VETERINARY REVIEW
Non-refereed articles and papers

OPERANT CONDITIONING AND ITS APPLICATIONS IN EQUINE PHARMACOLOGY

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SUMMARY

Studies of the effects of drugs on equine performance require access to sensitive methods of measuring subtle behavioral changes. Variable interval (VI) reinforcement scheduling is a specific type of operant conditioning that is sensitive to drug effects even when overt clinical signs of the drug have diminished. In our VI studies, horses were conditioned to break a light beam with a head-bobbing movement and this behavior was reinforced with a reward of clean oats (approximately 30 mg/reinforcement). Initial training procedures included acclimatization to the behavioral equipment and fixed-ratio reinforcement scheduling. To establish baseline rates of behavior the horses were converted to a variable interval (60 seconds) reinforcement schedule and were kept on this schedule for the remainder of the experiments. Daily sessions lasted 30 minutes and responding rates remained remarkably stable even after long periods of inactivity. Responses and reinforcements were recorded and dispensed by use of an electromechanical relay system wired to an electric eye, an automatic feeder and a programming and recording system. Recently, responding rates from horses administered detomidine, hordenine, xylazine and saline were monitored and compared to responding rates in horses from previous studies using acepromazine, cocaine, methylphenidate, phenylbutazone and reserpine. Rates of behavior in these operant-conditioned horses were remarkably stable over time and readily detected subclinical pharmacological effects of drug administrations.

INTRODUCTION

Behavioral pharmacology and toxicology are relatively new scientific disciplines that have sought to answer questions concerning overt as well as subtle effects of drugs. Numerous methods of behavior monitoring are well suited for quantifying obvious clinical effects of drugs but are not sensitive enough to detect subtle changes in behavior. Operant conditioning is a strategy that has been employed to measure behavioral changes in laboratory animals.[15] A specific type of this form of conditioning is variable interval (VI) reinforcement scheduling, which is especially effective in measuring changes in rates of behavior.[2,10]

In this type of conditioning, the animal is first trained to perform a specific task for an appropriate reinforcement. By definition, the task performed is a behavior that the animal learns to perform and is not part of the animal's normal behavior pattern. There is no eliciting stimuli preceding the behavior, but rather the behavior is enhanced or reinforced by the stimuli proceeding it. In this way, the operant behavior is maintained by its own consequences, i.e., the reinforcement. However, before the operant behavior can be maintained or increased, one must wait for, or manipulate the occurance of the behavior.

Since one must wait for the occurrence of the operant response before reinforcing it, the behavior must first be created. To create the desired response, a process known as shaping is employed. The animal's behavior is shaped by reinforcing behavior that approximates the desired response until the operant response is performed. Thereafter, only the operant behavior desired is reinforced.

The animal will quickly learn the operant response and that an increase in the rate of that behavior will earn more reinforcers. After shaping the operant behavior by reinforcing each response, a fixed-ratio schedule of reinforcement can be instituted. One begins by reinforcing every other response and then gradually increasing the ratio. Once the ratio is as high as 10 responses to 1 reinforcer, the animal can be converted to a variable interval schedule.

In the variable interval method of scheduling, a response is not reinforced until a random period of time has elapsed. The program equipment is designed to reinforce a response after a variable amount of time. In the case of a VI 60 second schedule, a response is reinforced after an average of 60 seconds. In this way, the animal does not know when a response will be reinforced and will develop a unique and stable rate of behavior.

Rates of behavior have been studied and used as parameters for measurement of central nervous system excitation or depression in numerous experiments using

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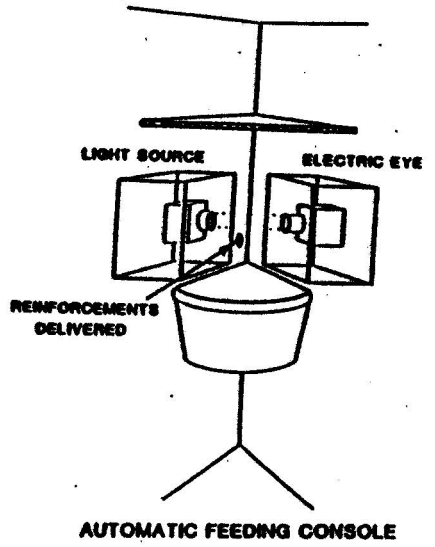

Figure 1. The automatic feeding console which was constructed in a standard box stall. All programming equipment was housed in an adjacent office.

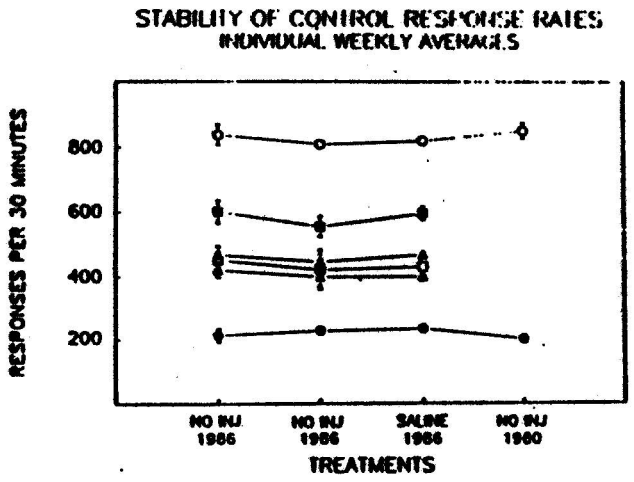

Figure 2. Stability of control rates measured as responses per 30 minute session. Each point represents the weekly average for one horse, with or without a saline injection. In addition, control rates for two horses that were used in studies in both 1980 and 1986 are shown.

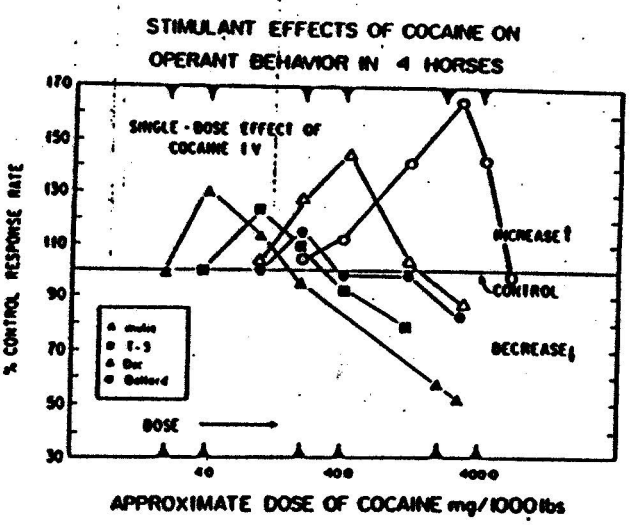

Figure 3. Effects of different doses of cocaine on operant behavior in horses. The points represent the percent change from control values for each horse. Behavioral sessions were conducted 20 minutes after an IV bolus injection. Reprinted with permission from Shults et al., *Am J Vet Res* 43:1143-1146,1982.

laboratory animals.[15] Few studies have been conducted using the horse as the subject animal, although promising results have been obtained.[16,17]

Our laboratory has conducted 2 series of drug studies using operant conditioned horses. The first was performed in 1980 and the second in 1986. We have analyzed and compared the data generated by both studies to review the applications of operant conditioning in equine pharmacology.

MATERIALS AND METHODS

Horses

The 6 horses used in 1986 represent different ages and breeds, as well as different individual responding rates (Table 1). Two horses (numbers 1 and 3) were previously

Table 1. Summary of the data on the horses used in 1986. Horses 1 and 3 were used in similar studies in 1980.

Horse	Weight (kg)	Age (years)	Breed	Control rate Responses/30 min
1	590	14	Standardbred	816
2	520	5	Thoroughbred	595
3	525	12	Mixed	235
4	480	15	Mixed	398
5	370	1	Thoroughbred	468
6	490	8	Thoroughbred	430

trained in this operant behavior in 1980. All horses weighed between 370 and 590 kg and were routinely maintained at pasture. They were brought daily to the research barn and allowed to relax in individual box stalls with hay and water before their experimental sessions.

Programming equipment and apparatus

The horses were trained initially to break a light beam with a head-bobbing movement by reinforcing this behavior with approximately 30 mg of oats dispensed directly into a feed bucket by an automatic feed dispenser. The light-activated feeding console was built into a corner of a box stall and consisted of a normal feed bucket, a light source, and an electric eye (Figure 1). The feed dispenser and the programming and monitoring equipment were housed in an adjacent office.

Programming the horse

In initial training sessions a horse, through random exploratory behavior, breaks the light beam and triggers the mechanism to dispense a reinforcer. If a horse was reluctant to break the light beam or was startled by the dispensing mechanism, then the behavior had to be shaped. This was accomplished by reinforcing any movement toward the feed bucket until the horse subsequently overcame its reluctance or fear of the mechanism. The operant response (bobbing of the head to break the beam) could then be reinforced.

The ratio of responses to reinforcements was gradually increased from 1/1 to 10/1, depending on how quickly the horse learned to operate the apparatus. The time course for training a totally naive horse to achieve stable responding rates at the 10/1 fixed-ratio schedule was, on average, five weeks. Once the horse was schooled to a fixed-ratio, the variable interval reinforcement schedule was instituted.

The previously trained horses did not require acclimitization and, in fact, began responding almost immediately when placed in the experimental stall. Their retained knowledge of the system allowed them to be placed on a VI schedule within a week. When changed to the variable reinforcement schedule, all of the horses used in 1986 required an additional three or four weeks of training to establish stable baseline rates of behavior.

RESULTS AND DISCUSSION

Figure 2 shows the baseline responding rates (with or without a saline injection) for the six horses used in the 1986 study. This ability of the horse to establish consistent baselines when presented with the VI reinforcement schedule allows for the detection of subclinical effects of pharmacological agents. Figure 2 also reveals the remarkably similar baseline rates of horses that were first trained in 1980 when compared to their baseline rates in 1986. Despite an interval of six years between training sessions, both horses had almost identical baseline rates as in 1980.

While the individual baseline rates were consistent, a problem arises when looking at the group as a whole. Figure 2 shows the large variability (a low of 200 to a high of 800 responses per 30 minutes) between baseline rates for each horse. This variability requires each horse to serve as its own control in drug administration studies. This is usually accomplished by employing within-subject experimental designs, with all horses receiving all treatment. Care must be taken, however, to counterbalance the treatment schedules to eliminate any confounding of the data associated with the time of the treatment. The data from the group can then be normalized and expressed as a percentage of control values.

In order for this practice of expressing the data from the group as a percentage to be statistically sound, each horse must have an equal opportunity to show an increase or decrease in responding rates. In the case of horse number 1, a drug or treatment might not produce a proportionally equal increase in behavior in a horse that responds at such a high baseline rate. In fact, recent studies in operant conditioning have shown that an individual animal's baseline rate can be a factor in its response to drug treatment.[3,8,10,14] This potential to confound the data (statisticians call this a nuisance variable) must be taken into account when interpreting the data.

While the horse's individual baseline appears to be less of a factor in the effects of depressant drugs, it would appear to be a confounding variable when studying stimulant drugs. This potential confounding of the data is illustrated in the effects of two stimulants, cocaine and methylphenidate.

Figure 3 illustrates the variability of responses that individual animals can exhibit after drug administration. In a 1980 study (Fig 3), four operant conditioned horses were administered various doses of cocaine 10 minutes prior to their behavioral sessions[17]. Two horses showed dramatic increases in responding rates after a relatively high dose, while the other two showed decreases in responding rates after the same dose. Examination of the baseline rates of these horses would indicate that this variability in response may be related to their normal rate of behavior as the horse with the lowest baseline rate was also the most sensitive to cocaine administration.

Recently, the effects of cocaine on brain neurotransmission have been shown to be far more complex or variable than previously thought[13]. The mechanism(s) of action of cocaine on equine brain tissue is unclear and the

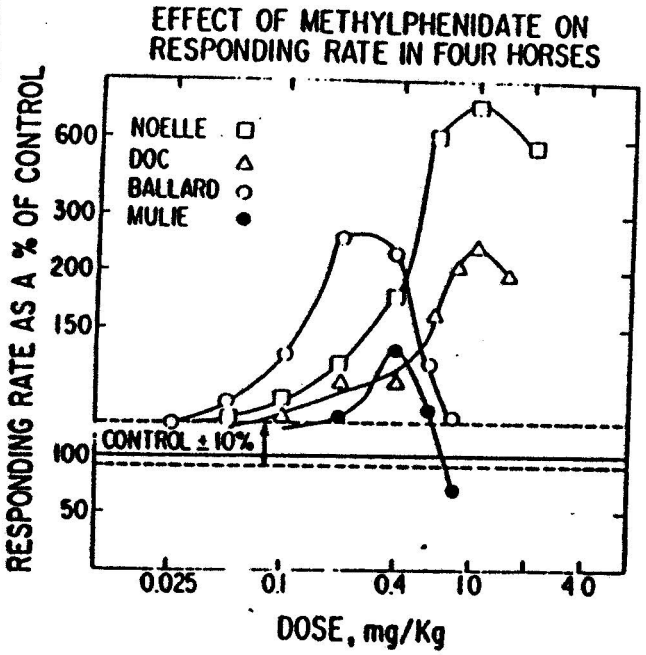

Figure 4. Effects of methylphenidate on responding rates in 4 horses. Each point denotes the percent change from control values for the different doses. The drug was administered by IV bolus injection 30 minutes prior to the experimental sessions. Reprinted with permission from Shults et al., *Am J Vet Res* 42:722-726, 1981.

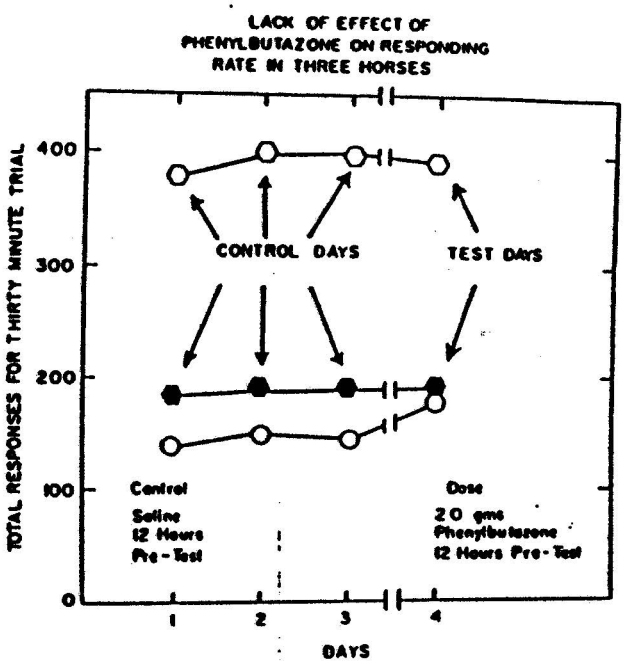

Figure 5. Responding rates were measured in 3 horses 12 hours after an IV bolus injection of 2 grams of phenylbutazone. The change in response rates were not statistically significant, even though the low responding horse did show a slight increase. Reproduced with permission from Tobin, *Drugs and the Performance Horse*, 1981.

reason or reasons for the different responses to cocaine from horse to horse remain unknown at this time. However, the use of operant conditioned response rates enabled the investigators to detect this variability of the effects of cocaine in the horse.

Figure 4 shows the effects of IV administration of methylphenidate (Ritalin®) on rates of behavior when given 20 minutes prior to testing[16]. Methylphenidate is known to have central nervous system stimulatory effects similar to amphetamine, but is believed to be more specific for thought processes than motor functions[8]. If, indeed, thought processes and not motor functions are stimulated, then the animal is more likely to show an increase in behavior rates measured in this manner. This may account for the fact that methylphenidate-treated horses showed the largest change in responding rates seen in any of the studies. This data would tend to support the theory that methylphenidate increases the mind's ability to concentrate on one task.

Of special interest is the fact that 3 of the 4 horses showed expected increases in responding rates, while 1 horse showed a decrease when the dosage was increased. The horse that was most sensitive to methylphenidate was also the most sensitive to cocaine and had a very low (< 200 responses per 30 minutes) baseline rate of behavior. Unfortunately, none of the horses tested with the stimulant drugs had exceptionally high baseline rates so that a comparison to a low-responding horse cannot be made.

We also examined the results of studies examining the effects on rates of behavior of 2 drugs that are not known as stimulants or depressants.

Phenylbutazone is a nonsteriodal anti-inflammatory agent that is widely used in veterinary medicine and is generally not considered as a stimulant or depressant.[19] The effects on the rates of behavior of 3 horses administered 2 grams of "bute" IV 12 hours' before their sessions are shown in Figure 5. As expected, there was little or no change in the rates, although the horse with the lowest control rate of behavior did show a slight increase. This would be consistent with the atypical response seen in the low-responding horse in the studies with cocaine and methylphenidate. It would appear that horses with unusually low baseline rates might react abnormally to drug administration and the following study shows that a high-responding horse may also respond atypically.

Table 2 shows the changes in response rates in our operant-conditioned horses after hordenine administration.[6] Hordenine is an alkaloid found in many plants, including grains and barley.[18] As such, it is likely that it

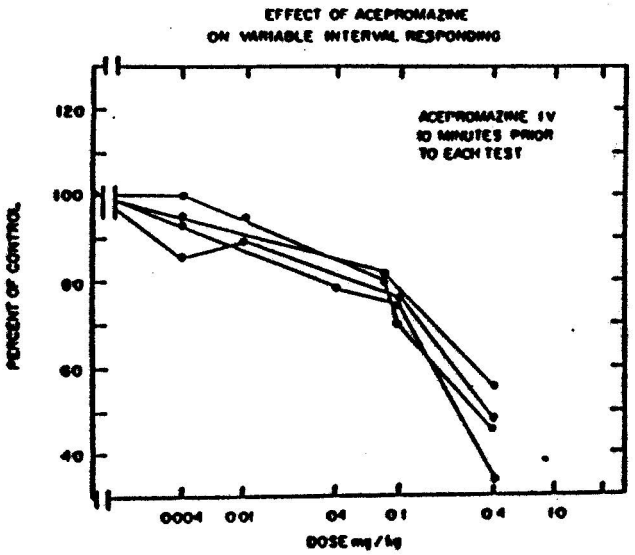

Figure 6. Dose-response effects of acepromazine measured by percent change of responding rates in 4 horses. Behavioral sessions were conducted 10 minutes after an IV bolus injection. Reproduced with permission from Tobin. *Drugs and the Performance Horse*. 1981.

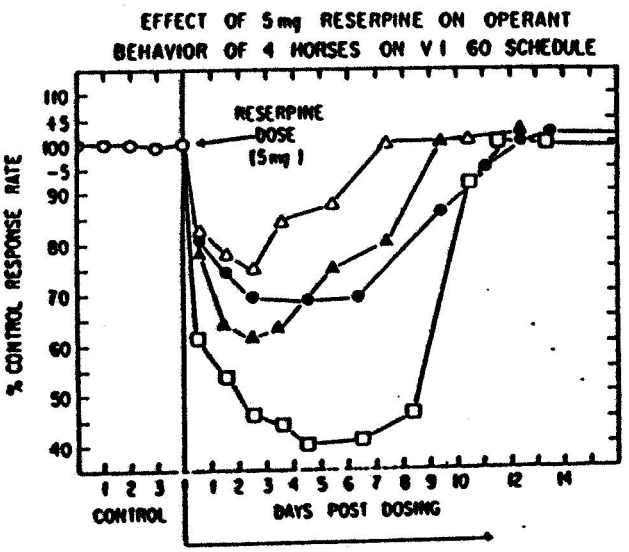

Figure 7. Decrease in rates of behavior seen in 4 horses after IV bolus administration of 5 mg of reserpine per horse. The data shows that responding rates can be depressed for as long as 10 days with this dose. Reproduced with permission from Shults et al., *Am J Vet Res* 43:1143-1146.1982.

would be found in some equine feeds and has been reportedly found in an occasional post-race urine sample.[1] At this time, many racing jurisdictions are undecided as to whether or not to consider hordenine a prohibited substance.

Our initial pharmacological studies with hordenine showed transient cardiovascular and respiratory stimulatory effects following IV administration.[6] However, when injected with the drug 30 minutes prior to experimental sessions, our horses showed decreased, but not statistically significant, changes in responding rates. Here again, the lesson of analyzing the data with a critical eye on the animals who respond at very high or low rates must be realized. The horse with the highest control rate did show a much larger decrease in its rate of behavior. It is reasonable to suspect, and this data supports, that a horse responding at a rate of above 700 responses per 30 minutes is capable of showing a subtle decrease in that rate regardless of the pharmacological agent used.

Studies on the effects of depressant drugs on equine behavior rates have provided more consistent results. One example of the this consistency can be seen in the results of the study illustrated in Figure 6.[11] This study was conducted in 1980 on the effects of acepromazine. Acepromazine is classified as a sedative for veterinary use and

Table 2. Effect of hordenine on variable interval responding.

Horse	Control	Saline	Hordenine
A	718±66	727±172	629±49
B	468±38	471±75	463±46
C	420±43	413±43	395±19
D	450±47	430±115	424±22
E	213±22	214±36	204±214

Horses were treated with 2.0 mg/kg hordenine or saline by bolus IV injection 30 minutes prior to their behavioral sessions. Each point represents the average number of responses per 30 minute session ± the standard deviation. Analysis of the data by ANOVA showed no significant differences at the P<.05 level. Reproduced with permission from Frank et al., Equine Vet. J. 1987.

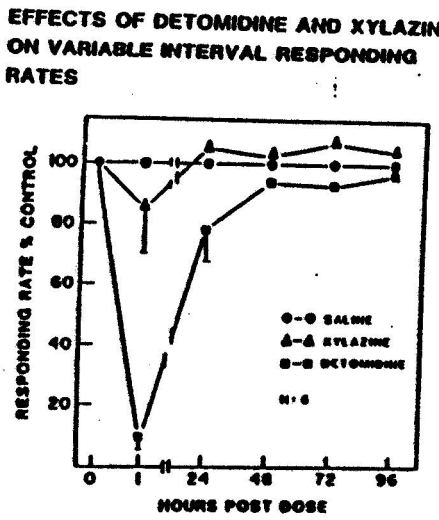

Figure 8. Percent of control response rates measured in six horses after IV bolus injections of 0.40 µg detomidine/kg body wt., 1.1 mg xylazine/kg body wt. and 10 ml saline per horse. All horses received all treatments in a Latin square cross over design. The 1 hour xylazine and 1 and 24 hour detomidine points are statistically significant at the $p<.05$ level. Reproduced with permission from Wood et al. *Equine Vet J* 20:320-322,1988.

causes sedation by blocking alpha-1 post synaptic receptors.[5] In this study, all horses showed decreased responding rates 10 minutes after IV injection in a dose-dependent manner. The baseline rate of the individuals apparently had little effect in this study as the decreases in response rates were similar in all horses. The lack of the baseline rates of the individual to be a confounding factor in response to sedative agents is illustrated in 2 other such studies.

Reserpine is a depressant drug that acts by depleting neuro-transmitters in the brain.[5] It is rarely used in human medicine at the present time, but has reportedly been used by trainers to "take the edge off" a nervous or fractious horse 4 or 5 days before an athletic event.[19] In 1980, the time course of reserpine's effects on behavior was examined. Figure 7 reveals that after a single IV dose of 5 mg of reserpine, responding rates were depressed for as long as 10 days.[17] Until the use of operant conditioning to quantify rates of behavior, such long term effects of reserpine could not be objectively detected in the horse.

Two other sedative agents were investigated for such long term effects in 1986. Xylazine (Rompun) is a widely used sedative in veterinarian medicine and causes a decrease in awareness of the animal by binding to alpha-2 presynaptic adrenoreceptors.[4] Detomidine (Domodesan) is a newly developed sedative agent that has not as yet been approved for use in the U.S. It is pharmacodynamically related to xylazine, although preliminary studies have shown that detomidine is more specific in its agonism for alpha-2 receptors in the brain, with less peripheral receptor binding than xylazine.[7,12]

Figure 8 illustrates the effects of IV administration of a therapeutic dose of xylazine and detomidine on responding rates.

Detomidine is clearly the more potent of the 2 agents and this study reveals that a therapeutic dose can have a depressant effect on behavior 24 hours after administration. In addition, the study shows that xylazine is a quick acting drug devoid of the long range effects seen with reserpine and, to lesser extent, with detomidine.

While there was variability seen in the individual responses to acepromazine, detomidine, reserpine and xylazine, there was no evidence to suggest that the horse's natural rate of behavior was an influence.

Operant conditioning, or more specifically variable interval responding, is a validated method for detecting subtle changes in behavior. It should be pointed out, however, that operant conditioning is not well suited for quantifying obvious or overt clinical effects of drug administration. Several techniques can be employed to study the clinical effects of drugs on behavior in the horse, but analysis of responding rates should be reserved for detecting long range or subtle changes. In fact, these subclinical effects are what is usually desired in cases of illegal drug use in the equine athlete.

The potential applications of operant conditioning in equine pharmacology or toxicology are numerous and promising. We have discussed several of these applications here, but there remain many that have not been fully explored. As a method of detecting subtle or subclinical changes in behavior the results have shown it to be both sensitive and informative. However, care must be taken to avoid a misinterpretation or oversimplification of important data. As pharmacological agents and their use become more sophisticated, the equine pharmacologist must also employ more sophisticated methods to accurately determine their effects on the equine athlete. The use of operant conditioning shows considerable potential in this area, as well as being a method of expanding mankind's knowledge of the behavior of the horse.

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POTOMAC HORSE FEVER NOW IN WESTERN CANADA

A serological diagnosis of Potomac horses fever, or equine monocytic ehrlichiosis (EME), was made in ten horses in Alberta. These horses were clustered within a six mile radius in an area east of Red Deer close to Red Deer River. They all had clinical signs of fever, depression, anorexia, and acute diarrhea.

Thirteen horses were affected; one horse died. Ten horses were tested serologically. Theses horses all had high titers to *Ehrlichia risticii* as tested by the indirect fluorescent antibody test (IFAT) at the University of Illinois.

Potomac horse fever was first recognized in Maryland in 1979 and is now seen in most parts of the United States . This disease may have occurred within Alberta before, but this was the first time it was confirmed by testing.

Horses selected for the serological test should have the clinical signs described above. Serum should be collected as acute and four week convalescent samples, and should be submitted as paired samples.

Treatment in suspected cases of Potomac horse fever is symptomatic and directed against the fluid and electrolyte imbalances. Potentiated sulfonamides have been beneficial. Tetracycline, which is used successfully in the treatment of canine ehrlichiosis, must be used with caution in horses because it can trigger acute enteric salmonellosis. A licensed vaccine against Potomac horse fever is available.

Potomac horse fever occurs in at least 32 states, in Europe and in parts of Canada. It is more common near larger rivers and has been reported along the Mississippi River in Iowa during the past 2-3 years. The vector is not known but may be a tick or other insect. Now is the season to begin client education programs. (From Iowa State University Extension Service).

OVULATION PREDICTION USING RAPID PROGESTERONE ASSAYS.

A quantitative enzyme-linked immunoassay (ELISA) for progesterone was evaluated for determining the day of ovulation during 143 estrous cycles of 81 fertile and 11 sub-fertile 10-year-old Quarter Horses mares. Estrous cycles were determined by regular testing and the days of ovulation were determined by rectal palpation and ultrasonography.

Plasma samples taken daily from beginning of estrus through 48 hours following ovulation were assayed with a rapid progesterone assay (Ovucheck, Cambridge Life Sciences, Cambridge, England) to determine if the kits could be used to accurately indicate the time of ovulation. By 24 hours following ovulation, progesterone levels had increased by at least .5 ng/ml over initial values in 55% of the fertile mares and 33% of the sub-fertile mares. By 48 hours following ovulation, progesterone levels had increased by at least .5 ng/ml over initial levels in 89% of the fertile mares and 50% of the sub-fertile mares. Rapid progesterone assays can be used to document that ovulation has occurred in mares. (From *Veterinary News*, Pennsylvania State University.

NEW TEST FOR LYME DISEASE

Lyme disease is the most commonly diagnosed tick-borne human disease AND has been documented in at least 37 states. The numbers that have been detected with this