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Cumulative Dose-Response Curves in Behavioral Pharmacology[1,2]

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WENGER, G. R. *Cumulative dose-response curves in behavioral pharmacology*. PHARMAC. BIOCHEM. BEHAV. 13(5) 647–651, 1980.—Cumulative dose-response curves have been widely used in many areas of pharmacology. To date, the applicability of cumulative dose-response curves has not been assessed in behavioral pharmacology. To determine the feasibility of this procedure, mice were trained to respond under a multiple time-out 5 min, fixed-ratio 30 (mult TO 5, FR 30) schedule of reinforcement. The FR 30 component consisted of 15 presentations of an FR 30 schedule of reinforcement. At the start of each TO 5 component, an intraperitoneal (IP) injection was given, and the effect on the response rate during the following 15 presentations of the FR 30 schedule was assessed. *d*-Amphetamine (0.3–30 μ moles/kg), pentobarbital (3–300 μ moles/kg), morphine (1–100 μ moles/kg), ketamine (3–300 μ moles/kg), and phencyclidine (1–100 μ moles/kg) all produced dose-related decreases in FR responding. In each case the lowest dose tested was without effect, and the highest dose tested essentially eliminated responding. As a control, the normal 4th dose in the ascending series of each drug was given preceded by 3 saline injections. Whether this dose of each drug was preceded by 3 separate saline injections or by 3 lower ascending doses of the same drug, the observed effect was identical. Five consecutive saline injections during the experimental session were without effect. The application of this procedure should greatly decrease the time required to examine the behavioral effects of a wide range of doses.

Cumulative dose-response Mice Fixed-ratio *d*-Amphetamine Pentobarbital Morphine Ketamine Phencyclidine

CUMULATIVE dose-response curves have been used for many years in pharmacology [3,4]. This technique has enjoyed its widest use in isolated tissue bath preparations, but has also been used in student laboratory exercises using whole animal preparations. In isolated tissue preparations increasing concentration of the drug are added to the bath without washing the tissue between doses, and the response measured is proportional to the concentration of the drug in the bath following the addition of the drug.

Cumulative dose-response curves in whole animal preparations have the additional features of distribution, metabolism and excretion which are dynamic processes that result in a continual change in drug concentration at the receptor site. This may be considered to function like a partial washing of an isolated tissue in the isolated tissue preparation described above. The end result is that the exact blood level of drug is not usually known when the next dose in the series is administered. This difficulty can be overcome if large increases in dose are used (for example, increasing the dose by log 10). The large increase in dose assures that the contribution to the measured effect of previously administered doses will be small.

Despite the history of cumulative dose-response curves in pharmacology, the technique has not been used in behavioral pharmacology. The use of the procedure would allow the investigator to assess the entire dose-response curve of a given drug during a single experimental session. This would appear to be of enormous practical advantage in behavioral experiments assessing the development of tolerance and cross-tolerance to a given drug. To assess the usefulness of cumulative dose-response curves in behavioral pharmacology, a series of experiments were designed to test the effect of *d*-amphetamine, pentobarbital, morphine, phencyclidine and ketamine when given in a cumulative fashion to mice responding under a multiple time-out 5 min, fixed-ratio 30 (mult TO 5, FR 30) schedule of food presentation. The behavioral effects of drugs are usually observed over a fairly narrow dose range. Typically there is less than a 2 log unit difference between the dose which has no effect and the dose which totally suppresses responding. Thus, in the construction of the cumulative dose-response curves the dose was increased by $^1/_2$ log unit increments rather than the 1 log unit increases described above.

METHOD

Subjects

Male CD-1 mice were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). When given free access to food and water they weighed 40–45 g. At the start of the experiment, they were food deprived to 80% of their free feeding weight, and they were maintained at that weight

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[2]A preliminary abstract of this study appears in *The Pharmacologist* **22**: 294, 1980.

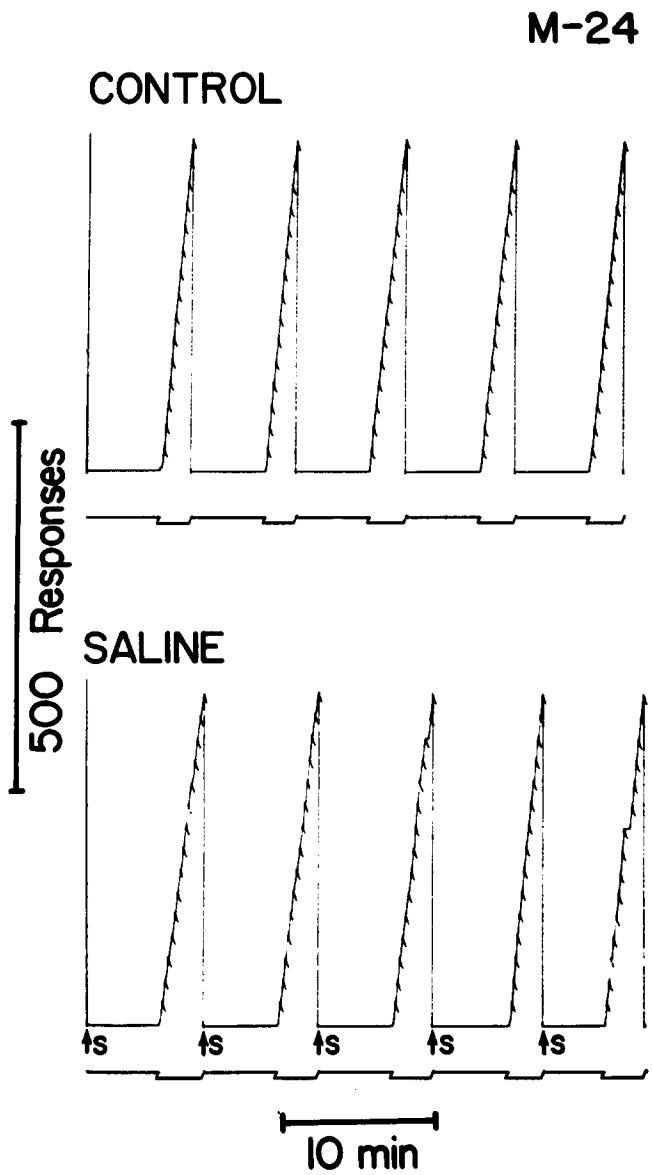

FIG. 1. Cumulative response records of the performance of mouse M-24 under the mult TO 5, FR 30 schedule. Top record shows performance under non-injection control conditions; bottom record shows performance following saline injections. Diagonal marks on the response pen line indicate the presentation of evaporated milk. Downward deflections of the lower horizontal line indicate the occurrence of the FR 30 component. Saline (S) injections were given at the times indicated by the arrows in the bottom recording.

throughout the experiment. Five mice were used: M21, M24, M48, M49 and M50. They were housed 2–3 mice/cage. Testing was conducted during the normal working day (0800–1700 hr).

Apparatus

The experimental chamber was identical to that described by Wenger [5]. Using this apparatus the mouse was trained to respond by interrupting a light beam which crossed the width of a blind corridor striking a photocell. Interruption of the light beam defined the response, and produced an audible click from an electromechanical relay (feedback). The experimental chamber was placed inside a sound attenuating chamber. Electromechanical relay programming and recording apparatus were used.

Schedule

The session started with a 5-min period during which no stimuli were programmed and responding had no consequences, time-out 5-min (TO 5). Responses during the TO 5 component were not recorded. Upon the completion of the TO 5 component, a clicking relay (4 clicks/sec) was started. During the presentation of the clicking relay, 30 responses produced 10-sec access to a small dipper of evaporated milk, fixed-ratio 30 (FR 30). If 30 responses were not made in 60 sec, a predetermining counter counted down 1, and the FR value was reset. Following 15 presentations of the FR 30 schedule component, the schedule changed back to the TO 5 component. Thus, the TO 5 component alternated with the FR 30 component, each FR 30 component consisting of 15 presentations of the FR 30 schedule. A single experimental session consisted of 5 presentations of the TO 5 and 5 presentations of the FR 30 component. In the absence of drugs, a session lasted approximately 50 min.

Drugs

The drugs used were: *d*-amphetamine·SO_4, Na·pentobarbital, morphine·SO_4, phencyclidine·HCl and ketamine·HCl. All drugs were dissolved in normal saline and administered by IP injection. All drug concentrations were made so that the desired dose could be given in a volume of 1 ml/100 grams of body weight. All doses are expressed as μ moles of free base/kilogram of body weight.

Cumulative dose-response curves were determined once per week. Doses of each drug were administered in an ascending order with the dose administered at the start of each TO 5 component. A control day was a day preceded by a non-drug day, and the control day usually preceded a drug day.

Measurement of Drug Effects

Average rates of responding during the FR 30 component were computed from digital counters and elapsed time meters. An average rate of responding was determined for each of the 5 FR 30 components during control sessions and during the determination of cumulative dose-response curves. Values are expressed as the mean±S.E.M. The S.E.M. for the control value was defined as the total standard deviation of all control data divided by the square root of n; where n is equal to the number of subjects studied at each dose of drug.

RESULTS

Control Performance

The control performance of the mice responding under the mult TO 5, FR 30 schedule was similar to that described for other species such as the pigeon [2]. A representative cumulative record of control responding in one mouse, M-24 is shown in Fig. 1. Very little responding was observed during the TO 5 components. During the presentation of the FR 30 component, responding occurred at a relatively high, continuous rate (~ 3 responses/sec) until the presentation of the dipper of milk upon the 30th response.

The effect of 5 consecutive saline injections, one at the

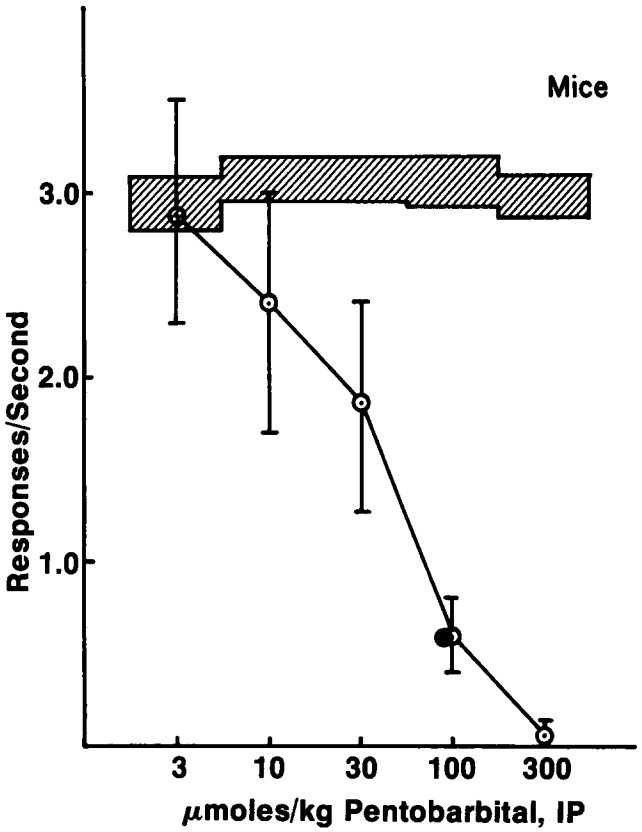

FIG. 2. Cumulative dose-response curve for the effect of pentobarbital on the average rate of responding under the FR 30 schedule. Abscissa: dose in μ moles/kg of body weight given by separate injections during a single experimental session; ordinate: average rate or responding in responses/second during the FR 30 component of the multiple schedule. Mean rate of responding±S.E.M. under control conditions for each FR 30 component period is shown by the shaded area. Vertical lines represent the S.E.M. for each dose determination. Open circles represent data obtained from the cumulative dose-response curve determination; filled circle represents the effect of 100 μ moles/kg preceeded by 3 separate saline injections. 10 μ moles of pentobarbital=2.48 mg of Na·pentobarbital.

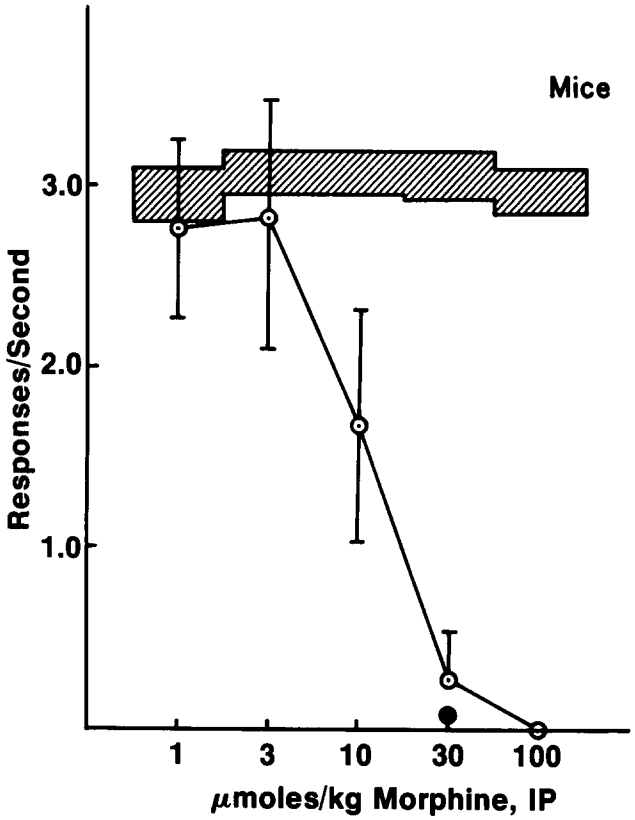

FIG. 3. Cumulative dose-response curve for the effect of morphine on the average rate of responding under the FR 30 schedule. Data presented as in Fig. 2. 10 μ moles of morphine=3.85 mg of morphine·SO_4.

beginning of each TO 5 component, is shown in the lower portion of Fig. 1. When saline was injected at the beginning of each TO 5 component, little effect on FR responding was observed.

Cumulative Dose-Response Curves

When five increasing doses of pentobarbital were given in an ascending order during a single session, FR 30 responding decreased in a monotonic dose-dependent fashion. Figure 2 shows that injection of 3 and 10 μ moles/kg pentobarbital had no apparent effect on responding. The third injection of 30 μ moles/kg reduced responding by about 40%, and the fourth injection of 100 μ moles/kg decreased responding by about 77%. It could be argued that the effect measured following the 100 μ moles/kg injection was due to the previous injections of 3, 10 and 30 μ moles/kg finally having an effect or adding to the effect of the 100 μ moles/kg injection. To determine the contribution of the previous doses to the effect measured following the 100 μ moles/kg dose, the following control experiment was conducted. For each drug the mice were divided into two groups of 2 or 3 mice each. In one group, the cumulative dose-response curve for the drug was established as described above. In the second group, the effect of the 4th dose was determined following 3 previous injections of saline instead of the 3 lower doses of the drug. The following week the groups were reversed. Thus, the effect of the 100 μ mole/kg dose of pentobarbital was determined in all subjects; once as part of the cumulative dose-response curve and once following 3 separate saline injections. The effect of the 100 μ moles/kg dose when preceded by 3 separate saline injections is shown by the filled point in Fig. 2. As can be seen the previous injections of 3, 10 and 30 μ moles/kg in the cumulative dose-response curve contributed very little to the effect measured following 100 μ moles/kg. This control was conducted for each drug studied.

Figure 3 shows the effect of five increasing doses of morphine when given as part of a cumulative dose-response curve. Doses of 1 and 3 μ moles/kg were without apparent effect on FR responding. Whereas, doses of 10 and 30 μ moles/kg reduced responding by 45 and 90 percent, respectively. A dose of 100 μ moles/kg morphine totally suppressed responding. When the 30 μ moles/kg dose was preceeded by 3 separate saline injections (filled point) the effect was not different than that observed as part of the cumulative dose-response curve.

When five ascending doses of *d*-amphetamine were given as a cumulative dose-response curve, responding was again decreased in a monotonic dose-dependent fashion (Fig. 4). Doses of 0.3 and 1 μ mole/kg had no apparent effect on

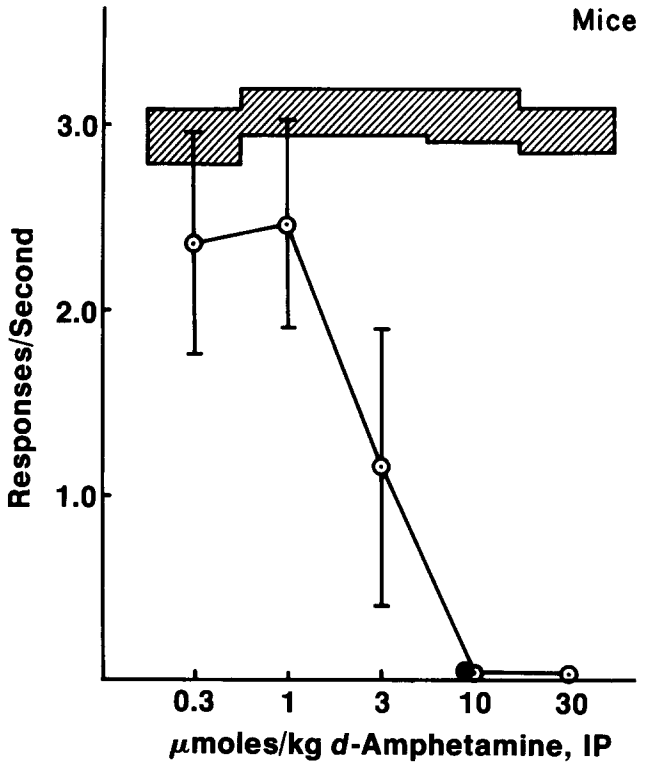

FIG. 4. Cumulative dose-response curve for the effect of *d*-amphetamine on the average rate of responding under the FR 30 schedule. Data presented as in Fig. 2. 10 μ moles of *d*-amphetamine= 1.85 mg of *d*-amphetamine·SO_4.

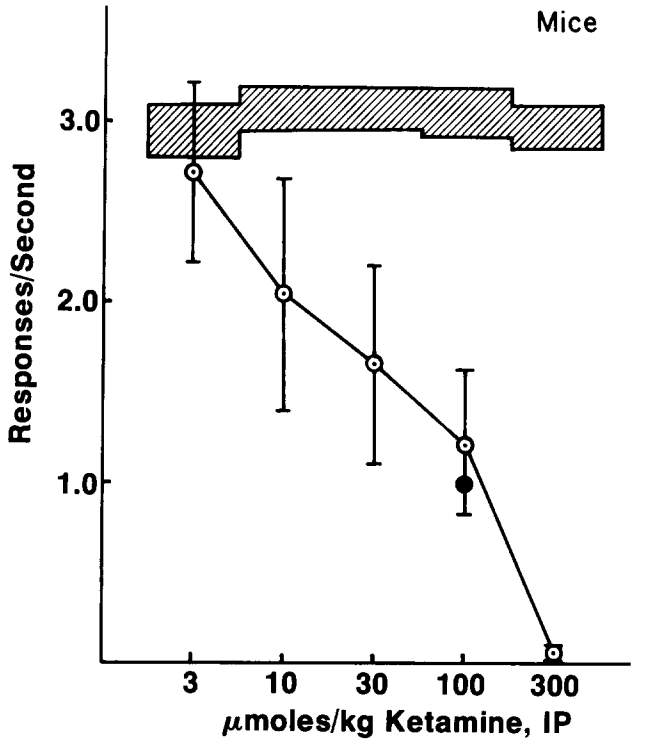

FIG. 5. Cumulative dose-response curve for the effect of ketamine on the average rate of responding under the FR 30 schedule. Data presented as in Fig. 2. 10 μ moles of ketamine=2.74 mg of ketamine·HCl.

responding. With increasing dose, the dose-response curve became very steep. The 3 μ moles/kg dose decreased responding by about 68%, and the 10 and 30 moles/kg doses suppressed responding. The suppression of responding was also observed when the 10 μ moles/kg dose was given preceded by 3 separate saline injections (filled point).

The dose-response curve obtained for ketamine was not as steep as that observed for *d*-amphetamine (Fig. 5). The 3 μ moles/kg dose of ketamine had no effect on responding. The 10, 30, 100 and 300 μ moles/kg doses each decreased responding in a dose-response fashion, and responding was totally suppressed at 300 μ moles/kg. The 100 μ moles/kg dose decreased responding by about 57 percent independent of whether this dose was preceded by doses of 3, 10 and 30 μ moles/kg ketamine or by 3 separate saline injections (filled point).

The effect of phencyclidine on FR responding is shown in Fig. 6. Graded effects were observed over a dose range of 1–100 μ moles/kg with 1 μ mole/kg having no effect and the 100 μ moles/kg dose suppressing responding. The 30 μ moles/kg dose decreased responding by 85% when preceded by 3 lower doses of phencyclidine, and by 77% when preceeded by 3 separate saline injections.

DISCUSSION

The series of experiments described here clearly demonstrate the applicability of cumulative dosing to behavioral pharmacology. The dose-response relationships demonstrated using the cumulative dose-response procedure agree

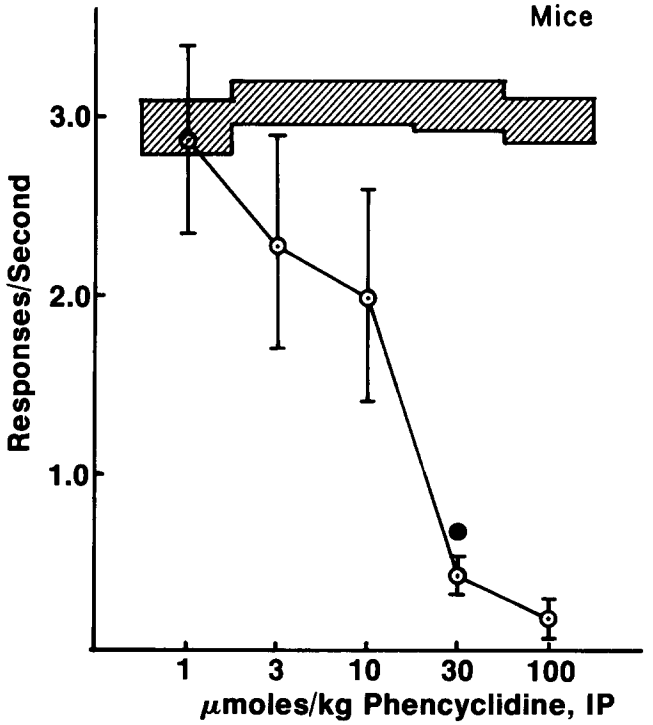

FIG. 6. Cumulative dose-response curve for the effect of phencyclidine on the average rate of responding under the FR 30 schedule. Data presented as in Fig. 2. 10 μ moles of phencyclidine=2.80 mg of phencyclidine·HCl.

very well with the effects of these drugs in mice responding under FR components which were part of other schedules such as a multiple fixed-ratio, fixed-interval [1,6]. It would also appear that the effects measured following individual doses are equivalent independent of whether they were measured following the acute administration of a single dose or as part of a cumulative dose-response curve.

The schedule selected to maintain responding during the determination of a cumulative dose-response curve is of some practical concern. The cumulative dose-response curve is designed to reduce the time required to determine an entire dose-response curve in a group of subjects. Thus, although certain schedules, such as long fixed-intervals, are very useful in behavioral pharmacology they are not ideally suited to meet this objective. To use a long fixed-interval schedule one must choose between running a very long session or determining the drug effect on a small number of schedule presentations. Neither of these options is desirable. Thus, one must choose a schedule which allows the investigator to assess the drug effect on a reasonable amount of behavior in a reasonable amount of time. The schedule selected for use in this study, mult TO 5, FR 30, would appear to satisfy these criteria.

Using behavioral procedures it is often extremely time consuming to assess the behavioral effects of a wide range of doses. The cumulative dose-response curve technique should greatly increase the speed of determination of the entire dose-response curve for a given drug, thus allowing the investigator to assess the entire dose-response curve in the time normally required to assess the effects of 1 dose using traditional dosing methods. This should be of great value in experiments which normally require great amounts of time, such as the assessment of tolerance development to a given drug and/or cross tolerance to other drug classes. In summary, the cumulative dose-response curve has been shown to be easily adapted to behavioral pharmacology, and should be of great value in decreasing the time required to examine the entire dose-response curve for a given drug.

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