Evaluation of a behaviour protocol for use in recording the behaviour of horses following administration of methadone

Utvärdering av beteendeprotokoll för användning vid registrering av hästars beteende efter administration av metadon

Maria Bornhede

Master in Animal Science

Sveriges lantbruksuniversitet
Institutionen för husdjurens miljö och hälsa
Avdelningen för etologi och djurskydd

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Nivå: A1E(tidigare avancerad nivå E), 15 högskolepoäng, Master i Husdjursvetenskap, Examensarbete i Husdjursvetenskap EX0718

Handledare: Lena Olsén. Sveriges Lantbruksuniversitet. Institutionen för biomedicin och veterinär folkhälsovetenskap
Biträdande handledare: Carina Ingvast Larsson. Sveriges Lantbruksuniversitet. Institutionen för biomedicin och veterinär folkhälsovetenskap
Examinator: Linda Keeling. Sveriges Lantbruksuniversitet. Institutionen för husdjurens miljö och hälsa

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Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för husdjurens miljö och hälsa
Avdelningen för etologi och djurskydd
Box 234, 532 23 SKARA
E-post: hmh@slu.se, **Hemsida:** www.slu.se/husdjurmiljohalsa

Abstract

There is a need for correct and adequate pain assessment in horses. Methadone is used in pain management in horses but the full pharmacokinetic picture of methadone is not yet known. The aim of this pilot study was to develop a behaviour protocol and suggest a reliable recording method for use in a larger research project with an aim to potentially correlate behaviours to concentration of methadone in blood. In this study two coldblooded trotters were given methadone intravenously with doses of 0.1 mg/kg for horse 1 and 0.2 mg/kg for horse 2. The horses were videotaped pre and post methadone administration. A behavioral protocol, with the purpose to find behaviours that were performed, was developed and evaluated. Following that different recording methods were tried to find the optimal recording method. The results showed that horse 2 had a pronounced peak in locomotory behaviour post methadone. Foraging behaviours had distinct peaks post methadone administration in both horses. Poor coordination was only seen twice and only in horse 2. Both horses scratched post methadone administration and this behaviour appeared frenzied at times. Instantaneous sampling with 10 second intervals was found to be the most rational way of observing behaviour. Instantaneous sampling and continuous recording could both be used during the same observation session, but it is suggested that the amount of time spent on continuous recording is standardized.

Sammanfattning

Det finns ett behov av korrekt och adekvat smärtbedömning på häst. Metadon används på häst vid smärtlindring men den fullständiga farmakokinetiska profilen av metadon är ännu inte fullständig. Syftet med denna pilotstudie var att ta fram ett beteendeprotokoll och föreslå en funktionell registreringsmetod som bägge skulle kunna användas i ett större projekt med syfte att korrelera beteenden till dos av metadon i blodet. I denna studie studerades två kallblodstravare som gavs intravenöst metadon i doser av 0.1 mg/kg för häst 1 och 0.2 mg/kg för häst 2. Hästarna filmades före och efter administration av metadon. Ett beteendeprotokoll, med syftet att finna vilka beteenden som visades, skapades och utvärderades. Därefter provades olika typer av registreringsmetoder för att hitta en optimal registreringsmetod. Resultaten visade att häst 2 hade en ökning i lokomotoriskt beteende och att födosöksbeteenden ökade tydligt hos bägge hästarna efter administration av metadon. Nedsatt koordination sågs endast två gånger och endast på häst 2. Båda hästarna kliade sig efter administration av metadon och detta beteende verkade ibland frenetiskt. Momentanregistrering med 10 sekunders intervall ansågs vara det mest rationella sättet att observera beteende. Momentanregistrering och kontinuerlig registrering kan användas under samma observationstillfälle, men det föreslås att den kontinuerliga registreringen standardiseras.

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Background

Studies in pain management could be considered to be important from several perspectives. In 1966 Brambell produced a report from where the so-called Five Freedoms used to define ideal states of animal welfare originates. Brambell (1966) states that every Act laying the foundation of animal welfare should make it an offence to cause preventable suffering, such as pain. As suggested by Broom (2011) feelings of pain are key parts in animal welfare estimation. Legislation is usually a result of the ethical opinions of a society and in the 2 § of the Swedish Animal Welfare Act (1988:534) it is stated that animals should be treated well and protected against unnecessary suffering. There have been improvements in the management and recognition of animal pain the last decades (Flecknell, 2008). Flecknell (2008) argues that analgesics used in humans have been helpful in animal pain management and that more research in animal pain could potentially benefit human pain management in the future. For pain management to be successful there is a need for correct and adequate pain assessment in clinics (Colvin and Lambert, 2008).

Horses have opioid receptors that play a part in the perception of pain and responses to pain (Hellyer *et al.*, 2003). Opioids are historically the most effective analgesic as they copy the action of encephalins and endorphins; the body's own painkillers and are pharmacologically active at low concentrations (Tobin, 1978). Opioids are thought to induce analgesia by activating pain control routes to the brain (Lüllman *et al.* 1999) and from the midbrain (Muir and Hubbel, 2009). Methadone (6-(Dimetylamino)-4,4-difenyl-3-heptanon) is an opioid agonist used as an analgesic (Muir and Hubbell, 2009). In horses' central nervous system excitation and sympathetic stimulation is observed when opioids are administered (Natalini *et al.*, 2006). Hubbell *et al.* (2010) claim that even though methadone is used in veterinary practice the basic pharmacological knowledge of methadone administration is insufficient. Adverse drug responses are a considerable risk in drug treatment (Davis, 1987). As stated by Muir and Hubbell (2009) "A great deal more research in horses is required before the full picture of the use and potential misuse of opioid agonists and agonists-antagonists can be appreciated". Clutton (2010) argue that opioid use for horses can be justified once the benefits of their sedative and analgesic properties compensate for the risk of adverse effects. In Sweden methadone is not an approved pharmaceutical for animals (FASS vet., 2011). According to the 2nd chapter 1§ in the Swedish legislation for use of pharmaceuticals (Jordbruksverkets föreskrifter om läkemedel och läkemedelsanvändning, SJVFS 2009:84 Saknr D9. Most recently altered 2011 as SJVFS 2011:13 Saknr D9:1) a veterinarian in Sweden should prescribe pharmaceuticals approved for the specific species of animal and for the specific condition of the animal. If there is no such pharmaceutical the veterinarian can prescribe pharmaceuticals for another species used for the same condition or a pharmaceutical used for humans or a pharmaceutical approved for animals in another EU country 2§, 3§, 4§ (SJVFS 2009:84 Saknr D9).

Introduction

The suggested methadone dose for analgesia in horses intravenously is 0.05-0.1 mg/kg (Muir and Hubbell, 2009). In humans methadone has similar pharmacodynamics properties to morphine (FASS, 2008). The pharmacokinetic profile of orally administered methadone in horses has been studied and is characterized by a short elimination half-life (Linardi *et al*., 2009). Several studies have been done in order to investigate the effect of methadone on the horse. For example, subarachnoidally administered hyperbaric methadone 0.01 mg/kg has no significant effect on parameters such as heart rate, blood pressure, blood electrolytes, respiratory rate or blood gases (Natalini *et al.*, 2006). Furthermore, orally administered doses of methadone 0.1mg/kg, 0.2 mg/kg and 0.4 mg/kg does not cause respiratory or cardiac rates to exceed or be less than normal values (Linardi *et al*., 2009). However, in a study by Muir *et al.* (1978) heart rate and cardiac output increased in pain free horses five minutes post intravenous administration of 0.12 mg/kg methadone. Post caudal epidural administration of 0.1 mg/kg methadone horses urinate and defecate normally (Olbrich and Mosing, 2003). On the other hand, a study by Combie *et al*. (1979) showed that horses given an intravenous dose of 1.0 mg/kg urinated frequently. Gut motility is not changed by doses up to 0.4 mg/kg methadone administered orally (Linardi et al., 2009) however; 0.1 mg/kg of morphine given intravenously increases the activity of the right ventral colon (Phaneuf *et al.,* 1972). Subarachnoidally administered 0.01 mg/kg hyperbaric methadone has no significant effect on locomotory behaviour (Natalini *et al.*, 2006) and caudal epidural administration of 0.1 mg/kg methadone do not cause signs of excitement ataxia, sedation or recumbence (Olbrich and Mosing, 2003). Orally administered doses of methadone 0.1 – 0.4 mg/kg do not induce excitement, locomotory activity or sedation in horses (Linardi *et al*., 2009). Though, a study by Combie *et al*., (1979) showed that locomotory activity peaked within one hour from intravenous methadone administration and took about five hours to reach baseline values at 1.0 mg/kg and 0.5 mg/kg. Signs of poor coordination, such as bumping into walls, were seen in some horses given 0.5 mg/kg methadone intravenously and in most horses given 1.0 mg/kg methadone intravenously (Combie *et al*., 1979). Intravenous administration of 0.1 mg/kg of morphine causes somatic movements such as stamping (Phaneuf *et al.,* 1972). Horses appear to have a desire to eat even though the locomotory behaviour is increased, as the locomotory behaviour decreases the eating behaviour increases (Combie *et al.*, 1979). Subarachnoidally administered hyperbaric methadone 0.01 mg/kg has no significant effect on head ptosis (Natalini *et al.,* 2006).

Behavioural reaction to drug administration is multifaceted and depends on the environment in which a drug is studied, the course of action of the drug and the individual receiving the drug (Combie *et al.,* 1979). The quality of a behavioural study is largely dependent on methods for observing, recording and registering the animals and their behaviour. As Bernstein (1991) puts it, “All techniques have their strengths and weaknesses and, as ever, the burden falls on the investigator to design a study specific to a research question”. Sampling rules indicate what kind of behaviour to record and when to record. Recording rules detail how behaviour is recorded. The effects of sampling and recording rules are of significant importance when comparing studies or species. Rose (2000) suggests that the recording method depends on the question of interest, the nature of the study, the field conditions and that most probably a mixed sampling regime is most useful. Ad libitum sampling is a sampling method where behaviours performed by the animal are registered without constrains of what or when (Martin and Bateson, 2007). This method is suitable for pilot projects and construction of ethograms. Ad libitum sampling does not produce true frequencies, but is useful for producing large amounts of data with no regard to frequencies (Bernstein, 1991). Time sampling is a recording rule where behaviours are recorded periodically, involving either instantaneous sampling or one-zero sampling. Instantaneous sampling is when the behaviours performed at a certain sample point are registered. Sample points are usually in an interval of 10 seconds up to 1 minute (Martin and Bateson, 2007). Instantaneous sampling is useful for estimating time spent in various states but not for estimating frequency of events (Zinner *et al.*, 1997). One- zero sampling also includes intervals and with this method specific behaviours performed during an interval are registered only once (Martin and Bateson, 2007). Continuous recording is also a recording rule and is used for registering the start and end of states resulting in a true duration and latencies or to register when events are performed and generate true frequencies (Martin and Bateson, 2007).

Objectives

The purpose of this project, which consisted of both an experimental part and a literature review, is to evaluate and develop a behavioural protocol that could be used for a main research project scheduled for autumn 2011. The purpose of the main project is to optimize the dose regimen of methadone and combination therapies with methadone in horses. The effects of methadone will be measured by different variables such as EKG, hematocrit, beta-endorphins, substance P, thermal threshold and behaviour and will then be correlated to the concentration of methadone in the blood. This project will present which behaviours are performed and when, to evaluate which behaviours are of interest in a behaviour study and propose a reliable and realistic sampling and recording method for the main project.

Material and methods

Experimental animals

The experiment was carried out in the facilities of the Norwegian School of Veterinary Science in Oslo. Two North Swedish trotters, with no available record of previous experiences were used in this study. Horse number 1 was an eight year old mare of 606 kg and horse number 2 was a six year old mare of 476 kg. An ethical permit was approved by the Norwegian Animal Research Committee.

Experimental design and data collection

Horse number 1 was given a dose of 0.1 mg methadone/kg and horse number 2 was given a dose of 0.2 mg methadone/kg both doses diluted to a total volume of 20 ml with sterile 0,09% NaCl. The methadone was administered during five minutes through an intravenous catheter from the jugular vein on one side of the neck. Blood was drawn from an intravenous catheter from the jugular vein on the opposite side before administration of methadone and 5, 10, 20, 30, 45, 60 and 90 minutes post methadone administration (Figure 1). The animals were videotaped 10 minutes prior to methadone administration, for one hour from the administration and for ten minutes one and a half hours after administration. Horse number 2 was also videotaped for ten minutes two hours post methadone. The camera, a Sony Hand cam HDR-CX130, was held by a person sitting on a ladder in close proximity to the box in order to get a good view. During the filming a thermal threshold testing system device attached behind the withers with a girth around the torso was used, giving the horses a sensation of heat near the withers when activated. The thermal threshold testing system was activated for 2 seconds about 1 minute before blood sampling.

Environment effects

The horses had not been fed hay since the night before, however there was some hay still left in the boxes. Throughout the trial the horses were not fed until four hours post methadone administration. The box in which the horses were observed was in a veterinary clinic and had walls on three sides and bars on one side permitting the horse to have visual contact with horses on the opposite side of the stable. The one horse that was not in testing that day was placed in a box next to the testing box. The tests were carried out the 21st and 22nd of June 2011. The temperature was about 20-25° C and there was a large amount of flies in the stable. The horses wore blankets all through filming to minimize the reactions to flies in order for the researchers to be able to see reactions to thermal threshold device. There was activity in the stable during the filming; personnel at the veterinary clinic walking past, activity of other horses, phones ringing, people talking and sounds of horses trotting in another part of the clinic possibly for lameness tests.

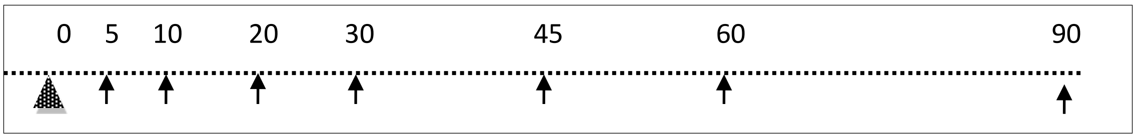

Figure 1. A time line in minutes indicating methadone administration ( ) and time in minutes time from the end of methadone administration to the heat treatment followed by blood sample ( )

Literature search

A literature search was done on the topic of methadone administration in horses, on sampling and registration methods and on methadone and horse behaviour in combination. Search words used were: methadone, horse, pain-assessment, pain, analgesia, welfare, attitude, veterinary, survey, opioid, sampling, recording, one-zero, continuous and ad libitum. Web of knowledge was used to search for scientific papers. In addition to searching in the databases scientific papers of interest, referred to in other papers were also included in the search. Some papers could be found electronically and some had to be collected in the Ultuna libraries at the Swedish University of Agricultural Sciences. The books used in this project were either found in the library previously mentioned or were owned by the author of this report.

Evaluation of data

All filmed material was reviewed and *ad libitum* sampling was used to obtain a record of behaviours shown pre and post methadone administration on one horse at a time. An ethogram of all behaviours was constructed (Table 1). The behaviours were only recorded the first time they were shown, thus the true frequency of the behaviours was not obtained. To find out what behaviours would be of interest when studying effects of methadone treatment and what behaviours would be of interest for correlation with blood concentration of methadone, sequences of the filmed material were reviewed again using *ad libitum* sampling and the number of occurrences of each behaviour in the ethogram was noted. Usually the frequency of all behaviours in the ethogram are recorded during ad libitum sampling (Bernstein, 1991) but when trying to do this, the large number of behaviours exceeded the observers' recording capacity. The trial was stopped since the observer considered the results to be inadequate and potentially misleading. The material was therefore reviewed several times and different recording methods were tried. The observer made notes during the observations if there was something of interest about a particular behaviour, for example whether it was only shown once, shown constantly or only shown by horse 1 or 2. Several ethograms were constructed to better fit the recording methods that were tried (Table 2, 3 and 4). Behaviours were grouped together, not changed at all or deleted. Reasons for rejection of behaviours in the instantaneous ethogram were that the behaviour only occurred a few times, that the behaviour could be due to something other than the methadone, that the behaviour was too short in duration or that the behaviour was difficult to see. Some behaviours had to be redefined to better fit the sampling methods chosen, e.g. 'position of body' used in instantaneous sampling or 'action' used in continuous recording. The data presented in this report were chosen as examples of what it might look like using different methods. During observations using the ethogram in Table 2 the observer noted whether the horse was standing or stepping. In addition, the other behaviours in that ethogram were registered if seen.

Results

Ad libitum sampling

32 behaviours (Table 1) were observed during the ad libitum sampling of the filmed material. Some behaviours such as drinking, failing to scratch and being unbalanced, where seen only once and seen only in horse number 2 who was administered with the higher dose of methadone. Behaviours such as neighing, scratching, skin shake, leg lift, being unbalanced, shaking neck, moving head upwards quickly, investigating inventory (not crib), defecating, seemingly drowsy by position of head, lips moving in assumed greeting of affiliate, having ears back close to neck and drinking were not seen before methadone administration. The greeting of affiliate occurred when the affiliate in the box next to the experimental box returned and was thus considered as a direct result of outer stimuli. This also indicated that the affiliate horse had not been in its' box during the whole experiment potentially affecting the behaviour of the experiment horse. Skin shake was performed some times during the thermal threshold treatment and regularly during the whole observation session when the thermal threshold treatment was not performed (Table 5). Pointing of ears occurred continuously during the whole filming session. Only seen in horse 2, but seen often, was ears back close to neck and muzzle slightly elevated.

Table 1. Behaviours performed during ad libitum sampling and explanations of the behaviours.

Behaviour	**Explanation**
Vocalizing	
Snorting	Letting out a snorting sound
Neighing*	Laryngeal vocalization, usually associated with arrival of food or return of affiliate (McGreevy, 2006)
Body	
Walking in a circle	Turning around in box about 360°
Step	Movement of foot on left side resulting in relocation of foot (Combie *et al.*, 1979)
Resting hindleg	One hind leg is in resting position with sacral part of back lower at side of resting hind leg
Standing still	Horse standing with all hoofs on ground and no leg resting
Scratching catheter*	Scratching body parts where catheters were attached against inventory or other body part
Scratching other*	Scratching body part against inventory or other body part
Scratching fail[1]*	Fail to scratch head with hoof - seems unbalanced
Foraging behaviour hoof	Moving hoof in caudal direction on ground
Skin shake[2]*	Skin shake, e.g. when shaking off flies
Swiching tail	Tail moving out from body rapidly
Leg lift*	Leg lifted rapidly, e.g. as when shaking off flies
Unbalanced[1]*	Resting body against wall or walking with staggering movements
Other	
Defecating[1]*	Defecating
Head	

Neck shaking*	Shaking neck and head
Head down	Putting head down with muzzle close to ground but not expressing other foraging behaviours
Head up*	Head and neck moved upwards rapidly in a jerking motion
Foraging behaviour	Investigating or manipulating material on ground with muzzle
Alert head	Rigid stance with neck elevated and head oriented toward the object of focus (McGreevy, 2006)
Pointing ears[3]	Pointing both ears or one ear in direction of noise
Turning head	Turning head to one side from median plane
Investigating waterbow[1]*	Placing muzzle in water bowl
Investigating crib	Placing muzzle in crib
Drowsie head*	Head still in distal position as compared to normal position. Relaxed neck muscles
Investigative*	Nibbling or sniffing inventory not on ground nor crib
Eating	Jaws moving in lateral and medial direction and/or feed seen in mouth
Lips moving[5]*	Head directed towards affiliate and lips moving but not touching any object, e.g. greeting
Overlooking	Having attention on activity outside of box
Ears back[4]*	Tip of ears pointing in caudal direction, ears close to neck and muzzle slightly lifted
Drink[1]*	Pressing water into water bowl and seemingly drinking
Reaction to sound	Reacting to sound by moving ears/head/body or vocalizing

1, Occurred only once. 2, Occurred during heat treatment. 3, Occurred often during most other behaviours. 4, occurred often in horse2. 5, Only seen in one horse when affiliate returned. * Not seen pre methadone.

Timeline

A timeline (Figure 2) was constructed in order to get a view of when methadone was administered and when blood samples were taken for the first 90 minutes. In the timeline possible four minute behaviour recording sessions pre blood samples were added. An additional four minute behaviour recording session prior to each blood sampling was added in case a more accurate timeline of behaviour would be wanted. As the behavioural recording sessions for the main project will continue for more than 90 minutes, this timeline is only an example.

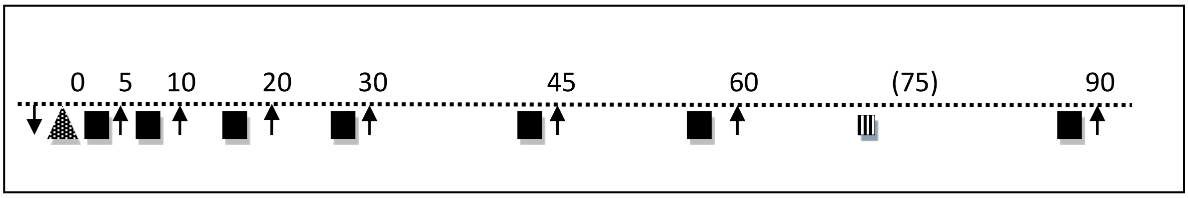

Figure2. A time line indicating methadone administration (▲) and time in minutes time from end of methadone administration for the heat treatment followed by blood sample (↑), pre methadone behaviour recording session (↓) four minute recording session(■) and a possible time for an additional four minute recording session (▥).

Instantaneous sampling, continuous recording and partial continuous

a) Instantaneous sampling

From trying out different recording methods on different behaviours an ethogram (Table 2) suitable for instantaneous sampling with 10 second interval for four minutes was constructed. These included behaviours grouped together, such as foraging, head move, step, standing still, scratching and unbalanced. The behaviours listed in the group 'unbalanced' were only seen once in the horse given the highest dose of methadone 0.2 mg/kg.

Table 2. Modified version of the ethogram suitable for instantaneous sampling with 10 second intervals during four minute sessions.

Behaviour	**Explanation**
Foraging	– Investigating or manipulating material on ground with muzzle – Moving hoof rapidly in caudal direction on ground – Placing muzzle in crib – Jaws moving in lateral and medial direction – Feed seen in mouth
Scratching	– Scratching any body part against inventory or other body part
Stepping	– Moving any leg in any direction resulting in relocation of foot
Standing	– Body in upright position and not moving any leg

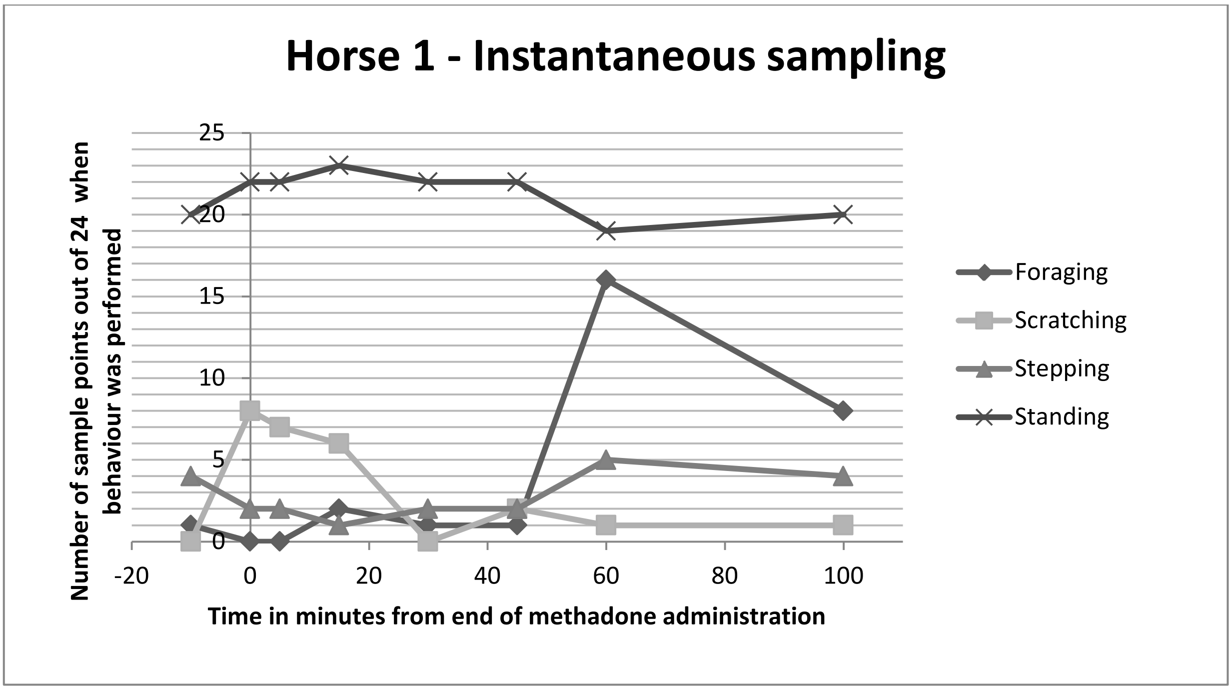

Figure 3. Behaviours performed by horse 1 receiving a dose of 0.1 mg/kg intravenous methadone, using instantaneous sampling with 10 second interval for 8 sessions of four minutes.

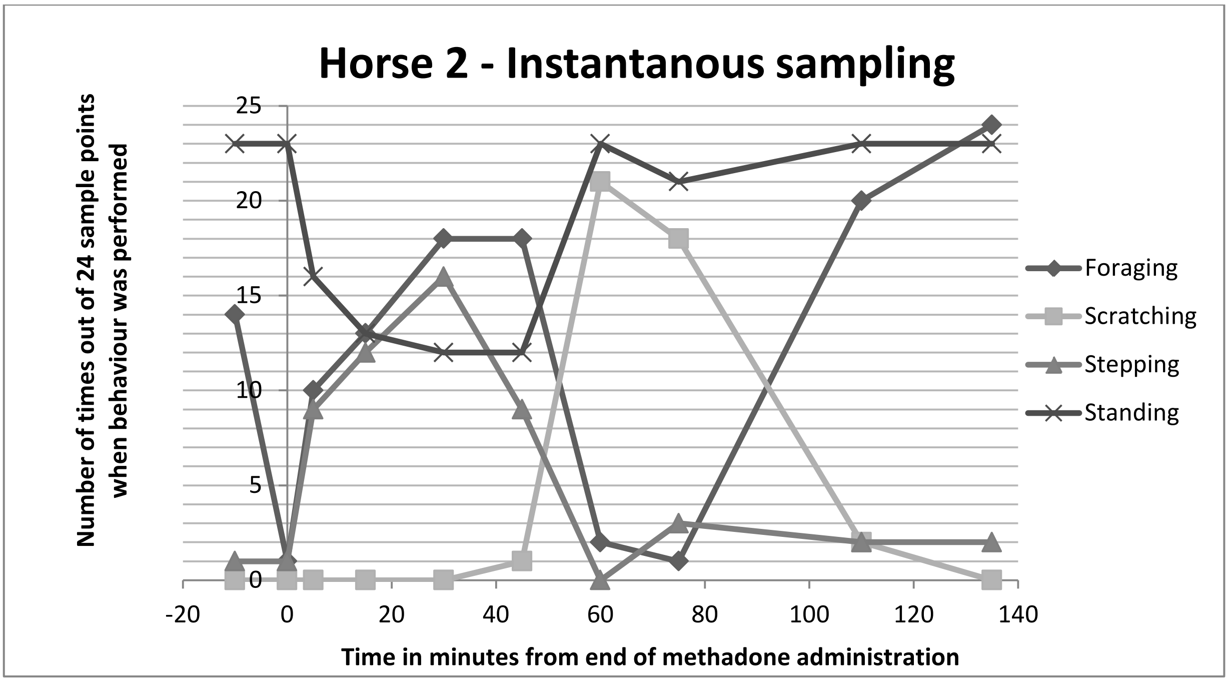

Figure 4. Behaviours performed by horse 2 receiving a dose of 0.2 mg/kg intravenous methadone, using instantaneous sampling with 10 second interval for 10 sessions of four minutes.

The locomotory behaviour of horse 1 peaked at 100 minutes and in horse 2 at 40 minutes and again at 135 minutes (Figure 3 and 4). As a consequence the number of sample points of when the horse was standing still decreased during these peaks. It can also be seen from Figure 3 and 4 that as scratching increases the foraging behaviour decreases. The scratching peaks were seen in horse 1 right after administration of methadone and in horse two at 60 minutes. Peaks for scratching, stepping and foraging were higher in horse number 2 receiving the higher dose of methadone 0.2 mg/kg maybe indicating that dose affects the behaviour.

Another ethogram (Table 3) was constructed to be able to differentiate different behaviour combinations. The amount of times a specific behaviour was performed at sample points can be extracted from this data.

Table 3. Modified version of ethogram for combination of behaviours suitable for instantaneous sampling. Table continuing on page 14.

Behaviour	**Explanation**
Standing foraging	Standing with legs not moving in any direction that results in relocation of foot and at least one of following: – Investigating or manipulating material on ground with muzzle – Moving hoof rapidly in caudal direction on ground – Placing muzzle in crib – Jaws moving in lateral and medial direction – Feed seen in mouth – Head in distal position as compared to resting position of head
Standing alert	Standing with legs not moving in any direction that results in relocation of foot and at least one of following: – Head and neck in elevated position with tense neck muscles and ears pointing forward – Head turned away from medial plane
Standing scratching	Standing with legs not moving in any direction that results in relocation of foot and scratching any body part against inventory or other body part
Standing other	Standing with legs not moving in any direction that results in relocation of foot and expressing other behaviours than mentioned above.
Stepping foraging	Moving any leg in any direction resulting in relocation of foot and at least one of following – Investigating or manipulating material on ground with muzzle – Feed seen in mouth – Placing muzzle in crib – Jaws moving in lateral and medial direction
Stepping other	Moving any leg in any direction resulting in relocation of foot and expressing other behaviours than mentioned on ‘stepping foraging’

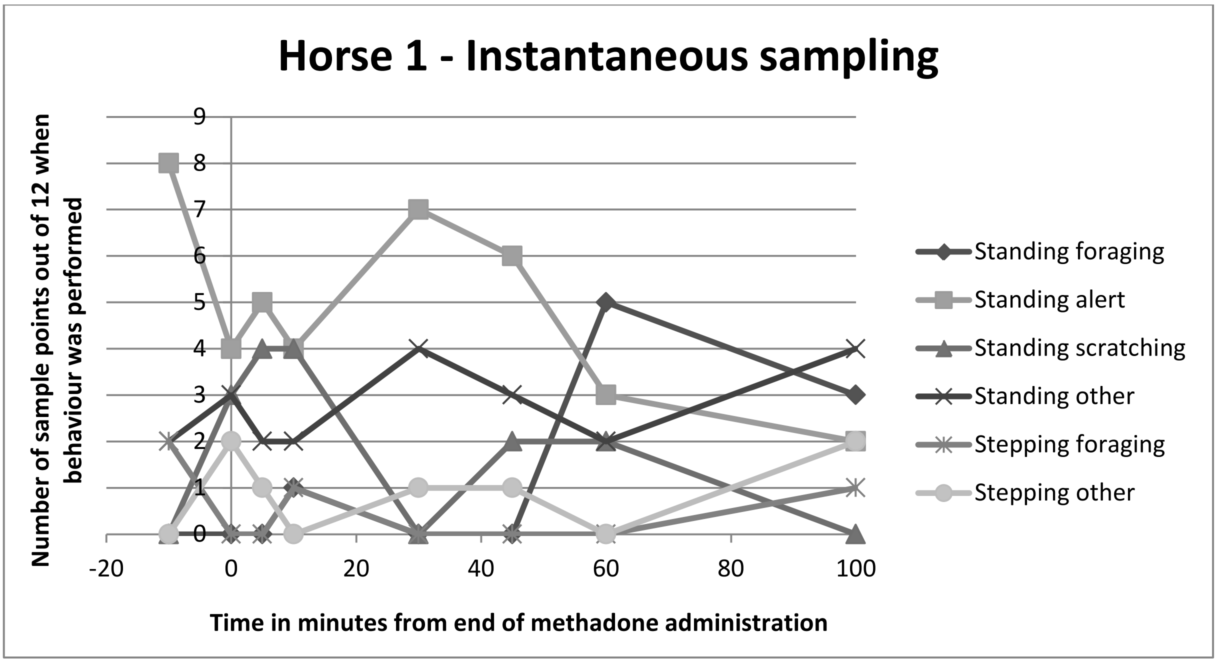

Figure 5. Behaviour performed by horse 1, receiving a dose of 0.1 mg/kg intravenous methadone, using instantaneous sampling with 20 second intervals for eight sessions of four minutes.

b) *Continuous recordings*

Horse 1 seemed to spend most of its time standing alert. Figure 5 shows a peak in 'standing scratching' very soon after administration of methadone and a peak in 'standing foraging' can be seen at 60 minutes post methadone. An ethogram (Table 4) was constructed for continuous recording of three different groups of behaviour.

Table 4. *Modified version of ethogram for combination of behaviours suitable for continuous recording.*

Behaviour	**Explanation**
Head move	– Head and neck moved upwards rapidly in a jerking motion, – Head moving in lateral direction
Irritation[1]	– Shaking neck and head – Tail moving out from body rapidly – Leg lifted rapidly (e.g. as when shaking off flies) – Skin shake (e.g. when shaking off flies)
Neighing*	- Laryngeal vocalization, usually associated with arrival of food or return of affiliate (McGreevy, 2006)

1. Lots of flies were present during this trial so it this behavior could be removed from the protocol .
   *Not seen pre methadone administration

Figure 6 shows that 'Neighing' occurred only in horse 2 and only a few times and peaked 5 minutes post methadone administration. 'Irritation' seemed to be somewhat constant over time in horse 2. 'Head move' decreased over time, with a dip at 60 minutes and then increased again. However, following the dip it did not reach levels of first peak during the time the horse was observed.

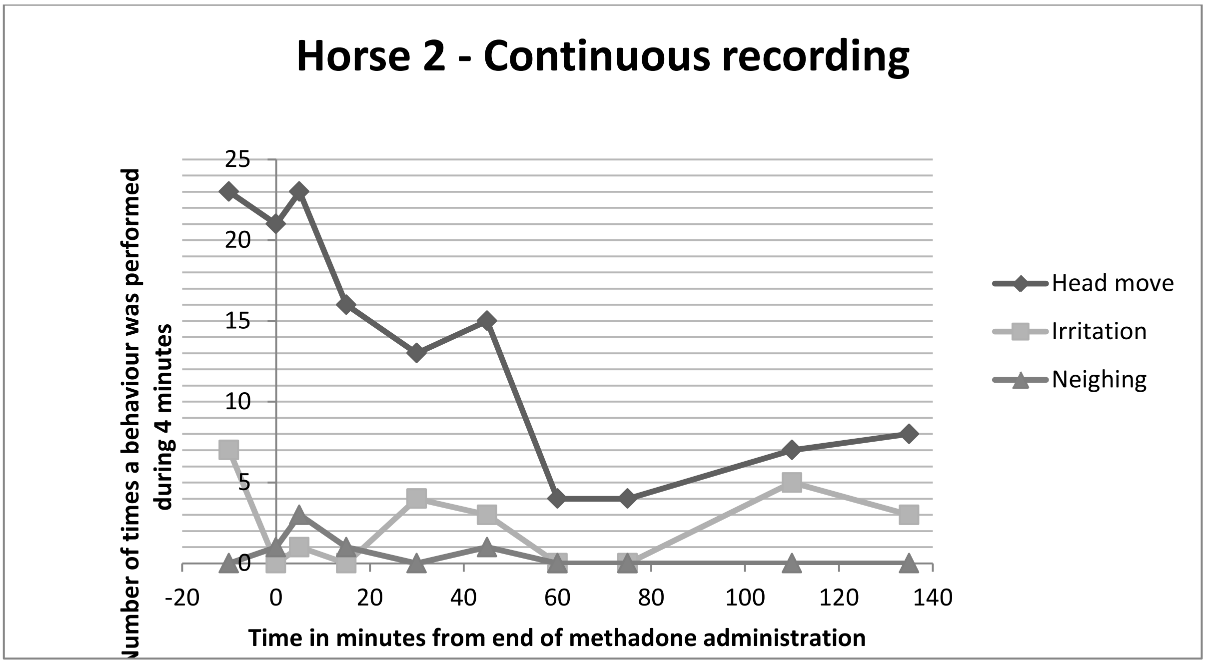

Figure 6. Frequency of behaviours, performed by horse 2, receiving a dose of 0.2 mg/kg intravenous methadone, using continuous recording during 4 minute observation sessions for 10 sessions.

c) *Partly continuous recording*

When behaviour was recorded with 20 second intervals using instantaneous sampling (Table 5) continuous recording was conducted in-between sample points. But as the time of when attention was not on horse after a sample point ranged from about two to eight seconds it was decided that a specific amount of time, 10 seconds, was to be reserved for continuous recording pre a sample point in order not to have biased results. A timeline stating the average time of instantaneous recording and the time for continuous recording was constructed (Figure 7).

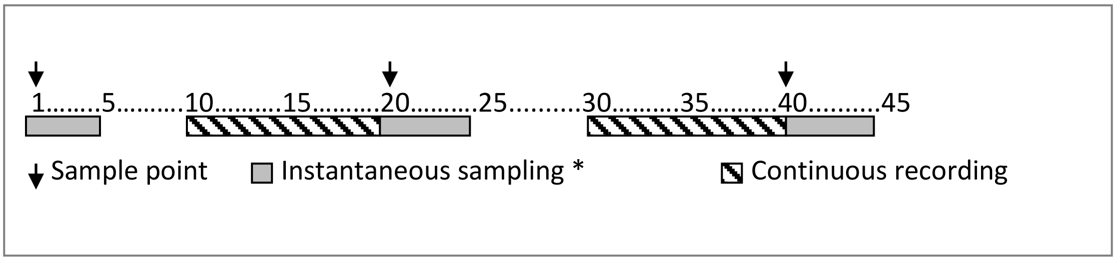

*Figure 7. Explanation of how continuous recording was performed on horse 1. *Estimated average time of attention away from horse during instantaneous sampling post sample point every 20 seconds.*

Table 5. Modified version of ethogram for continuous recording.

Irritation[1]	– Shaking neck and head – Tail moving out from body rapidly – Leg lifted rapidly (e.g. as when shaking off flies) – Skin shake (e.g. when shaking off flies)
Vocalizing	– Laryngeal vocalization, usually associated with arrival of food or return of affiliate – Letting out a snorting sound
Defecation	– Defecating
Unbalance[2]	– Resting body against wall – Walking with staggering movements
Drinking	– Pressing water into water bowl and seemingly drinking
Urination	– Urinating

1. Lots of flies were present during this trial, so this behaviour could be removed from the protocol. 2. Seen only ones but could be of importance

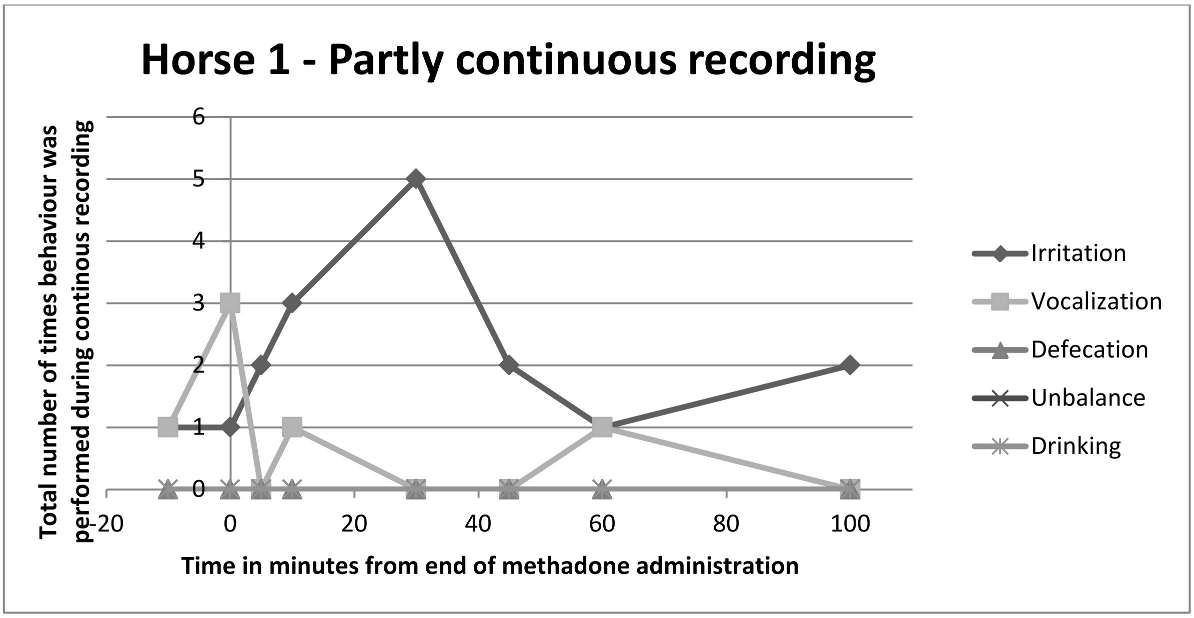

Figure 8. Number of times each behaviour was performed by horse 1 using continuous recording during a ten second period every tenth second for four minutes.

From the partly continuous recording explained in Figure 7 the behaviours 'unbalanced', 'defecation' and 'drink' was not seen once in horse 1 as can be seen from Figure 8. Irritation was seen five times during a total observation session of 120 seconds. In the ethogram for continuous recording (Table 5) vocalization included 'snorting'. This was heard only a few times, peaking right after methadone administration at three times during 120 seconds. The behaviour head move, potentially indicating alertness, was observed using two different recording methods to find the optimal way of integrating this behaviour in this behavioural study in a rational way. As the two different techniques require slightly different explanations of same behaviour these are stated in Table 6.

Table 6. Modified version of ethogram stating the different explanations of behaviour 'head move' for the two different recording methods.

Instantaneous sampling	– Head and neck in elevated position with tense neck muscles and ears pointing forward – Head turned away from median plane
Continuous recording	– Head and neck moved upwards rapidly in a jerking motion, – Head moving in lateral direction

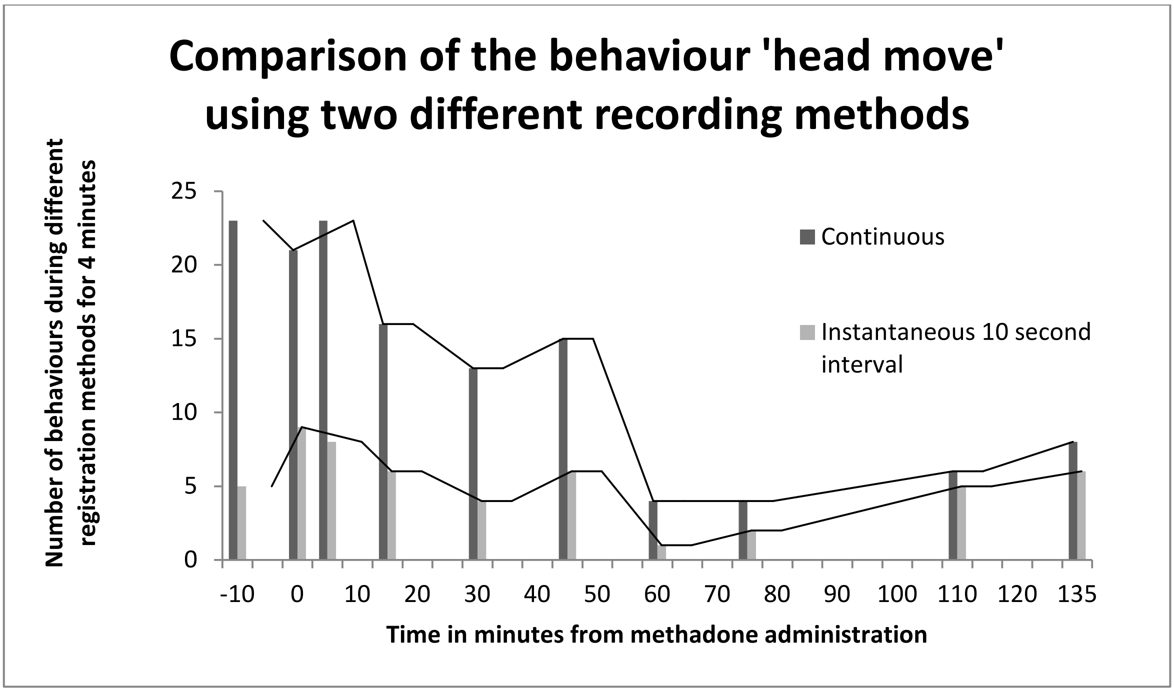

Figure 9. A comparison of the frequencies of the behaviour 'head move' using continuous recording for 4 minutes and the proportion of the behaviour 'head move' at sample points using instantaneous sampling with 10 second interval for 4 minutes on horse 2.

'Head move' was a behaviour that was performed regularly and was mostly performed post methadone administration. However the behaviour was also performed often pre methadone administration. The dip in 'head move' starting 60 minutes post methadone (Figure 6) coincided with the peak in scratching (Figure 3) and then 'head move' decreases slightly. Figure 9 shows that the two registration methods show about the same curve but at different levels with differences being larger the more times a behaviour is performed.

Discussion

It was found that some behaviours, such as head move and locomotory behavior was altered post administration of methadone administration Instantaneous sampling and continuous recording could both be used during the same observation session, but it is suggested that the amount of time spent on continuous recording is standardized.

Behaviours

Urination was never seen in this study. It has previously been seen frequently in horses administered 1.0 mg/kg methadone intravenously (Combie *et al.,* 1979). The lack of frequent urination in this study could be due to the doses of methadone being lower. Since methadone has to be absorbed into the bloodstream to affect the horse, different ways of administration affect the concentration in the blood at different times and thus the behaviour of the horse at different times.

In this study a pronounced peak in locomotory behaviour was seen in horse 2 that received intravenous methadone by 0.2 mg/kg. This is in line with a study by Combie *et al. (*1979) where the horses received 0.5 mg/kg. In contradiction, studies by Natalini *et al. (*2006), Olbrich and Mosing (2003) and Linardi *et al*. (2009) show that 0.1- 0.4 mg/kg orally administered methadone, 0.01 mg/kg subarachnoidally administered methadone and 0.1 mg/kg caudal epidurally administered methadone does not cause increased locomotory behaviour. Poor coordination such as leaning towards inventory or seemingly being unable to scratch head with hoof was only seen in horse 2 and only once. Poor coordination has previously been seen in horses given 0.5 mg/kg and more methadone intravenously Combie *et.al.* (1979). The low amount of poor coordination behaviours in this study is thought to be due to the relatively low doses of methadone administered. With increasing doses of methadone the importance of monitoring the horses increases. Thus the behaviours seen in the group ‘unbalanced’ could be an important behaviour parameter even though it was not shown more than twice in this study.

The distance of the head from the floor has been investigated post subarachnoidally administered hyperbaric methadone 0.01 mg/kg and did not differ from baseline (Natalini *et al.,* 2006) and caudal epidural administration of 0.1 mg/kg methadone did not cause signs of sedation (Olbrich and Mosing, 2003) that might be expressed by lowered head. Lowered head in resting position, maybe indicating sedation, was not seen on the filmed material, but was seen by personnel in the experiment box in horse 2 expressing potential sedation by lowering the head and blinking during methadone administration (pers. com. Olsén, 2011). Hence, some sedation might have occurred but as the camera was positioned from an angle above this behaviour might have been missed but may be important if potential sedation is to be recorded.

In this study horse 2 showed high levels of the behaviour that was called ’head move’. This was basically any movement of the head in any direction except for head in distal position from normal resting position, which was considered to be a foraging behaviour. It has not previously been studied, as to the authors’ knowledge, in this context. However, this was shown frequently and a decrease could be seen from administration and onwards indicating that this might be a good behavioural parameter for correlation with blood parameters. This behaviour was recorded using two different recording method, continuous recording for four minutes and instantaneous recording with 10 second interval for four minutes. It was found to be difficult to measure head movements using continuous recording due to the horse moving its head very little at times, but very often, and due to it being difficult to thoroughly define a ‘head move’. It was difficult to differentiate a movement of the head several times in the same direction from an oscillation head move. It was decided that when using instantaneous recordings, the position of the head away from medial plane or position of head above resting position would be considered a ‘head move’. This was considered to be easy to see and record.

Behaviours in the group ‘foraging’ also seemed to have pronounced peaks and somewhat follow the curve of the behaviour ‘step’. Combie *et al*. (1979) explained the foraging behaviour at high doses of methadone as giving the impression that the horse had a desire to eat but was too busy running. Foraging behaviour should be monitored since it seems to be affected by methadone. There was hay left in the boxes and this might indicate that the horses were not necessarily hungry.

Scratching was performed post methadone administration and appeared frenzied at times. When horses scratched it was mostly where catheters where attached, but other body parts were also scratched. When the horses scratched they did this for long periods of time and as an expected consequence, locomotory behaviour and foraging behaviours were interrupted at times of scratching. When the horses were scratching the neck against the crib this behaviour was interrupted by personnel in order for the horse not to scratch the catheter. This interruption of scratching will have affected the results of this behaviour parameter. It would most probably have been performed more if not interrupted.

Step and head move were at times performed so frequently that they could only be recorded one at a time using continuous recordings. If using continuous recording, no other behaviour can be registered at the same time as step or head move. Those behaviours occurred too often for the observer to have any chance to look at anything else than head or legs. In this situation a hand count would be suitable not to have to take eyes of film to register behaviour. These behaviours are found to be important due to their pronounced peaks in horse 2. As these can be recorded as position of leg or head using instantaneous sampling it is suggested that for the main study, behaviours such as step and head move are included in the instantaneous sampling since it will save time and provide somewhat correct result.

Pointing of ears occurred constantly during all recording sessions and could be considered a constant state of the animal. Accordingly the behaviour ‘pointing of ears’ was not considered to be of importance in this study.’ Irritation’ peaked in horse 1 but was constant in horse 2. However, the number of times it was performed overall was low and the peak may have been a coincidence. ‘Irritation’ could also have been due to flies so the group “Irritation” seems not to be a good behaviour parameter to correlate to different blood parameters. It could be of interest to observe the behaviours in the group ‘Irritation’ when thermal threshold treatment is not performed and when flies are not present.

Recording methods

Ad libitum sampling has previously been shown to underestimate quiet behaviours, bias towards dramatic events, and give inaccurate data on durations and rates of frequent events (Bernstein, 1991). Ad libitum sampling was very good for retaining information on what behaviours that were performed by the horses. But as mentioned previously the frequency of all behaviours could not be obtained using this method since it exceeded the observers’ capacity to observe. Ad libitum sampling may not be needed in the main study.

Instantaneous sampling does not give true frequencies or duration of behaviour if sample intervals are not short in relation to the duration of the performed behaviour (Martin and Bateson, 2007). In this study foraging, scratching and standing were behaviours of longer durations than 10 seconds. In a study by Rose (2000) comparing continuous recording to instantaneous sampling the time budgets did not differ significantly between methods on group level. Zinner *et al.* (1997) found similar results indicating that instantaneous sampling is a dependable method for the estimation of time budgets. The behaviours step and head move were usually of shorter duration in this study but was, at times post methadone, performed almost constantly. Zinner *et al.* (1997) claimed that instantaneous sampling tends to underestimate time of frequent states. If the sample interval, the time between sample points, is very short the result can be similar to those of continuous recording (Martin and Bateson, 2007) however, Zinner *et al.* (1997) found that the different lengths between intervals did not affect the results significantly. In this case 10 second intervals were used and were the shortest interval possible since it at some sample points took about 8 seconds to finish registration. It is believed, by the observer, to be a good enough estimation of the time budget. Events, behaviours of short duration, could be underestimated using instantaneous sampling, so called error of omission (Rose, 2000). Rose (2000) claimed that missing events of short duration, usually under instantaneous sampling, do not affect the overall time budget but will affect analyses of specific behaviours. Continuous recording would be suitable for behaviours considered to be events such as vocalization, defecation or the behaviours in group ‘irritation’. These behaviours were not performed frequently and were short in duration.

Whether or not using instantaneous sampling and continuous recording at the same time is optimal is worthy of discussion. In this study using instantaneous recording about 5 seconds at every sample point was used for registration of behaviours in the instantaneous ethogram and could thus not be used for continuous recording. With a sample interval of

20 seconds this meant that 25% of the time the behaviours of the supposed continuous ethogram were not recorded. However Martin and Bateson (2007) claim that instantaneous sampling and continuous recording can be used simultaneously to register different kinds of behaviour. One reason for not using these methods at the same time is that the recordings could not be performed in an actual stable at actual time since a clock would be needed to notify the observer of when the sample point is in order not to miss sample points. Horses are prey animals and have evolved to react to sudden sounds; a beep could affect the behaviour. If a clock is used the horse should be habituated to the sound and tests should be done to make sure that the horse does not react to the sound. However, sudden sounds could any time potentially bias the results. It is very wearing for the observer to use both continuous recording and instantaneous sampling at the same time and there is a risk of missing some behaviours if the horse performs many behaviours in a row. In this study no behaviours were found to be important for continuous recording that could not be recorded using instantaneous. However if there is an interest for recording behaviours of short duration continuous recording could be used at the same time as instantaneous sampling. If there is an interest for recording behaviours that are audible, ad libitum sampling can be conducted during the whole recording session. In this study a modified technique of continuous recording and instantaneous sampling was used (Figure 7). Using continuous recording with set intervals in-between sample points standardizes the amount of time used for continuous recording. If these intervals are not specified the amount of time spent on continuous recording could be biased towards shorter registrations during times when the animal is active plus the instantaneous sampling takes longer when there is more than one behaviour to record and find in the protocol. It is suggested that the standardized technique is used if infrequent events of short duration are going to be recorded during the same time as instantaneous sampling is conducted.

Strengths and weaknesses with this study

As the behavioural recordings were only done on two individuals the results may not be representative for how horses in general react to intravenous methadone. However some results are in accordance with previous studies. There was much activity in the stable and in the experiment box that could affect the behaviour of the horse; however this is the kind of environment where most horses receiving methadone will be stabled. The horses were frequently interrupted in their behaviour either by specific behaviours being actively interrupted by staff or any behaviour being interrupted by activity inside or outside of experiment box. This will most probably have affected the results. As there was a larger amount of recorded material on the horses post methadone than pre methadone it is difficult to tell which behaviours were due to methadone. It is suggested that horses are studied as much before the administration as after administration if wanting to compare behaviour the horse performs when it is not affected by methadone to the behaviours it performs when it is affected by methadone.

Conclusion

Results showed that locomotory behaviour peaked in one horse post methadone and that foraging behaviour peaked in both horses post methadone. Poor coordination was only seen twice and only in horse 2. Both horses showed a peak in scratching post methadone administration and this behaviour appeared frenzied at times. The most rational way of observing and recording behaviour pre and post methadone administration in horses was found to be instantaneous sampling at10 second intervals. However ethograms for continuous recording can easily be constructed. If instantaneous sampling and continuous recording is to be used simultaneously, it is important to specify the exact amount of time of when to record continuously pre sample point for instantaneous sampling.

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Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Environment and Health
P.O.B. 234
SE-532 23 Skara, Sweden
Phone: +46 (0)511 67000
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