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MUTUAL POTENTIATION OF AMPHETAMINE AND AMYLOBARBITONE MEASURED BY ACTIVITY IN RATS

BY

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Dose/response relations have been analysed for the actions of amphetamine-barbiturate mixtures on exploratory activity and ataxia in rats. Amphetamine sulphate and amylobarbitone sodium were studied separately and together (in a constant ratio of 1:20) in doses which ranged from those producing no effect to those which incapacitated the animals. Dexamphetamine and amylobarbitone were similarly studied in a ratio of 1:6.5; this corresponds to the ratio of a commercial preparation, Drinamyl. The results showed that mixtures could stimulate exploratory activity and their maximal effects were much greater than the effects produced by any dose of the separate drugs. The maximal effect with the first dose-ratio included conspicuous ataxia, but the maximal effect with the second ratio did not. Further experiments in which the dose of one drug was held constant and that of the other was varied showed that maximal effects on activity could be obtained with mixtures of dexamphetamine and amylobarbitone. Equivalent effects could be obtained both with relatively small and with relatively large amounts of the two drugs, in varying ratios; some constituent doses of the individual drugs were found to be optimal; whether the mixture effect was accompanied by ataxia depended largely on the constituent amount of barbiturate. For practical purposes mixtures producing maximal effects on activity with the smallest amounts of both drugs and not accompanied by ataxia might be most desirable, and these can be approximately read off from an isobol plotted from the results. It was concluded that the marked stimulant effects of the amphetamine-barbiturate mixtures on activity of rats could be regarded as due to true potentiation.

In previous experiments (Steinberg, Rushton & Tinson, 1961) it was found that small doses of amphetamine sulphate (0.75 mg/kg) and of amylobarbitone sodium (15 mg/kg) which individually had little effect could, when given together, markedly increase the spontaneous exploratory activity of rats in an unfamiliar environment, even though most of the animals were ataxic and moved clumsily. The ratio of the doses of the two drugs was different from the ratio of mixtures commonly used in psychiatry since there was several times more barbiturate. Experiments on man with amphetamine sulphate and cyclobarbitone in a similar dose-ratio to that used in the animal experiments showed that a mixture could produce a pattern of effects on various aspects of behaviour which was different from the pattern due to the separate drugs (Legge & Steinberg, 1962).

The present experiments were carried out to determine whether mixtures of the two drugs over a wide range of doses could indeed produce effects on the activity of rats which were greater than the effects produced by either drug separately, and to analyse the nature and limits of responses to mixtures further. The doses ranged from those producing no effect to those which incapacitated the animals, and the effects of various mixtures were compared with those of the individual drugs. The reactions tested were (*a*) exploratory activity in an unfamiliar environment, (*b*) ataxia, as indicated by irregularity of the rats' footprints. Preliminary accounts of some of these results have been published (Rushton & Steinberg, 1962, 1963).

METHODS

Subjects. Adult Lister hooded female rats were used, aged 115 to 140 days and reared under standard conditions since weaning. The animals had not been handled or used in previous experiments.

Apparatus for testing exploratory activity. The apparatus was similar to that previously described (Steinberg *et al.*, 1961). It consisted of a symmetrical wooden Y-shaped runway, 13 in. high and with arms 15 in. long and 5 in. wide. A trial consisted of placing a rat in the centre of the Y and leaving it in the apparatus for 5 min ; the number of times it entered the arms of the Y with all four feet was the measure of exploratory activity.

Apparatus for studying ataxia. Another Y-shaped runway was used which had the same dimensions as that already described, except that the arms were 30 in. long. The floor of the runway was covered with paper. Each rat's hind-paws were smeared with Vaseline, the animal was placed in the centre of the Y and removed as soon as it had reached the end of an arm. The paper over which the rat had run was dusted with powdered charcoal which adhered to the grease marks and so showed up footprints, and the irregularities of the spacing of these prints were measured (Rushton, Steinberg & Tinson, 1963). The method of obtaining footprints for studying gait was modified from that described by Shirley (1931) for infants and by Khairy (1961) for rats.

Plan of experiment. Three series of experiments were carried out. (*i*) Series 1: mixtures of amphetamine sulphate and amylobarbitone sodium in a ratio of 1:20 were given in a range of doses, and the effects were compared with the effects of the individual drugs in the same doses and with saline controls. A group of ten animals was used for each dose and sixteen animals received saline. (*ii*) Series 2: dexamphetamine sulphate and amylobarbitone sodium in a ratio of 1:6.5 were studied in a similar way. This ratio is used in a proprietary preparation Drinamyl (Smith, Kline & French). This is equivalent to a ratio of 1:4.3 in terms of amphetamine sulphate on the assumption, based on experiments with "jiggle cages" (Schulte, Reif, Bacher, Lawrence & Tainter, 1941), that dexamphetamine sulphate is 1.5 times as potent as amphetamine sulphate. This mixture therefore contains a much smaller proportion of barbiturate than that used in Series 1. There were eight animals in each group and fifteen saline controls ; the results for amylobarbitone were taken from Series 1 and were therefore based on ten rats. (*iii*) Other mixtures: various other drug mixtures were investigated in which the dose of one drug was held constant and the dose of the other was varied. The doses were chosen as follows: starting from the mixtures which had produced the maximal effect on activity in Series 1 and 2, the dose of one or the other drug was varied until an activity peak could be located. Mixtures which produced big effects were then studied in a similar way. The number of rats used varied and is indicated in the Figs. Twenty-three control rats received saline. All doses given refer to the sulphates of amphetamine and dexamphetamine, and to the sodium salt of amylobarbitone.

All drugs were given in single subcutaneous injections dissolved in saline so that the volume injected was 2 ml./kg. Rats were allocated to different treatments at random and tested 35 min after injection ; preliminary experiments had suggested that mixtures which produced

the highest scores in exploratory activity have their maximum effect at about this time. Each rat was given a single 5 min trial in the runway and immediately afterwards records of footprints were obtained in the larger runway. All experiments were carried out between 2.30 and 5.30 p.m. Animals were used once only.

RESULTS

Exploratory activity

Series 1. Fig. 1 shows the mean activity, expressed as mean differences from the activity of the saline control group, for various doses of amphetamine sulphate and amylobarbitone sodium alone and together in a ratio of 1:20. The activity of the

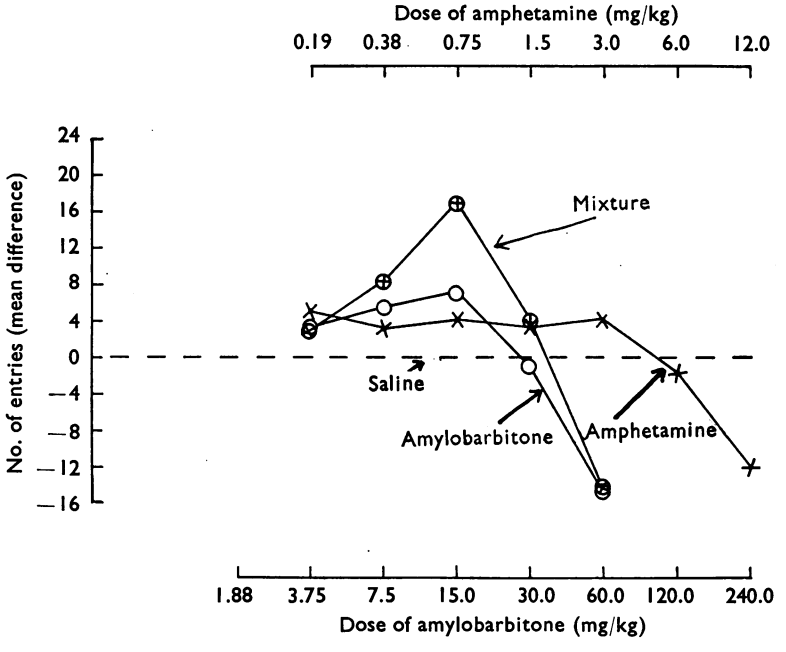

Fig. 1. Series 1: activity of rats influenced by amphetamine sulphate and amylobarbitone sodium, given separately and together, over a range of doses (abscissa). The ordinate represents the number of entries into the arms of a Y-shaped runway during 5 min, expressed as mean differences from the activity of the saline control group (interrupted line). The mixture contained the two drugs in a constant ratio of 1 : 20.

saline controls was 14.7 ± 5.8 entries (mean and standard deviation). The smallest doses of each drug produced effects on activity which were indistinguishable from those due to saline, and the largest doses, including those of amphetamine, inhibited exploratory activity almost completely. Qualitatively, the behaviour of the animals given the largest dose of amphetamine by itself was unique; rats given amylobarbitone or mixtures usually lay flat on the floor and seemed unable to move even though they sometimes tried; the rats given amphetamine on the other hand seemed hyperexcited and mostly stood in one place, often on their hind-feet, shaking their heads and upper body. The standard deviations of the various means shown in

Fig. 1 ranged from 4.0 (3.75 mg/kg of amylobarbitone) and 4.7 (0.19 mg/kg of amphetamine) to 12.3 (30 mg/kg of amylobarbitone); the large size of this last standard deviation reflects the observation that the activity of some animals was stimulated and that of others depressed by this dose of amylobarbitone.

The most marked stimulant effects on exploratory activity usually occurred over the middle range of doses. Amphetamine by itself produced only slightly more activity than saline. For the first five doses used the overall mean difference was 4.0 entries, which is statistically significant at the 5% level. Amylobarbitone by itself produced a curve which was similar to, but much flatter than, the mixture curve, with a hump directly below that of the mixture curve. At its peak the activity with the drug mixture was much greater than that with any amount of the separate drugs, and more than twice that with saline (mixture compared with amylobarbitone: $P<0.01$; with amphetamine: $P<0.01$; with saline: $P<0.001$). The mixture containing the highest doses of amylobarbitone and amphetamine was indistinguishable in its depressant effect from the barbiturate alone, although it contained enough amphetamine to produce slight stimulation by itself.

The mean numbers of entries in successive minutes of the 5 min trial with the peak mixture dose were calculated and the decline of activity was expressed by subtracting the number of entries in the fifth minute from entries in the first minute. There were no significant differences between the mixture, amphetamine and saline groups (combined average decline = 2.7 entries), but the activity in the amylobarbitone group fell off more steeply (average decline = 5.2 entries; compared with saline: $P<0.01$; with amphetamine : $P<0.01$; with the mixture: $P<0.05$).

Series 2. The activity resulting from dexamphetamine and amylobarbitone separately and together in a ratio of 1:6.5, expressed as differences from the effect of saline, is shown in Fig. 2. The saline mean was 14.9 ± 7.42 (standard deviation). The shape of the curve resulting from the mixture was similar to that in Series 1 (ratio 1:20) and the peak was, if anything, slightly higher. The peak occurred at half the previous peak dose of amylobarbitone and at a dose of dexamphetamine which was equivalent to about twice the previous dose of amphetamine. Again the largest doses of the individual drugs and of the mixture inhibited exploratory activity almost completely, but at the highest dose of the mixture there was more inhibition than with either constituent drug ($P<0.05$ for dexamphetamine, and $P<0.01$ for amylobarbitone). The variability in these experiments was similar to that in Series 1. Fig. 3 shows the mean activity in successive minutes at the peak mixture dose. The decline in activity for the mixture was significantly less than for saline ($P<0.05$) and for amylobarbitone ($P<0.05$), but not significantly different from that for dexamphetamine.

Other dose combinations. Fig. 4 shows the curves obtained by varying the dose of dexamphetamine and keeping the dose of amylobarbitone constant at any one of four levels (3.75, 7.5, 15.0 and 17.5 mg/kg). The curves show maxima which are approximately the same with different doses of amylobarbitone and are similar to the peak shown in Fig. 2. High activity over a wide range of doses of dexamphetamine was obtained if amylobarbitone was held constant at 15 mg/kg. This dose of amylobarbitone was also the most effective when given alone (Figs. 1 and 2),

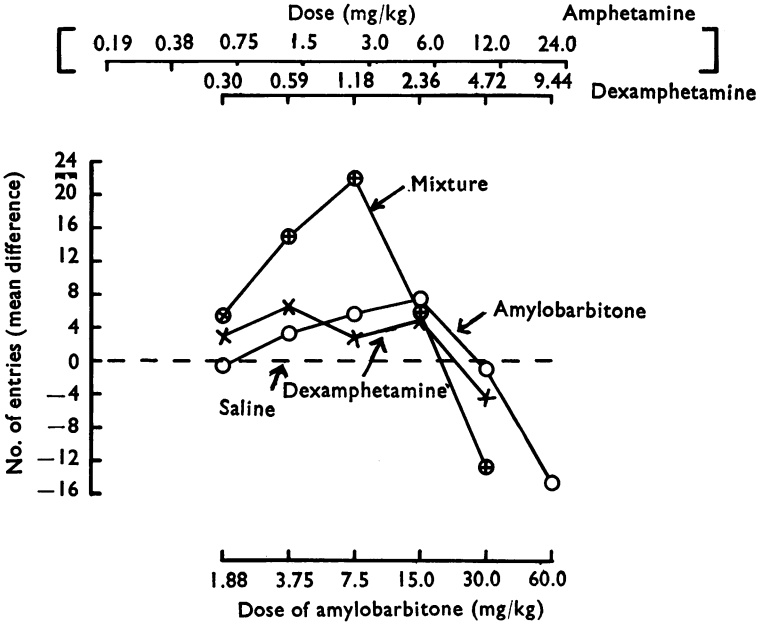

Fig. 2. Series 2. Conditions were similar to Series 1 (Fig. 1), except that dexamphetamine sulphate and amylobarbitone sodium were used, in the ratio of 1 : 6.5. The amphetamine sulphate scale (in square brackets) showing the doses used in Series 1 has been included for comparison. It has been assumed that dexamphetamine is 1.5 times as potent as amphetamine.

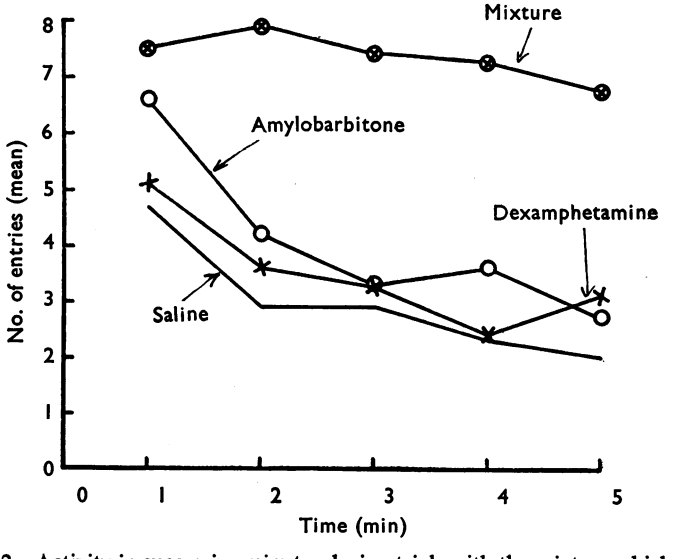

Fig. 3. Series 2. Activity in successive minutes during trials with the mixture which produced most activity (1.18 mg/kg of dexamphetamine and 7.5 mg/kg of amylobarbitone) in Fig. 2.

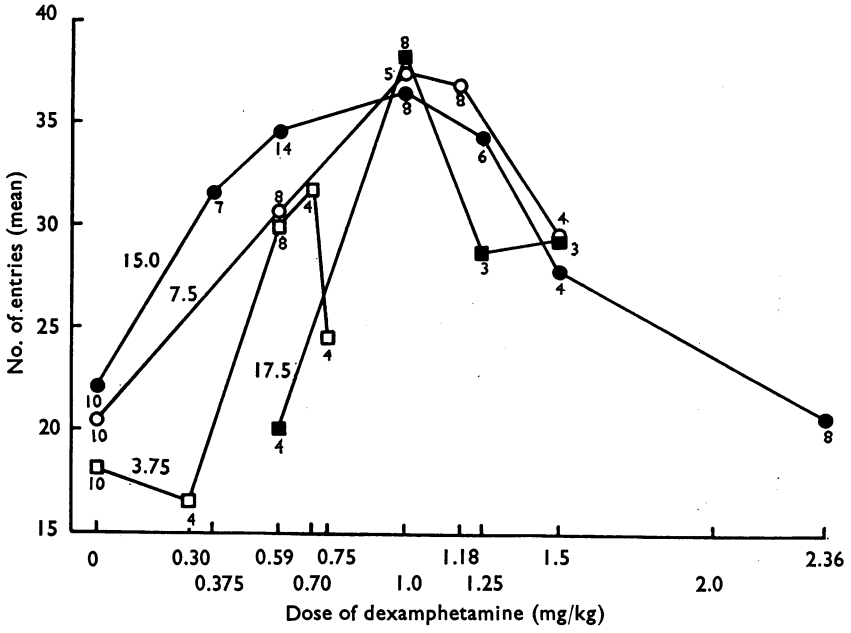

Fig. 4. Activity produced by four doses of amylobarbitone together with different doses of dexamphetamine sulphate (abscissa). The dose of amylobarbitone in mg/kg is shown in large figures for each curve. Each point represents mean results from a different group of rats; the number of rats in most groups was between four and ten (small figures).

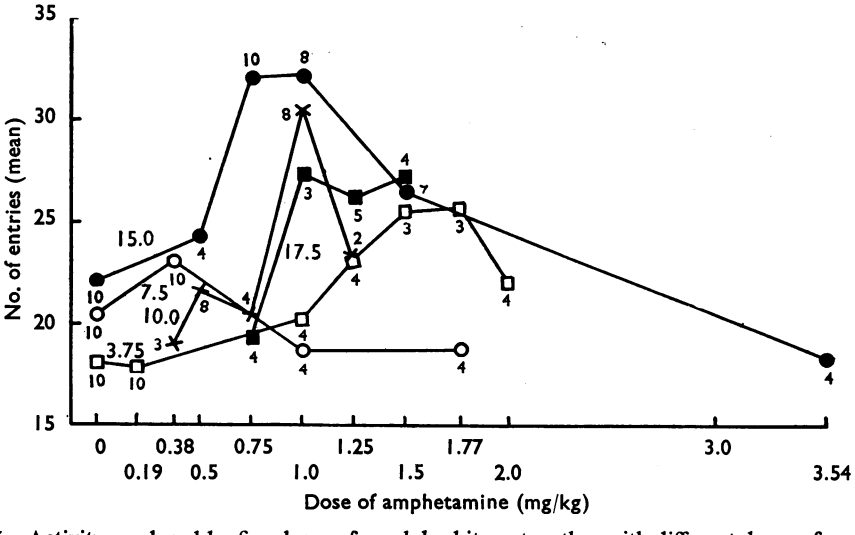

Fig. 5. Activity produced by five doses of amylobarbitone together with different doses of amphetamine sulphate, plotted in a similar way to Fig. 4. The number of rats in most groups was between four and ten (small figures).

though much less effective than when given with dexamphetamine. The optimal admixture of dexamphetamine was 1 mg/kg.

Fig. 5 shows corresponding curves for amphetamine. Again 15 mg/kg of amylobarbitone was optimal. The two doses of amphetamine which elicited maximal activity were 1 and 0.75 mg/kg. Thus although the potency of amphetamine as a stimulant of the central nervous system is generally estimated as at most two-thirds of that of dexamphetamine the two drugs are about equiactive in potentiating the action of amylobarbitone. However, the maximal scores produced by mixtures containing dexamphetamine were never reached by mixtures containing amphetamine.

Other mixtures of amphetamine and amylobarbitone were tried which are not shown in the Figs. All these gave mean activity scores between 20 and 30, except for 1 mg/kg of dexamphetamine with 10 mg/kg of amylobarbitone (38.8) and with 20 mg/kg amylobarbitone (36.8). The mean for rats receiving saline was 13.7 ± 4.9 (standard deviation).

Ataxia

The regularity of the spacing of the footprints was expressed as the mean log variances of the distance between the rats' hind feet for consecutive steps (Rushton *et al.*, 1963). Fig. 6 shows the results for Series 1 and 2. The spacing in the

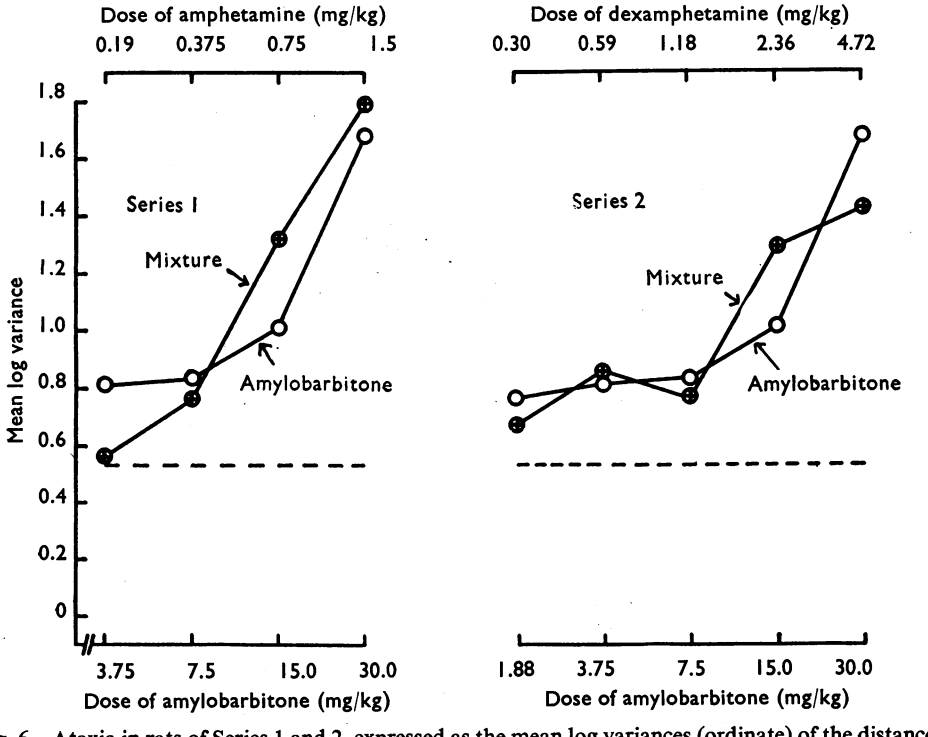

Fig. 6. Ataxia in rats of Series 1 and 2, expressed as the mean log variances (ordinate) of the distance between the rats' hind-feet for consecutive steps. The saline controls are shown by the interrupted lines (for Series 1, n=16; for Series 2, n=15).

animals treated with saline was regular, as shown by the small log variances, but the spacing for rats receiving mixtures was increasingly irregular with larger doses. The previous investigation had shown that animals which were made ataxic by a drug mixture gave mean log variances above 1.0, while the means of saline groups were 0.55 and 0.74, and these differences between ataxic and saline groups were significant at the 0.1% level (Rushton *et al.*, 1963). We therefore decided to treat all means above 1.0 as denoting ataxia in the present experiments. In the first series of experiments (ratio 1:20) the mean for rats receiving saline was 0.53 ± 0.38 (standard deviation) and the smallest dose of the mixture at which significant ataxia was detected was that which produced highest activity (saline compared with mixture, $P<0.001$). In Series 2 (ratio 1:6.5, dexamphetamine) ataxia was only detected at double the dose which produced most activity ($P<0.001$). Hence, in Series 1, where proportionately more barbiturate was used, the peak effect on activity was accompanied by significant ataxia, whereas in Series 2 with relatively

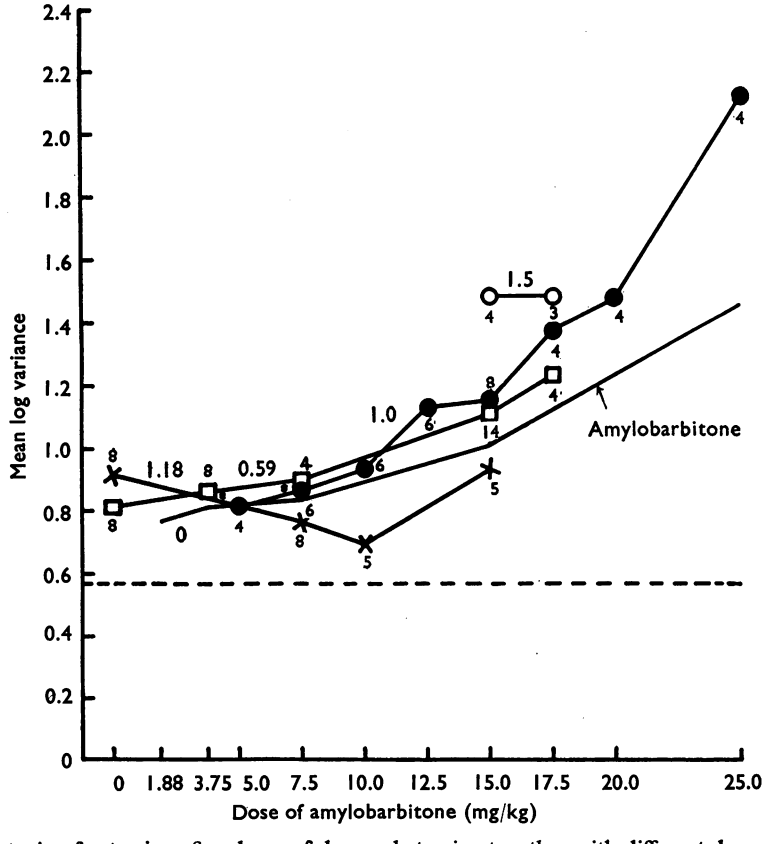

Fig. 7. Ataxia of rats given five doses of dexamphetamine together with different doses of amylobarbitone. The corresponding scores for activity can be found in Fig. 4. The saline controls are shown by the interrupted line (n=23). The results are presented as in Figs. 4, 5 and 6. For amylobarbitone alone, n=10. Most of the other points are based on results from groups of four to eight rats (small figures).

less barbiturate peak activity was not accompanied by significant ataxia. Although amylobarbitone by itself usually produced rather less ataxia than corresponding mixtures, the difference was not statistically significant. Neither amphetamine nor dexamphetamine alone produced observable ataxia. It was, however, difficult to obtain satisfactory footprint records from these animals since they tended to pause and retrace their steps and failed to make continuous runs, and their records were therefore not used.

Fig. 7 shows the effects on gait of the mixtures with dexamphetamine for which exploratory activity is plotted in Fig. 4. It shows that ataxia increased when the proportion of amylobarbitone in the mixture was greater, and that mixtures of dexamphetamine and amylobarbitone usually produced more ataxia than the corresponding dose of amylobarbitone alone. Similar trends were found when the gait was analysed for mixtures containing amphetamine, for which exploratory activity is shown in Fig. 5.

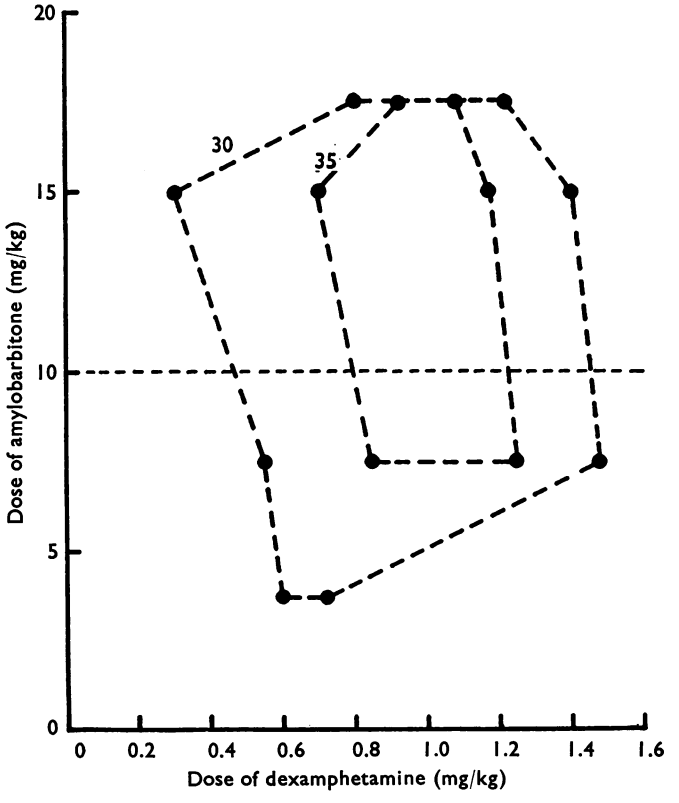

Fig. 8. Diagram showing doses of amylobarbitone and dexamphetamine mixtures which produced equal effects on rat activity. The outer contour was plotted by reading off points on the curves in Fig. 4 at the thirty entries level, and the inner control represents the thirty-five entries level. No ataxia was detected with mixtures which contained 10 mg/kg or less of amylobarbitone, and a line has been drawn at this level.

DISCUSSION

The results show that mixtures of amphetamine and amylobarbitone can produce a considerable increase of spontaneous exploratory activity in rats. The combined effects of the two drugs can be regarded as true potentiation, since the maximal effects produced by the mixtures are considerably greater than the maximal effects produced by any doses of either constituent alone and are greater than would be expected from simple addition. Maximal effects can be obtained over a limited range of drug combinations and they may or may not be accompanied by ataxia. Whether ataxia occurs seems to depend mainly on the amount of barbiturate in the mixture. This is not surprising since amylobarbitone alone produced ataxia though rather less than the corresponding mixtures.

Fig. 8 illustrates some of the findings when plotted as Loewe (1928) diagrams or isobols, representing equiactive drug combinations. The outer contour isobol represents a mean activity of thirty and the inner of thirty-five entries in 5 min. The inner isobol thus represents a greater degree of potentiation than the outer. Inspection of the inner contour shows that the same degree of potentiation can be achieved by giving together 0.6 mg/kg of dexamphetamine and 3.8 mg/kg of amylobarbitone as by giving 1.4 mg/kg with 15 mg/kg. Clearly the former combination is more desirable than the latter.

A further criterion is the tendency of mixtures to produce ataxia. The limits of ataxia are represented in Fig. 8 by a horizontal dotted line below which ataxia is always absent. Taking the various criteria into account, the mixture which gives most exploratory activity with least ataxia and least intake of drug would seem to be in the bottom left-hand corner of the smaller contour. The dose mixture corresponding to Drinamyl which produced most activity, is very near to this position: this mixture contained a little more dexamphetamine and it also produced a slightly higher activity score than 35. The highest scores for activity of all mixtures tried were produced by 1 mg/kg of dexamphetamine with 10 and with 17.5 mg/kg of amylobarbitone ; this last mixture produced considerable ataxia and the high exploratory activity was achieved in spite of this handicap.

The results of Series 1 and 2 seem in some respects similar to those recently obtained in man (Dickins, Lader & Steinberg, unpublished). A mixture of amphetamine and cyclobarbitone similar in ratio to that of Series 1 (1:20) produced more reports of feeling " sociable " than did the separate constituents, but it also produced more reports of feeling " unsteady." A mixture similar in ratio to Series 2 (1:6.5, dexamphetamine) also produced many reports of feeling " sociable," but it yielded more reports of feeling " alert " and " quick-witted," and fewer of being " unsteady " than the former mixture.

The effect of the mixtures seems to be primarily on the amount of co-ordinated walking. Maxwell (personal communication) has confirmed this interpretation. He has observed increased " ambulation " with mixtures of dexamphetamine and amylobarbitone as compared with the separate drugs in Hall's (1934) open-field test, a circular arena in which the distance walked by rats in a given time can be determined. Preliminary experiments with activity cages, using phototransistors, have suggested that activity in such cages is increased by amphetamine more than is activity in the

Y-shaped runway, probably because the cages can also record more undifferentiated movements like head shaking, body shaking, twitching and stepping on the same place. Mixtures can nevertheless sometimes be shown to increase activity in such cages significantly more than the separate constituents (Banna & Dickins, personal communication).

It has already been suggested (Steinberg *et al.*, 1961 ; Rushton *et al.*, 1963) that the effects of mixtures in experiments which make use of new environments may be so great because amphetamine by itself increases activity directly while barbiturates can reduce fear (Miller, 1961, 1963), including perhaps fear of new environments, and may so lead indirectly to more exploratory activity.

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