Behavioural Actions of Some Substituted Amphetamines

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Many of the substances which are known to have psychotomimetic effects in man, such as lysergic acid diethylamide (LSD) and certain tryptamine derivatives, contain an indole moiety. Mescaline, however, does not contain such a moiety despite its psychotomimetic activity, but it has been suggested by various authors that the side chain of the mescaline molecule might fold back towards the benzene ring to resemble the indole nucleus of LSD and thereby impart psychotomimetic activity.

A new group of psychotropic substances, which are substituted amphetamine derivatives, have recently aroused interest. Shulgin (1964) and Shulgin *et al.* (1969) have described a number of trimethoxyamphetamines and methylene-dioxyamphetamines which have been evaluated in man and many of them are psychotomimetic, often with potencies much greater than that of mescaline. As with mescaline many of these compounds may owe their psychotropic activity to an ability of the side chains to form indole rings. A potent substituted amphetamine, first synthesised by Shulgin, is DOM (2,5-dimethoxy-4-methylamphetamine) which has been informally named STP by American hippies and widely used by them as a psychotomimetic. Work by Snyder *et al.* (1967) on human subjects showed that DOM in doses of 2–3 mg produces euphoria and some perceptual changes. At doses in excess of 3 mg, hallucinations and mood changes are seen, comparable with those produced by mescaline, but with 60–100 times the potency of mescaline. Thus DOM is only about one-thirtieth the potency of LSD. The effects can last up to 24 h but usually are considerably shorter (Snyder *et al.*, 1967). It is also reported by Snyder *et al.* (1967) that the psychotropic effects can be attenuated by chlorpromazine given at the same time as DOM administration. The effects in man of the ethyl homologue of DOM, DOET (2,5-dimethoxy-4-ethylamphetamine) have also been described by Snyder *et al.* (1968). DOET is a potent drug for producing states of euphoria and enhanced self awareness, but there have been no reports of gross perceputal changes or hallucinations produced by this drug. It appears to be approximately twice as potent at DOM at producing euphoria.

Tissue distribution and neurochemical studies after DOM administration in mice and cats have been made by Idänpään–Heikkila *et al.* (1969) and Idänpään–Heikkila and McIsaac (1970). DOM appears to have a high affinity for brain tissue and accumulation in the brains of both cats and mice was greatest in the hippocampus. Brain amine levels appear to be little changed by DOM except when it is given in very

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large doses (Idänpään–Heikkila *et al.*, 1970). Behavioural changes produced in animals by these compounds have been little investigated, but Idänpään–Heikkila *et al.* (1969, 1970) noted that mice and cats were hypersensitive to touch and to auditory stimuli; and mice had body tremors and showed a slight increase in motor activity after 10 mg/kg and 50 mg/kg. Phillips and Wesley (1969) report some behavioural effects produced from a small sample of DOM. Open-field behaviour and a mouse "head-twitch" test were employed (Corne and Pickering, 1967) and showed DOM to have effects closer to those of mescaline than amphetamine.

An attempt has been made here to investigate extensively the behavioural changes produced in rats by DOM and DOET, and to compare these changes with those produced by LSD and mescaline as examples of known psychotomimetics and with (+)-amphetamine to which they are also closely related. A series of closely related compounds, substituted phenylaminobutanes have also been examined for behavioural effects. These differ basically from DOM and DOET only in side chain length.

METHODS

Male Porton albino rats, weighing approximately 200 g, were used throughout. All rats were experimentally naive at the beginning of the tests and were only used in one experiment each. They were maintained on an *ad libitum* food and water diet and housed in a room of constant temperature and constant day–night length for at least 7 days before the beginning of an experiment.

Behavioural test methods

i. Spontaneous activity measurement

Photocell activity cages measuring 56 cm × 24 cm × 16.5 cm were used. A single light beam (through infrared filter) crossed the middle of the box and interruptions of the light beam were recorded on decatron counters. Rats were placed singly in the cages 2 h before the beginning of the test period to permit exploration of the cage. In groups of 12, the rats were injected subcutaneously with the drug or an equivalent volume of the solvent (saline) and replaced in the cages. Fifteen minutes later recordings began and continued for 2 h. Recordings were always made between 1400 and 1600 h. Total activity scores were taken for each group and mean counts were compared with controls for significant differences by Student's *t*-test. Each drug was tested initially at a dose of 10 mg/kg, with the exception of LSD (0.3 mg/kg) and (+)-amphetamine (5 mg/kg). If this dose significantly changed activity the experiment was repeated with half the initial dose, and a new group of animals, and the procedure continued until a dose was used which produced no change. Thus an approximate minimal effective dose (MED) was obtained to produce a change in spontaneous activity for each drug.

ii. Open-field test

The procedure used was essentially that described by Brimblecombe (1963) except that the open field employed was similar to that of Broadhurst (1957). The arena was circular with a diameter of 82 cm, and was illuminated by four 150 W lamps, 137 cm above the base. White noise of 88 dB was used and the operator was screened from the rat by a muslin curtain.

Groups of 8 rats were injected subcutaneously with the drug or an equivalent volume of saline, 1.5 or 3 h before placing in the open field for a 3 min period, with the exception of LSD, which was injected 15 min before testing. During this period the rat was scored for number of times rearing, preening, defaecating, number of faecal boluses passed, number of floor squares crossed at the periphery of the arena and separately, the number of squares crossed in the central circle of the field. The mean occurrence of each of these aspects of behaviour for the drug groups was compared with that of control groups by Student's *t*-test. All drugs were tested initially at the same doses as for spontaneous activity measurement, and the same procedure was followed to obtain the lowest effective dose.

iii. Conditioned Avoidance Test

Conditioned avoidance experiments were performed in an automatic shuttlebox (Camden Instruments, London). A training session consisted of 50 trials presented at a frequency of two trials per min. A trial consisted of 6 sec of a tone (CS) followed immediately by a maximum of 6 sec of scrambled shock (US) to the grid floor (approximately 0.3 mA). The response of crossing from one side of the box to the other, which occurred during the CS presentation, was termed an avoidance, and a cross during the US presentation was termed an escape. Either response terminated the remaining tone or shock. Subjects were free to cross the box during the intertrial period and were given 5 min exploration time in the box before the beginning of each session.

Groups of 10 rats were injected subcutaneously with drug or saline, 15 min before placing in the shuttlebox, thus 20 min before the beginning of training, of the *first* training session. Further training sessions of 50 trials each were given at 24 h intervals. At least 4 training sessions were given to rats of each drug group. Ten animals per group were trained in sessions one and two and 5 per group of these animals were retained for further sessions. Avoidance scores were summed for each session and means compared for significant differences using Student's *t*-test.

An experiment was performed to investigate the effects of one of the drugs, DOM, on an already learned conditioned avoidance response. The drug was injected 15 min before the beginning of session four. Sessions five and six were performed on the following days. Five animals made up the test group and 5 the saline injected controls.

DOM, DOET (structures shown in Fig. 1), LSD, mescaline and (+)-amphetamine were tested in all 3 test situations and the series of substituted phenyl aminobutanes (structures shown in Fig. 2) were tested in open-field and spontaneous activity tests only.

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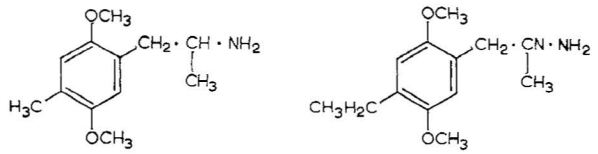
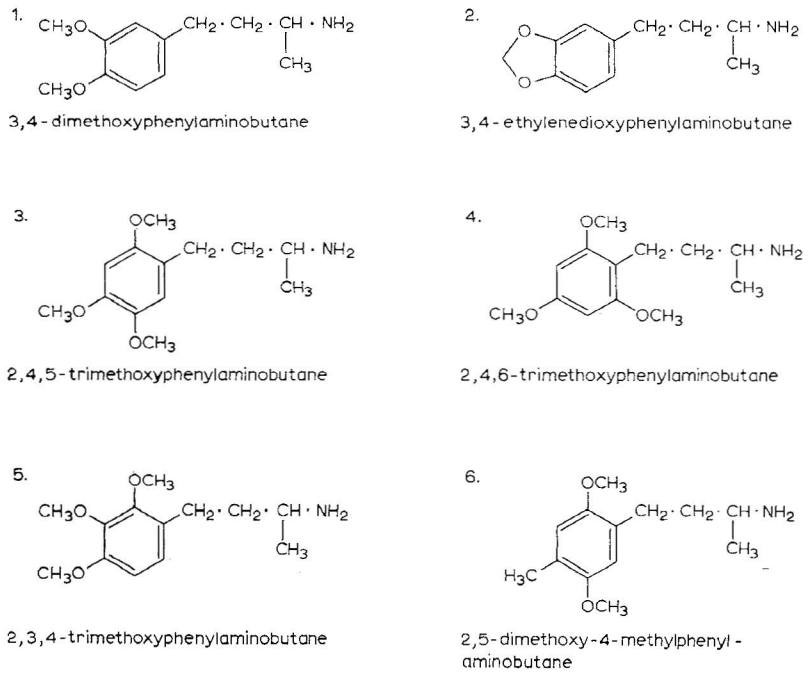
The doses used in the conditioned avoidance experiments were as follows; DOM, 2.0 mg/kg, DOET, 2.0 mg/kg, LSD, 0.3 mg/kg, mescaline, 10 mg/kg and (+)-amphetamine, 2 mg/kg.



2,5-dimethoxy-4-methylamphetamine (DOM or STP) 2,5-dimethoxy-4-ethylamphetamine (DOET)

Fig. 1. Structures of DOM and DOET.



3,4-dimethoxyphenylaminobutane

3,4-ethylenedioxyphenylaminobutane

2,4,5-trimethoxyphenylaminobutane

2,4,6-trimethoxyphenylaminobutane

2,3,4-trimethoxyphenylaminobutane

2,5-dimethoxy-4-methylphenyl-aminobutane

Fig. 2. Structure of the substituted phenyl aminobutanes.

RESULTS

The drugs DOM, DOET, LSD, mescaline and (+)-amphetamine all produced significant increases in rat spontaneous activity, mainly at low doses. The minimal effective doses (MEDs) are shown in Table I. Of the 6 substituted aminobutanes only two compounds produced significant increases in activity and these at the highest doses used. The MEDs for producing these increases are shown in Table II.

Almost all changes produced in open-field behaviour were manifested as increases in ambulation (squares crossed) and decreases in defaecation scores. All compounds which produced changes did so to one or both of these paramaters for at least one of

TABLE I

MINIMAL EFFECTIVE DOSES (MED) TO PRODUCE CHANGES IN OPEN-FIELD BEHAVIOUR AND INCREASES IN SPONTANEOUS ACTIVITY

Drug	*MED (mg/kg) open field*	*Effect in open field at this dose*	*MED (mg/kg) for increase in spontaneous activity*
DOM	0.05	Preens ↓	0.2
DOET	0.10	Ambulation ↑	0.5
LSD	0.01	Ambulation ↑	0.3
Mescaline	5.00	Ambulation ↑	10.0
(+) - Amphetamine	0.50	Ambulation ↑	2.0

TABLE II

MINIMAL EFFECTIVE DOSES (MED) TO PRODUCE CHANGES IN OPEN-FIELD BEHAVIOUR AND TO PRODUCE INCREASES IN SPONTANEOUS ACTIVITY

Drug no.	*MED (mg/kg) open field*	*Effect in open field at this dose*	*MED (mg/kg) for increase in spontaneous activity*
1	No Effect	—	No Effect
2	No Effect	—	No Effect
3	10.0	Ambulation ↑	No Effect
4	10.0	Defaecations ↓	No Effect
5	No Effect	—	10.0
6	5.0	Ambulation ↑	10.0

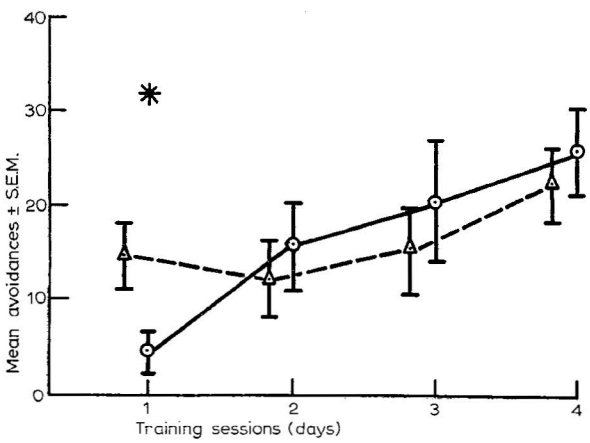


Fig. 3. Conditioned avoidance after mescaline (10 mg/kg) injected 15 min before the first session. (⊙) Control group; (△) drug group. (*) Significant difference between means in sessions marked.

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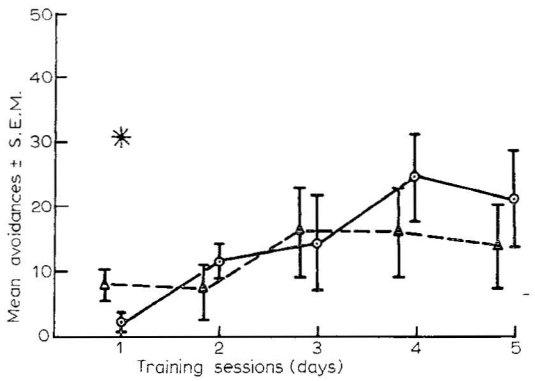

Fig. 4. Conditioned avoidance after (+)-amphetamine (2 mg/kg) injected 15 min before the first session. For symbols, see Fig. 3.

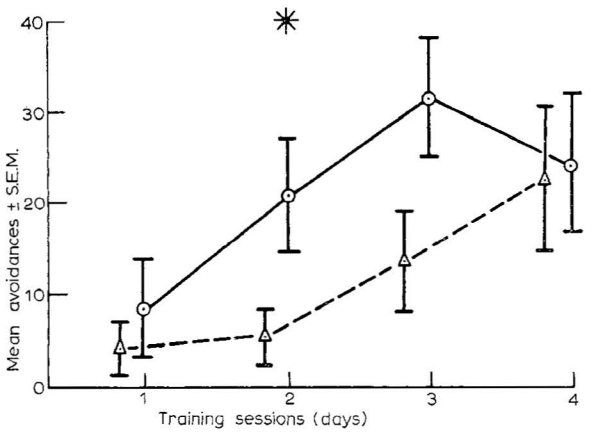

Fig. 5. Conditioned avoidance after LSD (0.3 mg/kg) injected 15 min before the first session. For symbols, see Fig. 3.

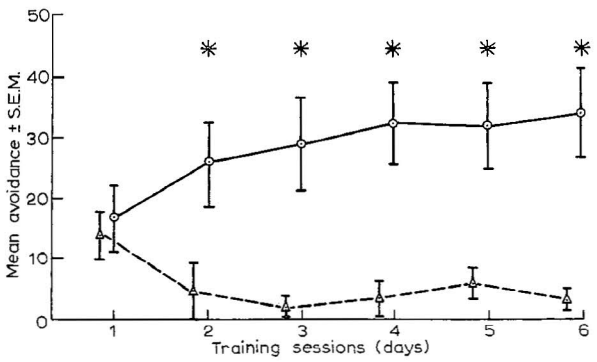

Fig. 6. Conditioned avoidance after DOM (2 mg/kg) injected 15 min before the first session. For symbols, see Fig. 3.

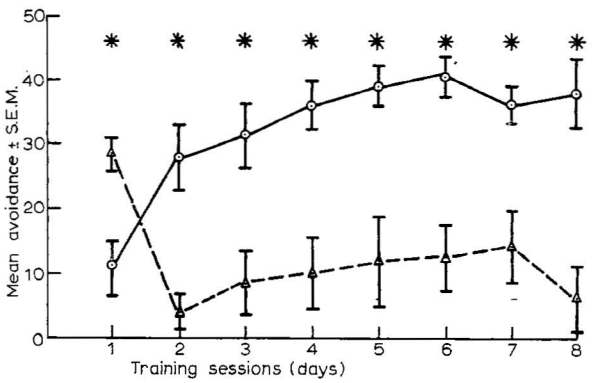

Fig. 7. Conditioned avoidance after DOET (2 mg/kg) injected 15 min before the first session. For symbols, see Fig. 3.

the doses used. The MEDs for producing any change in open-field behaviour are also shown in Tables I and II. The effects of the drugs on conditioned avoidance response are shown in Figs. 3–7.

Three drugs produced significant increases in avoidance scores in the first session. These were (+)-amphetamine, mescaline and DOET. The 3 groups receiving DOM, DOET, and LSD each attained significantly fewer avoidance scores in session two than controls, and two of these, DOM and DOET, continued to demonstrate fewer avoidances than controls for all further training sessions performed. This consisted, for DOM, of 6 sessions, and for DOET of 14 sessions, the latter spaced at irregular intervals over a period of several weeks, after the first 4 on successive days.

The group of rats which was pretrained for 4 training sessions before receiving DOM (2 mg/kg) showed a small reduction in avoidance scores for the day of injection which was not, however, significant. Further training sessions showed no differences between control and treated groups. This result is shown in Fig. 8.

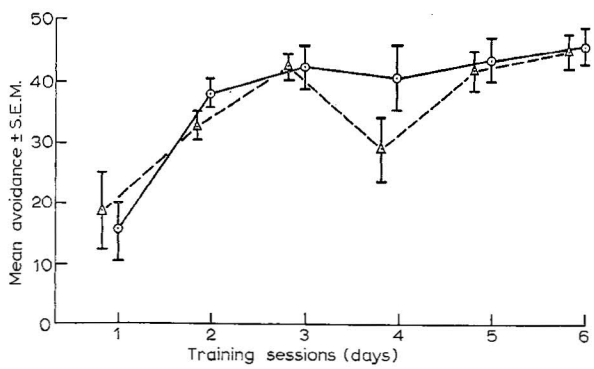

Fig. 8. Effect of DOM (2 mg/kg) injected 15 min before the fourth session only. For symbols, see Fig. 3.

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DISCUSSION

Both of the known psychotomimetics tested produced a state of hyperactivity, with LSD showing a potency of about 30 times that of mescaline; (+)-amphetamine, well-known to produce increased activity, did so at a dose 5 times less than mescaline. Both DOM and DOET appear to be very potent stimulants too and are comparable with LSD in this respect. Only two of the substituted phenylaminobutanes showed any ability at all to produce increases in activity but did so at a similar dose to that required for mescaline.

The open-field results show DOM and DOET to have similar actions on this behaviour to that demonstrated for other potent psychotomimetics, both here and in previous work by Brimblecombe (1963). Amphetamine in contrast to the psychotomimetics does not affect scores for emotional elimination that are seen with LSD, mescaline, DOM and DOET but, in common with the latter, does produce increases in ambulation scores. Three of the phenylaminobutanes produced effects in the open field of a similar type to those seen for psychotomimetic compounds but at relatively high doses. The most potent of this group, compound No. 6 (Fig. 2) produced effects at 5.0 mg/kg. This compound has the same ring substitution as DOM and differs only in chain length, but is about 100 times less potent than DOM at producing these behavioural changes. The other two compounds which produced open-field changes, 2, 4, 5 and 2, 4, 6 substituted methoxyaminobutanes, resemble psychotomimetic substituted amphetamines synthesized by Shulgin *et al.* (1964; 1969) except in chain length. It would appear, therefore, that the ethylamine or propylamine side chain is important as well as appropriate ring substitution, for potent psychotomimetic activity, possibly because the longer side chain does not permit convenient "bending back" to rejoin the benzene ring as may occur in other psychotropic phenylethylamines and propylamines.

In the conditioned avoidance experiments some striking effects were produced by DOM and DOET on rat learning. DOET produced a significant increase in avoidance responding during the first training session, so too did mescaline and (+)amphetamine. This increase was probably produced by the stimulant properties of these compounds. High intertrial activity in these experiments confirmed the hyperactive state of all the treated animals in the first training sessions. Because of the nature of shuttlebox avoidance learning it is possible for a hyperactive animal to have an advantage not possessed by a normal animal. A hyperactive animal is more likely to make non-conditioned avoidances early on in the session by virtue of this hyperactivity and thus, presumably, facilitate earlier, true conditioned avoidances. This effect has been described before for stimulants such as (+)-amphetamines and strychnine sulphate and experiments to investigate drug enhancement of learning for this reason usually employ post trial injections.

In session two, the avoidance scores of both mescaline- and (+)-amphetamine-treated groups had returned to control level and remained at control level for all further sessions of training. The LSD-, DOM- and DOET-treated groups, however, show significant decreases in avoidance scoring in the second session of training. This

phenomenon has been previously described (Evans and Patton, 1970) and it has been suggested that it is the result of state-dependent learning. The animals were trained under the influence of a drug which was not present when the animals were retested at a later stage. Thus the response had to be relearned in the absence of the drug. This would appear to explain the reduction in avoidance scores of the group previously treated with LSD, because in further training sessions there were no significant differences between this group and the controls. This was not the case, however, with the two groups treated with DOM and DOET, where there was apparently no relearning of the response, even in the case of the DOET treatment, after 14 training sessions over a period of several weeks. There have been no previous reports of such long-term effects after DOM or DOET in human studies. Without further experiments it is not possible to tell whether DOM and DOET specifically blocked the learning of conditioned avoidance in that situation or changed a wide range of behavioural characters of the animals. The animals appeared normal on qualitative observation and gained weight normally during the period of several weeks after the treatment. Snyder *et al.* (1967) report that their subjects in clinical studies with DOM had "a good memory for the drug experience", so these effects would appear to be absent from human experiences with the drug. It is difficult to see how such long-term effects could be brought about. Little is known about the central actions of the drugs. High doses of DOM are required to change brain amines. Thus Idänpään–Heikkila *et al.* (1970) report that it was necessary to give 50 mg/kg to produce a 22.5% rise in brain noradrenaline in mice which was undetectable after 2 h. The same author reports that over 60% of radioactive labelled DOM had been excreted in the urine of mice at 24 h after injection and in clinical studies. Snyder *et al.* (1967) showed that 20% of unchanged DOM had appeared in the urine. There is little yet, therefore, to suggest that DOM at least, stays in the body for an unusual length of time or produces changes that could account for the long-term effects seen in rats. The experiment in which DOM was given to trained rats shows the resistance of a well-learned response to DOM effects.

SUMMARY

1. The behavioural effects of 2,5-dimethoxy-4 methylamphetamine (DOM), 2,5, dimethoxy-4-ethylamphetamine (DOET) and a series of 6 substituted phenyl aminobutanes, were compared with the behavioural effects of lysergic acid diethylamide (LSD), mescaline and (+)-amphetamine.

2. DOM and DOET, in common with LSD, mescaline and (+)-amphetamine, caused an increase in spontaneous activity of rats. Two of the phenylaminobutanes, those most closely resembling DOM, also produced activity increases.

3. DOM and DOET, in small doses, produced changes in rat open-field behaviour comparable with those produced by LSD and mescaline. Three phenylaminobutanes produced changes of the same type, but at much higher doses than DOM or DOET.

4. DOET (2 mg/kg) enhanced conditioning of naive rats in a conditioned avoidance

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situation (shuttlebox) but these rats showed no retention or the ability to relearn in further training. DOM did not enhance the initial learning of naive rats, but did prevent further learning in the same way that DOET did. LSD, mescaline and (+)-amphetamine did not have long-term effects on conditioning.

5. DOM had no effect on already learned conditioned avoidance.

ACKNOWLEDGEMENTS

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DISCUSSION

HOLMES: You said that you did not get any learning even when you tested these animals after several months. What about their response to other tests? Did the animals behave differently from normal rats in these tests?

BUXTON: I cannot answer that yet. It is something we are going to look at. We still have the animals

from the DOET-treated group which were injected at the end of November 1970, and we propose to put these through other tests—not just conditioning tests.

KERKUT: Do you think that the DOET-treated rats were just "shuttlebox blind"? Was there any evidence that the rats could learn, say, the new position of a water bottle in the cage?

BUXTON: On casual observation the animals behave quite normally when they are in their cages, when they are being handled, and so on. This effect may be specific to the shuttlebox though how this can operate it is difficult to understand. I did put the rats in a different type of shuttlebox and I tried to retrain them using a light instead of a tone, presenting trials 3 times, instead of twice, per minute. These DOET-treated rats showed no learning at all.

RICK: Have you recorded the body weights of these rats or the amount of food they are eating or water they are drinking?

BUXTON: Accurate records have not been kept, but the animals seem to be gaining weight normally. They are certainly very big now.

BRADLEY: Did you give the drug at the start of training? If so, what possible effect would it have had on the training? Have you tried giving it 24 h before training?

BUXTON: We have tried one experiment. I did not report it because the results were not conclusive. We gave DOM 24 h before beginning training in the shuttlebox and 2 or 3 animals out of 7 learned very little. This may be within the bounds of the normal occurrence of poor performers and was not a statistically significant effect. I am going to look at this again.