QUANTITATION OF THE LOCOMOTOR EFFECT OF THERAPEUTIC MEDICATION IN A BEHAVIOUR CHAMBER: A PRELIMINARY REPORT

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ABSTRACT

To establish highest no-effect doses and no-effect thresholds for stimulant or depressant medications, sensitive methods of measuring behavioural responses to these agents must be developed. This paper reports the use of a behaviour chamber to quantify locomotor activity in the horse. A standard 3.4 x 3.4 m box stall was converted into a locomotor chamber. Outside stimuli were reduced by adding sound-proofing material and covering a portion of the window. Light entering through the upper window maintained the horse's normal circadian rhythm. An air-conditioner and fan ensured ventilation and created 'white noise'.

Locomotor activity was detected by 4 mini-beam sensors spaced equally around the stall, 45 cm above the floor. Each disruption of the beam yields a score; total output from the sensors was recorded on a data logger every 5 min. Normal activity varied throughout the day, horses being more active during daylight, reflecting activity in their surroundings. An 'average' horse yields approximately 15 counts/min.

A number of medications (isoxsuprine, xylazine, fentanyl, acepromazine, cocaine, amitraz and amitraz with yohimbine) were administered iv and locomotor activity was recorded during the following 24 h. Xylazine (1 mg/kg bwt) reduced and fentanyl (0.016 mg/kg bwt) increased locomotor activity significantly, validating the behaviour chamber as a suitable tool for measuring spontaneous locomotor activity in the horse.

Amitraz administration (0.05, 0.10 and 0.15 mg/kg bwt) resulted in virtual inactivity for up to 5 h at the higher doses. Yohimbine (0.12 mg/kg bwt) injected 1 h after amitraz (0.15 mg/kg bwt) reversed its sedative effect immediately. Isoxsuprine increased spontaneous activity significantly between 15 and 105 min after injection.

There was no significant effect on spontaneous activity following iv injection of 0.3 and 1.0 mg acepromazine. However, there was a significant decrease in activity when the dose was increased to 3.0 and 9.0 mg. Neither 15 nor 45 mg of cocaine affected locomotor activity significantly, suggesting that larger doses are required for a significant effect.

INTRODUCTION

To establish highest no-effect doses and no-effect thresholds for medications that may stimulate or depress physical activity, sensitive methods of measuring behavioural responses to these agents must be developed. To establish thresholds for therapeutic medications in performance horses, the cut-offs must be low enough to ensure that there is no effect on performance.

Ideally, the best method to determine the effect of a drug on a horse's locomotor ability is matched competitions against peers where all factors are equal except for the presence or absence of the drug in question. Because of the variability in weather, surface conditions, physical and mental condition of the horses, human influences (rider, trainer, groom, farrier and competitors), performance conditions can not be duplicated exactly. Although it is not possible to reproduce an athletic competition in the laboratory, the controlled atmosphere can eliminate many of the uncontollable factors associated with equine performance.

When horses are placed in a delineated area, they move around spontaneously and explore that environment. This movement is defined as spontaneous locomotor activity (Kamerling and Owens 1994). A method for quantitating the effect of stimulant and depressant drugs on spontaneous locomotor activity was first presented by Tobin *et al.* (1979). The tested horse was isolated in a partially-shielded box-stall. An observer, watching

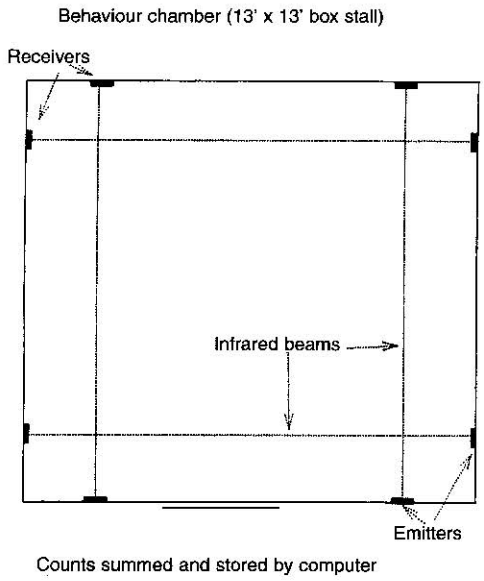

Fig 1: Diagram of behaviour chamber with sensors spaced around the stall to measure activity.

through a small, one-way glass window, counted the number of times the horse lifted its left front leg as a measurement of spontaneous activity during a 2 min period.

Kamerling *et al.* (1988) automated the activity measurement with photoelectric counters which emitted continuous beams of infrared light. When the light was interrupted by the horse moving, a count was scored. This paper reveiws the measurement of spontaneous locomotor activity in a locomotor chamber similar to that described by Kamerling *et al.* (1988).

MATERIALS AND METHODS

Horses

Six mature Thoroughbred mares weighing 413–602 kg were used. They were maintained on grass hay and feed (12% protein), which is a 50:50 mixture of oats and an alfalfa-based protein pellet. They were vaccinated annually for tetanus and de-wormed quarterly. A routine clinical examination confirmed that the animals were healthy and sound. During experimentation, horses were given water and hay *ad libitum.*

Behaviour chamber

A 3.4 x 3.4 m box stall was converted into the behaviour chamber. Outside stimuli were reduced by adding insulation for sound-proofing and covering the lower portion of the window to prevent the horse from seeing out of the stall. Outside light entered through the upper window so that the horse's normal circadian rhythm was not altered. An air-conditioner and circulation fan were used to provide adequate ventilation and create 'white noise'.

The activity of the horse was detected by 4 mini-beam sensors (SM31E and SM2A31R, Banner Engineering, Minnesota, USA) spaced equally around the stall and recessed into the wall 45 cm above the dirt floor (Fig 1). Each time the horse disrupted the beam of light, a count was scored. The total output from the 4 sensors was recorded on a data logger (CR10, Campbell Scientific, Inc, Utah, USA) every 5 min.

Experimental design

Horses were placed in the behaviour chamber at 06.00 h, 2 h prior to drug administration at 08.00 h. Locomotor data were collected from 07.00 to 08.00 h to determine a baseline for spontaneous activity. The total number of times the sensors were activated was averaged over a 15 min period, and the results were expressed as step counts per 5 min. Horses were used as their own controls. At least 7 days elapsed before a horse was used in a subsequent experiment.

Drug administration

Isoxsuprine HCl (Sigma Chemical Co, Missouri, USA) was dissolved in 50 ml sterile water and 25 ml 95% ethanol. The solution was filtered through a sterile millipore filter (Cameo 25NS, Micron Separations, Inc, Massachusetts, USA), and injected iv at 2.0 mg/kg bwt within 1 min. Control horses were injected with 50 ml sterile water and 25 ml 95% ethanol, also filtered through a millipore filter.

Amitraz (Sintesul S.A., Brazil) was injected iv at 0.05, 0.1 and 0.15 mg/kg bwt. Xylazine HCl (1 mg/kg bwt) was injected as the negative control, and fentanyl citrate (0.016 mg/kg bwt) was injected as the positive control. In a separate experiment, yohimbine HCl (0.12 mg/kg bwt) was injected following injection of amitraz (0.15 mg/kg bwt) to assess its reversal effect.

In other experiments, acepromazine maleate was administered iv at 0.3, 3.0 and 9.0 mg. Similarly, cocaine HCl was injected iv at 5.0, 15.0 and 45.0 mg to assess its effect on locomotor activity.

Statistical analysis

Analysis of variance with repeated measures was used to compare locomotor activity following

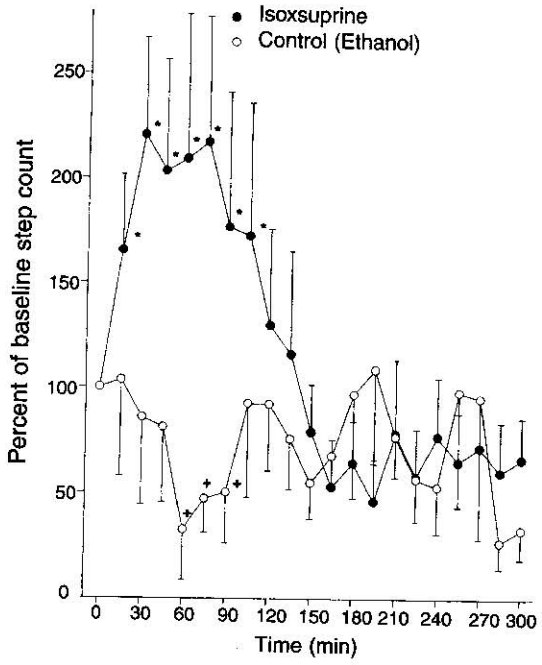

*Fig 2: % change in step count following injection of isoxsuprine. *Significantly different from control values; +Significantly different from control baseline values. (Reproduced with permission from* Equine vet. J.*)*

control and drug treatments. Significance was set at $P<0.05$.

Results

Table 1 shows the normal diurnal activity of pooled control data from 4 experiments (n=18). Normal activity of control horses was greater during daytime than the evening (Table 1). Normal activity data were obtained from the same horses used for this study.

Xylazine reduced spontaneous locomotor activity significantly immediately after injection. This effect persisted for about 60 min with peak reduction at 15 min. In contrast, fentanyl increased locomotor activity significantly for 45 min after administration.

Amitraz produced a significant and dose-dependent decrease in locomotor activity. All doses reduced activity to near-zero. The 0.05 mg/kg bwt dose lasted about 2 h, and the 0.15 mg/kg bwt dose for about 5 h after injection. In a seperate experiment, yohimbine (0.12 mg/kg bwt) was injected 60 min after amitraz treatment (0.15 mg/kg bwt) and immediately reversed its sedative effect.

Figure 2 shows the effect of iv isoxsuprine on spontaneous activity. There was a significant increase in activity from control values 15–105 min after administration. Peak activity occurred at 30–75 min. Compared to baseline values, there was a significant decrease in the activity of control horses from 60–90 min following administration.

There was no significant effect on spontaneous activity following injection of 0.3 or 1.0 mg acepromazine. However, there was a significant decrease in activity after administration of 3.0 and 9.0 mg. None of the cocaine doses affected locomotor activity significantly.

Discussion

The increased daytime activity of control horses was partially influenced, at least, by increased activity in the immediate area. Although the behaviour chamber was insulated, and white noise was provided by a fan and air conditioner, the horses were not completely isolated from outside stimuli. Control treatments (iv injections of saline) were administered at 08.00 h, which usually increased activity for several minutes. Average daytime activity (~70 counts/5 min) remained fairly constant from about 08.15 to 14.15 h. The horses were fed grain at about 15.00 h, and activity probably reduced during the time of feeding. However, horses in an adjacent paddock were normally fed earlier, and the fact that the experimental horses were aware of this was reflected by increased activity from about 14.15 to 15.00 h. After feeding, the staff left the area for the day. From about 15.30 to 06.00 h, activity decreased gradually or remained constant. These data were collected from late spring to early fall and sunrise during that period occurred at 05.45 to 06.30 h The horses were fed again at about 07.00 h, which again partially explains the

TABLE 1: Spontaneous locomotor activity of control horses (n=18) during a 24 h period

Time of day	08.00 h	10.00 h	12.00 h	15.00 h	17.00 h	20.00 h	02.00 h	04.00 h	07.00 h
Extraneous activity	Injection of water/ethanol			Feed					Feed
Step count (per 5 min)	130	70	65	150	45	30	15	40	200

increased activity at this time (Table 1).

Data from the isoxsuprine study (Fig 2) illustrate the rapid onset and short duration of effects following iv administration. In light of the long detection times (30 days) for isoxsuprine, this illustrates the need for a 'threshold value' for this agent.

Results obtained following administration of fentanyl and xylazine show that the behaviour chamber is adequate for measuring variations in spontaneous activity. It enabled investigators to determine the average time of onset and duration of an agent's effect on movement.

Amitraz produced almost total inactivity at all 3 doses administered. Because total inactivity is the maximal expression of sedation, it was the duration, rather than the intensity, of sedation that was manifested by the chamber.

The acepromazine study is on-going, and these data show only preliminary findings following administration to a small number of horses. The lack of any effect following administration of 0.3 and 1.0 mg was expected (Tobin 1981). The significant effect following administration of 3.0 mg persisted for about 3.5 h. There may be some increased activity several hours after administration of acepromazine. For the 3 smaller doses, the activity curves merge about 5 h after treatment.

The cocaine data are also preliminary. However, it appears that a dose greater than 45 mg is required to affect locomotor activity significantly. Harkins *et al.* (1996) showed that 45 mg injected as a nerve block was required for a significant local anaesthetic effect from cocaine. Interestingly, after the nerve block, there was increased pain accommodation in the contralateral leg for 2 of the 4 horses, 30 min post injection, which raised the issue of central effects from such a high dose. The other 2 horses demonstrated no increased pain tolerance. Therefore, there may be individual differences between horses regarding systemic sensitivity to cocaine, as suggested by Shults *et al.* (1982).

It is natural for a horse to explore its surroundings. Although there was a wide variation, the average activity of horses equated to about 90 counts/5 min. This is similar to an average of 20–30 counts/min reported by Kamerling and Owens (1994). The natural variation between horses can be used to the investigator's advantage. Horses showing limited activity are ideal for testing stimulant medications, and horses that are overly active are better suited for testing depresant agents.

Horses tend to be more active when first placed in the chamber. Therefore, a specific period is required for adaptation. Horses in this study were placed in the chamber 1 h before data collection.

A noticeable difference was observed between daytime and evening activity, with horses being more active during daylight. Table 1 illustrates the activity differences during a 24 h period.

In summary, the behaviour chamber is a useful and sensitive tool for measuring spontaneous locomotor activity in the horse and the effects of differing agents on this activity. As such, it should be applicable to determining highest no-effect doses and no-effect thresholds for therapeutic medication and environmental and dietary substances in racehorses.

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