory impairment already mentioned which was observed after AOAA[2], an indirect GABA agonist compound, and further supports a GABA involvement in memory processes.

The amnesic-like activity of muscimol may be imputed to events secondary to the modification of the activity of the GABAergic systems. Accordingly, it has been found that, in certain conditions, GABA-blocking agents such as picrotoxin and bicuculline, could elicit a release of vasopressin[7], the administration of which had been reported to cancel amnesia induced by various treatments in different animals[8]. Furthermore it has been established that memory deficits may be related to a decrease in central catecholamine levels. The fact that GABA-like drugs may reduce catecholamines turnover in the CNS[9,10], may account for the amnesic effect of muscimol.

However, when muscimol was injected before both the 'shock-session' and the 'test-session', the release of the inhibited behavior was no longer observed. Consequently, it seems unlikely that muscimol possesses amnesic properties, but rather impairs the transfer of the conditioned suppression from the drugged-state of the 'shock-session' to the saline-state of the 'test-session'. Such a state-dependent learning has previously been reported with baclofen[6], a compound structurally related to GABA.

Benzodiazepines, when given before the 'test-session' may enhance food or water intake[11]. As a consequence, an eventual dissociation of learning induced by these drugs – perhaps responsible for their amnesic-like activity – cannot be formally ruled out. The present experiment showed that, in animals given diazepam before the 'shock-session', muscimol injected before the 'test-session' did not modify the increased drinking time (situation A), but reduced the enhanced presses for food (situation B). Since muscimol (0.5 mg kg^{-1}) did not by itself reduce responding for food, our data suggest that (depending on the experimental conditions, the importance of which had been emphasized[12]) it is possible to obtain a cross-dissociation of learning between diazepam and muscimol.

In conclusion, such a cross-effect may be related to the GABA agonist activity of both diazepam and muscimol. This possibility may imply that the amnesic-like activity of benzodiazepines derive from a dissociation of learning. However, the results obtained in the situation A did not allow us to draw definite conclusions.

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2 R.J. Katz and L. Liebler, Psychopharmacology *56*, 191 (1978).
3 E. Costa, A. Guidotti and C.C. Mao, in: GABA in Nervous System Function, p. 413. Ed. E. Roberts, T.N. Chase and D.B. Tower. Raven Press, New York 1976.
4 P. Soubrié, P. Simon and J.R. Boissier, Experientia *32*, 359 (1976).
5 H. Möhler and T. Okada, Nature *267*, 65 (1977).
6 P. Soubrié, P. Simon and J.R. Boissier, Experientia *32*, 1323 (1976).
7 W. Feldberg and M. Rocha e Silva, Br. J. Pharmac. *62*, 99 (1978).
8 H.B. Bookin and W.D. Pfeifer, Pharmac. Biochem. Behav. *7*, 51 (1977).
9 D. Benton and J.T. Rick, Psychopharmacology *49*, 85 (1976).
10 G. Biggio, M. Casu, M.G. Corda, F. Vernaleone and G.L. Gessa, Life Sci. *21*, 525 (1977).
11 P. Soubrié, L. de Angelis, P. Simon and J.R. Boissier, Psychopharmacology *50*, 41 (1976).
12 B.D. Berger and L. Stein, Psychopharmacologia, Berl. *14*, 271 (1969).