Development and characterization of an equine behaviour chamber and the effects of amitraz and detomidine on spontaneous locomotor activity

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This report describes the development of a behaviour chamber and the validation of the chamber to measure locomotor activity of a horse. Locomotor activity was detected by four Mini-beam sensors and recorded on a data logger every 5 min for 22 h. Horses were more active during daytime than in the evening, which was at least partially related to human activity in their surroundings. To validate the ability of the chambers to detect changes in activity, fentanyl citrate and xylazine HCl, agents well-characterized as a stimulant and a depressant, respectively, were administered to five horses. Fentanyl citrate (0.016 mg/kg) significantly increased locomotor activity which persisted for 30 min. Xylazine HCl (1 mg/kg) significantly reduced locomotor activity for 90 min. Amitraz produced a dose-dependent decrease in locomotor activity, lasting 75 min for the 0.05 mg/kg dose, 120 min for the 0.10 mg/kg dose, and 180 min for the 0.15 mg/kg dose. In a separate experiment, yohimbine administration immediately reversed the sedative effect of amitraz. This suggests there is a similarity in the mode of action of amitraz, xylazine and detomidine, as yohimbine acts primarily by blocking central α 2 -adrenoceptors that are stimulated by agents like xylazine. There was also a significant decrease in locomotor activity following injection of detomidine (0.02, 0.04 and 0.08 mg/kg) for 1.5, 3.5 and 5.0 h, respectively. The locomotor chamber is a useful, sensitive and highly reproducible tool for measuring spontaneous locomotor activity in the horse, which allows investigators to determine an agent's average time of onset, duration and intensity of effect on movement.

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INTRODUCTION

When horses are placed in a confined space, they instinctively move around and explore their environment. This movement is defined as spontaneous locomotor activity (Kamerling & Owens, 1994). A method for quantifying the effect of stimulant and depressant medications on spontaneous locomotor activity in the horse was first presented by Tobin *et al.* (1979). The tested horse was isolated in a partially shielded box-stall while an observer watched the horse through a small, one-way glass window and counted the number of times the horse lifted its left front leg as a measurement of spontaneous locomotor activity.

Building on this approach, Kamerling *et al.* (1988) automated the activity measurement with photoelectric counters. The counters emitted continuous beams of infrared light. When the light was interrupted by the horse moving around the stall, a count was scored. The current paper describes the construction, evaluation and validation of a 'locomotor chamber' in our laboratory based on the initial description by Kamerling *et al.* (1988). Using this chamber, baseline locomotor activity was determined by measuring the spontaneous activity of 18 horses over a 22 h period. The chamber was validated by showing its ability to quantify previously characterized behavioural responses to fentanyl and xylazine (Tobin, 1981).

Amitraz is a formamidine insecticide used for the control of many species of ticks, mites and lice. In the United States, amitraz is used to treat demodectic mange in dogs and to control ticks, mites and lice on livestock. Use of amitraz as a spray or dip for parasite control on horses has had deleterious effects. Signs associated with topical exposure to amitraz in horses are related

to the central nervous system and the gastrointestinal system. They include colic, depression, incoordination, recumbancy, decreased gut sounds, impaction and weakness.

Although amitraz is dangerous when used as a topical acaricide in horses, the drug was selected for testing because there is always demand for better sedative and analgesic agents. As amitraz has the same pharmacological action (adrenergic agonist) as many commonly used agents (e.g. xyaline, detomidine, romifidine), it is an ideal drug to test. Furthermore, it is often used as an acaricide in many tropical countries outside the USA, and its use as such causes intoxication in many horses. Therefore, a better understanding of the agent and possible antidotes for it would greatly assist veterinarians in treatment of the toxicity.

The goals of this project were (1) to develop techniques and protocols for the measurement of locomotor activity in the freely moving horse and (2) to quantify both the degree of tranquilization and the duration of effect on spontaneous locomotor activity following administration of detomidine and amitraz.

MATERIALS AND METHODS

Horses

Five or six mature Thoroughbred mares weighing 463–554 kg were used for each experiment described in this paper. The animals were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. While in the behaviour chambers, horses were fed at 06.00 and 15.00 h. The animals were vaccinated annually for tetanus and were dewormed quarterly with ivermectin. A routine clinical examination was performed prior to each experiment to assure that the animals were healthy and sound. During experimentation, horses were provided water and hay *ad libitum*.

Locomotor chamber

Two 3.4 × 3.4 m box stalls in an isolated barn were converted into locomotor chambers. Outside stimuli were reduced by adding insulation in the walls for sound-proofing and obscuring the lower portion of the exterior window to prevent the horse from seeing out of the stall. Daylight still entered through the upper window to insure that the normal circadian rhythm of the horse was not altered by the isolation. An air-conditioner and circulation fan were used to provide adequate ventilation and to create 'white noise' in each stall to further reduce outside sounds.

Locomotor activity of the horse was detected by four Minibeam sensors (SM31E and SM2A31R, Banner Engineering, Minneapolis, MN, USA) spaced equally around the stall and recessed into the walls 45 cm above the dirt floor. Each time the horse disrupted the beam of light a count was scored. The output from the four sensors was summed and recorded on a data logger (CR10, Campbell Scientific, Inc., Logan, UT, USA). A schematic diagram of the behaviour chamber and its support system is presented in Fig. 1.

Experimental design

All behavioural experiments followed a rigorous standard protocol to reduce variability from extraneous effects. Horses were placed in the behaviour stall at 07.00 h. After 1 h to allow for acclimation to the stall, data collection was begun at 08.00 h. Baseline locomotor data was collected for 60 min before drug administration. At 09.00 h, the agent or control was administered, and data was collected until 06.00 h the following morning. The total number of times the sensors were activated was recorded every 5 min. To smooth the activity curve, recordings were averaged over subsequent 30 min periods, and the results were expressed as counts per 5 min. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Spontaneous locomotor activity

To validate the behaviour chamber, fentanyl citrate (Janssen Pharmaceutica, Titusville, NJ, USA; 0.016 mg/kg) was injected as the positive control. For the negative control, xylazine HCl (Miles Inc., Shawnee Mission, KS, USA; 1 mg/kg) was injected in the same horses on separate days with at least 7 days between the fentanyl and xylazine dosings. Following validation of the behaviour chamber, amitraz (Sintesul S.A., Pelotas, RS, 96080 Brazil) was injected at doses of 0.05, 0.1 and 0.15 mg/kg to assess the effect of this agent on locomotor activity. In a separate experiment, yohimbine HCl (Sigma Inc, St. Louis, MO, USA; 0.12 mg/kg) was administered following injection of amitraz (0.15 mg/kg) to assess the reversal effect of that agent. The order of dosings was randomized. In a subsequent experiment, detomidine HCl (Pfizer Animal Health, West Chester, PA, USA) was

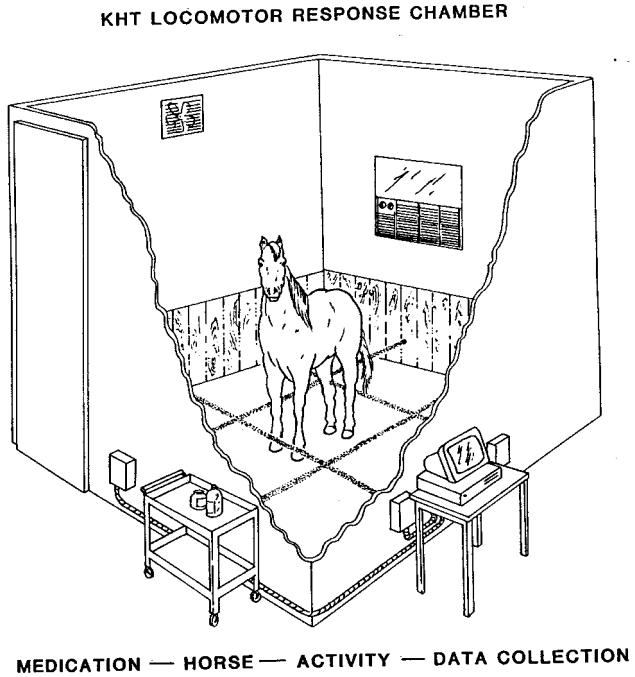

Fig. 1. Schematic diagram of behaviour chamber with infrared sensors spaced around the stall to measure activity.

injected at doses of 0.02, 0.04 and 0.08 mg/kg. The order of dosings was randomized. All horses served as their own controls, and at least 7 days elapsed before a horse was used in another experiment. All administrations were by rapid intravenous (i.v.) bolus to accentuate behavioural effects.

Statistical analysis

Data are presented as means ± SEM. Analysis of variance with repeated measures (SAS Institute Inc., 1985) was used to compare control and treatment values for each medication at each measuring time. Significance was set at $P < 0.05$. Dose–response curves were determined by calculating the differences between control and drug treatments based on the total number of counts recorded during the period of significant effects. These differences were plotted against dosage, and the linear regression was determined.

RESULTS

Figure 2 illustrates the pooling of control data from previous experiments to show normal diurnal activity. A total of 18 horses were used to compile this data. Normal locomotor activity of horses was greater during daytime hours than during the evening. There were obvious peaks at 15.00 h and at 06.00 h the following day. Both occurred because of feeding of horses in the adjacent paddocks.

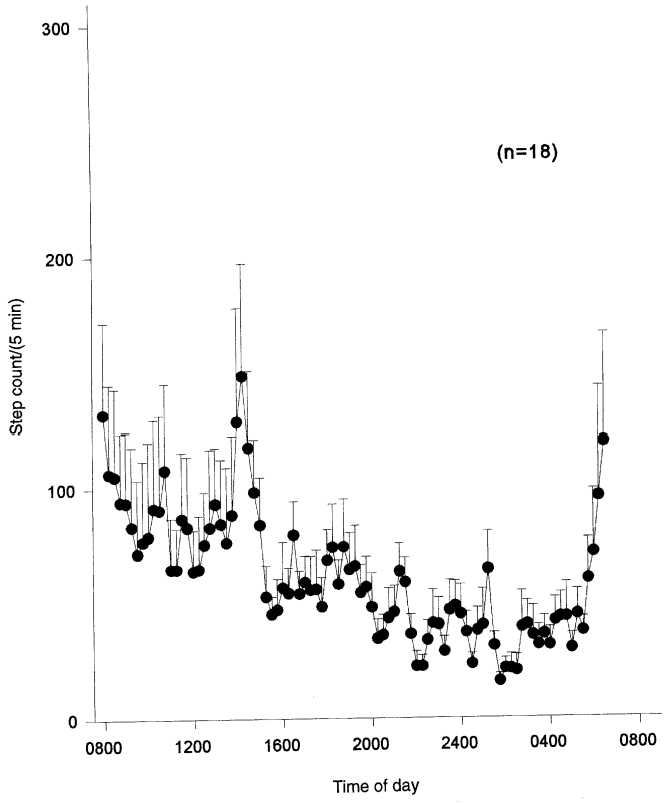

Fig. 2. Spontaneous activity of control horses ($n = 18$) during a 22 h period. Data were collected between May and Sept.

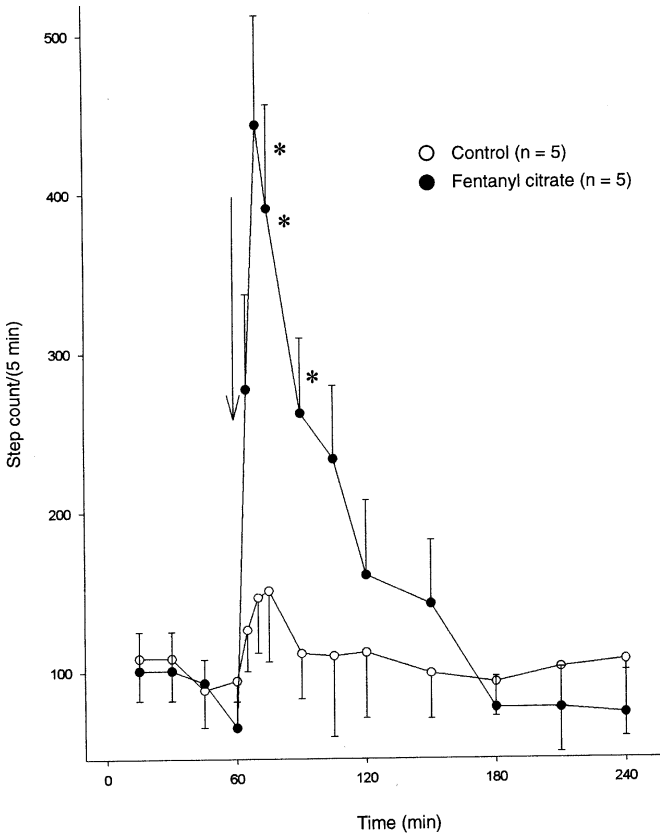

Fig. 3. Spontaneous locomotor activity following rapid i.v. injection of fentanyl citrate (0.016 mg/kg). Arrow indicates time of injection. *Significantly different from control values ($P < 0.05$).

Figure 3 shows that injection of fentanyl citrate (0.016 mg/kg) significantly increased locomotor activity, and the increased activity persisted for 30 min after administration. In contrast, i.v. injection of xylazine HCl (1 mg/kg) significantly reduced spontaneous locomotor activity immediately after injection, and the decreased activity persisted for 90 min (Fig. 4). Peak reduction of activity was evident 15 min after administration.

Intravenous amitraz produced a significant and dose-dependent decrease in locomotor activity (Fig. 5a-c). All doses reduced activity to near-zero, with the effect lasting about 75 min for the 0.05 mg/kg dose, 120 min for the 0.10 mg/kg dose, and 180 min for the 0.15 mg/kg dose. The slope of the dose–response curve (Fig. 6) was significantly different from zero ($P = 0.0142$). The regression was $y = 0.05–112x$.

In a separate experiment, yohimbine HCl (0.12 mg/kg) was injected intravenously 60 min after amitraz administration (0.15 mg/kg). Yohimbine administration immediately reversed the sedative effect of amitraz (Fig. 5d).

There was a significant decrease in locomotor activity following injection of detomidine (0.02, 0.04 and 0.08 mg/kg; Fig. 7). As with all of the behaviour chamber studies, there was increased activity associated with injection of the treatment and control medications (time = 0 h). For both control and treatment values, activity decreased rapidly following injections; however, detomidine treatment values decreased significantly lower than controls. Activity was significantly decreased for 1.5,

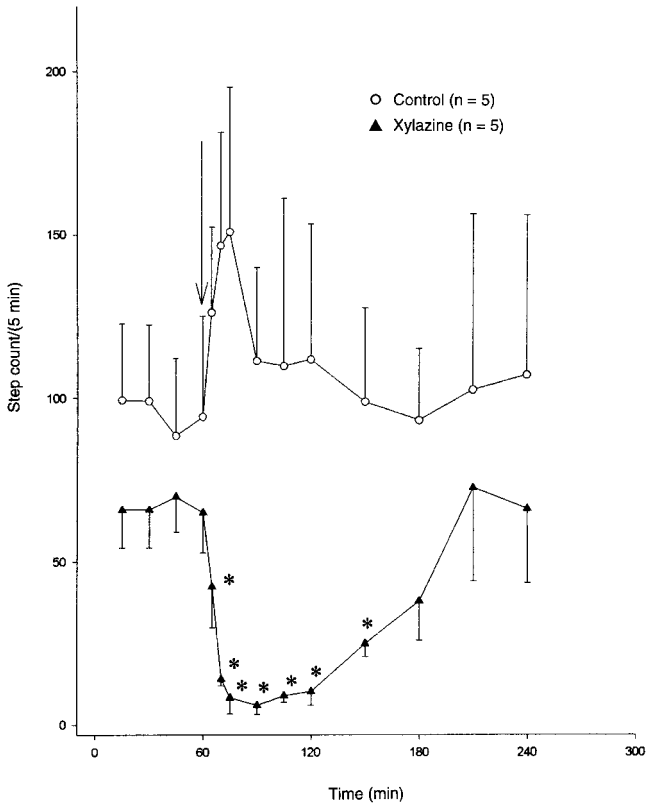

Fig. 4. Spontaneous locomotor activity following i.v. injection of xylazine HCl (1 mg/kg). Arrow indicates time of injection. *Significantly different from control ($P < 0.05$).

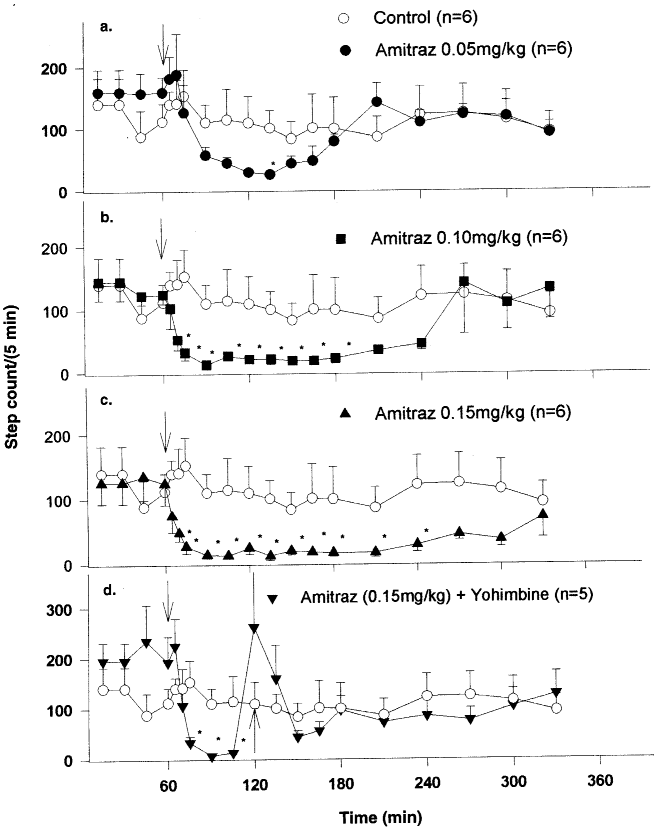

Fig. 5. Spontaneous locomotor activity of horses a–c, after injection of amitraz (0.05, 0.01 and 0.15 mg/kg) and d, amitraz (0.15 mg/kg) followed by yohimbine (0.12 mg/kg). Arrows indicate times of injection. *Significantly different from controls ($P < 0.05$).

3.5 and 5.0 h for the three doses, respectively. The slope of the dose–response curve was significantly different from zero ($P = 0.0423$).

DISCUSSION

Like most animals, it is natural for a horse to explore its surroundings. There was a wide variation in the activities of individual horses. Average daytime activity ($\approx$ 90 counts/5 min) remained fairly constant from about 08.15–14.15 h (Fig. 2). For the entire 22 h testing period, the average activity was about 65 counts per 5 min.

Although the behaviour chambers were insulated and white noise was present, the horses were not completely isolated from outside stimuli. The horses in the behaviour chamber were fed grain at about 15.00 h, and their activity was likely reduced during the time of feeding. However, horses in the paddocks adjacent to the barn were normally fed before the horses in the behaviour chambers, and apparently that activity did not go unnoticed by the horses being tested. This excitement was reflected by increased activity from about 14.15–15.00 h. After the 15.00 h feeding, there was no more human activity in the barn area for the day. From about 15.30–06.00 h the following morning, activity gradually decreased. Non-experimental horses in the adjacent paddocks were fed grain again at about 07.00 h

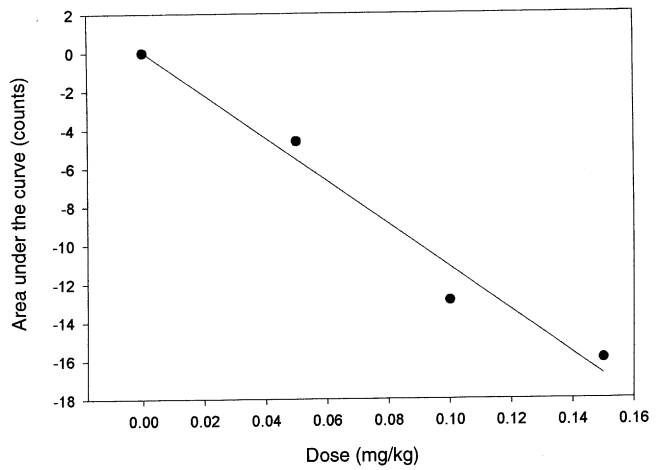

Fig. 6. Dose response curve for i.v. amitraz with the area of locomotor activity plotted against dose (y = 0.05–112x; $r^2 = 0.97$).

the next morning, which partially explains the increased activity in the behaviour chamber at the end of the experiment. The bulk of the data was collected from late spring to early autumn, and sunrise during this period occurred from 05.45 to 06.30 h.

In an earlier study, Kamerling & Owens (1994) showed an average activity of 20–30 counts per minute (100–150 counts/5

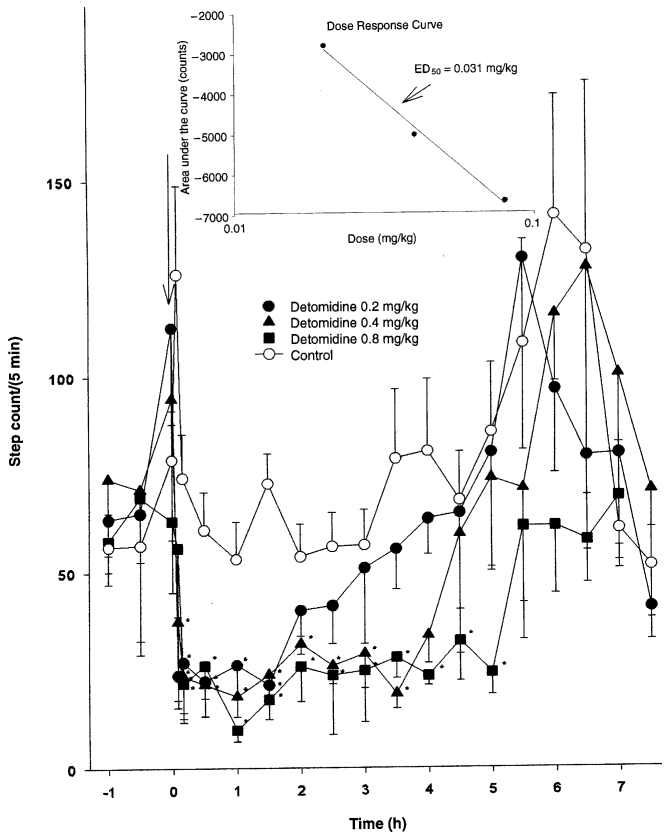

Fig. 7. Spontaneous locomotor activity following injection of detomidine. Inset graph shows dose–response curve with the area of locomotor activity plotted against dose ($r^2 = 0.92$). *Significantly different from control.

min) during daytime testing. The small variation in mean activity between the two studies could be because of differences in many factors such as measuring equipment, inherent activity of the horses selected, external stimuli, or experimental protocol.

The variation in locomotor activity between horses may be used to the investigator's advantage. Horses showing low activity may be suitable for testing stimulant medications, and horses that show higher than average activity may be better suited for testing depressant agents.

Horses tended to be more active when initially placed in the locomotor chamber. Therefore, a specific period was allowed for the horse to adapt to the novelty of the new surroundings. The testing protocol for this study was for the horses to be placed in the chamber 1 h before collection of baseline data and 2 h before injection.

The results obtained following administrations of a well-characterized stimulant and depressant showed that the locomotor chamber could measure drug-induced variations in spontaneous activity. The locomotor chamber enabled investigators to determine an agent's average time of onset, duration and intensity of effect on movement. Because of the unavoidable variation in normal activity, it is likely that the behaviour chamber would more sensitively measure changes in activity over short time periods, when the baseline is likely to be relatively stable.

Kamerling *et al.* (1985) administered fentanyl (0.01, 0.005 and 0.0025 mg/kg) and saline to horses and manually counted mean activities of 38, 27, 17 and 12 steps/2 min, respectively, which represented a three-fold increase of activity for the 0.01 mg/kg dose over baseline (saline) activity. In this experiment, there was also a three-fold increase in activity following administration of 0.016 mg/kg fentanyl (443 steps/5 min) when compared with control activity (146 steps/5 min; Fig. 3). Xylazine reduced activity to near-zero which persisted for $\approx$ 90 min after injection (Fig. 4).

Amitraz is a formamidine acaricide widely used to control ectoparasites in cats, dogs and cattle (Folz *et al.* 1984). Included among its pharmacological effects is α_2-adrenergic agonist activity (Bonsall & Turnbull, 1983), similar to the pharmacological effects of xylazine and detomidine (Lowe & Hilfiger, 1986). Amitraz produced almost total inactivity at the three doses (Fig. 5). As total inactivity is the maximal expression of sedation, it was the duration rather than the intensity of sedation that was observed in the locomotor chamber. The immediate reversal of the sedative effect following yohimbine injection illustrated the similarity in the mode of action of amitraz and xylazine and detomidine. Yohimbine acts primarily by blocking central α_2-adrenoceptors that are stimulated by agents like xylazine.

Yohimbine is a very specific α_2-blocking agent and is often used in scientific studies to pharmacologically determine the selective action on α_2-adrenergic receptors. The drug also has a small antagonistic effect on 5-HT receptors, but the scientific literature does not give support to the hypothesis that the effect on the locomotor activity is caused by this action (Gilman *et al.* 1990). It is more likely that this effect is because of its action on α_2-adrenoceptors.

Detomidine is a potent, non-narcotic tranquilizer/analgesic when administered to horses. Chemically, detomidine is an imidazole hydrochloride with pharmacological properties closely associated with xylazine. The dramatic and rapid decrease in spontaneous activity following detomidine administration (0.02, 0.04 and 0.08 mg/kg; Fig. 7) was in agreement with the findings of Kamerling *et al.* (1988). Although in our study the decreased activity persisted for a longer period (2.0, 4.5 and 6.75 h, respectively), Kamerling *et al.* (1988) also demonstrated a dose-dependent response of activity for 90, 120 and 180 min after injection, respectively.

The locomotor chamber provides a useful, sensitive and highly reproducible method for measuring spontaneous locomotor activity in the horse. We have used the behaviour chamber as a means of assessing the degree of effect, duration of activity, and the dose response for several agents (e.g. isoxsuprine, cocaine, pyrilamine, acepromazine) that can alter performance in racing horses.

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