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Bromolysergide and Methysergide Protection against ECS-Induced Retrograde Amnesia[1]

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Abstract. Bromolysergide (BOL 148) and methysergide (UML 491), 2 mg/kg intraperitoneally, and saline were administered to rats 45 min before one-trial passive-avoidance conditioning followed by electroconvulsive shock (ECS) or sham-ECS ($\overline{\text{ECS}}$). On test session (24 h later), the groups treated with both BOL 148 and UML 491 exhibited a clear-cut retention in comparison to saline-ECS rats. On the other hand, all drugged groups, regardless of their submission to ECS, showed a little less pronounced consolidation than saline-$\overline{\text{ECS}}$ rats. The antiamnestic effect brought about by the two drugs was discussed in terms of receptor antagonism against ECS-released brain serotonin, whereas the lower passive-avoidance level observed in treated animals was considered in relation to a possible antipunishment effect of antiserotoninergic treatment.

Introduction

It is known that a single electroconvulsive shock (ECS) given to mice and rats after a one-trial passive-avoidance conditioning impairs memory consolidation, as shown by testing animals, e.g., 24 h later (*McGaugh and Herz*, 1972; *Gibbs and Mark*, 1973).

Several studies indicate that drugs which modify ECS-induced changes in serotonin metabolism also protect against ECS retrograde amnesia (*Essman*, 1967, 1968a, b, 1973a). Similar protection was accomplished by inhibiting rat serotonin synthesis with *p*-chlorophenylalanine before administering ECS (*Riege*, 1971).

Many of the above mentioned studies demonstrated that the reduced incidence of retrograde amnesia obtained, counteracting the ECS-induced rise of brain serotonin level and turnover rate, was accompanied by reduction of the inhibition of brain RNA and protein synthesis brought about by ECS (*Essman*, 1973b).

Furthermore, intrahippocampal injections of physiological quantities of serotonin were described to cause retrograde amnesia and brain protein synthesis inhibition of similar magni-

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tude to that produced by ECS (*Essman*, 1973c). This investigation showed that both intracranial injection of serotonin and ECS produced their amnestic effects only if the treatments were temporally close to training, although the brain serotonin level following injection and amine changes induced by ECS remained elevated for a prolonged period after the treatments.

It seems, therefore, that the sudden rise in brain free serotonin, achieved by amine intracranial injection shortly after training, represents the earliest event responsible for lack of consolidation of the trained experience. The same consideration should hold true for ECS treatment, taking into account that electrical stimulation of the brain leads to a release of serotonin from its stores (*Chase et al.*, 1967). In other words, ECS might produce an increased concentration of the neurotransmitter in the synaptic cleft, followed by the above mentioned amine turnover changes, which could be considered adaptive consequences of that first event.

The interaction of increased serotonin with its receptor sites could, in turn, initiate the macromolecular events, such as inhibition of RNA and protein synthesis, which lead to reduced consolidation (*Essman*, 1973b).

In the framework of the above mentioned evidences, the present investigation was undertaken in order to evaluate whether two brain serotonin receptor antagonists, bromolysergide and methysergide (*Snyder and Bennet*, 1975), were able to reduce or abolish the amnestic effect of cerebral electroshock.

Method

Animals. 300 male rats of the Sprague-Dawley strain, weighing 200–230 g, were used for the whole investigation. They were individually housed in an air-conditioned room artificially illuminated from 7:00 a.m. to 7:00 p.m. and received a commercial diet and water *ad libitum*.

Apparatus. A 'step-down' apparatus consisting of a 40 × 40 × 30.5 cm cage with a platform of 7 cm height, located in the center of the grid floor, was used. Under the platform surface (measuring 12 cm in diameter), a microswitch, operated by the weight of the rat, activated a timer measuring the time spent by the animal on the platform.

Handling. Animals admitted to the investigation were gently handled in their home cages for few minutes on the first 2 days of the experimental period.

Pretraining. For 2 other consecutive days, the animals, bearing ear clip electrodes, were put on the platform of the experimental cage. After having stepped down on the grid-floor, they were allowed to explore the cage for 30 sec and then they were returned to their home cages.

Training. The rats, bearing ear clip electrodes, were put on the platform. 0.5 sec after stepping down, all the animals receiving a single 2-sec unavoidable scrambled foot shock (2 mA constant AC), 5 sec later followed by an ECS of 400 msec, 1,000 V, 50-mA current, administered through ear clips. Sham-ECS ($\overline{\text{ECS}}$) consisted of ear clip application only, without passage of current.

Test. A memory test was performed 24 h later and consisted of measurement of the time spent by the animals on the platform, up to a maximum of 120 sec. Handling and all the other phases of the procedure were carried out between 10:00 a.m. and 3:00 p.m.

Drug Treatment. Methysergide bimaleate (UML 491) and 2-bromolysergide (BOL 148) were administered intraperitoneally 45 min prior to the training session at a dose of 2 mg/kg. Controls received an equal volume of saline (5 ml/kg) at the same time. For each of the three treatments, 30 rats were employed. Half of the animals of each group received $\overline{\text{ECS}}$.

Statistical Evaluation. Reciprocal-transformed step-down latencies of training and test sessions were submitted to a hierarchical-factorial analysis of variance. Partition of degrees of freedom and choice of appropriate denominators for F ratios were made according to a repeated-measure design, taking into account that ECS was administered after the training session. For this reason, the main effect of ECS and its interaction with drugs were analyzed only within the higher level of the factor 'session' (test).

Some rats were discarded because of single technical failures occurring on the training or test session or

when a body weight loss was noticed during pretraining. Owing to the unequal number of replications for treatment, unweighted analysis of cell means (*Winer*, 1971) has been adopted. Analysis of variance was followed by multiple comparisons of means according to *Duncan*'s (1955) test.

Results obtained from statistical evaluation on reciprocal-transformed step-down latencies were interpreted in terms of comparisons among the medians of the data in the original scale, as emphasized by *Li* (1964).

Results

Figure 1 shows the medians (in seconds) of step-down latencies exhibited by the 6 experimental groups on training and test sessions.

Analysis of variance upon reciprocal-transformed data (table I) showed significant interactions of drugs both with sessions and with ECS within test session. The former comparison suggested that the animals' responses were influenced by pharmacological treatments in different ways, according to the session; the latter one indicated that the effect of ECS upon test response varied according to the drug administered 45 min before training. Owing to the significant interaction 'drugs × session' mentioned above, results of training and test session were analyzed separately.

Training

Possible drug effects upon unconditioned step-down response were examined by grouping the animals' transformed values according to their pharmacological treatment. *Duncan*'s (1955) test (table II) indicated that saline- and BOL-148-treated groups did not differ from each other, whereas the UML 491 group showed a significantly faster step-down time.

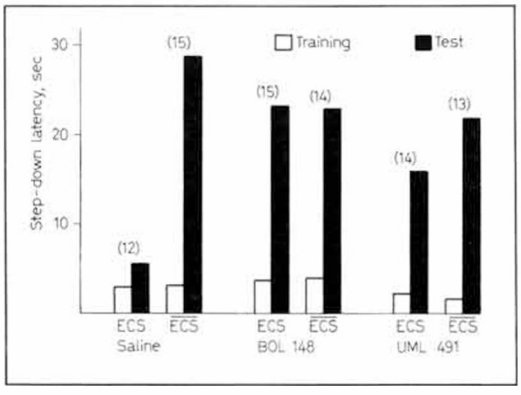

Fig. 1. Training and test median step-down latencies exhibited by male Sprague-Dawley rats injected with saline, BOL 148 (2 mg/kg intraperitoneally) and UML 491 (2 mg/kg intraperitoneally) 45 min before training and submitted to one-trial passive-avoidance conditioning followed by ECS or $\overline{\text{ECS}}$. Figures in parentheses indicate group sizes.

Table I. Unweighted-means analysis of variance for reciprocal-transformed training and test step-down latencies (sec^{-1}) of groups represented in figure 1

Source of variation	d.f.	Variance	F	p
Drugs	2	6.86711×10^{-3}	4.11	<0.025
Animals within groups	77	1.67059×10^{-3}	–	–
Sessions	1	5.73047×10^{-2}	99.63	<0.001
Drugs × sessions	2	2.03279×10^{-3}	3.53	<0.05
ECS within test session	1	8.01177×10^{-3}	13.93	<0.001
ECS × drugs within test session	2	5.15217×10^{-3}	8.96	<0.001
Animals within groups × sessions	77	5.75193×10^{-4}	–	–

On the other hand, when the same groups were examined for their reciprocal-transformed step-down values exhibited in the last pretraining session (without drug treatment), they did not significantly differ from each other ($F_{2;80} < 1$; n.s.), the overall median being 3 sec.

Apart from the above mentioned UML 491 influence upon training step-down latencies, no other differences related to drug treatment were observed in animal responses to handling, to foot shock (most animals showing vocalization and jumping) and to ECS (all animals exhibiting full tonic-clonic convulsions of about 20 sec of duration followed by a 60-sec postictal depression).

A possible drug-induced change in rat sensitivity to foot shock was excluded submitting 3 other groups of 10 animals each to a simplified version of the flinch-jump test (*Fennessy and Lee*, 1975). Rats tested 45 min after saline, BOL 148 (2 mg/kg intraperitoneally) and UML 491 (2 mg/kg intraperitoneally) exhibited almost identical jump thresholds with respect to their pretreatment values, the overall mean after treatment being 0.85 ± 0.9 (SEM) mA.

Table II. *Duncan*'s (1955) test ($\alpha = 0.05$) upon reciprocal-transformed latency means (sec^{-1}) of groups represented in figure 1

	Training	Test
BOL 148	0.20073	
Saline	0.23661	
UML 491	0.31146	
Saline-$\overline{ECS}$		0.04510
BOL 148-ECS		0.07143
BOL 148-$\overline{ECS}$		0.07233
UML 491-$\overline{ECS}$		0.10835
UML 491-ECS		0.13969
Saline-ECS		0.23391

Any two treatment means embraced by the same parenthesis are not significantly different.

Training values were grouped according to drug treatments, since ECS was administered after training.

Test

The already mentioned significant interaction 'ECS × drugs within test' indicated that the effect of ECS varied according to the pretraining drug treatment. In particular, *Duncan*'s (1955) test (table II) pointed out that saline-injected rats submitted to ECS immediately after training exhibited the shortest step-down time since this group showed results significantly different from all the others. Test step-down time of the saline-ECS group was very close to that exhibited on training session, thus confirming the amnestic effect of ECS in our procedure.

On the contrary, saline-$\overline{ECS}$ animals showed the highest step-down latency. BOL-148-treated rats did not differ from saline-$\overline{ECS}$ rats, regardless of their submission to $\overline{ECS}$ or ECS.

UML-491-$\overline{ECS}$ rats did not appear statistically different from the saline-$\overline{ECS}$ group, whereas UML-491-treated rats submitted to ECS exhibited a step-down time significantly longer with respect to the saline-ECS group, but they were faster than the saline-$\overline{ECS}$ rats.

In other words, BOL 148 brought about an almost complete protection of ECS-induced retrograde amnesia and UML 491 produced a similar but less pronounced effect.

Discussion

Our findings suggest that ECS-induced retrograde amnesia is prevented by pretreating rats with the centrally acting serotonin receptor antagonists bromolysergide and methysergide.

Some questions arise about the specificity of such an effect, i.e. if it represents a pharma-

cological influence upon ECS-induced mechanisms of impairment of consolidation, or rather upon other stages of memory formation and retrieval. In fact, drug administration before training represents a condition known as not easily allowing to rule out pharmacological effects on processes other than learning and consolidation, such as a possible enhancement of animal sensitivity to shock or degree of arousal during training, if not even a lasting drug effect on test session (*McGaugh*, 1973).

In our case, a possible drug influence upon foot shock sensitivity was excluded by the above mentioned pain threshold evaluation, and no prominent changes in gross behavior of treated animals were observed on training session.

However, the statistically significant shorter step-down latency exhibited during training by UML-491-injected rats with respect to the other two groups as well as to their own pretraining values requires to be explained.

In this regard, it is worthwhile to consider the step-down response as the resultant from opposite drives, like in every exploratory behavior which is sustained by fear and curiosity: in this specific instance the actual step-down latency might be determined by opposite components such as intolerance of the limited surface of platform vs. unwillingness of dealing with the gradient to descend.

An ancillary experiment performed by us in the context of the present investigation showed that step-down latencies of different groups of naive rats linearly increased by using platforms of increasing height and, conversely, the percentage of stepping-down animals significantly decreased within a range from 7 cm (100% of responses; latency median = 4.00 sec) to 10 cm (33.3% of rats descending from the platform; median from responding rats = 14.00 sec). When platform height was set at 13 cm, all animals refused to step down. UML-491- and BOL-148-treated rats (2 mg/kg intraperitoneally, 45 min prior to session) and saline-injected animals (15 subjects per group) were assayed for their latencies of responses from a 9-cm-high platform. Saline- and BOL-148-injected rats did not statistically differ, showing 53.3 and 47.7% of responses with medians of stepping-down animals' latencies of 10.5 and 12.0 sec, respectively. On the contrary, UML 491 rats showed 86.7% of responses with a latency median computed on descending rats of 7.5 sec; thus UML-491-treated rats were statistically more prone to step down than the two other groups.

The foregoing interpretation of unconditioned step-down response in terms of conflict situation and the well-known role of serotonin in behavioral inhibition (*Stein and Wise*, 1974) could suggest that antiserotoninergic treatment with UML 491 could have brought about a certain behavioral facilitation upon training step-down response.

In learning instances like the present one, another problem arising in evaluating pharmacological effects upon memory is represented by a possible 'state dependency'. However, if a state-dependent learning would occur when studying drug antiamnestic effects, such a pitfall would cause a lack of retention in drugged groups and not the prevention of ECS-induced retrograde amnesia observed by us.

Indeed, a less elevated level of conditioned passive avoidance was observed in antiserotoninergic-treated rats, concurrently with a clear-cut antiamnestic effect.

In our opinion, the two findings may have different explanations.

As far as the faster step-down time exhibited on test session by the drugged groups and chiefly by UML-491-treated rats is concerned, such findings could be related to the observa-

tions of *Graeff and Schoenfeld* (1970), according to which BOL 148 and UML 491 increased the rate of punished responses in multiple-schedule operant behavior of pigeons, under this respect UML 491 appearing more effective than BOL 148 in a dosage range of 1–3 mg/kg.

UML 491 was confirmed to have anticonflict effects also in the rat at doses of 1.25 and 5 mg/kg (*Cook and Sepinwall*, 1975) and 10 mg/kg (*Stein et al.*, 1973; *Graeff*, 1974).

Such data were obtained upon already established behaviors and, moreover, employing more complex passive-avoidance paradigms with respect to the present investigation.

In any case, if the serotonin system in the brain mediates the suppressive effect of punishment (*Stein and Wise*, 1974), it is not hard to accept that the administration of antiserotoninergic drugs before training could have established a little weaker conditioning in our one-trial passive-avoidance task. However, experiments with longer training-test intervals and drug administrations at different times before test session are needed in order to discriminate whether the obtained findings were also due to lasting drug effects on test session or to a 'state dependency' component.

On the other hand, as far as the antiamnestic effect observed in our drugged groups is concerned, such findings could be accounted for by a drug antagonism against ECS-induced serotonin changes. Possible influences of BOL 148 and UML 491 upon brain serotonin level and turnover should be considered before invoking their serotonin central-receptor-blocking properties as related to the observed protection of ECS-induced retrograde amnesia. In fact, the greater deal of evidences furnished up to now indicates that ECS-induced impairment of memory consolidation may be prevented by drugs that interfere with serotonin metabolism (see Introduction).

Unfortunately, extensive data concerning serotonin level and turnover modifications produced by drugs like bromolysergide and methysergide are lacking. A slight and transient decrease (−11%) in whole brain serotonin content was observed in guinea pigs 15 min after subcutaneous administration of 3 mg/kg of UML 491, but at 60 and 120 min following 3 and 10 mg/kg no significant alterations of brain amine level were observed; moreover, both doses failed to alter whole brain serotonin turnover (*D'Amico et al.*, 1976).

In the rat, a fall (−13%) in whole brain serotonin content 10 min following intraperitoneal administration of UML 491 (0.5 and 3 mg/kg) was observed (*Torre et al.*, 1974). However, an increase of rat whole brain serotonin turnover was obtained with UML 491 only by giving a dose as high as 40 mg/kg intraperitoneally (*Sofia and Vassar*, 1975).

The foregoing findings do not easily allow to interpret our present data in terms of drug interference with serotonin level and turnover, since it seems that at least UML 491 scarcely modifies brain serotonin content and does not alter amine turnover at the dose employed in the present investigation.

However, the conclusion that BOL 148 and UML 491 antagonize ECS-induced retrograde amnesia by blocking brain serotonin receptors necessitates further experiments to be valid.

On the one hand, it should be examined if and to which extent such central serotonin receptor blockers interfere with the release of serotonin and the amine turnover changes promoted by ECS. On the other hand, it should be assessed whether centrally acting serotonin antagonists succeed in preventing ECS-induced inhibition of RNA and protein synthesis, which are the neurochemical events considered the intimate basis of memory consolidation.

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