# The effectiveness of the twitch in donkeys

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**Abstract**

*Objective*: to assess the effectiveness of the muzzle twitch in donkeys
*Animals*: five donkeys serving as their own controls
*Procedures*: painful stimuli were applied to a donkey with and without use of a muzzle twitch. Effectiveness was measured by use of behavioural, cardiovasculair (HR, HRV) and hormonal (ACTH, cortisol and ß-endorphins) parameters.
*Results*: donkeys responded less to painful stimuli under influence of the twitch compared to the control group. Mean HR was significantly increased during the twitch procedure after applying the twitch. Administration of painful stimuli in the twitch procedure did not further increase mean HR. Mean HR during the procedure without the twitch significantly increased after applying painful stimuli. The use of the twitch resulted in a significant increase in mean plasma ACTH concentration.
The use of the twitch did not result in significant differences in mean plasma ß-endorphin concentrations.
*Conclusions and clinical relevance:* although twitching of donkeys led mainly to a hormonal stress response without concurrent increases in ß-endorphin concentration, the response to painful stimuli was clearly less. Therefore, it is advisable to use a twitch for restraining donkeys when to perform mildly painful and/or brief procedures.

**Keywords**
Donkeys, twitch, HR, HRV, ACTH, cortisol, endorphins, behaviour.

## 1. Introduction

Techniques for restraining horses have been used for many years by handlers, farmers and veterinarians who wanted to perform mildly painful and brief procedures. One of the most popular techniques is twitching, a time-honoured method of applying restraint in horses by means of a construction to the upper lip. It has the merits of being simple, effective and easy to apply, and it is safe for the horse and the operator. *(McGreevy 2004)*

After application of the twitch, the horse becomes quieter, appears somewhat sedated, the eyelids drop and its heart rate decreases even as its hostile attitude. The horse's interest in its surroundings diminishes, and the tolerance and acceptance of pain decreases. *(Lagerweij et al 1984)*

Several hypotheses have been proposed to explain the twitch's mode of action in history. So could distraction of attention play a role in the mechanism or a decreased perception and awareness of pain elsewhere by the pain induced by pressure on the upper lip. *(Lagerweij et al 1984; vanRee 1984; McGreevy 2004)*

The central mechanism by which the twitch is believed to work is examined by Lagerweij et al in 1984. They concluded that the twitch procedure activates the endorphin systems in the body owing to elevation of plasma ß-endorphins after twitch application. The same result of elevation of plasma ß-endorphins after twitch application was seen by McCarthy et al (1993). These results resemble the mechanism of classical acupuncture in that it stimulates mechano-receptors in the skin which result in an analgesic effect mediated by endorphins. *(Lagerweij et al 1984; McCarthy et al 1993; vanRee 1984)*

The donkey makes an attendance in popularity as a pet animal in West-Europe. Although genetically seen there are big similarities between the horse and the donkey it is not correct to ignore all distinctions. *(Taylor and Matthews 1998)*

One of the differences between donkeys and horses is the donkey's response to the twitch. It is said in different literature that the twitch has not got the preliminary effects on donkeys as it has on horses. There are no evidence based arguments named however. *(Matthews et al 1997; McGreevy 2004; Taylor and Matthews 1998; Trawford and Crane 1995)*

The aim of the current study was to assess the effectiveness of the twitch in donkeys. Therefore, a painful stimuli was applied to the donkeys with and without use of the twitch, like the way Lagerweij et al (1984) did with horses. Effectiveness was measured by use of behavioural, hormonal (cortisol, ACTH, ß-endorphins) and cardiovascular parameters (HR, HRV).

## 2. Materials and methods

*2.1 Animals and housing*

A total of 5 donkeys (3 mares, 2 geldings) mean aged 18.5 (± 3.87 SD and range 12-22) years and with a mean weight of 170.6 (± 42.5 SD and range 118-235) kg were used in this study. They were kept on pasture in a larger group for years. At night they are housed in straw-bedded boxes with access to outdoor areas. The donkeys had different breeds and different histories.

*2.2 Experimental preparation*

On the day of the experiment, each donkey was physically examined by a veterinarian. Following local anaesthesia a 14-gauge Intraflon 2 catheter (Mila, Mila international, Erlanger, KY, USA) was aseptically placed in a jugular vein before the experiment started. The catheter was flushed with heparinised saline to maintain patency and was affixed to the skin with sutures. An extension line was attached to the catheter for blood sampling during the experiment.

Ten minutes before the experiment started two electrocardiogram electrodes were placed on wet skin on the left lateral thorax wall and fixed thoroughly with a stable girth. The electrodes were directly connected to the storage device (Polar® Equine T52H Horse Heart Rate Transmitter) which was fixed onto the girth.

A record was kept of behaviour at the moment of catching the donkey before the procedure started. Levels of activity were graded as follows (defined by Lay et al 1992):

0 = recumbent

1 = standing quietly, docile (relatively small flight distance (< 1 m))

2 = grazing, intermediate responses
3 = active, easily excited (maintaining a large flight distance (> 3,3 m))
*( Lay et al 1992)*

*2.3 Experimental design*

The twitch experiment was divided in two consecutive test procedures. After preparation each donkey was tested two times in the morning at the same day (first time at 10h00 and second time 11h00) and served as its own control.

The first test contained a five-minute twitching procedure in which ten painful stimuli were applied with a sharp needle to the skin alongside the spinal column at a frequency of one per second (at T9). The pain stimuli were provided by the same person during the whole experiment. The reaction of the donkey was recorded, and HR and HRV were measured during this whole procedure, all starting at T0. Blood samples were collected for measuring plasma ACTH, cortisol and ß-endorphin concentrations at rest prior to the twitch application (T4), at three minutes during the five minutes of application (T8) and 5 and 25 minutes after the use of the twitch (T15 and T35 respectively). The blood samples were centrifuged and the plasma was stored at -80ºC until analysis. After an hour this procedure was repeated without the use of the twitch, blood samples were taken at the same time intervals. In two donkeys the sequence was reversed.

**Figure 1**
Time table

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| 0 | *4* | **5** | *8* | 9 | **10** | *15* | *35* |

T = 0: start heart rate measurement and recording
T = 4: blood sample 1 (basal)
T = 5: start of twitching (or not)
T = 8: blood sample 2
T = 9: painful stimuli with the help of needle
T = 10: end of twitching (or not)
T = 15: blood sample 3
T = 35: blood sample 4

*2.4 Behaviour recording and analysis*

To record the behaviour of the donkey, a video camera (Sony DCR-TRV 20E) was used, fixed on a tripod standing in the test chamber. Behaviour was recorded during the first fifteen minutes of each test and the recording coincided precisely with the ECG intervals selected for the heart rate determination and power spectrum analysis (PSA) of HRV starting at T0.

The behavioural response to the painful stimuli during the two test procedures was judged on a four-point scale as defined by Lagerweij et al (1984):

0. indicated no reaction;
1. local muscular twitching and a slightly rigid posture;
2. avoidance of the painful stimuli, looking around, and moving away;
3. fierce reaction, lashing out, or trying to escape.

*(Lagerweij et al 1984).*

*2.5 Heart rate and heart rate variability recording and analysis*

Heart rate (HR) and heart rate variability (HRV) were analysed using Polar 180i® receivers with Polar® Equine T52H Horse Heart Rate Transmitter. Both the transmitter and the receiver were fitted to an elastic girth and attached to the donkey according the guidelines of Polar® and the contact was enhanced by Chemolan® Contact gel.

Heart rate measurement started at T0 till T15 in both test procedures and therefore was determined before, during and after application of the twitch. The intervals between the R-peaks of the ECG were stored continuously in milliseconds. Five time points during the procedures were chosen to compare mean HR: 1: basal level mean HR (T1-4), 2: after one minute of twitching (T6), 3: after three minutes of twitching (T8), 4: after administration painful stimuli (T10), 5: five minutes after ending twitch use (T15)

*2.6 Blood sample collection*

All samples were taken between 10:00 AM and 11.30 AM to minimise the effects of diurnal rhythms. Blood samples were collected via the venous catheter: a basal at rest prior to the twitch application (T4), at three minutes during the five minutes of application (T8) and 5 and 25 minutes after ending twitch use (T15 and T35 respectively). Blood samples of the test without the twitch were taken at the same time intervals.

For cortisol and ACTH determination prechilled 4 ml. ethylenediaminetetraacetic acid (EDTA) tubes were used. For the assay of β-endorphin 2000 µl Trasylol® 0.5, a proteinase inhibitor, was specially added to the EDTA tube.

All blood tubes contained 4 ml blood and were immediately placed on ice and centrifuged (Hettich Zentrifuge, Universal 16R) at 3500 rpm for 5 minutes at 4ºC within 5 minutes of sampling. Subsequently the plasma was carefully separated and divided into 2 ml micro tubes and stored at -80ºC until analysis.

*2.8 Biochemical analyses*

Plasma cortisol concentrations were measured by use of a RIA (ACS: 180®Cortisol Assay, Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) that had been validated for use in samples obtained from horses. The mean intra-assay coefficients of variation were in low range of concentration (3.45-6.83 nmol/l) 8.3%, medium range of concentration (11.4-21.6 nmol/l) 8.1% and high range of concentrations (28.9 – 53.7 nmol/l) 9.0%. All samples were analysed within one assay.

Plasma ß-endorphin concentrations were assayed by an EIA (B-endorphin, bovine, camel, mouse S-1245, Peninsula Laboratories LLC, San Carlos, CA, USA) with a 100% cross reaction with equine ß-endorphin proteins. An intra-assay CV was calculated for donkey blood samples. Seven blood samples were pooled. This pooled solution was assayed six times. From the resulting concentrations intra-assay CV was calculated to be 6.6% for donkey blood samples.

The intra-assay CV for plasma ACTH concentrations was in the lower range (20.7-34.2 pmol/l) 19.3% and in the upper range (302.4 – 537.6 pmol/l) 23.9%.

*2.9 Statistical analyses*

Hormone and HR values were recorded as mean ±SD. A linear mixed-effect model (SPSS 16.0, SPSS Inc, Chigaco, Ill, USA) was used to determine significant hormone differences between the two groups, between different time points during one test procedure and the testing order. Significant differences in mean HR were determined of five given time points.

## 3. Results

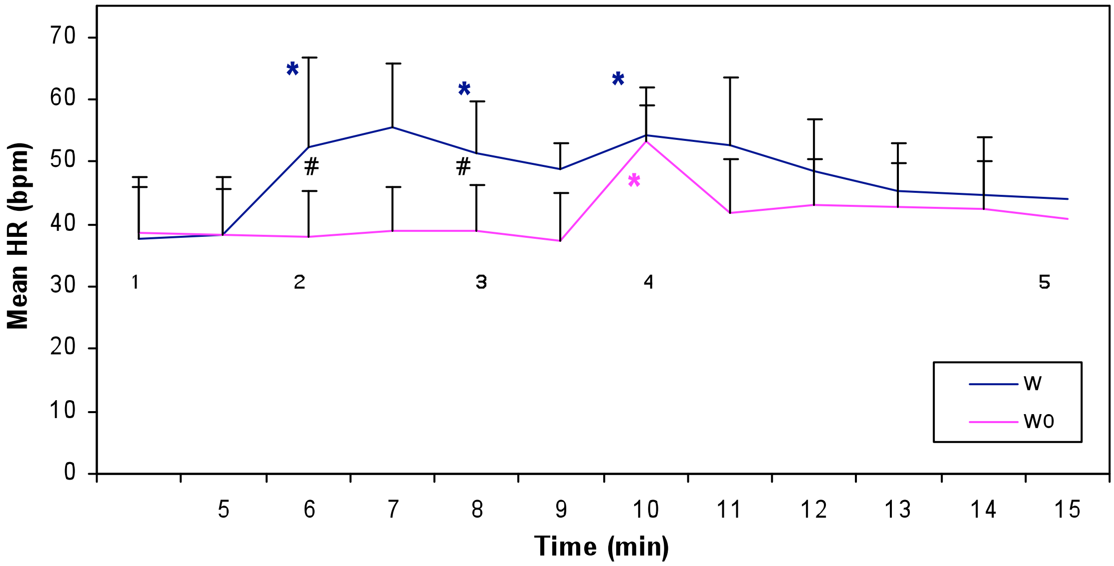
*3.1 HR and HRV*

The mean HR courses are shown in Figure 1. Mean HR was significantly increased during the twitch procedure after 1 minute of twitching (T6) (52±9 bpm, p=0.00), after 3 minutes of twitching (T8) (51±10 bpm, p=0.01) and after administration of painful stimuli (T10) (54±4 bpm, p=0.00) compared to the basal mean HR at T1-4 (38±8 bpm). In contrast with the control group in which painful stimuli significantly increased mean HR (53±8 bpm vs. 39±9 bpm, p=0.00), mean HR was not further increased by painful stimuli in the test group under influence of the twitch.

At T6 and T8 the mean HR of the procedure in presence with the twitch was significant higher compared to the procedure in absence of the twitch (p=0.00 at both points)

Statistical analyses of HRV parameters could not be done because the three intervals of measurements were too small to get reliable results. The tendency however showed an increased LF/HF ratio and a decreased mean RMSSD during the five minutes interval of twitching (which also included painful stimuli) compared to the basal first interval as shown in Tabel 1.

**Figure 1**
Response of mean HR+SD (bpm) for five donkeys to pain stimuli with (w) or without (wo) application of the twitch during 15 minutes of testing. The twitch was applied at T5 till T10 and pain stimuli were applied between T9 and T10.




The indicated numbers in the figure show chosen time points for measurement and comparing mean HR for statistics: 1: basal level mean HR (T1-4), 2: after one minute of twitching (T6), 3: after three minutes of twitching (T8), 4: after administration painful stimuli (T10), 5: five minutes after ending twitch use (T15). The symbols * and # refer to significant differences in mean HR course and between the two procedures at the indicated time points respectively.

**Table 1**
Response of mean heart rate variability parameters ± SD in five donkeys to pain stimuli with (w) or without use of the twitch (wo).

| HRV parameters | 0-5 min | 5-10 min | 10-15 min |
|---|---|---|---|
| RMSSD w (ms) | 153.68 ± 121.05 | 78.40 ± 22.46 | 140.18 ± 91.29 |
| RMSSD wo (ms) | 184.38 ± 120.18 | 165.36 ± 50.79 | 121.14 ± 39.78 |
| LF/HF w (%) | 132.18 ± 92.23 | 254.74 ± 145.75 | 151.08 ± 107.81 |
| LF/HF wo (%) | 103.04 ± 76.93 | 101.18 ± 55.79 | 111.24 ± 39.39 |

RMSSD w is an estimate of short-term components of HRV beat to beat variability during the test with use of the twitch, RMSSD wo is without the twitch. LF/HF is the sympatho-vagal balance, LF is index for sympathetic modulation and HF for vagal modulation of the heart rate.
0-5min: basal interval, 5-10min: interval including twitch and painful stimuli, 10-15min: interval after ending twitching

*3.2 Behaviour*

The temperament score and the pain scores are shown in Table 2.
The temperament score revealed one donkey standing quietly, two donkeys were active and easily excited and the other two showed intermediate responses towards catching them for the experiment.
In absence of the twitch the donkeys responded more to the painful stimuli as shown by the elevated pain score during the procedure without the twitch. The donkeys with the highest temperament score did not respond differently to the painful stimuli compared to the donkeys with the lowest temperament score. Informal observations indicated that the donkeys varied in their behavioural response to the twitch from standing still, shaking to trying to escape.

**Table 2**
Temperament score of five donkeys before start of the experiment and results of pain score as a response to painful stimuli

| Scores | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Temperament score | N = 0 | N = 1 | N = 2 | N = 2 |
| Painscore with twitch | N = 0 | N = 4 | N = 1 | N = 0 |
| Painscore without twitch | N = 0 | N = 0 | N = 3 | N = 2 |

Temperament score: 0: recumbent; 1: standing quietly, docile; 2: grazing, intermediate responses; 3: active, easily excited. Pain score: 0. indicated no reaction; 1. local muscular twitching and a slightly rigid posture; 2. avoidance of the painful stimuli, looking around, and moving away; 3. fierce reaction, lashing out, or trying to escape. N refers to number of animals. *(Lagerweij et al 1984; Lay et al 1992)*

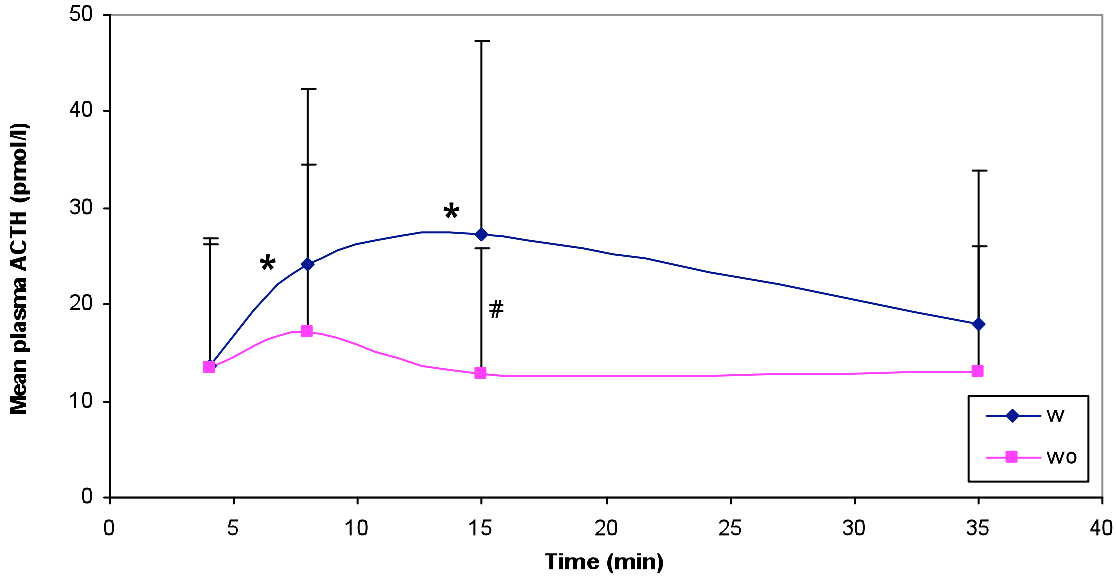
*3.3 Hormone concentrations*

Influence of painful stimuli in presence and absence of a twitch on the mean concentrations of plasma ACTH, cortisol and ß-endorphins are summarised in Figures 2, 3 and 4 respectively.

*3.3.1 ACTH*

The use of the twitch resulted in a significant increase in mean plasma ACTH concentration at T8 (23.94 pmol/l) and T15 (27.13 pmol/l) compared to T4 (12.39 pmol/l) with p=0.03 and p=0.00 respectively. Plasma ACTH concentration was also significant higher in presence of the twitch compared to absence of the twitch at T15 (p=0.00). At T8 plasma ACTH levels in the group with twitch almost reached significance (p=0.05) compared to the group without twitch. There were no significant differences at time points during the procedure in absence of the twitch.
Starting sequence had no significant influence on plasma ACTH concentration.

**Figure 2**
Influence of painful stimuli with or without twitching on the concentration of plasma ACTH of five donkeys




Mean plasma ACTH levels +SD (pmol/l) during the procedure with the twitch (w) compared to the procedure without the twitch (wo) at T4 (basal), T8 (after 3 minutes twitching), T15 (5 minutes after twitch use) and T35 (25 minutes after twitch use) The symbols * and # refer to significant differences in concentration course at T8 and T15 and between the two procedures at T15 respectively.

*3.3.2 Cortisol*

The use of the twitch resulted in a significant increase in mean plasma cortisol concentration at T15 (106.71 nmol/l) and T35 (134.32 nmol/l) compared to T4 (63.24 nmol/l) and T8 (70.85 nmol/l) with p=0.00 at both points. T35 has compared to T15 also a significant increase in mean plasma cortisol concentration (p=0.02).

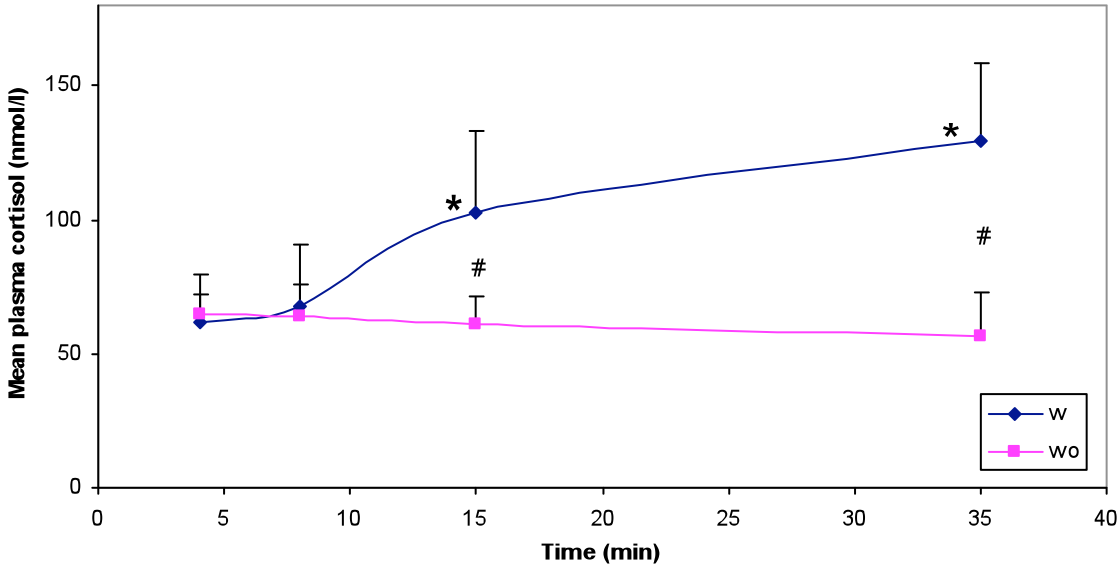
Mean plasma cortisol concentration was also significant higher in the group with the twitch compared to the group without the twitch at T15 and T35 (p=0.00 at both time points).

There were no significant differences during the procedure in absence of the twitch.

Starting sequence had significant influence (p=0.04) on mean plasma cortisol concentration. (85.22 nmol/l if started without twitch vs 69.91 nmol/l if started with twitch.

**Figure 3**

Influence of painful stimuli with (w) or without (wo) twitching on the concentration of plasma cortisol of five donkeys.



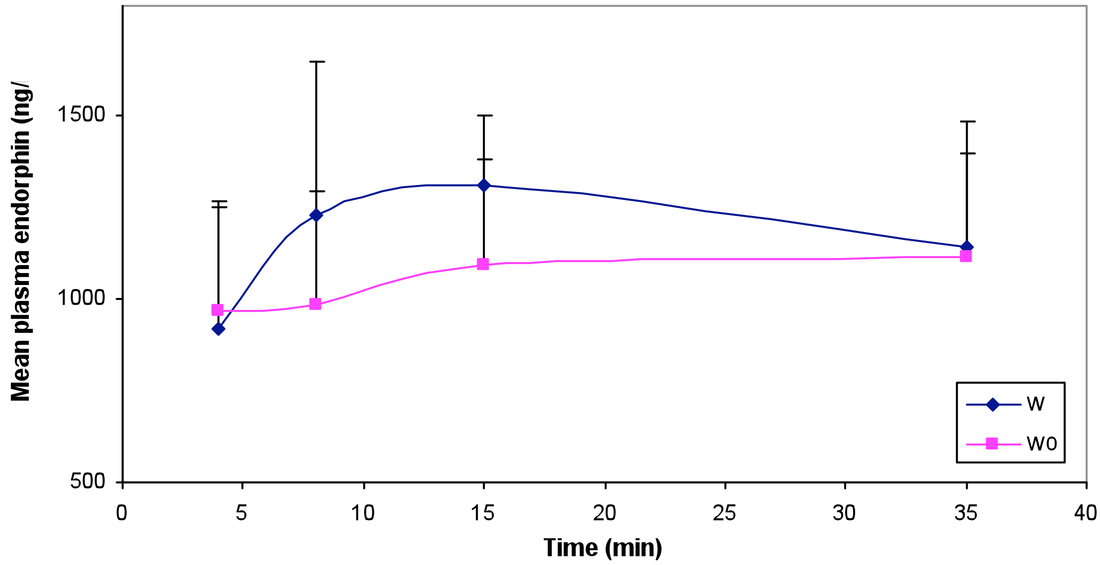

Mean plasma cortisol levels +SD (nmol/l) during the procedure with the twitch (w) compared to the procedure without the twitch (wo) at T4 (basal), T8 (after 3 minutes twitching), T15 (5 minutes after twitch use) and T35 (25 minutes after twitch use). The symbols * and # refer to significant differences in concentration course at T15 and T35 and between the two procedures at the same points respectively.

*3.3.3 Endorphins*

The use of the twitch did not result in significant differences in mean levels of plasma ß-endorphin concentration, although the mean concentrations during the procedure with the twitch were at a significant higher level (1215.08 compared to 1013.35 ng/l, p=0.03).

Starting sequence had no significant influence on plasma endorphin concentration.

**Figure 4**
Influence of painful stimuli with (w) or without (wo) twitching on the concentration of plasma endorphin of five donkeys




Mean plasma endorphin levels +SD (ng/l) during the procedure with the twitch (w) compared to the procedure without the twitch (wo) at T4 (basal), T8 (after 3 minutes twitching), T15 (5 minutes after twitch use) and T35 (25 minutes after twitch use).

## 4. Discussion

The aim of current study was to assess the effectiveness of the twitch in donkeys by measuring behavioural, hormonal and cardiovasculair responses to pain stimuli in presence or absence of the twitch.

HR (heart rate) represents the net effect of the parasympathetic nerves that slow it down and the sympathetic nerves that accelerate it. Different stressors can induce a shift of the autonomic balance towards either the sympathetic or a parasympathetic dominance. *(Visser et al 2002)*
HRV (heart rate variability) describes the variation between consecutive heartbeats of an ambulatory ECG and is a reliable quantitative marker of autonomic nervous activity. RMSSD, the root mean square of successive beat to beat differences, is an estimate of short-term components of HRV. HR increases under influence of sympathetic activity through which RMSSD decreases, meaning in stressful situations. *(Task Force of the European Society of Pacing and Electrophysiology, 1996)* Changes in the LF/HF ratio are an indicator of alterations in the sympathetic-vagal balance with LF reflecting the sympathetic tone and HF reflecting the parasympathetic tone. In stressful (mental or physical) situations the parasympathetic nerve activity will decrease in favour of higher sympathetic activity resulting in a LF/HF ratio increase. *(Bachmann et al 2003; Rietmann, Stauffacher et al 2004; Rietmann, Stuart et al 2004; Visser et al 2002)*

In the current study using a muzzle twitch in donkeys revealed significant elevation of the mean HR, increased LF/HF ratio and a decreased mean RMSSD which indicates a shift towards sympathetic dominance after applying the twitch associated with a stress response. HR values showed a significant raise the first minute after applying the twitch and a little fall after three minutes of twitching but HR did not return to basal level. This is in contrast with the 8% lower HR compared to basal HR found in horses after twitching indicating a parasympathetic effect of the twitch. In the same horses HR returned to basal level after application of painful stimuli in presence of the twitch. The donkey's heart rate did not return to basal level at all, although there was no significant difference at three minutes of twitching or at the end compared to the basal level. *(Lagerweij et al 1984)*

Also the behavioural response towards the twitch was different for the donkey compared to the horse. Donkeys showed no signs of the quieter and what sedated attitude with dropping eyelids as described for horses in presence of the twitch *(Lagerweij et al 1984)* This fits to a relative more increased sympathetic dominance in the donkeys compared to an increased vagal dominance in the horse. In contrast, the response towards the painful stimuli was equal for the donkeys and the horses. In absence of the twitch the donkeys and the horses responded more to the painful stimuli. *(Lagerweij et al 1984)* In the current study the temperament score seemed to have no influence on pain behaviour seeing the outcome that donkeys with a high temperament score did not react different compared to donkeys with a lower temperament score. Lay et al (1992) found that mean hormone concentrations (cortisol and catecholamine) as well as heart rate responses in their frozen or hot-iron branded calves were not affected by the temperament score as well. Facing these results one could presume that prediction of an animal's response to stress or pain could not be made on the basis of temperamental characteristics.

The use of the twitch resulted in a significant increase in mean levels of plasma ACTH concentration after three minutes of twitching till five minutes after using the twitch. This shows resemblance with plasma ACTH levels reaching peaks at five minutes after exercise in the studies of Kurosawa et al (1998) and Schwarz and Kindermann (1990) although in their studies the mean plasma ACTH levels were in humans and horses respectively a 70 and 6 fold higher. *(Kurosawa et al 1998; Schwarz and Kindermann 1990)* In our study plasma levels were only 2 fold higher, but in contrast to the previous named studies it did not concern physical stress. Because blood samples were taken at three minutes twitching and five minutes after ending use of the twitch it could be that due to the relatively short halflife of ACTH of 3 minutes *(Schwarz and Kindermann 1990)* the peak level of plasma ACTH was missed. It was expected that peak levels were soon over after ending twitching because of the short halflife of ACTH.

In the present study mean levels of plasma cortisol started to increase significantly five minutes after using the twitch. Studies in horses and humans showed also a delayed raising of cortisol levels ten minutes after the plasma ACTH peak. *(Kurosawa et al 1998; Schwarz and Kindermann 1990)* The relative long halflife of cortisol (70-100 minutes) *(Harbach et al 2007)* could be the reason that the starting sequence showed a connection with the mean level of plasma cortisol. If cortisol concentration stays at a high level for at least 70 minutes due to twitching than there is a possibility that in the following procedure without the twitch, the basal plasma cortisol is still at a higher level. In two animals this was the case, but as we only had five animals to randomize for order we had uneven numbers (3 started with twitch, 2 started without) and it would be incorrect to draw any conclusions.

Although plasma ß-endorphin levels did not show significant differences between time points or groups after application of the twitch in the donkeys, it seems that plasma endorphin increases after application of the twitch indicated by the significantly increased total mean plasma ß-endorphin concentration in the test group with twitch compared to the control group without twitch.
This correlates with studies in horses in which application of a twitch significantly increased levels of plasma ß-endorphin. *(Lagerweij et al 1984; McCarthy et al 1993)*
The basal ß-endorphin concentration in the testgroup with twitch is estimated on the basis of four animals because of an extraordinary basal plasma endorphin level in one of the donkeys. This result was considered as an outlier, because considering the endorphin halflife of 20 minutes *(Schwarz and Kindermann 1990)* this result was left out as three minutes after the basal sample the plasma endorphin concentration was a 9 fold decreased in this animal. Since plasma ß-endorphin concentrations can be used to monitor stress and pain in horses *(Fazio and Ferlazzo 2003; Harbach et al 2007; Lebelt et al 1998; McCarthy et al 1993)* the slightly elevated plasma concentrations of endorphins in the present study could be caused by stress in stead of the more common explanation of the analgesic mechanism of classical acupuncture. *(Lagerweij et al 1984; Ree van 1984)* This hypothesis is strengthened by the fact that there were no sedated signs as result of twitching.

The presence study allows a number of conclusions. Twitching donkeys led to an increase of HR and LF/HF ratio, a decreasing RMSSD, no sedated attitude, and elevated plasma ACTH and cortisol concentrations all of which point to an increased stress response. However, twitching clearly lowered the reaction of the donkeys to painful stimuli and it is therefore advisable to use a twitch for restraining donkeys when to perform mildly painful and/or brief procedures possibly in combination with painkillers. A possible explanation for the lowered pain reaction in combination with an elevated stress level could be the donkeys nature. Donkeys tend to have different responses to fear inducing stimuli compared to horses (they tend to freeze more readily in stead of flee) and thereby they tend to have a better tolerance for pain than most horses do. *(Matthews et al 1997; McGreevy 2004; Taylor and Matthews 1998)*

For better and more precise results in effectiveness of the twitch in donkeys it is advisable to use larger animal groups and to spread out the time interval of application painful stimuli in presence of a twitch. When using bigger intervals statistical analyses could be made of the HRV parameters RMSSD and LF/HF ratio to determine more precise the reaction to the twitch and the pain stimuli separately, which could give more information about pain tolerance. It could not be said that the donkeys stress reaction is remarkable as there are no data published of stress responses to twitches of horses. Caution should be taken though when comparing (stress) hormone values from horses to values from donkeys. Baseline values of hormone concentrations for donkeys are not known and variables as sex, (medical) history and age could be of influence.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## References


1. Bachmann, I., Bernasconi, P., Herrmann, R., Weishaupt, M.A., Stauffacher, M., 2003. Behavioural and physiological responses to an acute stressor in crib-biting and control horses. *Applied animal behaviour science,* **82**: 297-311

2. Fazio, E. and Ferlazzo, A., 2003. Evaluation of stress during transport. *Veterinary Research Communications, supplement, 27*: 519-524

3. Harbach, H., Moll, B., Boedeker, R.H., Vigelius-Rauch, U., Otto, H., Muehling, J., Hempelmann, G., Markart, P., 2007. Minimal immunoreactive plasma β-endorphin and decrease of cortisol at standard analgesia or different acupuncture techniques. *European journal of anaesthesiology,* **24**: 370-376

4. Kurosawa, M., Nagata, S., Takeda, F., Mima, K., Hiraga, A., Kai, M., Taya, K., 1998. Plasma catecholamine, adrenocorticotropin and cortisol responses to exhaustive incremental treadmill exercise of the thoroughbred horse. *Journal of equine science,* **9**: 9-18

5. Lagerweij, E., Nelis, P.C., Wiegant V.M., van Ree J.M., 1984. The twitch in horses: a variant of acupuncture. *Science,* **225**: 1172-1174

6. Lay Jr., D.C., Friend, T.H., Randel, R.D., Bowers, C.L., Grissom, K.K., Jenkins, O.C., 1992. Behavioural and physiological effects of freeze or hot-iron branding on crossbred cattle. *Journal of animal science,* **70**: 330-336

7. Lebelt, D., Zanella, A.J., Unshelm J., 1998. Physiological correlates associated with cribbing behaviour in horses: changes in thermal threshold, heart rate, plasma ß-endorphin and serotonin. *Equine veterinary journal, supplement,***27**: 21-27

8. Matthews, N.S., Taylor, T.S., Hartsfield, S.M., 1997. Anaesthesia of donkeys and mules. *Equine veterinary education,* **9**: 198-202

9. McCarthy, R.N., Jeffcott, L.B., Clarke, I.J., 1993. Preliminary studies on the use of plasma ß-endorphin in horses as an indicator of stress and pain. *Journal of equine veterinary science,* **13**: 216-219
10. Mc Greevy, P., Handling and transport. In: Equine Behavior, a guide for veterinarians and equine scientists. Saunders, London, 2004: 313-329

11. Ree, van J.M., 1985. Pijn en endorfinen. *Tijdschrift voor diergeneeskunde,* **110**: 3-11

12. Rietmann, T.R., Stauffacher, M., Bernasconi, P., Auer, J.A., Weishaupt, M.A., 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. *Journal of veterinary medicine,* **51**: 218-225

13. Rietmann, T.R., Stuart, A.E.A., Bernasconi, P., Stauffacher, M., Auer, J.A., Weishaupt., M.A., 2004. Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters. *Applied animal behaviour science,* **88**: 121-136

14. Schwarz, L. and Kindermann, W., 1990. β-Endorphin, adrenocorticotropic hormone, cortisol and catecholamines during aerobic and anaerobic exercise. *European journal of applied physiology,* **61:** 165-171

15. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation,* **93**: 1043-1065

16. Taylor, T.S. and Matthews, N.S., 1998. Mammoth asses selected behavioural considerations for the veterinarian. *Applied animal behaviour science,* **60**: 283-289

17. Trawford, A.F. and Crane, M.A., 1995. Nursing care of the donkey. *Equine veterinary education,* **7**: 36-38

18. Visser, E.K., Reenen van, C.G., Werf van der, J.T.N., Schilder, M.B.H., Knaap, J.H., Barneveld, A., Blokhuis, H.J., 2002. Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiology & behaviour,* **76**: 289-296