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The Effect of Mescaline upon the Conditioned Avoidance Response in the Rat

By

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With 10 Figures in the Text

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In the study of the mode of action of the psychotomimetic drugs, the effect of these compounds on animal behaviour is one important source of information. A suitable technique for use in Psychopharmacology has been the conditioned avoidance response (CAR). However, reports in the literature on the effect of mescaline on this response have been conflicting: Cook and Weidley (1957) using a pole-jump escape technique and oral doses of mescaline of up to 100 mg/kg, failed to block the CAR in rats. The oral route, however, is not a very reliable mode of administration, and it is difficult to assess the amount of active mescaline eventually reaching the C.N.S. Courvoisier and Julou (1956), on the other hand, found that the CAR and often the unconditioned response (UR) were abolished by mescaline; unfortunately they do not mention any details of the dose or of their experimental design.

Chorover (1960), using a shuttle box with sound as the conditioned stimulus and shock as the unconditioned stimulus, showed that, with intraperitoneal injections of 25 mg/kg of mescaline sulphate, there was immediate extinction of the CAR, and that the response was disrupted for eleven days afterwards. This only occurred, however, if the animal was tested immediately after the injection; if an interval of a day was left between the injection and the testing no effect was observed. Locomotor activity, as tested in the open field, did not appear to be affected under either condition. Chorover also noted that animals given a dose of 50 mg/kg, and paced in an activity wheel, became markedly paralysed and ataxic. Since Schopp *et al.* (1961) have shown that mescaline has a direct curare-like effect upon muscle, it would appear that the i.p. dose should not be greater than 25 mg/kg in any experiment. At this dose level, we did not observe ataxia in any of the animals used in the following study.

There do not appear to be any reports in the literature on the effect of DL-trimethoxyphenylalanine (the amino-acid derivative of mescaline) on any biological system. In the present study an attempt has been made to clarify the effects of mescaline on the CAR, and behavioural tests have been conducted on DL-trimethoxyphenylalanine.

Method

Subjects

Seventeen male hooded rats, supplied by the National Institute for Medical Research, London, were used in this study. The animals were approximately thirty weeks old at the beginning of the experiment and were allowed food and water ad libitum.

Apparatus

A modified Levine Shuttle box was used. Two chambers measuring 25.5 cms by 23.0 cms deep were separated from one another by a metal sheet with a doorway (11.5 cms by 10.0 cms) in the centre. The floor of each chamber consisted of a grid made of 0.75 cms diam. chromium plated brass rods placed 1.8 cms apart. Each grid was hinged at the central end, and at the further end, was suspended on springs over a series of microswitches. Any weight of over 100 grams placed anywhere on the grid caused these microswitches to be closed. Thus, an animal crossing from compartment A to compartment B caused the microswitches in compartment B to close, thereby terminating either the conditioned or the unconditioned stimulus. The walls of the chambers were made of zinc sheet and the lids were clear perspex. A buzzer and a houselight were situated midway between the two compartments. All the metal was a uniform grey and was buffed to prevent reflection.

The time intervals, the duration of the sound and shock, and the trials were controlled automatically, by means of a control unit placed in an adjacent room.

Procedure

The preliminary training period for each rat varied in the number of days required for any given animal to reach the criterion of 80% correct. Normally ten days was adequate, and most animals easily attained this criterion. On the first day, the rat was allowed ten minutes in the box for exploration and acclimatisation to the new environment. This was followed by a two-hour training period. In this conditioned avoidance schedule the buzzer (conditioned stimulus or CS) sounded for five seconds; if the animal did not cross from one compartment to the other within that time an electric shock of 1.0 ma. (unconditioned stimulus or US) was received at the end of the five seconds. Both the US and the CS could only be terminated by the animal crossing.

Each two-hour period consisted of seven runs of twenty trials each. The trials, randomly spaced, occupied eight minutes and were followed by a five minute time-out. The houselight was switched on at the beginning of the two hours and remained on until the end of the experiment. The results were recorded in terms of the number of shocks received, the

number of crosses made and the reaction time i.e. the time that elapsed from the beginning of the CS to its termination by the animal. Besides the numerical record of the crosses and shocks, a kymograph enabled the reaction times to be measured from a graphic record.

When the animals had achieved a stable number of correct responses, a saline control injection was given intraperitoneally during the interval between the second and third runs. This appeared to be the optimum period for administration, taking into consideration the period of time that must elapse before each experimental session to allow the animal to become re-orientated to the shuttlebox procedure; otherwise the initial readjustment period, with its increased number of shocks, may obscure the effects of the drug under investigation.

In the first series, nine animals were given 25 mg/kg of mescaline hydrochloride intraperitoneally on the following day, with a second saline control on the fourth day. Two weeks later the series was repeated. In the second series 25 mg/kg and 12.5 mg/kg mescaline hydrochloride were administered to eight animals in a latin square design at two weeks' interval.

Preliminary tests were performed, using two animals, on the mescaline analogue, DL-trimethoxyphenylalanine. A fortnight after the mescaline series, 25 mg/kg was administered, followed at weekly intervals by doses of 50 and 100 mg/kg.

The results for each run of 20 trials were expressed in M-S scores, i.e. the mean mescaline score minus the mean saline score for all animals. The statistical significance of the results was tested by Wilcoxon's non-parametric ranking method for paired replicates and a 2×2 factorial design in the analysis of variance.

Results

First Series 25 mg/kg Mescaline HCl.

It was apparent from the results that 25 mg/kg mescaline had a biphasic effect upon CAR behaviour. Qualitatively, an animal appeared to become confused at first; the CS was heard and the animal responded by movement of the head towards the source of sound and an increased rate of breathing. The CAR, however, appeared to be blocked, and the animal remained stationary until shock was administered; there was a notable lack of activity between trials. The US caused the animal to squeak and dash violently arond the test compartment, banging its head against the walls, apparently in an effort to find the doorway; under non-drug conditions, on the other hand, the animal leapt straight through into the other compartment. During the period of increased excitability, the confusion described above apparently disappeared, to be replaced by alertness; the animal reacted very shortly after the onset of the US,

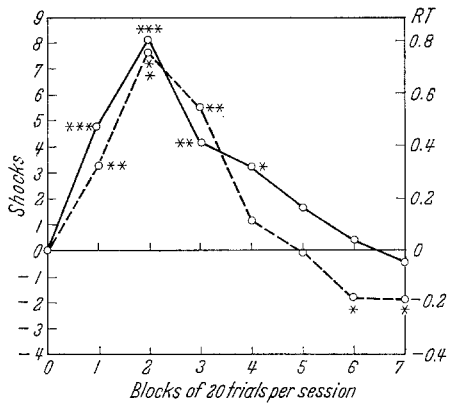
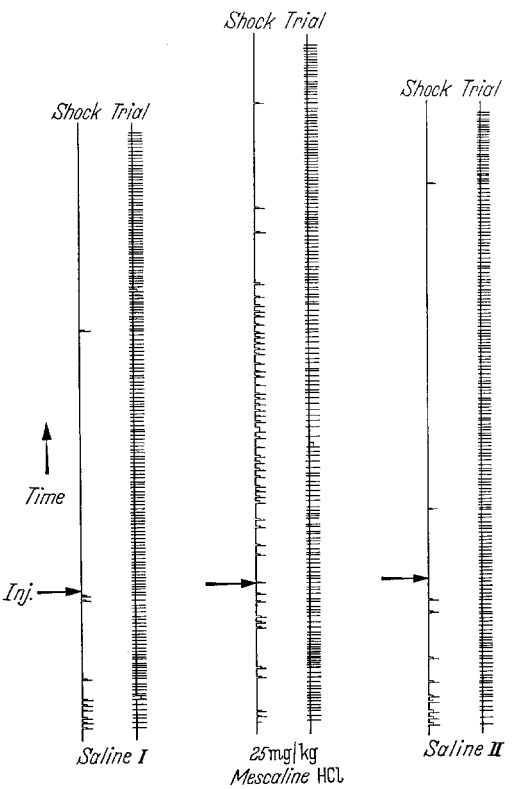


Fig. 1. Graphs showing the effect of 25 mg/kg mescaline intraperitoneally upon the reaction times and number of shocks for nine rats in the first series, expressed in mescaline-saline (M-$\bar{S}$) score. Ordinate (left): number of shocks. Ordinate (right): reaction time in mms (recording speed = 25 mms per min). Abscissa (bottom): time in runs of 20 trials. Line through 0: mean saline score. ● —— ● Shocks, ● ----- ● RT, * * * $p<0.001$, * * $p<0.01$, * $p<0.05$



crossing into the other compartment at once. Exploratory activity between trials increased, together with the amount of grooming that took place. This excitability usually lasted until the end of the session; since no session was continued for more than two hours, it is not known how long this second phase persisted. Behaviour was, however, normal during the second saline control.

Quantitatively, it was apparent that this dose of mescaline had the effect of increasing the reaction time and the number of shocks received; this effect lasted for approximately forty minutes, and was succeeded by a period of decreased reaction time, which lasted until the end of the experimental session (see Figs. 1 and 2). This decrease in reaction time is not reflected in a similar decrease in the number of shocks, since, as so few shocks

Fig. 2. A sample record showing the effect of 25 mg/kg mescaline upon one animal's response. Saline I: saline control on Day 1. Mescaline HCl: mescaline hydrochloride (25 mg/kg intraperitoneally) on Day 2. Saline II: saline control on Day 4. Inj.: time of injection. Shock: number of shocks. Trial: period of time during which buzzer sounded

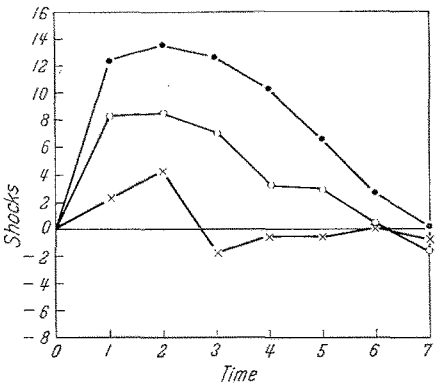


Fig. 3

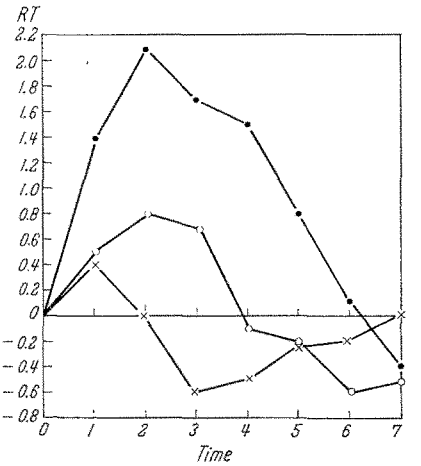


Fig. 4

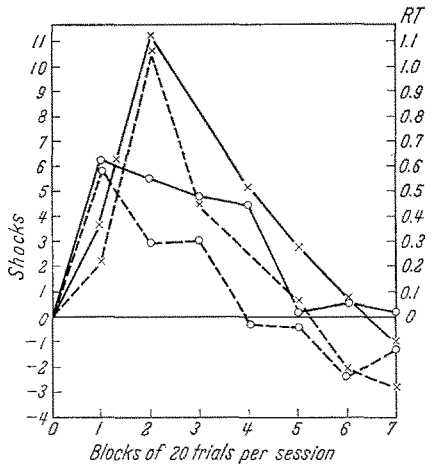


Fig. 5

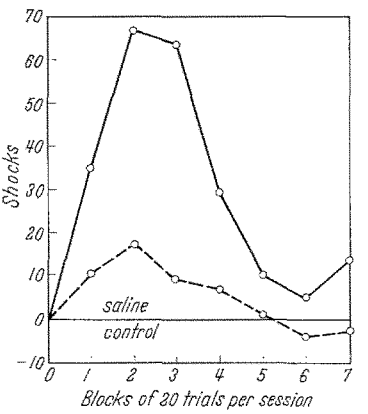


Fig. 6

Fig. 3. Graphs showing individual differences, expressed in M-$\bar{S}$ scores, in the number of shocks received by three animals at a dose of 25 mg/kg mescaline intraperitoneally. Ordinate: number of shocks. Abscissa (bottom): time in runs of 20 trials. Line through 0: mean saline scores. Rat: ●——● 16 LS, ×——× 15 LS, ○——○ 22

Fig. 4. Graphs showing individual differences, expressed in M-$\bar{S}$ scores, in the reaction times of three animals at a dose of 25 mg/kg mescaline intraperitoneally. Ordinate: reaction time in mms (recording speed = 25 mms per min). Abscissa (bottom): time in runs of 20 trials. Line through 0: mean saline scores. Explanation see Fig. 3

Fig. 5. Comparison between the first injection of mescaline and the second, for animals in Series I (N = 9) expressed in M-$\bar{S}$ scores; interval between injections (25 mg/kg i.p.) = two weeks. Ordinate (left): Number of shocks. Ordinate (right): reaction time in mms (recording speed = 25 mms per min) Abscissa (bottom): time in runs of 20 trials. Line through 0: mean saline scores. ×——× M 1 Shocks, ○——○ M 2 Shocks, ×-----× M 1 RT, ○——○ M 2 RT

Fig. 6. *Class I animals.* Total number of shocks received during an CAR experiment under two different doses of mescaline (— 25 mg/kg, - - - 12,5 mg/kg, N = 4), expressed in M-$\bar{S}$ scores. Ordinate: number of shocks. Abscissa: time in runs of 20 trials. p for difference between 2 curves: 1st 3 runs: <0.01, last 3 runs: <0.01

were being received under saline conditions, it was impossible for the number to drop any further. However, there were marked individual differences between the animals, as shown in Figs. 3 and 4; the number of shocks received and the amount of increase in reaction time varied considerably. Nevertheless, all animals exhibited some reaction to the drug; this reaction was normally biphasic, but could be predominantly an increase or a decrease in reaction time. Of the nine rats used here, two showed mainly an increase, two a decrease, and the remainder were biphasic.

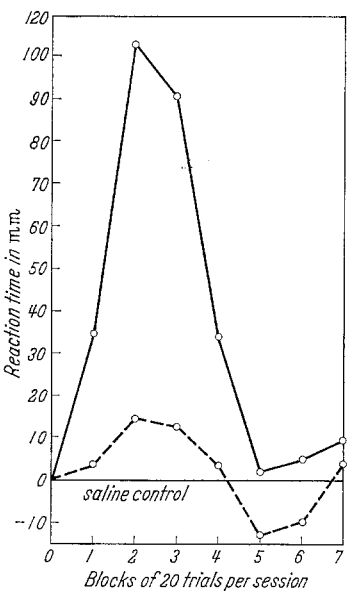


Fig. 7. *Class I animals.* Total reaction time to the sound stimulus in a CAR experiment under two different doses of mescaline (explanation see Fig. 6), expressed in M-$\bar{S}$ scores. Ordinate: reaction time in mms (recording speed = 25 mms per min). Abscissa: time in runs of 20 trials. p for difference between 2 curves: 1st 3 runs: <0.01, last 3 runs: <0.05

The results of the second mescaline injection appeared to show that some tolerance of the drug persisted for at least two weeks. The rats received fewer shocks during their second mescaline run than during their first, compared with their respective saline controls (see Fig. 5) but this did not reach statistical significance. The experimental design precluded any learning or series-effect being responsible for this. The increase in reaction time was also less on the second occasion, but again not to a significant degree. Two animals were tested eight weeks later, by which time any observable tolerance had disappeared.

Second Series 25 mg/kg and 12.5 mg/kg mescaline HCl.

In the second part of this study eight more rats of the same stock and approximately the same age were trained and tested under similar conditions, but at two dose levels 25 mg/kg and 12.5 mg/kg. The results of the 25 mg/kg dose were substantially the same as those observed earlier, namely the biphasic effect and the wide individual variation. At the lower dose level of 12.5 mg/kg, however, individual variations were marked; in some animals the second phase was potentiated, that is, there was no initial "confusion" phase and reaction times were decreased throughout the experimental session. In others, both phases were present. One animal appeared quite unaffected. It was apparent that some animals were more sensitive to the drug than others, and so they were divided into two groups on the basis of their performance under the influence of the *higher* dose. Those animals most sensitive to

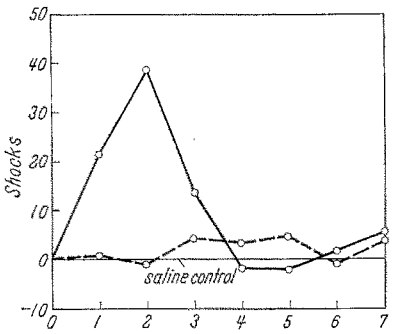


Fig. 8

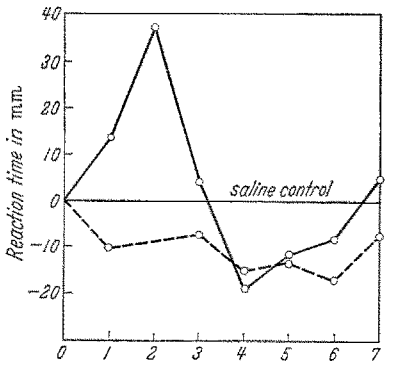


Fig. 9

Fig. 8. *Class II animals.* Total number of shocks received during a CAR experiment under two different doses of mescaline (explanation see Fig. 6), expressed in M-$\overline{S}$ scores. Ordinate: number of shocks. Abscissa: time in runs of 20 trials. p for difference between 2 curves: 1st 3 runs: <0.01, last runs: n.s. [*Class I and II* (shocks) Drug runs v. saline controls. 1st 3 runs: 25 mg/kg: $p<0.001$ (+), 12.5 mg/kg: $p<0.05$ (+), last 3 runs: 25 mg/kg: p n.s. (—), 12.5 mg/kg:p n.s. (—)]

Fig. 9. *Class II animals.* Total reaction time to the sound stimulus in a CAR experiment under two different doses of mescaline (explanation see Fig. 6), expressed in M-$\overline{S}$ scores. Ordinate: reaction time in mms (recording speed = 25 mms/min). Abscissa: time in runs of 20 trials. p for difference between 2 curves: 1st 3 runs: <0.01, last 3 runs: <0.05; [*Class I and II* (reaction time). Drug runs v. saline controls. 1st 3 runs: 25 mg/kg: $p<0.001$ (+), 12.5 mg/kg: p n.s. (+); last 3 runs 25 mg/kg: p n.s. (—), 12.5 mg/kg: $p<0.05$ (—)]

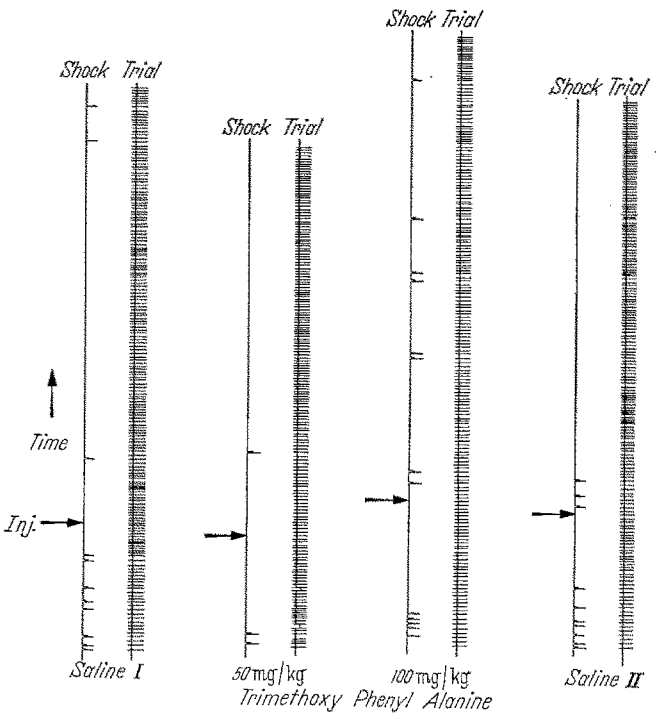


Fig. 10. A sample record showing the effect of DL-trimethoxyphenylalanine (50 and 100 mg/kg) upon one animal's response (c.f. Fig. 2). Saline I: Saline Control on Day 1. Trimethoxyphenylalanine: 50 mg/kg on Day 2 and 100 mg/kg i.p. on Day 11. Saline II: Saline control on Day 12. Inj.: time of injection. Shock: Number of shocks. Trial: Period of time during which buzzer sounded

mescaline were classified as Class I animals (with an increase of more than 35 shocks i.e. 99, 86, 55, 51), and those less sensitive as Class II (with an increase of less than 35 shocks, i.e. 22, 22, 17, 10). The difference between the two groups in the total number of shocks and in the total reaction time is apparent (Figs. 6—9).

Third series. With the phenylalanine compound, no behavioural effects were observed in either animal at any of the three dose levels (see Fig. 10). These animals had reacted well to mescaline.

Discussion

The results of this study indicate that mescaline has a biphasic effect upon the behaviour of rats trained on a CAR schedule. Initially, the drug at 25 mg/kg depresses the CAR to a sound stimulus, as measured by the number of shocks received and by the reaction times; this is followed by a period of excitation. The depression of the CAR in phase 1 does not appear to be caused by locomotor failure. The animals injected with 25 mg/kg were able to move in a co-ordinated manner, and paralysis and ataxia were only observed at 50 mg/kg. It would appear, therefore, that the confusion is central in origin; the sound is registered, as evidenced by the behavioural reaction described earlier, but the animal seems to be unable to translate this recognition into the required pattern of activity. It is therefore possible to say that the CAR is specifically depressed, rather than that the animal is unable to respond.

The second phase has not been mentioned in previous reports on the effect of mescaline on animal behaviour, and it is suggested that this is due in part to the length of the experimental session used in this investigation; most workers do not study the aninal's behaviour for longer than half an hour after the injection, in which case, taking into account the fact that the drug does not take effect for ten minutes, and that the depressant phase lasts for a further forty minutes, the second excitatory phase would be unobserved. It might be argued that the greater number of shocks received during phase 1 is responsible for increasing the level of agitation in the animal in phase 2, but this is not borne out by comparison of the drug trials with the training trials. The earlier training trials produced a large number of shocks but this did not increase the amount of observable activity or of exploratory behaviour. It therefore seems likely that this activity increase is a secondary effect of mescaline. At 12.5 mg/kg the first phase is present but attenuated in Class I animals and practically absent in Class II animals. The second phase is more prominent and comes on earlier, particularly in Class II animals. Individual variation in reaction to this dose is again marked. It is interesting that some tolerance of the drug should appear to last as long as two weeks. It was found, from further testing, that this tolerance

had completely disappeared after eight weeks, but the limits have not yet been more closely defined. It is, however, apparent that it is essential to define the tolerance limits of a drug under investigation, and also to investigate the possible cross-tolerance of drug A with drug B; otherwise, in this type of behavioural study, the results can be biased.

It is perhaps worth mentioning here that the full activity of mescaline is not necessarily demonstrated in this particular experimental situation since it is known that stress can have a blocking effect on the action of hallucinogens. We are therefore investigating the effects of mescaline upon non-stress situations, such as social behaviour and certain types of conditioned reinforcement.

The results of the preliminary tests of trimethoxyphenylalanine were particularly interesting. By analogy with the relationship between DOPA and dopamine, and between 5 HTP and 5 HT, this might be expected to cross the blood-brain barrier more easily than mescaline, and once in the brain, it might be decarboxylated to form mescaline. It might therefore be expected to be more active than mescaline. However, it was found that even in a dose of 100 mg/kg, it had no effect on the CAR, a difference so large as not to depend for its significance on any dose-response analysis. Therefore, either this compound does not cross the blood-brain barrier, or it does not become decarboxylated.

The next stage is to determine the dose-response relationships of mescaline, using other behavioural techniques besides the CAR. Structure-activity relationships can then be investigated in more detail.

Summary

Seventeen male hooded rats were trained on a CAR schedule in a shuttle box, using sound as the CS and shock as the US. After training, nine animals were injected intraperitoneally with 25 mg/kg mescaline hydrochloride, and eight animals with 25 mg/kg followed by 12.5 mg/kg. The results were recorded in terms of the number of shocks received, the number of crossings made and the reaction time from the onset of the CS. The results showed that mescaline exerted a biphasic effect, initially depressing the CAR, and then giving rise to a prolonged excitatory phase. When the experimental series was repeated two weeks later, it seemed that, although the behavioural pattern was basically the same some. tolerance of the drug had persisted. Further tests showed that this tolerance has disappeared eight weeks after the last injection. The smaller dose depressed the CAR less, and in fact the CAR was not depressed in less sensitive (Class II) animals, whereas the excitatory phase was increased and significantly so in Class II animals.

Tests with trimethoxyphenylalanine indicated that this compound did not appear to affect CAR behaviour, even in doses of 100 mg/kg.

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