VARIABLE INTERVAL CONDITIONING IN THE HORSE: A SENSITIVE MEASURE OF BEHAVIOR

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SUMMARY

Most information available on the behavioral effects of drugs in horses comes from subjective evaluations. Because of this, these studies are often difficult to replicate and involve drug dosages which produce clear-cut clinical signs of medication. Operant conditioning, however, provides an objective and reproducible means of evaluating drug effects in horses at doses which produce no overt clinical effects.

Variable interval (VI) scheduling is a specific type of operant conditioning now being used in our laboratory. This method yields a stable baseline of behavior which is sensitive to drug effects on the CNS, and the results obtained are objective and easily reproduced.

Horses were trained to break an electric eye for food (30 ml of oats) reinforcements. A constant rate of responding was established in horses when reinforcements were given on a random time schedule (Variable Interval). This response rate was shown to be significantly (50%) depressed for up to 12 days after a single dose of 5 mg reserpine (Serpasil^{R}) intravenously (IV) to a 500 kg horse. Despite this protracted behavioral depression, all horses eventually returned to the pre-drug control rate of responding. This behavioral effect persisted long after the clinical signs of reserpinization, which had abated by 72 hours post dosing.

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Publication No. 58 from the Kentucky Equine Drug Research and Testing Program Department of Veterinary Science, University of Kentucky, Lexington, KY 40546. Published as Kentucky Agricultural Experiment Station Article No. 78-4- with the permission of the Dean and Director, College of Agriculture.

Supported by a grant from the Kentucky Equine Research Fund.

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The rate of responding in VI was significantly increased by cocaine. Cocaine administered directly before 30-minute sessions, at doses as low as 0.001 mg/kg IV, significantly increased the overall response rate. This dose is far below that required to elicit subjective signs of CNS stimulation.

Operant methods can, therefore, be a valuable tool in objectively measuring the CNS effects of "subclinical" doses of a wide spectrum of psychotropic agents and may unmask currently undetected central effects of other therapeutic agents and procedures.

INTRODUCTION

The most pertinent type of behavioral study on which equine medication rules might be based is the performance study. Unfortunately, performance studies are both expensive to carry out, rarely yield useful data, and are not often independently replicated to confirm results.[1] As a part of the Kentucky Equine Drug Research program, we are developing alternate methodologies which may be sensitive and objective indicators of behavioral effects of drugs in the horse.

The operant method we are using examines drug effects on a controlled rate of conditioned responding maintained by a variable interval schedule of food presentation. A behavior such as this, maintained by its consequences, is defined as operant behavior.[2] The technique of studying operant behavior was developed by B. F. Skinner in the early 1950's and since that time a great deal of work has been done on a variety of different animals. However, this is the first report of such methodologies being applied to the study of drug effects in the horse.

MATERIALS AND METHODS

HORSES: Mature Thoroughbred and Standardbred mares and geldings, weighing 400-500 kg, were used throughout. These horses were maintained at pasture and were brought daily into the operant conditioning stall for their conditioning or experimental sessions. Pertinent information on the six horses used in the operant behavior studies is presented in Table I. All IV injections were into the jugular vein and all blood samples were drawn from the opposite vein. Intramusucular injections were made deep into the muscles in the side of the neck.

BEHAVIORAL EXPERIMENTS

1. OPERANT CONDITIONING APPARATUS

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

The light beam was installed four inches above the rim of the bucket in such a way that the horse could not eat out of the bucket without breaking the beam. However, the horse could easily break the light beam without putting his head in the bucket. Reinforcements were delivered through a small hole in the console between the light beam and the bucket, as shown in Fig. 1.

(b) Programs and programming equipment: The function of the programming equipment is to determine when a horse has earned a reward. The requirements for reward can be determined by the researcher. These requirements are known as the 'schedule of reinforcement'. Common types of reinforcement schedules are the fixed ratio, fixed interval, variable ratio and variable interval.[2] [3] We have used both the fixed ratio and variable interval.[4]

A fixed ratio schedule was used when the animals were first trained to use the automatic feeder. On a fixed ratio schedule the horse received his reinforcement after a set number of responses. When the animals establish a stable rate of responding on the fixed ratio schedule they are switched to the variable interval schedule. All of our drug studies were with the variable interval schedule.

In a variable interval schedule the apparatus is programmed so that there is no direct relationship between the rate of responding and the delivery of a reinforcement, as with the fixed ratio schedule. A reinforcement is delivered when a response is made after a randomly determined time interval. The duration of the no reinforcement interval is variable, hence the name variable interval. In the VI 60 schedule the average duration of the no reinforcement interval is 60 seconds. Since the horse has no way of knowing exactly when a response will yield a reinforcement, it selects a steady rate of responding which assures it that the maximum number of reinforcements are earned.

All of the programming equipment required for this study was installed in a tack room adjacent to the behavioral stall. In addition to the electronic relay board, we used an automatic feeder.* The feeder consists of a compartmentalized treadmill attached to an electric motor. The oats are loaded into the compartments and when the feeder receives a signal from the programming equipment it advances one compartment. The oats are then ejected into the feeding bucket in the behavioral stall. The whole sequence of events, from the breaking of the electric eye to the oats falling into the bucket, takes about 1 ½ seconds.

* Frank Gerbrand Company

(c) Cumulative recorder: A Harvard* recorder was used to make a hard copy of all the data. It produced a chart which records the horse's response rates and reinforcements as a function of time.

(d) Reinforcements: Good quality oats, about 30 ml per reinforcement, were used as the reinforcement throughout these experiments.

*Harvard Instrument Company

(e) Drugs: Reserpine was administered as injectable Serpasil®**, 2.5 mg/ml. Cocaine*** was obtained as pure flake and made up fresh in normal saline immediately before administration. Normal saline was used as the control injection for both studies.

(f) Experimental procedure: Horses were brought in from pasture and placed in a holding box where hay and water were available *ad libitum*. For the reserpine study, the horses were given 5 mg of reserpine IV in the evening 12 hours before the start of testing the next day. Tests were run on consecutive days, once a day for 30 minutes. Cocaine was administered directly before the animals entered the operant behavioral stall. A minimum of 3 days of control preceded each dose. During the control studies saline was injected immediately before the horse entered the behavioral stall.

**Ciba/Geigy Corp., Summit, NJ
***Merck & Co., Ltd., Philadelphia, PA

RESULTS

It turned out to be relatively easy to train horses in the use of the feeding apparatus. The oat reinforcements fall into a typical feed bucket which the horses inspected regularly. The first time the horse breaks the electric eye, the combined sound of the beeper and the oats falling down the pipe startle the animal. Oats, however, are very strong reinforcers and our horses overcame their normal cautious behavior and habituated to the apparatus quickly. It took about 2 weeks for the horses to master the fixed ratio schedule and respond at a high rate. At this point the horses were switched over to a variable interval schedule, and it took about 3-4 weeks for the horses to establish a stabilized response rate. The variable interval schedule was used because it had been reported to be a sensitive indicator of drug action in other animals.[5,6]

As shown in Fig. 2, the rates that were finally established on the variable interval schedule were different for each animal, some horses establishing high rates while others established much lower rates. This does not mean that the horses received different amounts of oats since each rate shown in Fig. 2 yielded about the same number of reinforcements over the 30-min test period. More important is the fact that after a horse established a rate it appeared to maintain it over a long period of time, as shown in Fig. 2. In this figure, the rates are recorded as responses per minute, and each one of the lines in Fig. 2 represents the response rate of an individual horse.

The sensitivity of the response rates to drug effects has been demonstrated in studies with reserpine and cocaine. The reserpine study was designed to determine the magnitude and duration of the effect of a single therapeutic dose, and in the cocaine study we wished to examine the acute effects of a wide range of doses administered directly before entering the operant box.

In the reserpine study, 4 horses were administered a single 5 mg dose IV the evening before the first test. When first tested, 12 hours post dosing, all horses had a depressed response rate and this depression continued to develop for about three days. This gradual onset of reserpine's effect is clearly shown in Fig. 3. The maximum depression in response rate occurred about 3 days post dosing the rates remained depressed for 7-10 days. Figure 3 also shows that all the horses returned to their original rates within 14 days. At this dose of 5 mg per horse, all the clinical signs of reserpinization had disappeared within 48-72 hours.

The data in both the reserpine and cocaine study is reported as percent change from control rates, and all the rates are mean rates. Mean response rates, by themselves may not tell the whole story since a rate may change during the 30-min test and/or there may be periods when the horse does not respond at all. To allow examination of these important variations a Harvard recorder was used, which produces a graphic representation of responses with time. Typical tracings of the Harvard recorder data is shown in Fig. 4 and represent typical experimental results from one horse.

In the experiment of Fig. 4, the horse made a total of 399 responses following a saline injection and its rate was relatively consistent throughout the 30-min test. The record labeled 40 hours post-reserpine has a lower slope, which reflects the depressed response rate and lower total of 246 responses after reserpinization. The record labeled 0.01 mg/kg cocaine shows a stimulated response rate where the total number of responses in 30 minutes was about 460, a 15% increase on control values.

In studies with higher doses of cocaine (0.75 mg/kg) we saw depressed rates of responding and, immediately after injection of the drug, no responding at all. This period where the animal fails to respond is referred to as 'lag time'. The lag time shown in Fig. 4 is about 5 minutes long. Longer lag times have been seen with higher doses; the overall effect of the lag time is to lower the total number of responses made by the horse.

The relationship between the dose of cocaine administered and the overall response rate is shown in Fig. 5. All the horses tested had the same type of biphasic dose-response curve, in that increasing the dose first caused an increased response rate, and then decreased it. The data also shows that there is a clear-cut difference in the sensitivity of individual horses to cocaine's effect. Further, the sensitivity of the method is such that the lowest doses of cocaine produced increases in VI responding rates without any change in the clinical appearance of the animals.

DISCUSSION

To date, few quantitative methodologies of measuring equine behavior and drug effects have been reported, and the data presented

here apparently represent the first application of operant conditioning to the study of drug effects in the horse. The data suggest that the method of variable interval responding is a sensitive and reproducible means of objectively measuring the effects of low doses of drugs having prominent CNS effects.

The data indicate that horses can be easily trained to respond on a variable interval schedule for food; that the response rate is a very sensitive indicator of drug effect; and that there are significant behavioral effects that are not readily detectable by clinical observation. The method is objective and quantitative and should be readily adaptable to studies of other drugs. The objectivity and reproducibility of the operant method creates a solid foundation for the results, regardless of their interpretation. The method also exposes to quantitative examination subtle drug effects not readily observable clinically.

Veterinary clinicians have long been aware that the clinical and behavioral effects of reserpine "fade into" normal behavior over a period placed by some[2] at more than two days. On the other hand, horsemen, familiar with reserpine in single horses over long periods believe that doses of reserpine of the magnitude used in this study affect horses over longer periods. The VI method reported here shows that clear-cut behavioral depression due to reserpine takes between three and five days to peak after dosing with reserpine and then up to 10 days for the effect to decay. These data clearly demonstrate behavioral effects which are easily missed on simple clinical inspection unless the observer is familiar with and inspects the animal over a long period of time.

This operant method also clearly demonstrates the differing sensitivity of individual horses to cocaine. Peak responses were produced by widely different doses, 0.001 mg/kg in the case of one horse, while 0.8 mg/kg was needed to produce peak response in another animal. This variability in dose required to produce peak response is substantial and emphasizes the importance of determining dosages of stimulant drugs for horses on an individual basis.

The second characteristic which shows clearly in the VI 60 system is the biphasic nature of the response to stimulant drugs. While low doses of cocaine produce stimulation, high doses produce inhibition of responding. This effect is characteristic of stimulant drugs and has been reported in other species.

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Table 1.

CONDITIONED HORSES

NUMBER, SYMBOL	NAME, BREED	WEIGHT	SEX	HISTORY
I ○	BALLARD STANDARDBRED	449 Kg	GELDING	HAS RACED
II ●	BEA STANDARDBRED	490 Kg	MARE	HAS RACED
III △	DOC STANDARDBRED	410 Kg	GELDING	HAS RACED
IV ▲	MULIE —	478 Kg	MARE	— ?
V □	NOELIE THOROUGHBRED	430 Kg	MARE	HAS RACED
VI ■	T-3 —	423 Kg	MARE	— ?

Figure 1 *Automatic Feeding Console.*
An oak console was built around a feed tub installed in the corner of a 13' x 13' box stall. Horses were conditioned to break the light beam for food reinforcements which were delivered through the small opening above and behind the bucket.

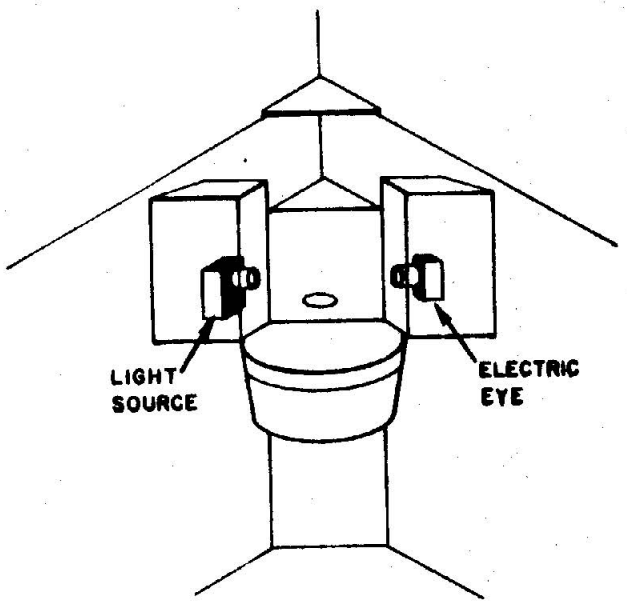


Figure 2 *Stability of Control Response Rates.*

Each symbol represents an individual horse and the horse's response rate for a particular day. Recording of control data began after these horses demonstrated less than a 10% difference over three consecutive days on a VI 60 schedule.

These horses were run at various times between August and December 1978. Datum points are missing except for the periods where the horses were used for drug studies.

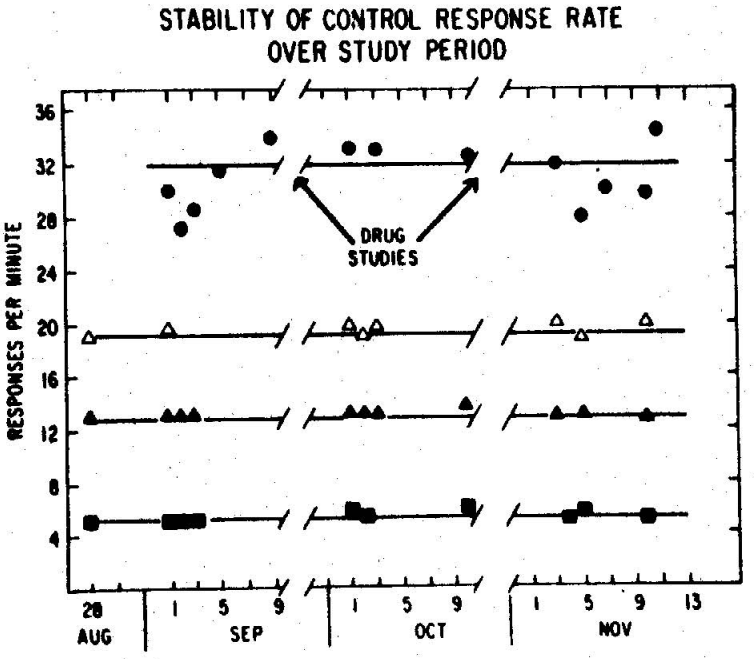


Figure 3 *Effects of reserpine on variable interval responding.*

The open circles (○-○) show operant behavior, normalized as 100% for four horses for five days prior to dosing. The open squares (□), solid circles (●-●) and open (△) and solid (▲) triangles show the reduction in operant behavior in these horses after a single dose of 5 mg of reserpine IV on indicated day zero.

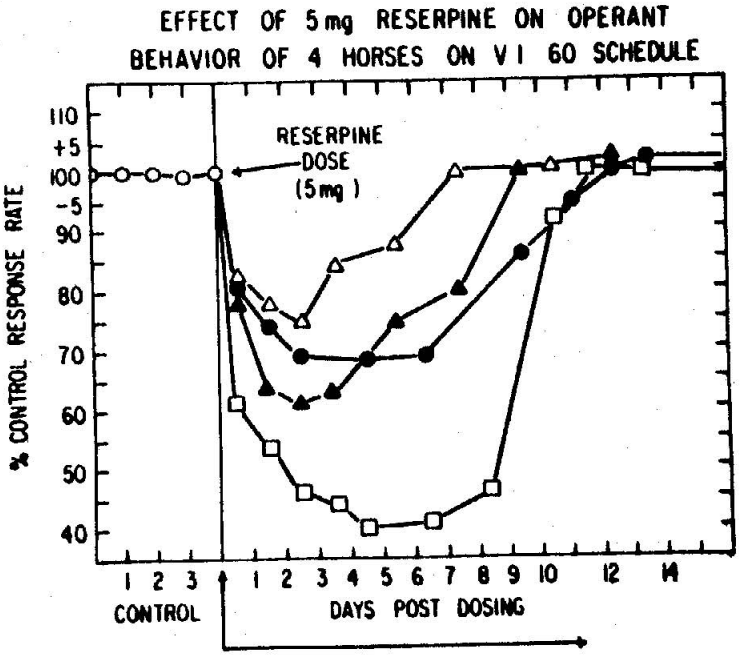


Figure 4 *Examples of drug effects on the control response rate.*

The Harvard recorder records the horse's performance over the 30-min test period. Each response the horse makes causes the recorder pen to move slightly upward on the slowly moving chart paper. The faster the response rate the steeper the slope. The delivery of reinforcements are recorded by the same pen as small downward deflections.

A typical tracing of one horse's control response rate and reinforcement frequency is labeled. Records of the same horse's response rate following drug administration are presented for comparison.

Two different doses of cocaine are administered, 0.01 and 0.25 mg/kg, immediately before entering the operant box, and a single 5 mg IV dose of reserpine 40 hours before testing.

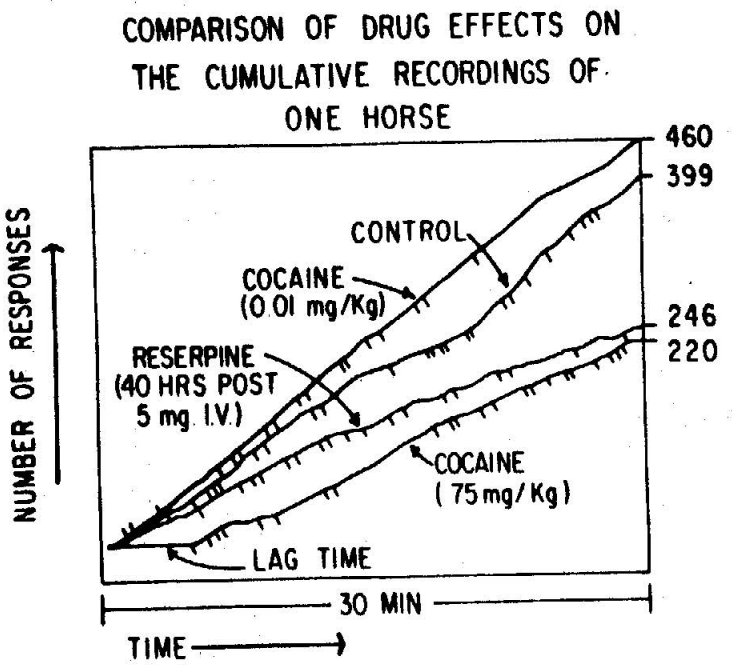
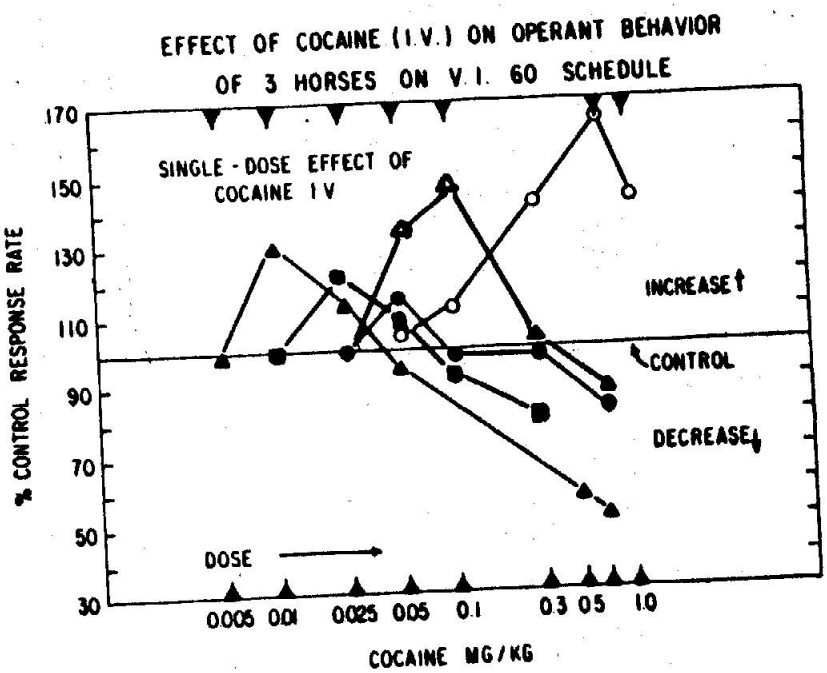


Figure 5 *Acute effects of cocaine (IV) on variable interval responding.*

A wide range of doses were administered (IV) to horses before they entered the operant box. The x-axis is in log dose units.

Before each dose was administered a three-day saline control was established. The results are reported as percent change from individual control rates and show that cocaine caused both increases and decreases in the overall response rate.



DISCUSSION

ALMASY: Since this is a conditioned behavior study, I just wondered if the control animals were given an injection of saline at the appropriate times?

SHULTS: Yes, there are quite a few behavioral pit-falls you can fall into. You can have behavioral tolerance to a situation, or you can have habituation. For all the drugs studied, we did an appropriate saline control with the appropriate volume and time indication. Before each dose of cocaine was administered we had a minimum three day control period with saline controls. If, during that control period, the horse's behavior fluctuated outside of an acceptable range which in most behavioral work was 10%, we held up the dosing.

MAYLIN: Have you used other innocuous drugs such as phenylbutazone, in this trial?

SHULTS: Yes, we have. What I am looking for in particular are treatments such as you mentioned which are thought to not have a central behavioral effect. I would like to uncover some untoward effects of typical medication practices. It is too early to comment on the phenylbutazone results.