and similarly assayed for measurements of adrenal epinephrine levels[11].

Table I presents the locomotor activity in terms of number of lines crossed by the test and control mice at 5 min intervals during the 0–40 min period. It is apparent that during the first 5 min, TMA initially caused a transitory stimulation of locomotor activity followed by decreased activity which tended to be most marked during the 16–20 min period. None of the alterations were statistically significant by Student's *t*-test procedures[12], although *p*-values were low with both doses during the 16–20 min period (Group A – 0.08; Group B – 0.06).

Thereafter, revealing further relationships to dosage, inhibitory effects on locomotor activity declined and at the 31–35 and 36–40 min periods significant increases were observed in the locomotor activities of the 100 mg/kg treated mice. Corollary increases induced by the 50 mg/kg dose at those periods were not statistically significant. It should be noted that evaluation of total locomotor activity during the 0–40 min period would not have indicated the various changes in the locomotor activity patterns of the TMA-treated mice since the summed effects in both test groups revealed increases in locomotor activity which were not statistically significant for the total 40 min period.

In contrast, continued evaluation of the effects of the hallucinogenic agent TMA on locomotor activity of different populations of test vs. control mice after 40 min to $2^1/_2$ h intervals, demonstrated consistent and in general significant increases in the subsequent locomotor activity of both test groups with the 100 mg/kg dose causing significantly greater increases than even the 50 mg/kg dose. Table II presents some representative samplings of locomotor activity patterns observed at 5 min periods during the 40 min to $2^1/_2$ h post-injection and observation periods. It should be noted that at 80 min and thereafter, the locomotor activity of the control group had declined considerably due to the generalized lesser activity in the control group. Thus, 11 of the control mice during the 80–85 min period were relatively inactive, assuming quiet and resting postures as compared to 7 in Group A (50 mg/kg) and 1 in Group B (100 mg/kg). These findings would indicate that TMA significantly stimulated locomotor activity during the 40–150 min observation period and appeared to attain peak levels at the 80–85 min interval suggesting an amphetamine-like effect on locomotor activity.

Table III presents plasma corticosterone and glucose as well as adrenal epinephrine levels of the test and control groups sacrificed after 40 min and $2^1/_2$ h. It is evident that at 40 min both doses caused significant increases in plasma corticosterone and glucose values. Although decreases were observed in adrenal catecholamine titers of both test groups, a significant reduction in adrenal epinephrine was only caused by the higher dose.

After $2^1/_2$ h, persistent increases were still evident in plasma corticosterone and glucose test group levels but only the 100 mg/kg dose showed a statistically significant increase. In contrast, none of the decreases in adrenal epinephrine induced by both doses at $2^1/_2$ h were statistically significant. Indicative of the greater activity of the higher dose, the percent changes caused by the 100 mg/kg dose in glucose, corticosterone and epinephrine were approximately 2 times greater than the percent changes evoked by 50 mg/kg, although the differences between Groups A and B were not significant.

It is apparent that acute administration of 3,4,5-trimethoxyamphetamine in the male mice produced stimulatory effects on adrenocortical and adrenomedullary activities causing respective release of corticosterone and epinephrine. The adrenocortical effects resemble actions of non-specific stress agents similarly noted in our prior LSD-25[1,2] and mescaline[3,4] investigations. The significant increase in plasma glucose is likewise in accord with non-specific stress reactions and reports that adrenal catecholamine release stimulates hyperglycemia[13]. In conclusion, 3,4,5-TMA evoked significant alterations in locomotor activity and stimulated adrenal activity.

[11] R. F. C. Vochten, J. Hoste, A. L. Delaunois and A. F. De Schaepdryver, Analyt. chem. Acta *40*, 443 (1968).
[12] G. W. Snedecor, *Statistical Methods*, 4th edn. (Iowa State Univ. Press, Ames, Iowa 1950).
[13] A. S. Milton, Br. J. Pharmac. *26*, 256 (1966).

An Amnesic Effect of Benzodiazepines in Rats?

P. Soubrie, P. Simon and J. R. Boissier

Unité de Neuropsychopharmacologie, INSERM, 2, rue d'Alésia, F-75014 Paris (France), 18 September 1975.

Summary. In rats, benzodiazepines seem to induce some amnesia as reported in man. This effect is apparent during the learning of both noxious events and extinction only if the rats are under the drug influence during the conditioning session.

It has recently been reported that diazepam[1–3], a benzodiazepine derivative, has an amnesic effect in man. Although such an activity has never been demonstrated in animal studies, it could contribute to the effect of benzodiazepines in some experimental procedures. In animals given chlordiazepoxide prior to learning a conditioned emotional response (CER), no behavioural inhibition was seen when a further conditioned stimulus appeared[4,5]. This effect does not seem to result only from a state-dependent learning[4,5], but it could be explained by the attenuation of the conditioning properties of the shock, since chlordiazepoxide has been shown to modify pain sensitivity or the emotional state elicited by pain[6].

In a similar protocol of CER we have examined the effect of 3 benzodiazepines (lorazepam, diazepam and chlordiazepoxide) and 1 neuroleptic (chlorpromazine) on behavioural inhibition by administering these agents before the learning phase (protocol A). In order to test if

[1] J. M. Gregg, D. E. Ryan and W. H. Levin, J. oral Surg. *32*, 651 (1974).
[2] P. A. Foreman, Oral Surg., oral Med., oral Path. *37*, 337 (1974).
[3] L. S. Mundow and S. V. Long, Irish J. med. Sci. *143*, 101 (1974).
[4] S. S. Tenen, Psychopharmacologia *12*, 1 (1967).
[5] S. R. Scobie and G. Garske, Psychopharmacologia *16*, 272 (1970).
[6] V. P. Houser and W. P. Pare, Psychopharmacologia *32*, 121 (1973).

Number (M ± SEM) of squares crossed by rats during the test session of the protocol A or B

Protocol	Doses ($mg \cdot kg^{-1}$)	Drugs			
		Lorazepam	Diazepam	Chlordiazepoxide	Chlorpromazine
A.	0.6	5.2 ± 2.4 (90)			
	1.25	15.4 ± 2.2[a] (98)			
	2.5	19.2 ± 1.3[a,b] (90)	5.2 ± 2.1 (100)		2.2 ± 1.6 (75)
	5	20.4 ± 1.1[a,b] (85)	14.2 ± 2.0[a] (95)	4.8 ± 1.6 (98)	2.4 ± 1.4 (66[d])
	10		15.4 ± 0.8[a] (82)	13.0 ± 2.0[a] (98)	4.8 ± 2.4 (35[e])
	20		20.2 ± 1.8[a,b] (80)	16.2 ± 0.6[a] (98)	
	40			21.0 ± 2.2[a,b] (68[d])	
B.	5	5.2 ± 1.2[c]			
	10		6.0 ± 1.2[c]		
	20			3.6 ± 1.8[c]	

Control rats without shocks: 23.0 ± 2.4; control rats with shocks: 5.0 ± 2.0; control rats with shocks + extinction: 11.5 ± 1.9; [a] $p < 0.01$ between control and treated rats with shocks. [b] ns between treated rats with shocks and control rats without shock. [c] $p < 0.01$ between control and treated rats with shocks + extinction. The numbers in brackets indicate the shock reactivity of treated rats as a percent of controls. [d] $p < 0.05$. [e] $p < 0.01$.

attenuation of the shock is responsible for such an effect, we have evaluated during the learning phase the reactivity of animals receiving benzodiazepines. Since rats exposed after the learning phase to a single extinction session exhibit a reduced behavioural inhibition, we have also studied the effects of benzodiazepines given prior to this extinction session (protocol B).

Material and methods. Male Wistar A.F. rats (200 ± 20 g) maintained 8 per cage on standard food and water ad libitum and a 12 h day cycle were used. The test-apparatus consisted of a translucent enclosure (36 × 36 × 30 cm) with an electrified floor divided into 4 equal squares by black lines. Drugs were administered (1 ml/100 g i.p.) in distilled water: solution or suspension with acacia gum (diazepam and lorazepam).

Protocol A. Each rat was placed in the test apparatus 30 min after injection (drug or distilled water). After 20 sec of free exploration, unavoidable shock (2 mA – 1 sec) was delivered with no prior signal every 10 sec for 1 min (shocks phase). Unshocked control rats were given a 1 min placement in the enclosure without any shock. Then the animals were placed back in their home cage. 4 days after, each rat was tested for 3 min in the test apparatus without any shock (test session).

Protocol B. 30 min after the 'shocks phase', rats were injected (drug or distilled water) and 10 min later were again placed in the test apparatus for 1 h without shock (extinction session). 4 days later, each rat was tested for 3 min in the test apparatus (test session).

The total number of squares crossed by each rat during the test session of the protocol A or B was recorded and considered as an index of the degree of behavioural inhibition. During the 'shocks phase' of the protocol A, the number of jumping reactions and the number of squares crossed during the 5 sec following the beginning of each shock were recorded as a measure of reactivity to shocks.

The statistical comparisons between treated and untreated groups (8 to 10 rats per group) were done using the Student's *t*-test or Darmois's *t*-test.

Results. A 78% reduction was observed in the exploratory behaviour of control rats receiving shocks as compared to control rats receiving no shock. This effect could be due to the conditioned properties acquired by the whole experimental apparatus: the reduction in exploratory behaviour is limited (30%) if the control rats receiving shocks were tested in another enclosure; the extinction session, as in protocol B, also decreased the behavioural inhibition of these rats.

A dose related reversal of the behavioural inhibition was observed with lorazepam, diazepam and chlordiazepoxide while chlorpromazine did not induce such an effect (Table). A kinetic study with diazepam (10 $mg \cdot kg^{-1}$) indicated that this effect could even be seen when diazepam was administered 2 min before testing (number of squares crossed: controls: 3.8 ± 1.6; diazepam: 12.2 ± 1.6). Diazepam showed a maximum effect when administered 30 min (16.0 ± 2.0) or 60 min (15.4 ± 1.8) before shocks and no effect when administered 4 h before (5.4 ± 2.6). When given 2 min after shocks, diazepam failed to modify the behavioural inhibition (number of squares crossed: controls: 3.8 ± 1.6; diazepam: 3.0 ± 0.9).

The attenuation in pain sensitivity or the emotional response to pain cannot fully explain the effects of benzodiazepines in the protocol A: in agreement with the results[7] showing the persistance of escape responses in shuttle-box, the benzodiazepines (except for the highest dose of chlordiazepoxide) did not alter, in our experimental conditions, the reactivity to shocks; chlorpromazine caused a significant reduction in reactivity to shocks without modifying the rats' behaviour during the test session; benzodiazepines counteract the reduction of the behavioural inhibition induced by 1 h extinction session.

Discussion. This latter effect could be related to some results[8] showing after chlordiazepoxide a prolonged resistance to extinction of an avoidance response.

Rather, the concordance of the effects of benzodiazepines on the learning both of noxious events (protocol A) and of extinction (protocol B) could be in favour of an amnesic action of these agents as reported in man[1–3]. However, further experiments would be needed to test whether such an effect was due to a learning defect or a state-dependent learning – the role of which although minor importance in the above mentioned experiments[4,5], must still be weighted in our experimental design – or rather to a memory defect. If it really is amnesia it would remain to elucidate which memory processes are affected by benzodiazepines. The fact that benzodiazepines administered after the shock session did not prevent the behavioural inhibition favour the hypothesis that the memory processes most altered are either the registration phase or those events occurring immediately after it.

[7] L. O. RANDALL and B. KAPPELL, in *The Benzodiazepines* (Eds. S. GARATTINI, E. MUSSINI and L. O. RANDALL; Raven Press, New York 1973), p. 27.

[8] D. ZISKIND, Z. AMIT and M. BAUM, Psychopharmacologia *38*, 231 (1974).