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Discrete versus cumulative dosing in dose–response discrimination studies

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Abstract

This study describes the results of a 'side-by-side' comparison of two measurement techniques and two dosing regimens in a discrimination study using rats trained to either 10 mg/kg cocaine or 2 mg/kg 3,4-methylenedioxymethamphetamine (MDMA). The measurements employed were either quantal or quantitative; the former an all-or-none correct lever selection measure and the latter a measure of all responses made at the time that the criterion for selection was met. The dosing regimens were either a discrete single injection of lower doses than used in training or a cumulative dose administration sequence, in an ascending order, during one session on two separate occasions. Results indicate that the cumulative dose–response relationships, as indicated by both the slope of the curve or the generated ED_{50} value, for the discrete and cumulative dose–response curves do not significantly differ. In addition, both the quantal and quantitative measurements yield almost identical ED_{50} values, thus allowing for accurate comparability of drug-discrimination data using different techniques. The present experimentation employed two drugs known to produce heightened response rates which would not allow for behavioral suppression at the highest doses used either in discrete or cumulative regimens. The pharmacokinetics of the two drugs employed in the discrimination tests are considered and discussed in light of the advantages and disadvantages of each of the two methods employed.

Keywords: Drug discrimination; Cocaine; MDMA (3,4-methylenedioxymethamphetamine); Dose response; Cumulative dosing; (Rat)

1. Introduction

Drugs have been successfully employed to establish interoceptive cues that can be distinguished from the absence of these cues produced by imposing a non-drugged state. These two conditions can, subsequently, be used to control differential responding in laboratory animals. This behavioral paradigm, known as drug discrimination or stimulus properties of drugs, engenders the ability of the animals to indicate, by performing one of two alternative responses, the presence or absence of the effects of the drug to which they are trained. This task becomes, in reality, a 'drug-detection' test that, once acquired, allows for reliable responding by the animals in accordance with the presence of the drug. Later, when trained animals are tested with a novel drug, comparison can be made between it and the drug used in training. Results from drug discrimination testing in animals have shown that the classification of drugs capable of producing this phenomenon is parallel to human reports of subjective drug effects (Schuster and Balster, 1977; but see Lamb and Henningfield, 1994).

When doses lower than the training drug are administered under test conditions, they generally produce a lower proportion of drug-appropriate responses. Thus, a generalization gradient is obtained to the trained drug state. Discriminative stimulus properties of drugs have been widely used in studies of the pharmacological properties of a great number of centrally acting drugs (Stolerman et al., 1982). Therein, numerous schedules of reinforcement, as well as varied types of measurements, have been employed to differentiate between the drug and non-drug state and to maximize the amount of information that can be garnered from this behavioral technique. Two of the cardinal functions applicable to this paradigm are the discriminability of the drug and its ability to produce a generalization gradient to its own dose–response effect (Colpaert, 1995). Discriminability may be defined as the percentage of animal subjects that reach a set criterion and is measured as the number of sessions to reach a criterion. Generalization gradient refers to the typical dose–response relationship that is generated when doses lower than the dose used in the initial training produce decreasing effects, in this case, decreased discriminative performance upon the drug-ap-

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propriate lever. This relationship, in turn, allows for determination of ED_{50} values, as well as measurement of the slope of the dose–response curve.

The most widely used schedule of reinforcement in the myriad of studies employing this paradigm has been the fixed-ratio (FR) schedule of positive reinforcement (Stolerman, 1989). In this task, the food-deprived and, thus, motivated animal subject is trained to press a lever ten times to receive one food reinforcement. In addition to the different schedules of reinforcement employed in this paradigm, two measurements of discriminative performance have appeared in the literature. These are known as the quantal and quantitative indices. The former simply allows for the number of animals that select the drug-appropriate lever to be calculated as a percentage of the total number of animals that are tested. The quantitative index consists of the number of responses on the lever appropriate for the training drug calculated as a percentage of the total number of responses made on both levers. The issue of the value of each of these measurements over the other continues to be a matter of debate in the drug-discrimination literature (e.g., Gauvin and Young, 1987; Harris et al., 1988; Mathis and Emmett-Oglesby, 1990). Whichever measurement is used, there has been general agreement that there is a stability to the dose–response gradient over time in that rats have been observed to discriminate the training and lower doses of drugs without significant change in dose–response slope or ED_{50} values over as long as a year (Colpaert et al., 1978; Schechter et al., 1989) and pigeons for as long as 5 years (McMillan, 1987).

Dose–response determinations are generally made by administering doses lower than the training dose to well-trained, discriminating subjects and allowing them access to the two levers. In some laboratories, these discrete, single-dose tests result in reinforcement given on the FR10 schedule when either lever accumulates 10 responses (e.g., Lal and Fielding, 1984), whereas in other laboratories, the subjects are immediately removed, without receiving reward, after either lever accumulates 10 responses (e.g., Schechter et al., 1989). By contrast, cumulative dose–response tests in whole animal preparations allow assessment of an entire dose–response range during a single experimental session in behavioral pharmacological experimentation (Wenger, 1980). This methodology has been used in drug discrimination testing (Bertalmio et al., 1982; Emmett-Oglesby et al., 1988; Hiltunen and Järbe, 1989) in a two-lever discriminative task, as well as in a three-lever choice procedure (France and Woods, 1985). In at least two of these cases, the cumulative dosing procedure was compared to the single, or discrete, administration of lower doses of the training drug (France and Woods, 1985; Hiltunen and Järbe, 1989); both of these studies employed drugs with depressant actions on the central nervous system. This property of the training drug allowed for the testing of drug-appropriate lever responding with increasing doses until the rate of responding became markedly suppressed (Bertalmio et al., 1982). It was, therefore, the aim of the present study to train rats to discriminate stimulus properties of two putative stimulant drugs, cocaine and 3,4-methylenedioxymethamphetamine (MDMA), and to compare the dose–response discriminative gradients after discrete tests of lower doses vs. the cumulative dosing schedule using the same doses in ascending order, with the suggestion that larger cumulative dosing would, in fact, not necessarily cause response suppression.

2. Materials and methods

2.1. Subjects

Sixteen male, Sprague-Dawley, rats purchased from Zivic-Miller Laboratories (Allison Park, PA, USA) were randomly divided into two equal groups with one group set to be trained to discriminate the interoceptive cues produced by 10 mg/kg cocaine from its saline vehicle and the second group trained to discriminate 2.0 mg/kg MDMA from its vehicle. All rats were individually housed and kept in an AAALAC-accredited vivarium facility with an ambient temperature of 20–22°C and maintained on a 12 h light/dark cycle with lights on at 06:00 h. Tap water was continually available in their hanging wire cages but commercial rat chow was rationed to approximately 16 g per day so as to allow maintenance of their body weights at 85–90% of free-feeding weights as determined by a growth chart provided by the supplier. This procedure was in place to facilitate motivation of operant performance for food reward.

2.2. Apparatus

Twelve standard rodent operant chambers (Lafayette Instruments, Lafayette, IN, USA), each containing two levers situated 7 cm apart and 7 cm above a metal grid floor, were located in a separate room from the vivarium facility. The number of chambers was greater than each of the trained groups of animals ($n = 8$) to allow for random assignment of the rats to a particular chamber on any given day. This decreased the possibility of olfactory cues between animals (Extance and Goudie, 1981). Each chamber had a food receptacle that received delivery of a 45 mg Noyes (Lancaster, NH, USA) food pellet which was located equidistant between the two levers. The chamber was, in turn, enclosed in a sound-attenuating cubicle with an exhaust fan that provided a masking noise and was lighted by a 9 W houselamp.

2.3. Discrimination training

All 16 food-deprived rats were first trained to press one of two identical levers with half of each of the eight

animals in each group assigned to the vehicle lever as its left-lever and half as its right-lever. This was to preclude any possibility of innate position preference. Training sessions were conducted once a day, 5 days a week, with 0.9% saline vehicle lever training starting after the intraperitoneal administration of 1 ml/kg of the vehicle on a fixed ratio of 1, i.e., one response resulted in delivery of one food pellet as reinforcement. During eight consecutive training sessions, the FR schedule, after saline administration, was gradually incremented to attain an FR10 schedule of reinforcement in that ten responses on the saline-lever produced one food reinforcement. The rat was removed from the operant chamber and returned to its home cage after making 400 responses and, thus, receiving 40 reinforcements on this FR10 schedule.

Once an FR10 schedule was established on the saline lever, training began on the opposite lever either 15 min, in the case of cocaine, or 20 min after administration of MDMA, administered intraperitoneally in an equal volume of 1 ml/kg saline. In these experiments, the rats were rewarded for responding only upon the drug-appropriate lever. The initial reinforcement schedule started at an FR1 and was gradually increased, over seven consecutive training sessions, to an FR10 schedule of reinforcement. Once FR10 lever-press performance was established on both levers, discrimination training began in which daily injections of either saline (S) or drug (D) were administered on a 2-week, repeating, administration schedule: D,S,S,D,D; S,D,D,S,S. The first lever upon which ten responses were accumulated at the beginning of each of these sessions was considered the 'selected lever' for that daily session. At the time of the tenth response, presses on both the selected and unselected lever were recorded but incorrect responses produced no programmed consequence. The session for that day's training was continued, regardless of the correctness of the selected lever, until 400 responses were made on the correct lever and, therefore, until 40 reinforcements were received. The training criterion, i.e., the discriminative performance that had to be attained to adjudge the animal as capable of discriminating between the drug state and the non-drug state, was a minimum of eight correct lever selections appropriate for the substance injected on that day during ten consecutive sessions.

2.4. Discrete dose–response tests

After all the rats reached the discrimination criterion, the discriminative training regimen was limited to every other day. The rats were administered the dose of either cocaine or MDMA employed in their training or saline and were allowed to freely choose between levers. They were reinforced only for presses upon the lever appropriate for the substance injected prior to that daily session. On intervening days, rats were tested with doses of cocaine in the cocaine-trained animals, or MDMA in the MDMA-trained animals, different than their training dose. Cocaine-trained animals received 1.25, 2.5 and 5.0 mg/kg cocaine, whereas the MDMA-trained animals received 0.062, 0.125, 0.5, 0.75, 1.0 and 1.5 mg/kg MDMA. During test trials, all rats were immediately removed, without receiving reinforcement, when ten responses were accumulated on either lever. Each novel dose of drug was administered twice in a random order, once following a maintenance session with the dose used in training and once following a saline maintenance session. This counter-balanced procedure was employed to control for any possible residual influence from the previous day's maintenance session. If, at any time during the maintenance test days, a rat's discrimination fell below the 80% criterion, i.e., less than eight correct state-appropriate lever selections in ten consecutive maintenance sessions, the data on that rat were to be dropped from the results. This, however, did not occur during the discrete dose–response tests.

2.5. Cumulative dose–response test

The cumulative dosing test followed the discrete dose–response testing. In this series, the drug was generally injected at the same doses as used in the discrete tests on 2 days separated by at least 2 weeks of re-training with the maintenance dose regimen. In this test, the animals were administered saline at time 0 and allowed to choose between the two levers with immediate removal upon accumulating ten responses on either lever. They were then administered the lowest dose of either cocaine or MDMA previously tested in the discrete trials and returned to their home cage for either 15 or 20 min, respectively. At that time, they were allowed access to the two levers and, once again, removed upon accumulating ten responses upon either lever without receiving reinforcement. This was immediately followed by another injection of a dose of either cocaine or MDMA, in most cases, to allow for the cumulative dose to equal the next highest dose used in discrete testing. Thus, the dose schedule for cocaine was saline, 1.25, 1.25, 2.5 and 5 mg/kg of cocaine tested at 15 min intervals. The large number of intraperitoneally injections with MDMA used in the discrete dose–response trials precluded cumulative dosing of every dose of MDMA. Nonetheless, the sequential dosing schedule for the MDMA-trained animals was saline, 0.0625, 0.0625, 0.625, 0.25 and 1.0 mg/kg MDMA tested at 20 min intervals. This allowed for a total of six total administrations during the cumulative testing of MDMA. In all cases, the rat was immediately removed upon accumulating ten responses on either lever without receiving reinforcement or after 2 min in the experimental chamber, whichever came first. In addition to cumulative drug testing, five consecutive saline injections were tested in a similar fashion in order to ensure that the successive trial drug lever responding did not develop as a result of multiple trial testing procedures. These multiple saline administrations

were also done in two sessions in each of the two trained groups of animals separated by 2 weeks of maintenance sessions.

2.6. Data analysis

The data collected in the drug discrimination session are expressed as both quantal and quantitative measurements. Each of these individual measurements provides a different indicator of lever preference before reinforcement. The quantal measure is the percent of animals ($n = 8$) that select the drug-appropriate lever, i.e., this lever was the first of the two available levers to accumulate ten presses. The quantitative measurement is the number of responses on the drug-lever divided by the total number of responses on both the drug and the saline lever at the time that ten responses are accumulated on either lever; this fraction is expressed as a percentage. Unlike the all-or-none quantal measurement, the quantitative measurement allows for responses on both the selected and unselected lever to be considered; thus, it provides a relative measure of magnitude, as well as direction of lever preference. A computer-based (Tallarida and Murray, 1987) formulation of the Litchfield-Wilcoxon procedure (Litchfield and Wilcoxon, 1949) which employs probits vs. log-dose effects was used to generate ED_{50} values with 95% confidence limits from the quantal dose–effect relationship for both the discrete and cumulative dose–response experiments. Lastly, the amount of time between placement into the test chamber and lever selection was recorded. This was then employed as a measure of the rate of responding, i.e., responses/minute, in both discrete and cumulative dose–response test responding.

2.7. Drugs

The drugs used were cocaine hydrochloride and 3,4-methylenedioxymethamphetamine (MDMA) hydrochloride, both supplied by the National Institute on Drug Abuse, made fresh daily by dissolving in a 0.9% sodium chloride distilled water vehicle solution and administered in equal volumes of 1 ml/kg intraperitoneally. Each dose was calculated as salt.

3. Results

The administration of increasing cocaine doses in eight rats trained to discriminate between saline and 10 mg/kg cocaine on two occasions each produced increasing quantal responding, whereas the maintenance saline allowed for 6.7% of responses on the cocaine-appropriate lever, and the training dose of 10 mg/kg led to 93.8% of all selected lever responses on that lever. This is represented as closed circles in Fig. 1. The quantitative measurements during each of the two sessions also indicated increasing means

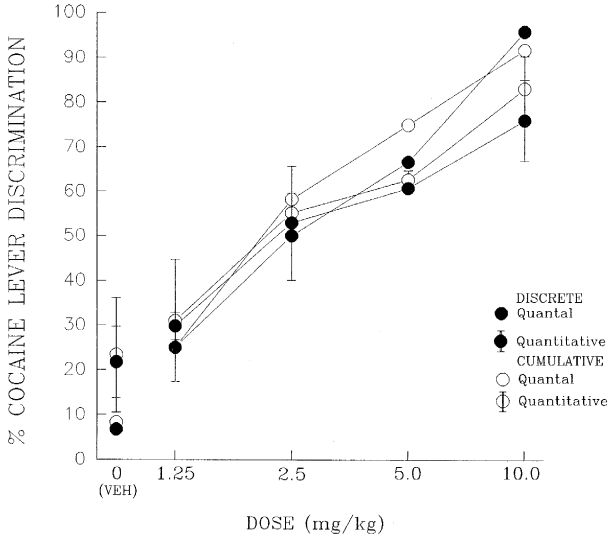

Fig. 1. Discrete single injection vs. cumulative injection dose–response to cocaine in rats trained to discriminate the effects of 10 mg/kg cocaine from its vehicle 15 min after intraperitoneal administration. Abscissa: dose in mg/kg of cocaine administered either singly on two occasions each or cumulatively in an ascending order in one session on two separate days. Ordinate: percent cocaine lever discrimination with quantal and quantitative (±S.D.) in eight rats.

with increased doses used. Analysis (Tallarida and Murray, 1987) of the dose–response curve (with 95% confidence limits) indicates an ED_{50} value of 2.524 (1.665–3.826) mg/kg for the quantal data of the discrete trials and 2.804 (2.197–3.580) mg/kg for the quantitative cocaine data. The two sessions of cumulative dosing with the same doses of cocaine administered in ascending order starting with saline and going from 1.25 to 2.5 to 5 to the training dose, likewise, produced incremental quantal responding. This ED_{50} value was 2.332 (1.444–3.767) mg/kg for the quantal cumulative data. Likewise, there was an increased quantitative measurement as progressively increasing doses of cocaine were administered and analysis yielded an ED_{50} value equal to 2.496 (2.023–3.050) mg/kg. Analysis of the quantitative measurements at each treatment dose between discrete and cumulative dosing indicated no significant differences at each of the doses employed. Lastly, the rates of responding ranged from a mean of 11.16 responses/min with saline to 16.44 responses/min at the highest dose of cocaine employed in the discrete dose–response curve with no significant difference between any dose administered. Likewise, the cumulative dosing rate of responding went from 14.28 responses/min with saline to a maximum of 19.38 responses/min at 10 mg/kg cumulative dose. Once again, there was no significant difference in response rates between saline and any of the cumulative doses administered.

Administration of MDMA to eight rats at doses of 0, 0.062, 0.125, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/kg in two discrete trials generally produced increased percent selection upon the MDMA lever as indicated by the closed

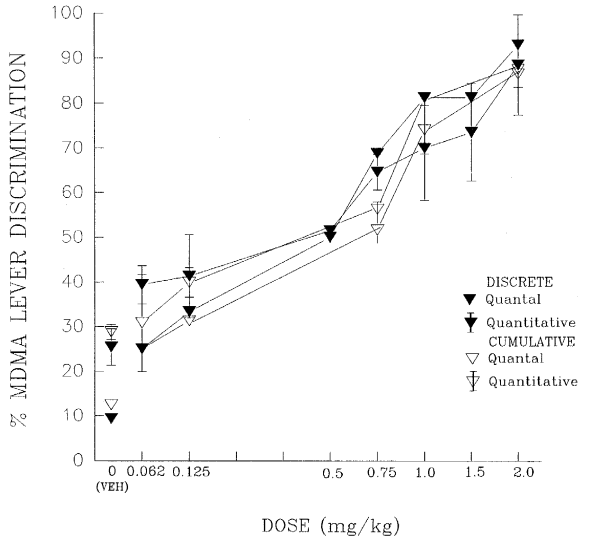

Fig. 2. Discrete single administration of various doses of MDMA to animals trained to discriminate 2.0 mg/kg MDMA from its vehicle. Coordinates as above with $n = 8$ and post-administration time of 20 min. Note: not all discrete doses were employed during cumulative testing; exclusion of a cumulative dose of 0.5 and 1.5 mg/kg to preclude excessive number of repeated injections in one day.

inverted triangles in Fig. 2. These data allowed for a calculated ED_{50} value (with 95% confidence limits) equal to 0.366 (0.082–1.622) mg/kg. There was a similar increase in the quantitative measurement with increasing doses of MDMA that allowed for an ED_{50} value of 0.345 (0.036–3.354) mg/kg. The rates of responding with vehicle were a mean of 19.8 responses/min with a decrease to 6.9 responses/min at the 2.0 mg/kg MDMA dose. Nonetheless, there was no systematic or significant effect in any of the doses employed as to rate of responding. Likewise, cumulative dosing with saline and cumulative doses of 0.062, 0.125, 0.75, 1.0 and 2.0 mg/kg allowed for increasing quantal responding and the generation of an ED_{50} value equal to 0.501 (0.048–5.269) mg/kg. Likewise, there was an increasing quantitative response on the MDMA-appropriate lever with increasing cumulative doses yielding an ED_{50} value of 0.511 (0.0475–5.507) mg/kg. Comparison of the ED_{50} values between the discrete and cumulative dose–response curves with MDMA showed a potency ratio of 0.73 with the discrete dose–response ED_{50} value being somewhat, but non-significantly ($P = 0.67$), different.

4. Discussion

The present work intended to compare the discriminative gradient of increasing doses of cocaine in animals trained to discriminate between saline and 10 mg/kg cocaine, as well as in other rats trained with 2.0 mg/kg MDMA. When the discrete single injection of different doses, on two occasions, were compared with the administration of approximately the same doses cumulatively administered in a 1-day session, the results indicate almost identical ED_{50} values in both treatment regimens. This similarity exists no matter which one of the two much-used discriminative measurements, i.e., quantal or quantitative measures, is employed. Thus, the 'side-by-side' comparisons of the two dosing regimens using the two measurement techniques indicate a close comparability of drug-discrimination data. The advantage of the cumulative dose procedure exists in the savings in the amount of time and quantity of drug needed for the multiple discrete test procedure. A disadvantage may exist when the acute administration of a drug produces sedation or causes the cumulative dose, if the drug is not metabolized quickly enough, to severely compromise the response rates; this occurrence is, in fact, often cited as the end point for cumulative dosing of slowly metabolized drugs (Bertalmio et al., 1982). In the present experimentation, neither of the two drugs, cocaine or MDMA, caused behavioral disruption at the highest dose used in either the discrete or the cumulative dose–response curve and yet, there was no major difference in the pattern of drug generalization gradient between the discrete and cumulative procedures.

In effect, the discrete dosing procedure may have differed from the cumulative dose regimen if either cocaine or MDMA had a very long onset of action, i.e., beyond 15 min and 20 min, respectively. This would have disallowed the ability of the animal, assumed to require a critical level of drug to reach the brain, to determine the full interoceptive stimulus cue and, thus, permit discriminative performance to occur. In addition, if either of the drugs had a very short half-life, the drug's potency during cumulative administration may have been underestimated. Thus, the pharmacokinetic variability may affect the dose–response curve obtained by cumulative dosing. A published example of this variability exists in which the drug used for discrimination performance was fentanyl as it was tested during a single day using an ascending series of drug injections (Emmett-Oglesby et al., 1988). In this particular case, fentanyl was shown to require twice the cumulative dose to produce the same effect as the training dose, suggesting to the authors that there was a degree of tolerance developed within the cumulative test session or that fentanyl was, in fact, metabolized more rapidly during the cumulative dose procedure. In addition, the discriminative performance level in rats trained with 32 mg/kg caffeine has been shown to directly increase with plasma caffeine levels (Modrow et al., 1981). In these cases, the results highlight the need for a thorough understanding of the pharmacokinetics of the drug used in discriminative training.

The cumulative dosing procedure using five consecutive administrations of saline (referred to as 'epochs'; Gui-Hua et al., 1992), to delineate the fact that the animal is tested, removed and re-injected, did not produce any variation in the amount of quantal responding on the drug-appropriate

lever. Thus, from the first to the fifth cumulative dose on two occasions, each with saline, no more than one of the eight animals in either of the two groups of rats chose the drug-appropriate lever, a quantal finding similar to that seen during maintenance sessions with the discrete dose–response experimentation. The present results also indicate that the progressive dose–response data outcome, from both discrete and cumulative testing, may be obtained with either quantal or quantitative indices of discriminative responding. The two types of indices have often been found to yield similar results (Boja and Schechter, 1987; Stolerman and D'Mello, 1981; Stolerman et al., 1984). Thus, either discriminative index will allow for valid results when the FR10 schedule of reinforcement is employed (Stolerman, 1989).

In conclusion, the results suggest that the current methods to assess dose–response experimentation in discriminative stimulus properties of drugs yield close agreement no matter if either the quantal or quantitative measurement is used and with either the discrete or cumulative dose–response schedule of administration. Nonetheless, there are statistical advantages in using the quantitative over the quantal measurement (Stolerman and D'Mello, 1981) and pharmacokinetic factors must be considered when using the cumulative vs. the discrete dose–response regimen.

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