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Variable-interval responding in the horse: A sensitive method of quantitating effects of centrally acting drugs

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SUMMARY

An operant conditioning apparatus for studies in equine pharmacology was constructed. Horses interacted with this apparatus by breaking a light beam and were rewarded with 30 ml of oats. Horses readily learned to use this apparatus and were trained to respond on a variable-interval-60 schedule. With this schedule, there was no direct relationship between the rate of light beam breaking and the reward. Horses thus developed their own individual response rates (ie, light-beam breaking rates), and these rates remained stable at between 5 and 35 responses/min for each horse over a period of months.

The effects of 2 drugs on this paradigm were tested. Reserpine (5 mg/horse, IV) depressed the response rate in all horses tested. This depression was maximal between 3 and 5 days after treatment and lasted for up to 10 days. After small doses of cocaine (0.01 mg/kg of body weight IV), the response rate of 1 horse was stimulated, whereas 1 mg of cocaine/kg was required for maximal stimulation of response rate in another horse. Larger doses of cocaine inhibited response. Variable-interval response was a sensitive method of measuring drug effects in the horse and allowed accurate quantitation of drug effects that were not detectable by clinical observation.

Behavioral pharmacology and behavioral toxicology have developed into independent disciplines that can answer many important questions concerning subtle and long-term effects of drugs.[1] Unfortunately, little behavioral pharmacology has been done in horses, despite the use of behavior-altering drugs in performance and show horses. As part of the Kentucky Equine Drug Research Program, a systematic study of the behavioral effects of drugs in horses was begun, and attempts were made to develop reliable and sensitive methods for objectively quantitating the effects of central-acting drugs in horses.

One such behavioral technique is a step-counting method.[2] This method has proved to be a reliable and apparently sensitive method for measuring the locomotor effects of drugs with a component of dopaminergic activity, such as apomorphine and narcotic analgesics. However, it does not appear to be useful for measuring the behavioral effects of drugs that act on the adrenergic system, such as cocaine or amphetamine.

A simple and sensitive method for measuring drug effects on the adrenergic system was needed, and therefore, an operant conditioning model for use in horses was developed. In this model, horses were conditioned to break a light beam located above their feed bucket. Breaking this beam was linked to positive reinforcements for the horse. Each horse established its own response rate that became stable over a period of months. After treatment with various drugs, horses were then reintroduced to the apparatus, and changes in their response rates from control rates were taken as an indication of drug effect. The effects of cocaine and reserpine were studied. Cocaine has been used in racing horses, as evidenced by racing chemists reports,[a] and reserpine has been used to calm nervous horses in a variety of situations. The purpose of the present report was to develop a dependable and sensitive method for measuring the effects of cocaine and reserpine in horses.

Materials and Methods

Mature Thoroughbred, half Thoroughbred, and Standardbred mares and geldings, weighing 410 to 490 kg each, were used (Table 1). These horses were maintained on pasture and were brought daily into the operant conditioning stall for their conditioning or experimental sessions.

Operant conditioning apparatus—Automatic feeding console—The light-activated feeding console (Fig 1) was built into a corner of a box stall adjacent to a tack room in which the monitoring and programming equipment was installed. The feeding console consisted of a feed bucket, an electric eye, and a beeper that sounded when the light beam was broken.

The light beam was installed 10 cm above the rim of the bucket in such a way that the horse could not eat from the bucket without breaking the beam. However, the horse could break the light beam without putting its head in the bucket. Reinforcements were delivered through a small hole in the console between the light beam and the bucket (Fig 1).

Programs and programming equipment—The function of the programming equipment was to determine when a horse had

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[a] Blake JW, University of Kentucky, Lexington: Personal communication, 1979.

TABLE 1—Information on six horses used in the operant conditioning and drug studies

Horse No.	Weight (kg)	Sex	History
1	449	Gelding	Raced
2	490	Mare	Raced
3	410	Gelding	Raced
4	478	Mare	Not known
5	430	Mare	Raced
6	423	Mare	Not known

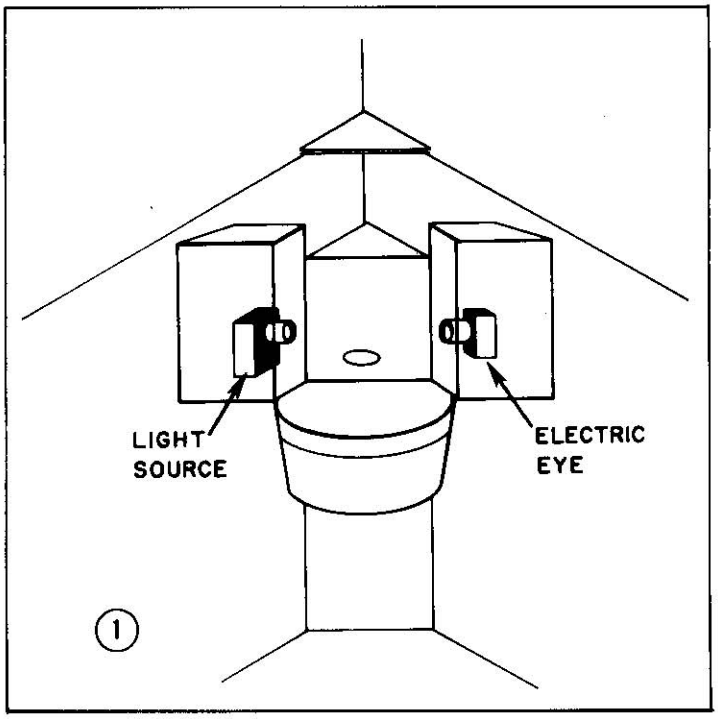

Fig 1—Oak automatic-feeding console built around a feed bucket installed in the corner of a 4 × 4 m box stall. Horses were conditioned to break the light beam for food reinforcements that were delivered through the small opening above and behind the bucket.

Tobin T: *Drugs and the Performance Horse*, 1981. Courtesy of Charles C Thomas, Publisher, Springfield, Ill.

earned a reward. The requirements for reward are determined by the research worker and are known as the schedule of reinforcement. Reinforcement schedules may be fixed-ratio, fixed-interval, or variable-interval schedules.

When a certain number of responses yields a reinforcement, the schedule is referred to as a fixed ratio. A fixed-ratio schedule was used when the animals were initially trained to use the equipment. When the animals demonstrated a stable rate of response on a fixed-ratio-8 schedule (after 8 breaks of the electric eye, a reinforcement was made), they were switched to a variable-interval schedule.

In the variable-interval schedule, a reinforcement was earned when a response was made any time after a randomly determined no-reinforcement interval. After a reinforcement was earned, more reinforcements could not be earned until another no-reinforcement interval (again of random length) had passed.[3] The duration of the no-reinforcement periods were variable, so the horse could not determine when a response would yield a reinforcement. In the variable-interval-60 (VI-60) schedule, the average duration of the no-reinforcement interval was 60 s. Consequently, if a horse had developed an adequate response rate, at the end of the 30-minute test the horse would have earned approximately 30 reinforcements.

The programming equipment was installed in a tack room adjacent to the behavioral stall. The programming equipment consisted of a board of electromechanical relays wired to the electric eye, an automatic feeder, a cumulative recorder, and a programming board. The relays were wired in such a way that

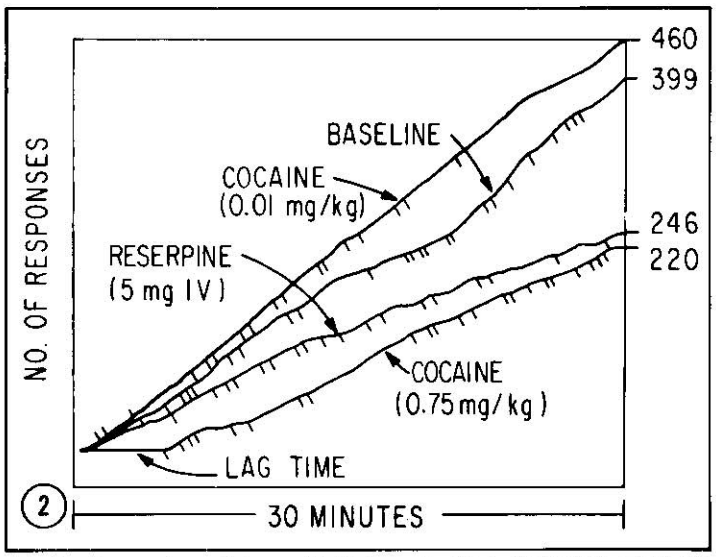

Fig 2—Comparison of drug effects on cumulative recordings. Each line represents the rate of responding and reinforcement after administration. The figures on the left axis are the number of responses made by the horse and determine the slope of the line. Each downward deflection represents an earned reinforcement. The indicated doses of cocaine were administered immediately before the horse entered the operant box, whereas the reserpine was administered 12 hours before the test.

Tobin T: *Drugs and the Performance Horse*, 1981. Courtesy of Charles C Thomas, Publisher, Springfield, Ill.

when the light beam was broken, the beeper sounded and the event was recorded on a counter and paper chart. The program determined whether a reinforcement had been earned. If it had, a 2nd circuit was activated that triggered the automatic feeder to deliver 1 reinforcement. Delivery was recorded on the chart paper.

The automatic feeder[b] consisted of a compartmentalized treadmill attached to an electric motor. When the treadmill advanced by 1 compartment, the contained reinforcement was released into the feeding bucket in the behavioral stall. The total sequence of events, starting with the breaking of the electric eye and ending with the delivery of a reinforcement, required about 15 s.

Cumulative recorder—A recorder[c] yielded a hard copy of all data, and some typical records are presented in Figure 2. The paper moved at a fixed rate along the horizontal axis while each response moved the pen 1/500 of the paper width up the vertical axis. The slope of the curve was thus the response rate, with the pen resetting back to the base line after each 500 responses. Delivery of a reinforcement was recorded on the chart as a transient downward marking deflection of the pen.

Reinforcements—Good quality oats, about 30 ml/reinforcement, were used as the reinforcer during these experiments.

Drugs—Reserpine[d] (5 mg/horse) was administered IV. Cocaine[e] was obtained as the pure flake form and was freshly prepared in isotonic saline solution before injection. Cocaine at 0.005 to 1.0 mg/kg was administered by rapid IV injection just before the animals entered the apparatus. Isotonic saline solution was used for the control or sham injection in both studies.

Experimental procedures—In a typical experimental procedure, the horses were brought from pasture into a holding box where hay and water were available ad libitum. For the actual experimental run, each animal was walked from the holding box into the operant conditioning box. All experimental sessions lasted 30 minutes, were held between 9 and 12 AM, and no animal was used more than once a day.

[b] Ralph Gebrands Co, Arlington, Mass.
[c] Harvard Apparatus Co, South Natick, Mass.
[d] Serpasil Injectable, Ciba/Geigy Corp, Summit, NJ.
[e] Merck & Co Ltd, Philadelphia, Pa.

Each experiment with cocaine was preceded by a 3-day control period in which saline injections were given. Cocaine was administered IV at doses of from 0.005 to 1 mg/kg immediately before the animal entered the operant conditioning box. Only the first 30-minute period after injection of cocaine was recorded. Because of its long duration of action, reserpine (5 mg/horse) was administered 12 hours before the first test session. Horses were then tested daily until the response rate returned to the base-line rate.

In all drug studies, the total number of responses and reinforcements were logged as cumulative recordings, but only responses per minute as a mean rate per session are presented.

Results

The first schedule used during our training period called for the horses to break the light beam twice for a food reinforcement. All horses rapidly learned to break the light beam for food, and then the number of responses required to obtain food was increased. Next, a variable-interval-30 (VI-30) schedule was introduced. On this schedule, the horses broke the beam for reinforcement, but the ratio between breaks and reinforcement was not rigidly linked. When the animals had stabilized on VI-30, the schedule was changed to VI-60. With a VI-60 schedule, it took 3 weeks for the response rate to stabilize. Once established, the response rate was unique for each horse, and in general, remarkably stable (Fig 3). The response rate for the horses used in the present study remained stable for each horse over the 4-month period, and each horse's rate yielded the maximum number of reinforcements.

It was noticed, during equipment malfunctions in early experiments, that the horses continued to respond at their base-line rate in the absence of reinforcement. It proved impossible, however, to carry out studies on the extinction of this behavior, as withholding of reinforcement for a period led to destructive assault on the console by the horses.

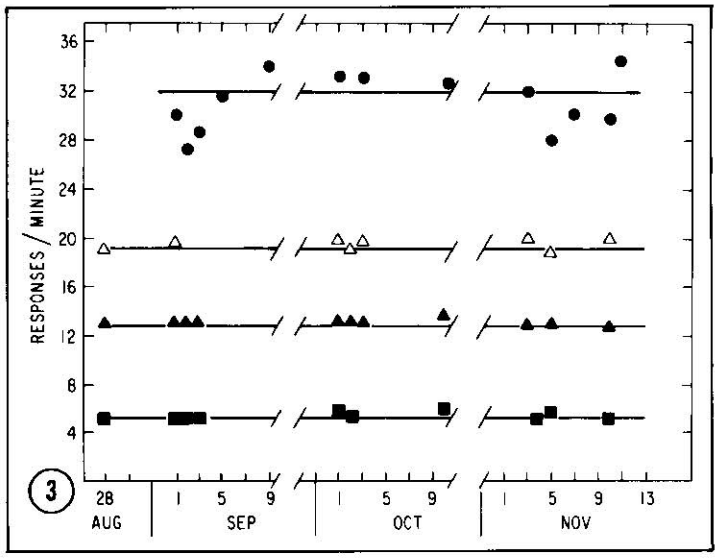

Fig 3—Stability of control response rates over experimental period. Response rate of individual horses on the indicated drug. The recording of control data began after these horses demonstrated 10% variability in response rate over 3 consecutive days on a VI-60 schedule. These horses were tested at various times between August and December 1978. The broken lines indicate the time of drug trials. Symbols represent individual horses.

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Typical control and drug-induced responses obtained on the VI-60 schedule are shown in Figure 2. Time (in minutes) is represented on the horizontal axis against the number of responses on the vertical axis. Each time the light beam was broken, the pen moved a notch in the vertical direction, so the slope of the curve indicates the rate of response. The short downward strokes represent delivery of individual reinforcements.

In the experiment presented in Figure 2, the horse tested made 399 responses after a saline injection, to give a mean response rate of 13.3 responses/min. The record of the horse's performance after administration of 0.01 mg of cocaine/kg shows that the response rate has a greater slope and thus a stimulated rate of response. Administration of 0.75 mg of cocaine/kg produced a period in which the horse failed to respond and caused an overall depression in the response rate. The period at the beginning of the cocaine test in which the horse failed to respond is referred to as a lag time, which in this case lasted about 5 minutes.

This horse's record after administration of reserpine is also included in Figure 2, showing that reserpine caused marked depression in the response rate, without any apparent lag time. The horses given reserpine made 246 responses, which was 60.9% of this horse's base-line rate.

The overall dose-response relationship for cocaine is presented in Figure 4. The data showed that, for each horse, a small dose exists that has no effect on the response rate, although increasing the dose level stimulated the rate of response in all 6 horses. However, the dose that produced the maximal increase in response rate varied from horse to horse, and the response decreased if the dose of cocaine was increased beyond that producing the maximum response. Inspection of the recordings indicated that larger doses of cocaine produced lag times and that the lag times increased in duration as the dose of cocaine was increased. Clinical observations made during the operant testing indicated that the lag time was associated with clinical signs of marked CNS stimulation. These signs include hyperexcitability, increased reactivity to extraneous stimuli, head bowing or shaking, and increased locomotor activity.

The sensitivity of this behavioral technique to the effects

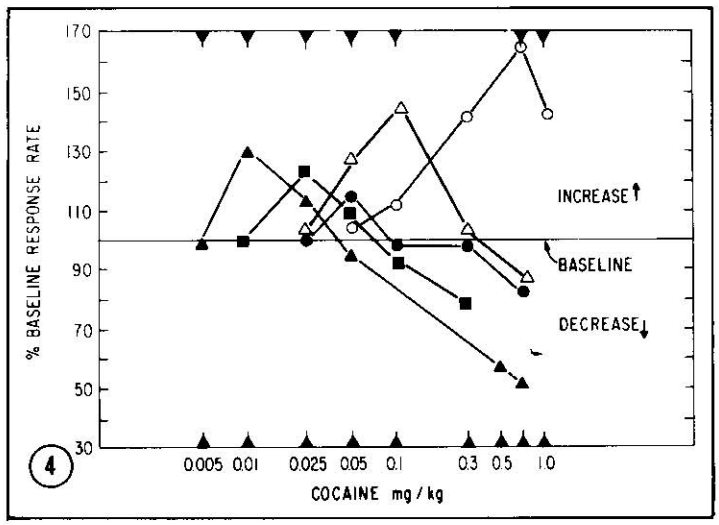

Fig 4—Acute effects of cocaine on VI-60 responding schedule. The symbols represent the percentage of change in responding rates from control for each animal as the dosage of cocaine was increased.

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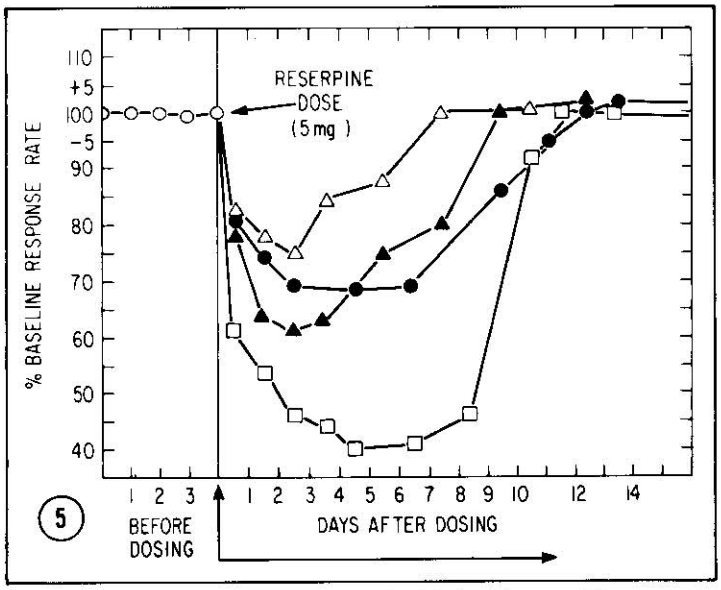

Fig 5—Effects of reserpine on VI-60 schedule in 4 horses. The operant-behavior rates of 4 horses were normalized as 100% for 5 days before dosing. The symbols show the changes in responding rate observed in 4 horses after administration of 5 mg of reserpine IV to each horse at the indicated time.

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of CNS depressants is exemplified by the reserpine study. The effect of a single injection of 5 mg of reserpine IV on the response rates of 4 horses is shown in Figure 5. The drug was administered 12 hours before the first test and thereafter the horses were tested daily. All horses took at least 3 days to develop their maximal response to reserpine, and in 1 horse, the maximal response took 5 days to develop. The action of this dose of reserpine lasted for 6 to 10 days. By 12 days after dosing, however, all horses returned to their base-line values.

All of these horses showed the effects of reserpine early in the study. These effects, which consisted of sweating, depression, ptosis of the upper eyelid, and diarrhea, abated by 48 to 72 hours after dosing, which was about the time the maximum depression in response rates occurred. By 3 days after dosing, horses given reserpine were clinically indistinguishable from normal horses.

Despite the marked depression in the response rates, all horses maintained a response rate sufficient to ensure that they obtained close to the maximum number of reinforcements per session, and during all the drug studies, horses never failed to eat all the oats earned during the 30-minute test.

Discussion

The present data indicated that variable-interval responding in the horse is a sensitive and reproducible means of measuring subtle drug effects. Horses readily learned to operate the apparatus, and judging from their behavior on test mornings, valued the rewards (oats) provided by this system. In the absence of drugs, their response rates were stable for a period of months (Fig 3). Administration of doses of cocaine that produced no overt or clinical signs of drug effects in these horses clearly affected their response rate (Fig 4). Similarly, their response rate was depressed by reserpine (Fig 5) long after clinical signs of its administration had abated.

As established in the present study, the experimental protocol of one 30-minute session/day is useful for studying the actions of long-lasting tranquilizers such as resrpine. In previous studies quantitating the pharmacologic effects of reserpine, using a modification of the step-counting method, the locomotor effects of reserpine were only detectable for 3 days after the relatively large dose of 12 mg/kg.[4] Similarly, Lowe[f] reported that after doses of reserpine, the more obvious effects will disappear within 24 hours and the more subtle effects may last up to 72 hours. These observations are also in agreement with the observed clinical signs caused by reserpine seen in the present experiments.

In contrast with these clinical observations are reports of veterinarians and trainers who believe that clinical (2 to 5 mg) doses of reserpine can calm horses for periods of more than 5 days and up to 10 days. These reports are in agreement with the data of Figure 5, which shows clear-cut effects of reserpine lasting for up to 10 days in some horses. The experiments show that operant conditioning is capable of detecting and quantitating drug effects which are visible only to experienced observers familiar with individual animals over long periods.

Similar results were observed with cocaine. In studies in which a step-counting method was used to quantitate behavioral responses to cocaine, high doses of cocaine were required to elicit a locomotor response and the response was erratic and difficult to quantitate.[4] However, with this variable-interval response method, the stimulant effects of small doses of cocaine (0.01 mg/kg) could be quantitated, and the ability of stimulant drugs to stimulate and then inhibit a performance was demonstrated.

One main problem in equine pharmacology is determining the relationship between blood concentrations of drugs and the possible stimulant or depressant actions of drugs.[4] To determine this relationship, accurate methods of quantitating the subtle effects of drugs acting on the CNS of horses are required.

Variable-interval responding should be readily adaptable to studies on other drugs in horses. Because of the high base-line rate of responses, this method would appear to be suited uniquely to studies on depressant drugs. Because depressant drugs used in equine medicine sometimes have periods of action measured in hours, rather than in days, the testing schedule may have to be modified for these drugs. Thus, a possible schedule might be 15 minutes of reinforcement in each hour of test, with horses remaining in the test box for up to 8 hours. Under these conditions, a serial time course of action could be obtained in 1 day's experiment, which would allow accurate quantitation of the time course of pharmacologic effects of drugs.

[f] Lowe J, Cornell University, Ithaca, NY: Personal communication, 1979.

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