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MESCALINE-INDUCED MOTOR IMPAIRMENT IN RATS, ASSESSED BY TWO DIFFERENT METHODS

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Summary

Motor impairment, especially ataxia, is often mentioned as a 'side effect' of doses of psychoactive drugs which depress animal behaviour; it is difficult to determine it accurately from visual observation, but relatively few attempts have been made to measure it objectively and quantitatively. Mescaline, in moderate to large doses, can induce biphasic -- depressant followed by stimulant -- effects on learnt and other performance of laboratory rodents. Motor impairment, using three doses, was accordingly measured during the depressant phase by two methods. An 'ataxia' test, involving analyses of footprints, showed few irregularities of gait splay due to mescaline, but the drug markedly reduced the length of steps ('stride') in a dose-related manner. In a 'tilt plane' test for general motor control, the animals' ability to cling to a tilted plane decreased with 25 mg/kg mescaline, at 30 and 40 minutes after administration. Deficits of this kind can be relevant to interpreting drug actions on forms of behaviour which involve movements for responding, and they also have interesting potential in their own right.

Deficits in various kinds of learned or 'spontaneous' performance which occur in animals given mescaline and other drugs may be due to direct effects on the performance and/or to more indirect effects, such as motor impairment. Motor impairment can take many forms; the method of assessment selected in any one case is often influenced as much by practical as by substantive needs.

'Ataxia', loosely defined as impaired co-ordination of voluntary movement, is in practice applied especially to staggering, irregular walking, that is, locomotor ataxia. It is regarded as a prominent and useful index of unacceptably high doses of drugs, and is widely mentioned in the literature, often in passing, as a side effect. It is difficult to assess ataxia accurately from visual observation alone, and there appear to be surprisingly few objective, quantitative and detailed studies. Research assessments typically consist of subjective ratings from observation. Where objective test methods have been used, they have depended, for example, on loss of righting reflex, startle responses or on apparatus such as rotating bars ('rotarods'), strain gauges, treadmills or other forms of moving belt, and different kinds of activity cage (e.g. 1, 2, 3). Most of these probably test for various aspects of general motor impairment rather than for ataxia specifically. Rotarods, unless sensitively used (4), are apt to be particularly unsatisfactory since the outcome can be heavily influenced by features of the apparatus and training procedures.

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Mescaline has 'biphasic' -- depressant then stimulant -- effects on rats in a conditioned avoidance test at a single 25 mg/kg dose (5). At other doses, of the order of 5 to 80 mg/kg, and/or with other test methods, biphasic or only depressant effects on performance have also been reported (e.g. 6, 7, 8, 9, 10, 11). Since the predominant effect of mescaline on behaviour seems to be facilitatory, it is particularly pertinent to determine how far such depression or disruption as does occur may be associated with motor impairment.

Rats were therefore tested during the depressant phase of mescaline treatment by two different methods: first, by a test for ataxic or 'staggering' gait, based on a method developed by Rushton et al (12). This method depends on quantitatively analysing the spacing of animals' footprints; it works without previous training or food or water deprivation and is direct, quick, simple and free of contaminating variables, gives consistent results in normal animals (cf. 13, 14), and has been shown sensitive to ataxia induced by increasing doses of a barbiturate (15). The second test was for general motor control, using a tilting plane, which Arvola et al (16) had found the most reliable of 9 tests for 'alcohol intoxication', and versions of which have often been used to measure effects of drugs (e.g. 17, 18, 19, 20).

Methods

Experiment 1: Ataxia Test: Six naive female hooded rats, approximately 100 days old, which had been housed singly with food and water ad libitum on a reversed light (20.00 : 8.00 hr) schedule, were tested in an open-topped wooden Y-maze, with arms 100 cm long, 15 cm wide and 28 cm high (12). The maze was placed on white paper, below constant illumination of 400 lux. Each animal was tested under the influence of two different doses of mescaline hydrochloride, six weeks apart, to prevent it acquiring tolerance to the drug; the two tests were treated as independent trials for statistical analysis. Doses, selected on the basis of previous experiments as moderate to large (5, 9), were 25, 50 and 75 mg/kg in 1 cc/kg isotonic saline i.p.; their allocation was randomised, with the constraints that each dose was given four times, and that 75 mg/kg was always given last, to prevent a possible long-term carry-over effect from this large dose affecting other trials. The schedule for each dose was:

TABLE 1

Day 1	2	3	4	5
No injection	Saline	Mescaline HCl	Saline	No injection

This sequence was repeated on 4 animals at each dose. Thus there was a control both for injection and for drug effects. Before each trial the rat's hind paws were smeared with vaseline; since the hindpaws are almost superimposed on the tracks made by the forepaws (14), only the hindpaws were used. The animal was placed in the centre of the Y-maze, and as soon as it had run to the end of an arm, which normally happened within seconds, it was removed. The paper under that arm was dusted with powdered charcoal which adhered to the greasy footprints, and varnished to fix the prints. Two 'runs' were obtained per trial. On days 1 and 5 of each experiment, when no injection was given, the rats were tested immediately on being taken from the home cage. On days 2, 3 and 4, the appropriate injection was given i.p. and the rat then returned to its cage for 15 minutes before being tested; after this time depressant effects of mescaline are usually observed in a CAR situation.

Experiment 2: General Motor Control - Tilt Plane: Eight naive male hooded rats, of similar strain and age as in Experiment 1, were singly housed with food and water ad libitum on a normal light (8.00 : 20.00 hrs) cycle. A tilting hardboard plane, 45 cm long by 40 cm wide, with the rough side upwards, was mounted on 'tufnol' and hinged to a blockboard base, and was placed on a table below constant illumination of 500 lux. Each animal acted as its own control, to allow for individual differences in baseline behaviour, body weight and in length of toe-nails, which could affect tilt plane performance. 25 mg/kg mescaline hydrochloride in 1 cc/kg isotonic saline was given i.p.; this was the lowest dose used in the ataxia test and had reliably affected behaviour in previous experiments (5, 9). Because at this dose in Experiment 1 no significant differences had been found between days 1, 2, 4 and 5, each animal was tested with the tilt plane with 'no injection' on day 1 and with mescaline on day 2. 10 minutes after injection, each rat was placed in the centre of the plane, which was angled at 20° from the horizontal, with the animal's head facing the upward slope. The plane was slowly tilted through a further 70° in 5 seconds (cf. 16, 17), and the angle at which the animal slid down was noted. The apparatus was placed at the edge of the table to prevent the rat from seeing anything below but the floor, and so learning to "let go" earlier than was necessary. During both control and drug trials, animals were tested 8 times at 10 minute intervals. The control trials on day 1 served to establish individual baseline performance.

Results

Experiment 1: Ataxia Test. The footprints for each animal were measured and analysed as by Rushton et al (12): the 'splay' for each rat was computed as the mean width over all steps in two runs, measured from the point between the second and third interdigital pads, at right-angles to the direction of travel. These distances can be read off directly by using transparent graph paper or a set square. The number of steps available for analysis varied from 10-38 per trial per animal, with a mean of 20. Ataxia was computed as the log variance of the width over all steps in the two runs, so as to determine irregularity of splay; the larger the log variance, the greater the irregularity of splay, and the more ataxic the animal's gait (15). Inspection of the results (Table 2) failed to suggest any consistent trends among log variances for the drug: at the two lower doses, log variances were smaller than the corresponding saline variances, though these differences were not significant (paired t-tests, pre- and post-drug saline combined vs. 25 mg/kg, $p=0.695$; vs. 50 mg/kg, $p=0.293$). There was, however, a significant difference at the highest dose, 75 mg/kg, specifically between the drug (log variance 1.25) and post-drug saline (log variance 0.85) $p=0.002$, though not between the drug and pre-drug saline (log variance 1.24) $p=0.948$. Although this result may have occurred by chance, as the result of several comparisons by paired t-tests, it is more likely to reflect a genuine effect, to be discussed later. Performance on day 5, with no injection (log variance 1.22), had returned to pre-drug values (75 mg/kg post-saline vs. no injection, day 5: $p=0.029$).

Observation of the animals during these and other experiments, and inspection of the footprint records, suggested that gait was nevertheless affected in a consistent manner by mescaline at all dose levels. The mean length of steps, or 'stride', that is the distance between successive steps with the same hindpaw in the direction of travel, was therefore computed for each rat over all steps in the two runs. Length of step over all 6 pre- and post-saline trials (Table 2) was found to increase significantly ($p<0.01$) as the drug dose increased (Jonckheere's trend test, 21). Using paired t-tests, drug and saline (pre- and post-drug combined) trials were compared within each of the three dose groups. The mean length of step at all three doses of mescaline was significantly less than that of the corresponding saline trials; with the biggest dose, mean step length was reduced by about 43% (Table 2 and Fig. 1).

TABLE 2

VARIABILITY OF STEP WIDTH (log variances of 'splay') and MEAN STEP LENGTHS ('stride') with 3 different doses (mg/kg) of mescaline

	Sal 1	Mesc 25	Sal II	Sal I	Mesc 50	Sal II	Sal I	Mesc 75	Sal II
Mean splay (mm)	33,68	29,61	32,42	32,43	25,49	31,32	31,93	32,35	31,72
log (s.d.)^2	1,22	1,08	1,10	1,28	0,94	1,02	1,24	1,25	0,85
Mean stride (mm)	55,21	47,05	59,55	61,56	48,36	62,13	67,37	38,63	68,91
s.d.	4,08	5,95	2,35	8,41	5,68	8,40	3,02	8,73	7,83
p($\bar{S}$ vs M)		0,022			0,008			0,006	

Each dose group (n=4) was tested 3 times, 1 day before (Saline I), during, and 1 day after (Saline II) mescaline treatment. 10-38 steps per animal per trial were available for statistical analysis, by paired t-tests: $\bar{S}$ = mean of Saline I and Saline II, M = Mescaline.

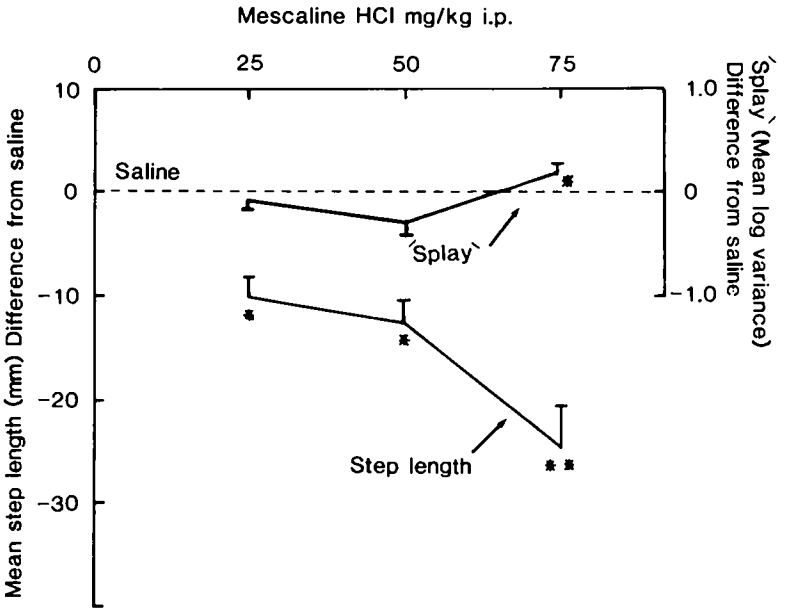

FIG. 1

*Measures of gait in Table 2, expressed as differences (mescaline minus mean of pre- and post-drug saline) from saline performance. Vertical bars = s.e.'s. Mescaline affected variability of 'splay' only at the highest dose (75 mg/kg), and then indirectly, by decreasing the post-drug saline log variance. Mean step length or 'stride' was markedly decreased by the 3 doses of mescaline. (*p<0.05, **p<0.01)*

Experiment 2: Tilt Plane. The mean scores for the 8 animals at each of the 8 trials under drug and control conditions were computed. For the control condition, the eight successive mean 'slipping off' angles from the horizontal (n=8 rats) ranged from 57.3°, s.d. 8.7, to 63.1, s.d. 9.1; for the drug condition they ranged from 53.3°, s.d. 7.2, to 56.8°, s.d. 7.2. Thus, drug-treated animals slipped off at a shallower inclination of the plane than they had done under control conditions, and there was no overlap between the means under control and drug conditions; there was also little evidence of practice effects under either condition. The differences between the 8 pairs of means reached statistical significance at 30 (p=0.035) and at 40 (p=0.007) minutes after drug administration (paired t-tests). At 40 minutes, when the drug had its maximal effect, the mean 'slipping off' angles were 63.1°, s.d 9.1, for the saline and 54.5°, s.d. 8.6, for the drug, a difference of nearly 9°, or about 14% (Fig. 2).

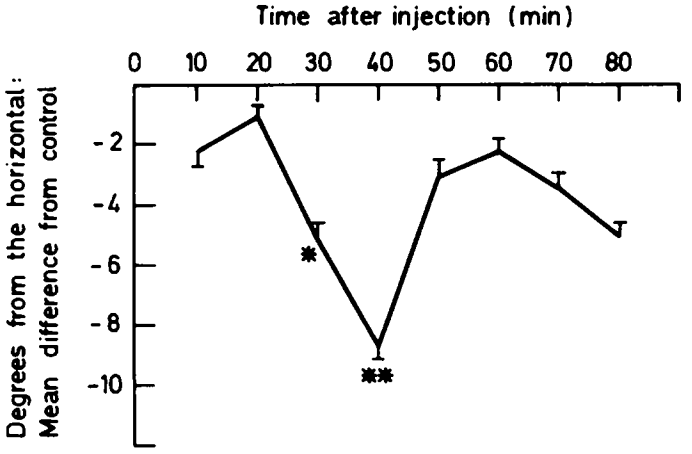

FIG. 2

*Effect of Mescaline (25 mg/kg) on tilt plane clinging (n=8), expressed as differences from control performance. The angle at which animals slid off the plane decreased markedly at 30 and 40 minutes after injection (*p<0.05 and **p<0.01), hence at these times the drug had impaired the ability to cling.*

The animals did not appear unduly 'fearful' during the experiments and few defaecated during control or drug sessions. Some animals scrambled to the top edge of the plane and held on to it by their forepaws before the plane was tilted; they were removed, replaced in the centre and the trial repeated.

Discussion

The results of the two experiments described show that 3 doses of mescaline, 25, 50 and 75 mg/kg i.p., which have repeatedly been found to impair learnt (e.g. conditioned avoidance) behaviour in rats, can also induce motor impairment: they shorten the rats' steps and reduce their ability to cling to a tilting plane. At the largest dose, 75 mg/kg, the mean step length was almost halved; step length was also significantly reduced at the two lower doses, in a dose-related manner. Variability of 'splay', which is a direct measure of locomotor ataxia or irregular gait, was unaffected by mescaline, however, except at the largest dose: with 75 mg/kg mescaline, there was no significant difference between pre-drug saline and drug trials, or between pre- and post-drug saline trials, but there was a significant difference between the drug and the post-drug saline trials, i.e. variability was reduced at post-drug saline. Since all the saline log variance values, apart from this post-75 mg/kg saline, not only did not differ significantly from the drug results but were also comparable with those obtained by others (12, 15), it is possible that this apparent single significant difference reflects a constriction of normal splay variability as a genuine after-effect caused by the high dose of mescaline given the day before. Such an after-effect would be consistent with other findings (to be published). The results also show for all three doses a consistent, though small, increase in step length between pre- and post-drug saline trials. It has been found that length of stride increases with speed (13), and it is possible that the greatest speed and length of stride produce the least variability in gait. Perhaps, therefore, the animals ran fastest at the 75 mg/kg post-drug saline trial, since this trial had both the longest mean step length and the smallest variability in gait.

The tilt plane test, though only making use of one dose, 25 mg/kg, was repeated 8 times during a single testing session, and so threw light on the time course of mescaline action after i.p. injection. The clearest impairment of clinging ability occurred at 30 and especially at 40 minutes after the drug injection. In conditioned avoidance experiments, 40 minutes was approximately the time when disruption changed to facilitation, although there was individual variation (5, 9). The nearly 9° maximum drop in clinging ability due to mescaline is roughly comparable with Arvola et al's effect with 3.6 mg/g alcohol using a hardboard plane, and 4.5 mg/g alcohol using a wire netting plane (16).

From the results presented, it is suggested that at doses of 25 mg/kg i.p. and upwards, and at post-injection intervals of more than 20 minutes, the possibility of motor impairment with mescaline should at least be considered. A fuller profile would require a combination of the two experimental designs used, that is, systematic varying of both doses and post-injection times with both test methods. It is, for example, possible that the bigger the dose, the later peak impairment occurs (cf. 22). Bourn et al (23), using smaller doses of mescaline (2 to 20 mg/kg i.p.), found increased general activity at all doses during 20-60 minutes post-injection, facilitated conditioned avoidance performance with 5 and 10 mg/kg mescaline tested at 25 minutes after injection, but no effect at any dose on rotarod performance tested at 15 minutes after injection.

The time course of drug effects on behaviour and, in particular, the optimising of the lengths of post-administration intervals, is becoming of increasing interest in research. For this, simple, quick, objective and quantifiable laboratory test methods are needed which yield reasonably stable baselines with repetition, which measure forms of behaviour relevant to everyday functioning, such as walking, clinging or grasping, and which are sensitive to moderate doses of psychoactive drugs. Motor tests of the kind described can yield not only indices of 'side effects' of high doses, but also routine markers for predicting time and dose characteristics of drugs, much as 'general activity', determined by activity cages, does.

The ataxic gait test, using footprints from unrestrained intact animals, has great potential for analysing changes in locomotion in a variety of ways which so far seem to have been little exploited with drugs (cf. 12, 15, 24, 25, 26, 27). It can of course also be used to study normal locomotion (13) and deficits due to non-drug factors such as irradiation (14), anoxia and cerebellar or basal ganglia lesions. In order to obtain footprints from untrained rodents, a Y-shaped runway should be used rather than a straight alley (cf. 12), in which animals usually fail to run, though tilting straight alleys upwards may overcome this (14, 15). The test has been adapted for man (28) and for mice, with computerised scoring and analysis, which saves time and effort, and allows large numbers of subjects to be tested (to be published). It is also possible to mechanise and simplify the tilt plane test, although the manual method used here seems to give adequate results with simple equipment (16, 17).

Drug-induced motor impairment can, moreover, be a flexible research tool, for example in the study of motivation, where animals can sometimes be found to persevere in behaviour despite considerable motor disability (e.g. 15, 26). Similarly, in aggression research, it has recently been demonstrated that ataxic movements induced in rats by various drugs, including mescaline, elicited attack behaviour with biting from undrugged opponents; this diminished as ataxia wore off (29).

The effects of mescaline on animal behaviour are being found to be increasingly complex (30, 31). Some of the apparent inconsistencies may be due to differences in procedure, including the wide range of doses and post-administration intervals used by different investigators. Since almost any behaviour being tested involves some kind of movement, more emphasis on testing for concurrent motor-impairment might also help with clarification.

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