*BEHAVIORAL EFFECTS OF CAFFEINE,
METHAMPHETAMINE, AND METHYLPHENIDATE
IN THE RAT*

FRANCIS MECHNER¹ AND MIKLOS LATRANYI

DEPARTMENT OF NEUROPHARMACOLOGY, SCHERING CORPORATION

It was possible to distinguish three closely-related psychomotor stimulants, caffeine, methamphetamine, and methylphenidate, by means of two operant behavior procedures, fixed interval and fixed number. Under the fixed interval procedure, the percentage change in the number of R_{BS} per reinforcement was significantly smaller with caffeine than with methamphetamine or methylphenidate ($p < .001$). Under the fixed number procedure, the percentage change was significantly smaller with methamphetamine than with caffeine or methylphenidate ($p < .001$). Thus, methylphenidate had a methamphetamine-like effect under fixed interval and a caffeine-like effect under fixed number.

The purpose of this study was to take an initial step in the development of a methodology for distinguishing closely-related drugs on the basis of their effects upon operant behavior. By this methodology, subjects are trained under certain behavioral procedures until their performances are stable. The performances are then analyzed in terms of as many indices as can be obtained. The selection of these need not be based on any elaborate theoretical preconceptions concerning their usefulness. Using a computer, one can separately examine each index for its sensitivity to the effects of the drugs tested. Of a large initial set, some indices prove to be sensitive to the differences between the drugs, and most do not. Those that do may then be considered as potential categories in a system of drug classification, and as routine measures in a drug evaluation program.

Three widely-used stimulant drugs—caffeine, methamphetamine, and methylphenidate—were compared in rats at four or five doses per drug under four different behavioral procedures. The drugs were chosen because of their pharmacological similarity as psychomotor stimulants.

METHOD

Subjects

Thirty-two male, albino rats of Charles River stock were used, eight being assigned to each of four "rat rotors."

Apparatus

The rat rotor is a device which cycles rats through a testing station automatically and continuously without the need for daily human attendance. It consists of a set of eight trapezoid-shaped cages arranged circularly on a turntable. Each cage is permanently occupied by a rat. At any given time, one of the eight cages is opposite the testing station. The rat that occupies this cage has access, through suitably-placed holes and slots, to two bars that it can press, and to the cup of a liquid reinforcement dispenser. Through another hole, the animal can see a stimulus light. When a rat's scheduled work session is over, the entire turntable indexes one position, bringing the next rat into the testing position. A more complete description of the rat rotor can be found in Mechner, Guevrekian, and Mechner (1963).

Procedure

Three to six weeks of training were generally required before an animal's performance was sufficiently stable for the drug study to be undertaken.

The rats were always fasted for 7 hr prior to receiving treatment. All treatments (physiological saline or drug) were administered orally in aqueous solution 1 hr before testing. The volume administered (0.5 ml) was

¹Reprints may be obtained from Dr. Francis Mechner, Basic Systems Incorporated, 2900 Broadway, New York 25, N. Y.

Fig. 1. Normal resulting performance under fixed interval procedure.

approximately 5 per cent of an animal's total daily liquid intake, the other 95 per cent being delivered, 0.03 cc water at a time, in the form of reinforcements. This volume of physiological saline produced negligible effects on the animals' motivational state. The changes observed were minimal and of varied direction. The treatments administered were as follows: caffeine (2.5, 5.0, 15.0, 30.0, and 60.0 mg/kg), methamphetamine (0.2, 0.4, 0.8, 1.6, and 3.2 mg/kg), and methylphenidate (6.0, 12.0, 24.0, and 48.0 mg/kg). All treatments were completely crossed over, so that each animal received all of the drug dosages. Treatments were administered in random sequence over a 15-week period. Only the results for animals that survived the study and received all the treatments are presented in this paper.

A minimum of four or, in the case of higher doses, eight days were allowed for recovery between consecutive drug administrations. For comparison, the pre-drug baseline performance of the animals was collected for six sessions (three sessions per day) before administration of the drug. Collection of the baseline data for a drug run was never begun until the behavioral effects of the previous treatment had disappeared.

PROCEDURES AND RESULTS

Four behavioral procedures were used: (1) fixed interval, (2) fixed number, (3) fixed minimum interval, and (4) fixed consecutive number. Each procedure involved the pressing of two bars; these responses are referred to as R_A and R_B .

Following each description are the results obtained under that procedure. The fixed interval and fixed number procedures, (1) and (2), proved to be the best discriminators of differences between the behavioral effects of

caffeine, methamphetamine, and methylphenidate. The fixed minimum interval and fixed consecutive number procedures, (3) and (4), while also sensitive to the behavioral effects of the drugs, were less useful for the purpose of differentiation.

Fig. 2. Dose-response curves for five animals with caffeine, methamphetamine, and methylphenidate. The horizontal dotted line represents the control level of R_B s per reinforcement for each animal. All treatments were administered in random order.

Fig. 3. Peak changes in number of R_B s per reinforcement under caffeine, methamphetamine, and methylphenidate. Six rats were tested under the fixed interval procedure (left graph) and five rats under the fixed number procedure (right graph). Every rat received all treatments in random order. Under the fixed interval procedure the smallest increase is produced by caffeine; under fixed number by methamphetamine. Each point represents the peak of the dose-response curve.

(1) *Fixed Interval (FI)*. An R_A (response on bar A) initiated a 30-sec fixed interval. The first R_B following the end of this interval was reinforced. In addition to initiating the time interval, the R_A also produced a light which remained present until reinforcement was delivered. Upon delivery of the reinforcement, the light went out and was illuminated again only by another R_A . R_B s made prior to the termination of the 30-sec time interval were not reinforced, but did not reset the interval.

After initiating the fixed interval with an R_A , the animal generally paused awhile before it began to respond on bar B. This pause ranged from about 10 to 25 sec. Once an animal began to respond on bar B, a comparatively high rate was maintained until reinforcement was received. The general pattern was an acceleration of responding on bar B throughout the course of the 30-sec fixed interval. Once the interval started, further R_A s were extremely rare.

Fig. 4. Results for two rats trained under the fixed interval procedure. The figure shows response rate changes during the 30 sec of the fixed interval under caffeine, methamphetamine, and methylphenidate. Every rat received every dose of the three drugs in random order.

Fig. 5. Results for two rats trained under the fixed interval procedure. The figure shows response rate changes during the 30 sec of the fixed interval under caffeine, methamphetamine, and methylphenidate. Every rat received every dose of the three drugs in random order.

To show the acceleration pattern, the 30-sec fixed interval is subdivided into eight subintervals, as shown in Fig. 1. These subintervals are marked off along the abscissa of the graphs in Fig. 4 and 5. The rates within corresponding subintervals are averaged for the entire 1-hr session. The resulting curve shows the average response rate as a function of the fixed interval's subintervals. This average curve accelerates smoothly.

The complete dose-response curves for the three drugs are shown in Fig. 2 for five animals. As this figure shows, there is animal-to-animal variation in the dose at which the peak response occurred, as well as in the magnitude of the response. Analysis of the data reveals a proportionality between baseline performance and response to drug. In the left graph of Fig. 3, the *peak* responses irrespective of dose are shown and expressed as the per cent increase in the number of R_{BS} per reinforcement over saline control levels. The compari-

sons represent treatment crossovers in the same animals. The figure reveals an increase in the number of R_{BS} per reinforcement with all three agents, but less with caffeine than with methamphetamine or methylphenidate. This was highly significant statistically ($p < .001$) by either the sign test or analysis of variance.

Another effect of methamphetamine and methylphenidate which caffeine did not appear to produce is destruction of the temporal discrimination at the higher doses. This can be seen by inspection of Fig. 4 and 5, especially in rats 11 and 81. As discussed above, the average rate function is normally a smoothly accelerated one. Under increasing doses of methamphetamine and methylphenidate, the curves rise, straighten out, and then begin to drop back down, becoming horizontal. A horizontal rate function indicates absence of temporal discrimination.

Fig. 6. Normal resulting performance under fixed number procedure.

(2) *Fixed Number (FN)*. After the initial R_A , which turned on the stimulus light, the animal had to make an additional 45 R_A s before an R_B would be reinforced. As in the fixed interval procedure, premature R_B s did not reset the count but were also not reinforced.

As shown in Fig. 6, animals generally made a large number of the 45 required R_A s before the first R_B , which was usually premature. The animal then continued to alternate between R_A and R_B , making fewer and fewer R_A s between the consecutive R_B s until the stipulated 45 R_A s had been accumulated and R_B was finally reinforced. Some animals were, of course, more accurate than others. After reinforcement, the rats generally paused quite a long time, often as long as 2 min, before resuming work.

The graphical description of this performance (Fig. 8) is analogous to that of fixed interval. The run of 45 R_A s is subdivided into seven segments, as in Fig. 6. These seven segments are shown along the abscissa of Fig. 8. Again, the average frequency with which R_B s were made in each of these segments is shown on the ordinate. This frequency is expressed as the ratio of R_B s to R_A s rather than as the time rate of occurrence of R_B s, since the abscissa dimension in this procedure is number of R_A s rather than time. Also, as in the fixed interval procedure, an average is taken across the corresponding segments of all the reinforcement cycles in the hour. For example, a value of 0.4 in the seventh segment would mean that the animal interspersed an average of 2 R_B s among the 5 R_A s of that segment. The curve showing R_B s per R_A describes the internal organization of the performance rather than the rate at which responses occur in time.

The dose-response curves for three of the five animals are shown in Fig. 7. As was the case in the fixed interval procedure, the dose

at which the peak response occurred varied considerably from animal to animal. The right-hand graph of Fig. 3 shows the peak responses irrespective of dose, again expressed as the per cent increase in the number of R_B s per reinforcement over saline baseline control levels. The figure reveals an increase in the number of R_B s per reinforcement with all

Fig. 7. Dose-response curves for three animals with caffeine, methamphetamine, and methylphenidate. The horizontal dotted line represents the control level of R_B s per reinforcement for each animal. Every animal received all treatments in random order.

Fig. 8. Results for three rats trained under the fixed number procedure. The figure shows changes in R_B frequency during the series of 45 R_A s under caffeine, methamphetamine, and methylphenidate. The first R_B following the 45th R_A was reinforced. The frequency of premature R_B s is expressed in terms of R_B s per R_A . Every rat received every dose of the three drugs in random order.

three agents, but less with methamphetamine than with caffeine or methylphenidate ($p < .001$). The complete functions relating the frequency of R_B s to the number of R_A s

made since the previous reinforcement are shown in Fig. 8.

(3) *Fixed Minimum Interval (FMI)*. This procedure is the same as the fixed interval,

Fig. 9. Normal resulting performance under fixed minimum interval procedure.

Fig. 10. Results for two rats trained under the fixed minimum interval procedure. The figure shows the relative frequency (proportion of times) with which the rat waited various periods of time between R_A and R_B . The vertical dotted line is drawn at the 12-sec requirement. Every rat received every dose of caffeine, methamphetamine, and methylphenidate in random order.

Fig. 11. Results for two rats trained under the fixed minimum interval procedure. The figure shows the relative frequency (proportion of times) with which the rat waited various periods of time between R_A and R_B . The vertical dotted line is drawn at the 12-sec requirement. Every rat received every dose of caffeine, methamphetamine, and methylphenidate in random order.

Fig. 12. Normal resulting performance under fixed consecutive number procedure.

Fig. 13. Results for Rat 6CN under the fixed consecutive number procedure. The figure shows the relative frequency (proportion of times) of various run durations, the run duration being the time from the first R_A to the final R_B . The rat received all doses of the three drugs in random order.

except that the time interval initiated by R_A was 12 sec rather than 30 sec long, and a premature response R_B reset the interval; i.e., the stimulus light went out and a new R_A was required to turn it back on and initiate a new 12-sec interval.

Under the conditions where the required waiting time between R_A and R_B was 12 sec, as in the present experiment, the time the animal actually paused between R_A and R_B usually averaged very close to 12 sec. In any case, it was almost never higher than 13.5 sec or lower than 11.0 sec. Once an animal started an interval with an R_A , any additional R_A s before the R_B were extremely rare, even though such R_A s would not be penalized with a reset. The animal generally spent the R_A - R_B interval in some repetitive activity, such as

licking the dipper cup, nosing bar B, or swaying back and forth.

The performances are described by the frequency distributions shown in Fig. 10 and 11. Each bell-shaped distribution shows the relative frequency with which the animal waited various lengths of time between R_A and R_B . A vertical dotted line through the twelfth category indicates the waiting time stipulated for reinforcement. The portion of the distribution to the right of that line represents R_B s that were reinforced; that to the left corresponds to unreinforced R_B s.

As Fig. 10 and 11 show, animals were normally quite accurate in waiting the required interval between R_A and R_B . After caffeine, methamphetamine, and methylphenidate, however, this accuracy was destroyed.

Fig. 14. Results for Rat 1CN under the fixed consecutive number procedure. The figure shows the relative frequency (proportion of times) with which the rat made various numbers of consecutive R_A s before pressing bar B. The vertical dotted lines are drawn at the eight-response requirement. All doses of caffeine, methamphetamine, and methylphenidate were administered in random order.

Fig. 15. Results for Rat 4CN under the fixed consecutive number procedure. The figure shows the relative frequency (proportion of times) with which the rat made various numbers of consecutive R_A s before pressing bar B. The vertical dotted lines are drawn at the eight-response requirement. All doses of caffeine, methamphetamine, and methylphenidate were administered in random order.

Methamphetamine and methylphenidate shift a part of the frequency distribution to the left, indicating more frequent premature responding. As a consequence, the animals received fewer reinforcements. While caffeine also produced this effect in some cases (Rat 5MI), it sometimes produced a shift of the frequency distribution to the right (Rats 3MI, 4MI, and 8MI), indicating longer waiting than necessary. These findings are generally consistent with those obtained under the fixed interval procedure.

(4) *Fixed Consecutive Number (FCN)*. This procedure differs from fixed number in that after the first R_A , eight rather than 45 additional R_A s were required for an R_B to be reinforced. Also, a premature R_B reset the count,

requiring the animal to start over with a new series of eight consecutive R_A s.

The performances were analyzed and described in terms of two relative frequency distributions:

(a) The distribution showing the relative frequency with which the animal made various numbers of consecutive R_A s before switching to bar B (Fig. 14 and 15). The abscissas labeled "run length" represent this number. The vertical dotted lines drawn at eight R_A s show the point which the animal had to exceed for reinforcement.

(b) The distribution showing relative frequency of "run durations." Run duration is the time between the first R_A in the run and the next R_B , that is, the time in seconds the

animal consumed in executing the run (Fig. 13).

The most consistent effect which the three drugs produced was a decrease in run duration. In a few rare cases, however, caffeine also produced increases in run duration. Since the decrease in run duration due to the drugs was so consistent, the data for only one typical animal are shown (Fig. 13). This animal was selected for the stability of its run duration baseline, rather than for its response to drugs.

The effects of the drugs on run length, on the other hand, were inconsistent. There appeared to be two distinct patterns of response: an increase and a decrease in run length. Seven rats were about evenly divided between these two patterns. Figures 14 and 15 show the clearest examples of the two modes of response. Rat 1CN (Fig. 14) showed an increase in run length with increasing dose, while Rat 4CN (Fig. 15) showed a marked decrease.

DISCUSSION

The results suggest that two behavioral procedures—fixed interval and fixed number—and one behavioral index which can be obtained under both of them—the number of R_B s per reinforcement—are sufficient to distinguish three closely related psychomotor stimulants, caffeine, methamphetamine, and methylphenidate. Fixed interval “picks out” caffeine and fixed number, methamphetamine. At the peak of its dose-response curve, methylphenidate had the behavioral effects of methamphetamine under fixed interval and

the behavioral effects of caffeine under fixed number. Doses of caffeine that produced a manifold increase in the frequency of R_B s per reinforcement under fixed number produced a negligible increase in the same measure under fixed interval. On the other hand, doses of methamphetamine that produced maximal increases under fixed interval had a very small effect under fixed number. These results were all significant at the .001 confidence level, by the sign test as well as by analysis of variance.

The two other behavioral procedures employed—fixed minimum interval and fixed consecutive number—differed from fixed interval and fixed number respectively in that premature R_B s were penalized with a reset. These procedures were also sensitive to the behavioral effects of the three drugs but seemed somewhat less reliable than fixed interval and fixed number for the purpose of drug differentiation.

The methamphetamine results obtained under the fixed minimum interval procedure were generally consistent with those reported by Dews and Morse (1961) and by Segal (1962).

REFERENCES

- Dews, P. B. and Morse, W. H. Behavioral pharmacology. *Ann. Rev. of Pharmacol.*, 1961, **1**, 145-174.
- Mechner, F., Guevrekian, L., and Mechner, V. A fixed interval schedule in which the interval is initiated by a response. *J. exp. Anal. Behav.*, in press.
- Segal, E. F. Effects of *dl*-amphetamine under concurrent VI DRL reinforcement. *J. exp. Anal. Behav.*, 1962, **5**, 105-112.

Received May 7, 1962