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Aetiology and treatment of crib-biting behaviour in horses

PhD dissertation

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Glossary

CART	– Classification and regression tree
CB	– Referring to horses crib-biting in the traditional way
CHIN	– Referring to horses using chin instead of incisors
CNS	– Central nervous system
G	– Gelding
GLM	– Generalised linear model
GLMM	– Generalised linear mixed models
H	– Hobby or leisure horses
HPA-axis	– Referring to the pituitary-adrenocortical system
HR	– Heart rate
HRV	– Heart rate variability
HS	– Hungarian sport horse
LF/HF ratio	– The ratio of the low- and the high-frequency spectral component of the HRV
M	– Mare
n.s.	– Not significant
n.u.	– Normalised units
Q	– Quarter horse
PCA	– Principal Component Analysis
R-R	– Beat-to-beat intervals
R.S.C.P.A.	– Royal Society for the Prevention of Cruelty to Animals
S	– Stallion
Sp	– Sport or competition horses
THB	– Thoroughbred
WB	– Warmblood
WS	– Referring to horses wind-sucking without grasping fixed objects

Summary

Stereotypies are repetitive behaviours induced by frustration, repeated attempts to cope and/or central nervous system dysfunction, and may signify suboptimal welfare conditions. Crib-biting is probably the most detrimental abnormal stereotypy in horses, prevention of its development is very important. Once fixed, crib-biting is difficult to eliminate by behaviour therapy, therefore several methods to inhibit its performance have been worked out. However, if crib-biting is a strategy to cope with stress, the effects of inhibition should be carefully estimated.

The first study discusses the potential risk factors for equine stereotypic behaviour. By conducting a questionnaire survey on 287 horses, we found that there is a possible influence of neighbours on stereotypic behaviour. The common belief that exposure to a stereotypic horse increases the risk of stereotypic behaviour has never been substantiated. This study reports the first empirical evidence for that hypothesis.

The second study compares the performance of tree-based methods to the logistic regression model using the data set from the first study. Our results suggests that tree based methods can be a new alternative in revealing risk factors even used alone or accompanying the logistic regression.

The third study compares ‘Nervousness’, ‘Trainability’ and ‘Affability’ temperament traits of 50 crib-biting and 50 control horses by using a previously validated questionnaire survey on equine temperament. Results showed that the passive coping strategy of crib-biters is responsible for the lower level of ‘Nervousness’ in crib-biting horses compared to control horses. No differences were found regarding ‘Trainability’ or ‘Affability’, which demonstrates that the previously reported impaired learning of stereotypic horses does not affect the horse’s performance or trainability in a negative way.

The aims of the fourth study were to develop a crib-biting triggering stress test and with the help of that to compare the stress coping ability amongst control and crib-biting horses and crib-biters treated with collar or the modified Forssell’s procedure. The test triggered crib-biting behaviour successfully and gave reliable and valid information about the severity of crib-biting, therefore it is a useful in veterinarian practice. Overall our results suggest that performance of oral stereotypies in a stress situation successfully diminishes stress, while inhibition of such stereotypy elevates it. Thus crib-biting may be a true coping strategy. It is emphasised that improving welfare (e.g. with environmental enrichment) should be as important as reducing the abnormal stereotypic behaviour of horses.

Összefoglalás

A sztereotípa egy ismétlődő viselkedés, melyet frusztráció, a stresszel való folyamatos küzdelem és/vagy a központi idegrendszer működésének zavara vált ki, valamint az állat életminőségének alacsony szintjének a mutatója. A lovak egyik legnagyobb gyakorlati jelentőséggel bíró sztereotípiája a levegőnyelés, mely súlyos egészségügyi problémákat is okozhat. A levegőnyelés gátlására számos módszer létezik, leszoktatni a lovat azonban nagyon nehéz róla. Amennyiben a levegőnyelés valóban segíti a ló stresszel szembeni küzdelmét, a sztereotípa gátlásával körültekintően kell bánni.

Az első vizsgálatban kérdőíves felmérést végeztem kilenc magyarországi lovardában 287 ló viselkedéséről, tartási körülményeiről. A sztereotípiát mutató ló szomszédsága jelentősen növelte a sztereotíp viselkedés előfordulási gyakoriságának az esélyét. A lovasok között általánosan elterjedt elképzelés, hogy a lovak eltanulhatják egymástól a levegőnyelést, de jelen vizsgálat az első, mely empirikus bizonyítékot szolgáltat erre vonatkozóan. A második vizsgálatban két klasszifikációs módszert, a döntési fát és a logisztikus regressziót hasonlítottam össze az első vizsgálat adatai segítségével. Kimutattam, hogy a döntési fa módszer hatékonyan alkalmazható az állatorvos-tudomány területén belül, és új alternatívát jelent a hajlamosító tényezők feltárásában.

A harmadik vizsgálatban egy korábban már validált kérdőív segítségével összehasonlítottam 50 levegőnyelő és 50 kontrol ló temperamentumát. Igazoltam, hogy a levegőnyelő lovak passzív megküzdési stratégiát használnak, és ezért a lovasok kevésbé „Jedősnek” minősítik őket a kontrol lovakhoz képest. A „Képezhetőség” és a „Barátságosság” tekintetében nem volt különbség a kontrol és levegőnyelő lovak között. Ezzel kimutattam, hogy levegőnyelő lovak viselkedés-tesztekben mutatott „gyengébb” tanulási képessége és a sztereotípa jelenléte nem befolyásolja negatív irányba a ló teljesítményét vagy képezhetőségét

A negyedik vizsgálatban elsőként kidolgoztam egy levegőnyelést provokáló stressz-tesztet, mely megbízhatóan igazolja a levegőnyelés jelenlétét. Majd a teszt segítségével vizsgáltam a nyakszíjjal és a módosított Forssell-féle műtéttel gátolt, valamint a nem gátolt levegőnyelő és kontrol lovak stressz-kezelési különbségeit. Az eredmények igazolják, hogy a levegőnyelés segíti a lovat a stresszel való megküzdésben. A levegőnyelés gátlásának hatására szignifikánsan romlik az állat stressz-kezelési képessége. Ezért a gátlás önmagában nem elegendő, a levegőnyelés gyógykezelése során a ló motivációját is csökkenteni kell (pl. környezetgazdagítással).

Chapter 1.

General introduction

Stereotypies are repetitive, relatively invariant actions considered abnormal when they occur without any primary function (Mason, 1991). Since function of any behaviour is seldom obvious, Mason and Rushen (2006) redefined stereotypy as repetitive behaviour induced by frustration, repeated attempts to cope and/or central nervous system dysfunction. This new definition poses new challenges to research, as the development of stereotypies is a long process and little is known about their origin; possible central nervous system dysfunctions are difficult to study; and coping function of a behaviour detrimental to the health of the animal is questionable.

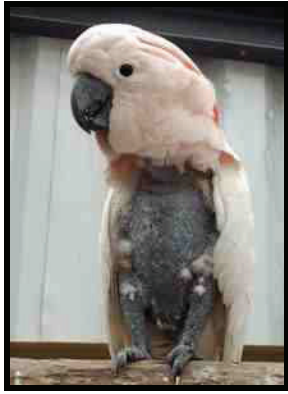
Stereotypical behaviour is widespread amongst captive animals and ungulates are the most prevalent mammalian stereotypies. Different orders of mammals typically favour different types of stereotypies. Amongst ungulates, oral stereotypies are more frequent, whereas carnivores usually suffer from locomotor stereotypies (e.g. pacing). Examples of stereotypic behaviour include pacing in captive large cats, circular swimming in captive polar bears, bar-biting, slat-chewing, chain-chewing and chain manipulation in tethered pigs, tongue-rolling, sham-chewing, vacuum-chewing and self-sucking in cattle, object-licking in giraffe and okapi, trunk-waving in elephants, bar-mouthing jumping and barbering in rodents, pacing and pecking in birds, paw-licking in cats and paw-chewing and tail-chasing in dogs (Bodó and Hecker, 1992; Mason and Rushen, 2006, **Figure 1.**).

Most common stereotypies in horses involve weaving, box-walking (locomotor stereotypies), crib-biting and wood-chewing (oral stereotypies). Among horse populations worldwide (United States, Canada, United Kingdom, Italy, Switzerland and Sweden) the mean prevalence of weaving, box-walking, crib-biting and wood-chewing are 4% (range 0-9.5%), 2% (0-5.5%), 4% (0-10.5%) and 18% (0-35%) (Vecchiotti and Galanti, 1986; McGreevy et al. 1995a, 1995b; Luescher et al., 1998; Redbo et al. 1998; McBride and Long, 2001; Mills et al., 2002; Waters et al., 2002; Bachmann et al. 2003a; Parker et al., 2008a; Albright et al., 2009).

As the definition of crib-biting and wood-chewing are sometimes ambivalent, differentiating them is important (Nagy and Bodó, 2009a, **Figure 1.**). Crib-biting usually involves seizing a projection with the incisors or supporting the chin, contracting the strap muscles of the ventral throat region, and emitting a grunt called “wind-sucking”. Radiographic and endoscopic study of horses performing crib-biting revealed that the characteristic noise of wind-sucking coincided with the in-rush of air through the cricopharynx, and appears to be more likely a burp than a gulp. During crib-biting, very little air goes down the oesophagus, and movement of air is not related to deglutition. Most probably it is provoked by the pressure gradient in the soft tissues surrounding the oesophagus created by the contraction of the strap muscles of the neck (McGreevy et al., 1995a). The ‘wind-sucking’ component makes crib-biting behaviour typical and distinguishable from wood-chewing (McGreevy et al., 1995a, 1995b; Albright et al., 2009). Some horses would wind-suck even without the use of a solid object. Morphological variation of this stereotypy exists, e.g. some horses would wind-suck without the use of a solid object or would press their chin against a fixed object and arch their neck and grunt (Turner et al., 1984; Lami, 1986).

Wood-chewing is often confused with crib-biting although the two behaviours are different in form and consequences to health. During wood-chewing the wood is eaten by the horse from a number of different sites within its stable or in the paddock, and is never accompanied by the grunting sound called wind-sucking (McGreevy et al., 1995b; Nicol et al., 2002; Waters et al., 2002; Albright et al. 2009). It has been debated whether wood-chewing should be considered a stereotypy as it can be frequently seen in the field and does not have such detrimental effects on health as crib-biting does (Mills and McDonnell, 2005). However, it has been suggested that wood-chewing and crib-biting might be related, because most horses chewed wood prior to developing crib-biting (Nicol et al., 2002; Waters et al., 2002).

Vacuum-chewing in cattle resembles the most to wind-sucking in horses, whereas bar-biting in sows shares the most similarities with wood-chewing. Interestingly, little information is available in the literature on the prevalence of stereotypies in other members of the Equidae family (asses, zebra), but there is evidence for a pacing zebra kept in zoo (Mason and Rushen, 2006).



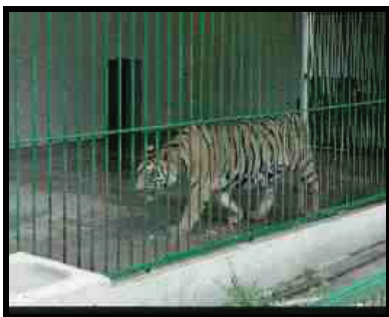
Self-plucked cockatoo
(www.aps.uoguelph.ca)



Bar-biting sow
(www.aps.uoguelph.ca)



Trunk-waving elephant
(www.aps.uoguelph.ca)



Pacing tiger
(www.aps.uoguelph.ca)



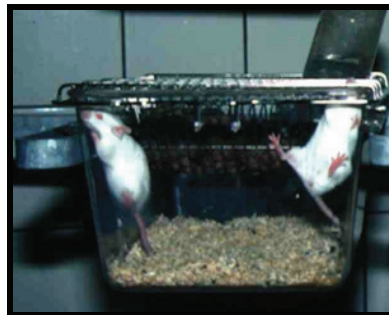
Self-sucking dairy cow
(*Servet Bademkiram*)



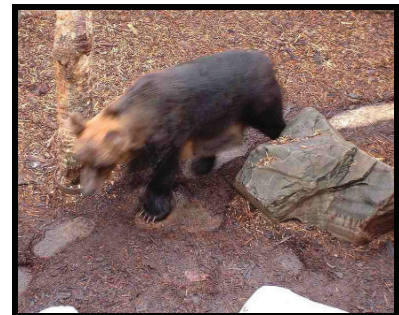
Paw-chewing dog
(www.aps.uoguelph.ca)



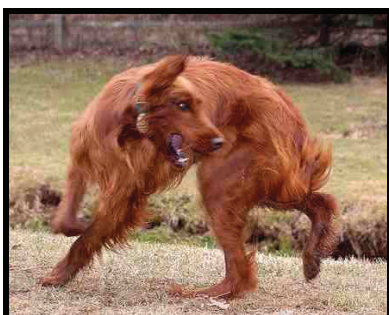
Wall-licking giraffe
(www.aps.uoguelph.ca)



Bar-mouthing and jumping mice
(www.aps.uoguelph.ca)



Pacing bear
(www.aps.uoguelph.ca)



Tail-chasing dog
(www.dogguide.net)



Wood-chewing horse
(www.aps.uoguelph.ca)



Crib-biting horse
(*Krisztina Nagy*)

Figure 1. Examples of animal stereotypic behaviour

Stereotypies are proved to be extremely challenging to study and, as a result, there is still much disagreement as to their origins, proximate causes and final consequences.

1.1. Origin

Long lasting stereotypies together with a variety of other abnormal behaviour can be precipitated by chronic stress as a consequence of restricted milieu at early age in many species (Mason, 1991). This process is reversible by environment enrichment but only at early life which, together with neurobiological evidence suggests a critical period for the neuroprotective effects of complex social and physical milieu (Hadley et al., 2006).

Stress may induce changes in mesoaccumbens dopaminergic activity resulting in an imbalance between the direct and indirect pathways of the basal ganglia and an enhanced efferent neural transmission from the striatum to the cortex (McBride and Hemmings, 2009). In concordance with this, crib-biting horses were reported to have more dopamine D1 and D2 receptors in the ventral striatum as well as lower density of D1 receptor subtypes in the caudate nucleus compared to control horses (McBride and Hemmings, 2005). The mesoaccumbens dopamine pathway is the primary neural center for initiation and control of goal-directed behaviours (either attaining rewarding or avoiding aversive stimuli). Therefore, the upregulated mesoaccumbens dopaminergic system can cause a highly motivated state directed toward goal attainment even in the absence of any goal-oriented situation, which may later serve as a basis for stereotypy development (McBride and Hemmings, 2009).

On the other hand, repetitive behaviours as normal displacement behaviour are often elicited by acute stress caused by inhibition of the appetitive phase of a goal directed behaviour (Hughes and Duncan, 1988). For example, non-stereotypic horses often performed weaving, nodding, and oral stereotypies when their companions were fed concentrate (Cooper et al., 2005). Although, such displacement behaviours are not abnormal, they are considered prerequisite for stereotypies (Waters et al., 2002). Sequences of behaviour starting with displacement followed by stereotypic behaviour can be observed even in stereotypic horses (Ninomiya et al., 2007). As most caretakers do not note such mild symptoms, studies are usually narrowed to true, fixed stereotypic behaviour.

1.2. Proximate causes

The development of stereotypic behaviour involves an interplay of genetic and environmental mechanisms. For instance, marked strain differences in mice interact with several environmental factors affecting the development of barbering. Even in inbred strains of mice consisting of genetically identical individuals, barbering and stereotypy vary considerably between individuals suggesting that small environmental perturbance acting upon similar genetic predisposition can make individuals differentially vulnerable to the effects of the type of housing (Garner et al., 2004). Regarding crib-biting in horses, certain breeds, like Thoroughbreds are more likely to be affected (Vecchiotti and Galanti, 1986; Luescher et al., 1998; Redbo et al., 1998; Mason and Rushen, 2006; Albright et al., 2009).

Management methods restricting natural behaviour have been suggested as major risk factors for developing stereotypic behaviour. Oral stereotypies (crib-biting/wind-sucking and wood-chewing) are mostly associated with rich diet and the restriction of normal grazing behaviour (McGreevy et al., 1995b; Waters et al., 2002; Bachmann et al., 2003a, Parker et al., 2008a; McBride and Hemmings, 2009), whereas locomotor stereotypies such as box-walking and weaving might be activated by social isolation or inadequate physical exercise (McGreevy et al., 1995b; Bachmann et al., 2003a).

1.2.1. Gastrointestinal dysfunction

Epidemiological and empirical studies revealed that crib-biting is primarily associated with feeding of concentrates; performed most frequently during and particularly following consumption of meals. The peak cribbing frequency begins usually one hour after feeding and the cribbing frequency may approximate 1470 events per horse per day (Henderson and Waran, 2001; McBride and Cuddeford, 2001; Cooper et al., 2005; Clegg et al., 2008).

The nature of the relationship between feeding concentrate and crib-biting is being widely investigated, although the exact mechanism is not well known. Crib-biting may represent an unsatisfied foraging need (Henderson and Waran, 2001; McGreevy et al., 1995b) caused by feeding low fibre, high carbohydrate concentrate diets. However, several studies suggest that stereotypies are not a response to nutrient deficits or reduced foraging time per se, but instead can represent coping with the gastro-intestinal consequences of consuming concentrates. Grain feeding can cause large intestinal acidosis, and periods of food deprivation increase gastric acid secretion and can cause foregut ulcers (Johnson et

al., 1998; Nicol et al., 2002). Such lesions are more prevalent in some breeds, e.g. Thoroughbreds, and management groups, e.g. racehorses (McGreevy et al. 1995c; Albright et al., 2009).

Thus, oral stereotypies may reflect, among others, a visceral discomfort (Johnson et al., 1998; Nicol et al., 2002; Hemmings et al., 2007). Function of crib-biting might be to lower the gastric acidity by increasing saliva production (Nicol et al., 2002; Hemmings et al., 2007; Moeller et al., 2008) or to reduce gut transit time from levels which otherwise would be even longer (McGreevy et al. 2001). Recent studies suggest that upregulation of transmission in midbrain dopaminergic pathways has important role in equine stereotypy development (McBride and Hemmings, 2009). The impaired basal ganglia function might be also associated with visceral discomfort (McBride and Hemmings, 2005; Hemmings et al., 2007), especially in horses with predisposed genotype or with early life experiences involving stress (Albright et al., 2009; McBride and Hemmings, 2009).

1.2.2. Stereotyping neighbours

According to anecdotal evidence, exposure to other horses engaged in stereotypies is a risk factor for developing similar habits (Haupt and McDonnel, 1993). Although controlled experiments or epidemiological studies have never supported such beliefs (Cooper and Albentosa, 2005). The belief that stereotypies are contagious is so widespread in owners that they often isolate stereotypic horses, in fact 39% of crib-biters were isolated from other horses according to one study (McBride and Long, 2001).

The effect of neighbours on horses with established stereotypies is controversial. On the one hand, it has been suggested that a poster image of a conspecific (Mills and Riezebos, 2005), a mirror in the paddock (McAfee et al., 2002; Mills and Davenport, 2002), or visual contact with other horses (Cooper et al., 2000; Mills and Davenport, 2002) effectively reduced the time spent in weaving. However, any treatment in those studies was limited to 2–5 days, a short period of time for habituation. Possible facilitation of weaving by exposure of weavers to non-stereotypic horses (Cooper et al., 2000) was also limited by the experimental design, because the two individuals with highest level of stereotypies were exposed to each other rather than to non-weavers in that study. On the other hand, observation of horses visually isolated or being able to see each other as a consequence of housing revealed that weaving was higher among horses whose boxes faced that of other horses than among horses whose boxes did not (Ninomiya et al., 2007).

1.3. Consequences

Stereotypies are believed to be indicators of suboptimal welfare conditions and it is questioned whether it is a maladaptive response detrimental to the animal, or a coping mechanism relieving some of the stress. In previous studies the evidence for a general coping function of crib-biting has been described as weak (Cooper and Albentosa, 2005; Mason and Rushen, 2006). Even though crib-biting behaviour was shown to be associated with a reduced plasma cortisol level (McBride and Cuddeford, 2001), and a decreased heart rate (Lebelt et al., 1998; Minero et al., 1999), the differences in stress physiology between crib-biting and control horses were unequivocal. Lebelt et al. (1998) found higher basal plasma β -endorphin level in crib-biters than in controls, however, McGreevy and Nicol (1998a) and Pell and McGreevy (1999) found no differences at all. Plasma cortisol level has been reported to be higher (McGreevy and Nicol, 1998a) or similar (Lebelt et al., 1998; Pell and McGreevy, 1999; Bachmann et al. 2003b) in crib-biters as compared to control horses. Minero et al. (1999) suggested higher basal sympathetic activity (LF component), and Bachmann et al. (2003b) reported lower vagal tone (HF component) in crib-biting than in control horses in rest. However, convincing correlation between HPA-axis activity (e.g. cortisol level) and coping style have not been always found, as it may depend on the type of stressor used and it is assumed to be more related to the emotionality than to the coping style (Koolhaas, 2008). It was also suggested that differences between crib-biting and control horses are only expected when crib-biting is prevented and horses are confronted with a stressor which triggers crib-biting (Bachmann et al., 2003). Cooper and Albentosa (2005) concluded that further research is needed to investigate whether the coping effect is truly causal in the development and persistence of crib-biting or it is merely a beneficial side effect.

A model introduced by McBride and Hemmings (2009) summarizing the interaction of the putative factors is presented in **Figure 2**. The effect of a stereotypic neighbour and visceral discomfort were added as further factors to the model.

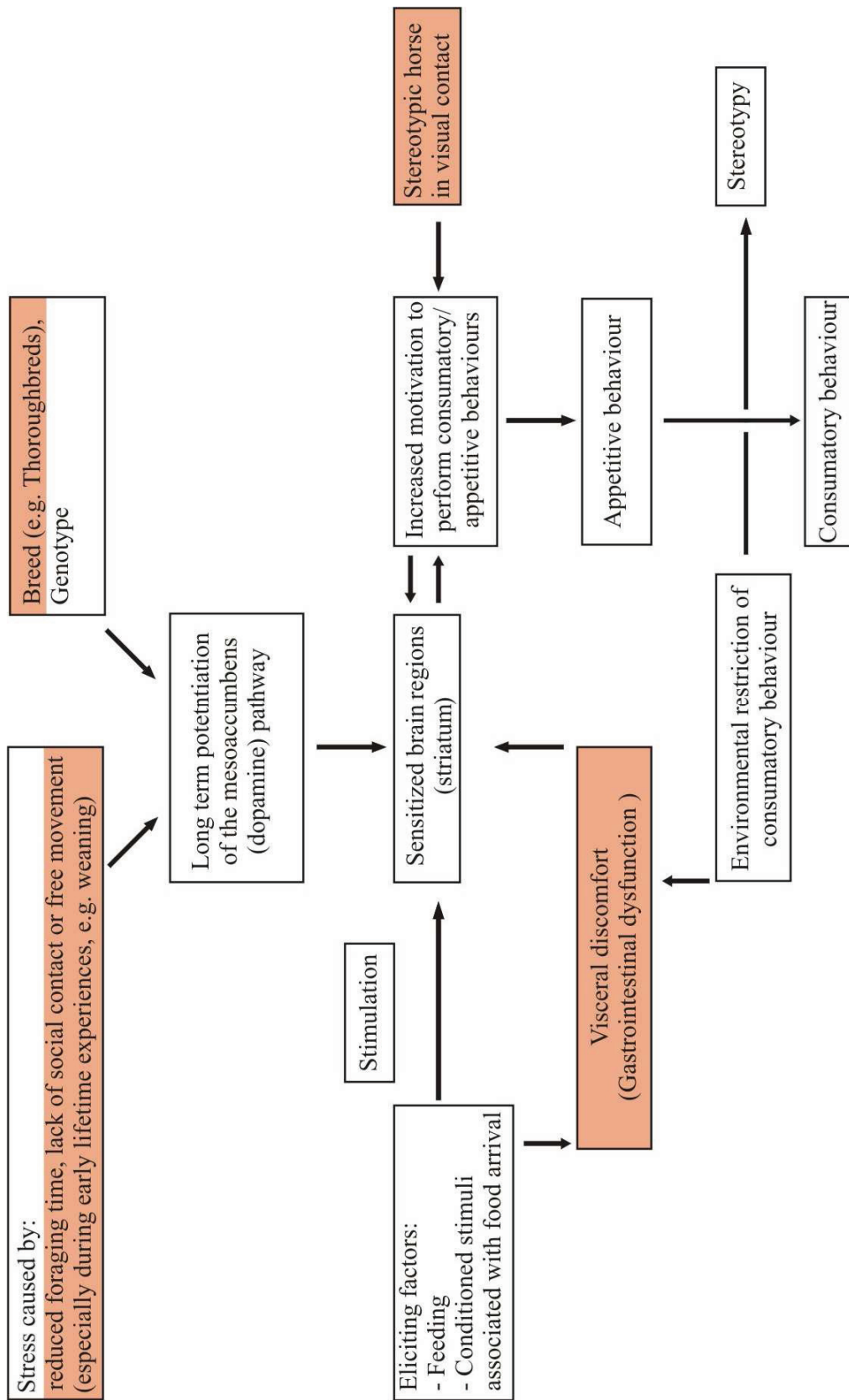


Figure 2. Considered causal factors of crib-biting in horses (McBride and Hemmings, 2009).

The coloured risk factors are incorporated to the model

1.4. Health, behavioural and cognitive correlates of crib-biting

Crib-biting is associated with tooth-erosion (**Figure 3.**), weight loss, altered gut function, such as decreased intestinal motility (McGreevy and Nicol, 1998a; Clegg et al., 2008), gastric inflammation/ ulceration (Nicol et al., 2002) and epiploic foramen entrapment colic (Archer et al., 2004, 2008). Because of such unwanted consequences of crib-biting, the economical value of the horse may decrease, and it is also considered an unsoundness (McBride and Long, 2001; Albright et al., 2009).



Figure 3. *Profound erosion of the incisor teeth of a severe crib-biter*
(Photo: Dr. Gábor Bodó)

Basal ganglia dysfunction may diminish learning abilities. For example, experimental inhibition of dopamine D1 receptors inhibit passive avoidance learning in chicks (Kabai et al., 2004), whereas stimulation of the dopaminergic system results in stereotypic behaviour in the same species (Kabai et al., 1999). In horse, crib-biters were less successful, required longer time to perform an instrumental task (Hausberger et al., 2007), and learned association persisted longer during extinction (Hemmings et al., 2007) compared to control horses. Furthermore, crib-biting horses failed to recognise differences in short versus long delays of a reinforcement in an instrumental choice procedure (Parker et al., 2008). Contrary to control horses, crib-biting horses seem to favour stimulus-response learning rather than response-outcome learning (Parker et al., 2009). Impaired learning abilities in crib-biting horses observed in behavioural tests raise the question whether trainability of crib-biting horses has also been affected. Previous studies suggested that crib-biting horses were more sensitive to stress (Minero et al., 1999; Bachmann et al., 2003) and spent less time eating and resting compared to control horses, which may further affect their performance (McGreevy et al., 2001; Hausberger et al. 2007).

1.5 Prevention of crib-biting

Because crib-biting can be detrimental to the animal, several methods have been worked out to diminish its occurrence. Treatment methods usually include attempts to prevent grasping of objects, to interfere with wind-sucking and to introduce punishment for grasping and neck-flexion, or suggest the use of acupuncture, pharmaceuticals, operant feeding and environmental enrichment (Nagy and Bodó, 2009a). Still, with any of the recently available treatment alternatives it is hard to abolish this stereotypy (**Figure 4.**). It can be speculated that the alteration of basal ganglia activity in stereotypic horses is persistent even after the stressor is removed (McBride and Hemmings, 2005; Hemmings et al., 2007; McBride and Hemmings, 2009) because of the involvement of endogen opioid - serotonin system in the process (Dodman et al., 1987; Gillham et al., 1994; McGreevy and Nicol, 1998a; Pell and McGreevy, 1999; McBride and Cuddeford, 2001).

The most widespread treatment is the application of crib-biting strap (collar), which makes flexion of the neck difficult and therefore the terminal grunting less easy to perform. Horses often adapt to the pressure applied by the collar, which is subsequently tightened, occasionally to the extent that skin trauma becomes apparent (**Figure 4.**). McBride and Cuddeford (2001) found that collar wearing increased heart rate, beta-endorphin and plasma cortisol level both in control and crib-biting horses, indicating that collar wearing per se imposes severe stress to the horse. Moreover, the first day after fitting the collar frequency of crib-biting significantly increased suggesting that the motivation to perform wind-sucking rises when prevented (McGreevy and Nicol, 1998b, **Figure 5.**).



Figure 4. *Horse with a collar covered with bandage around the occipital area*
(Photo: Krisztina Nagy)



Figure 5. *Horse performing crib-biting behaviour in an exaggerated fashion following inhibition (Photo: Krisztina Nagy)*

The modified Forssell's operation, which is a surgical veterinary procedure designed to treat crib-biting, is gaining popularity among horse-owners (**Figure 6.**). The method which was developed by Forssell in 1926 has been modified several times to improve efficiency and cosmetic appearance (Auer and Stick, 2006). Recent technique in use involves the removal of a 10 cm section of the ventral branch of the spinal accessory nerves (which innervate the sternomandibularis muscles), and 34 cm sections of the paired omohyoideus and sternothyrohyoideus muscles in order to reduce the distracting forces acting on the oesophagus. Operated horses are unable to draw the larynx caudally and consequently cannot emit the grunting sound (Auer and Stick, 2006).

The main complications of the modified Forssell's procedure are the development of swan-like neck or laryngeal hemiplegia. The assessment of its success-rate has revealed inconsistent results varying between 30 - 100% (Turner et al., 1984.; Hakasson et al., 1992; Schofield and Mulville, 1998; Delacalle et al., 2002). Previous studies on the success rate of the modified Forssell's procedure relied usually on the owner's reports and focused solely or mainly on the degree of stereotypy-inhibition, postoperative complications and side-effects. The implications of the treatment for the general welfare of the horse have not been studied until to date. Only Schofield and Mulville (1998) noted that stereotypy elimination may increase stress. Considering the low (30%) success rate reported by them,

they also suggested this method no longer to be recommended for the treatment of oral stereotypies in horses. The need to evaluate not only treatments itself but also the implications of a treatment for the welfare of the animal has become more apparent (Christiansen and Forkman, 2007).



Figure 6. *The modified Forssell's operation involves the myectomy of the sternohyoideus, sternothyroideus and omohyoideus muscles and the removal of the ventral branches of the accessory nerves (XI. cranial nerve).*

(Photo left: Krisztina Nagy, right: Simon Izing)

Aim of the studies

1. To investigate the risk factors of stereotypic behaviour in horses, and to see whether exposure to a stereotypic neighbour has a significant effect;
2. To compare the performance of classification methods used in risk factors analysis, such as the logistic regression model, the classification tree and the conditional inference tree methods;
3. To conduct a questionnaire survey on equine temperament and compare 'Nervousness', 'Trainability' and 'Affability' between crib-biting and control horses;
4. To develop a crib-biting triggering stress-test in order to prove the presence of this behaviour more accurately
5. To assess the success-rate of the modified Forssell's procedure, its implication for the welfare, and to compare the stress coping ability of surgically treated horses to that of collar treated, crib-biting and control (non-stereotypic) horses

Chapter 2.

Possible influence of neighbours on stereotypic behaviour in horses

2.1. Introduction

Previous studies indicated that breed type, feeding regime, housing, and management conditions have a strong effect on the development of stereotypies, and it is a commonly held belief that horses may learn to crib-bite from affected horses (Haupt and McDonnel, 1993). Although controlled experiments or epidemiological studies have never supported such beliefs (Cooper and Albentosa, 2005), McBride and Long (2001) reported 39% of crib-biters being kept isolated from other horses.

The aim of this study was to investigate risk factors of stereotypic behaviour in horses. We show that exposure to a stereotypic neighbour may have significant effect on the odds of horses performing abnormal stereotypies.

2.2. Methods

2.2.1. Data collection

We performed a questionnaire survey to detect potential risk factors of stereotypic behaviour (crib-biting/wind-sucking, wood-chewing, weaving, and box-walking) on 287 horses by visiting nine riding schools in Hungary. Before asking the horse-owners to complete the questionnaire, we explained them the definition of stereotypy and other behaviour problems (according to McGreevy et al., 1995b) During our 4–6 h stay at each riding school we observed stereotypic behaviour in all horses reported to show that. The survey items focused on subject variables, housing, management conditions, food regime, stereotypies, and problematic behaviour performed by the individual horse or by a horse in its visual contact.

2.2.2. Data analysis

Following a data-quality check, variables likely to be subject to recording bias and variables with a frequency more than 50% of missing values were excluded from further investigation. The remaining variables were classified as being recorded on a binary or an ordinary scale.

Stereotypies were dependent variables in five separate models (crib-biting/wind-sucking, wood-chewing, weaving, box-walking, and stereotypies in general, a derived variable for the occurrence of any of the four listed abnormal behaviours). The independent variables included the usage of the horse (competition or non-competition), subject variables, housing, and management conditions (as summarized in **Table 1.**), problematic behaviour performed by the given horse (aggression towards horses and people, door opening or knot-untying behaviour, and behaviour problems during riding), stereotypies and problematic behaviour of the “neighbour horses”. Position of the horses in the stable was also recorded so spatial proximity of stereotypic horses could be calculated. Horses not further than three boxes away and in three boxes across the aisle facing the given individual were considered neighbours. Riding school was considered as a random variable, and its effect was calculated within the statistical models.

Table 1. *Items of the questionnaire survey*

Age	3-5 years (9)	5-15 years (101)	15-20 years (15)	Missing value (1)
Gender	Male (16)	Gelding (69)	Mare (41)	
Breed	Thoroughbred(6)	Halfbred ^a (61)	Other (30)	Missing value (29)
Number of horses on the yard	10-30 (62)	30-60 (55)	More than 60 (9)	
Type of housing	Stall (21)	Box (105)		
Bedding	Straw (124)	Shavings (2)		
Frequency of feeding roughage (hay) per day	2 times(30)	3 times (65)	5 times (31)	
Frequency of feeding concentrates (oats) per day	1-2 times (100)	3 times (26)		
Daily amount of oats in kg	Less than 7 (93)	More than 7 (33)		
Tactile contact with other horses	Just sniff or see (17)	Touch (109)		
Move freely in the paddock weekly	Less than once (13)	1-2 days (18)	3-5 days (27)	More than 5 days (68)
Weekly box-rest	Less than 1 day (103)	More than 1 day (23)		
Weekly riding	Less than once (9)	1-2 times (0)	3-5 times (29)	More than 5 times (88)
Number of riders using the horse	No riders (5)	1-2 riders (58)	More than 2 riders (63)	
Daily grooming time (minutes)	Less than 10 (42)	10-30 (53)	More than 30 (30)	Missing value (1)

Levels of the independent factors (subject variables and housing and management conditions), and the number of non-competition horses in each level in brackets.

^a *Cross between thoroughbred and Hungarian native breeds.*

2.2.3. Statistical analysis

To determine which of the survey answers were the best predictors of the presence or absence of a stereotypic behaviour, first we performed a univariate analysis for the five stereotypic categories independently, using a single logistic mixed regression (generalised linear mixed models, GLMM) separately for all factors obtained from the questionnaire. Variables with $P < 0.10$ were considered for initial inclusion in the mixed-effect multivariate logistic regression model. Variables lacking a statistically significant association ($P > 0.10$) with the particular outcome variables were excluded from the given model, however, the same variables could have been included in the other models.

In the GLMM the effect of the riding school was treated as a random factor to control for possible yard effects. In case of crib-biting the variance of the random factor was negligibly small compared to the variance of the error term, so in this case we have used the generalised linear model (GLM) instead of the GLMM. GLM and GLMM were applied with binomial error distribution and logit link function. The penalised quasi likelihood method (PQL) was used to estimate the parameters in the GLMM (Breslow and Clayton, 1993). The models were manually built using a backward elimination process. Variables with a $P > 0.05$ were excluded, except in the case of crib-biting and stereotypies in general, where $P > 0.10$ was chosen to ensure better-predicted values by the models. The removal of the non-significant factors resulted models with lower Akaike information criterion in each case, interpreted as that the explanatory power of the initial and final models are the same. The exponentials of b-coefficients in the final models were interpreted as odds ratios of the five outcome variables. All analysis was carried out using the R statistical software 2.2.0 (Ihaka and Gentleman, 1996).

2.3. Results

2.3.1. Housing conditions and prevalence of behavioural disorders

In the population of 287 horses sampled, the prevalence of crib-biting/wind-sucking was 4.53% (N = 13), wood-chewing was 10.10% (N = 29), weaving was 2.79% (N = 8), and box-walking was 3.83% (N = 11). The occurrence of any of the four listed abnormal behaviours (stereotypic behaviour in general) was 16.70% (N = 48). Aggression towards horses was noted in 17.07% (N = 49) of individuals, aggression towards people was 9.41%

(N = 27), behaviour problems during riding was 9.06% (N = 26), and door or tier opening behaviour occurred in 5.23% (N = 15).

Stereotypies were more common in non-competition horses compared to competition horses (33 out of 126 non-competition horses and 12 out of 161 competition horses showed abnormal stereotypic behaviour; $\chi^2_1=13.264$, P = 0.0003). Although the risk factors revealed by the final models were consistent concerning non-competition horses (N = 126) and all horses (N = 287), to ensure reliable classification we present the data on risk factors among non-competition horses.

Housing conditions among non-competition horses were similar (**Table 1**). There was no variation in forage type (only hay was offered), all horse's diet contained grains, no individual was socially isolated, and all horses except two had straw bedding. All horses but nine individuals were ridden at least three times a week, most of them had no rest day and could move freely in the paddock 3 or more days a week. All horses but six individuals were non-thoroughbred, and the total number of horses in a yard were less than 60 in most cases.

2.3.2. Risk factors

Univariate GLMM statistics revealed 11 factors (presence of a weaving, or crib-biting, or box-walking or aggressive neighbour, performing aggression towards horses, receiving oats more than two times a day, more than two riders using the horse, performing box-walking, crib-biting, wood-chewing or door and tier opening behaviour) associated ($p < 0.10$) with any of the four stereotypies (**Table 2**). Eight of those factors (presence of a weaving, or crib-biting, or aggressive neighbour, performing aggression towards horses, receiving oats more than two times a day, more than two riders using the horse, performing wood chewing or box-walking) remained significant in the final model of the multivariate GLMMs (**Table 2**). The overall classification accuracy of the final models was 81–97%. Each model classified horses without stereotypic behaviour more accurately (96–100%) than stereotypic horses (30–65%) suggesting that risk factors are not absent in normal horses.

Table 2. *Risk factors of stereotypies in the final multivariate models*

Outcome variable	Risk factors	OR	CI at 95%	P
Crib-biting /wind-sucking	presence of a weaving neighbour	20.81	1.74-315.12	0.018
	aggression towards horses	11.36	2.36-82.89	0.005
	presence of a crib-biting neighbour	6.59	0.63-66.7	0.099
	presence of a box-walking neighbour	–	–	n.s.
	presence of an aggressive neighbour	–	–	n.s.
	door and tier opening behaviour	–	–	n.s.
	box-walking	–	–	n.s.
	wood-chewing	–	–	n.s.
Wood-chewing	receiving oats more than 2 times a day	24.82	2.66-231.47	0.006
	box-walking	33.44	4.19-266.52	0.001
	more than two riders using the horse	–	–	n.s.
Weaving	presence of a weaving neighbour	14.14	1.75-113.98	0.014
	presence of an aggressive neighbour	20.21	3.30-123.62	0.002
	more than two riders using the horse	33.05	4.44-245.83	0.001
Box-walking	aggression towards horses	6.11	1.51-24.75	0.013
	wood-chewing	35.84	7.95-161.65	<0.001
	more than two riders using the horse	0.02	0.00-0.19	0.001
	crib-biting	–	–	n.s.
	presence of an aggressive neighbour	–	–	n.s.
Stereotypies in general	presence of a stereotypic neighbour	10.14	2.22-46.29	0.003
	aggression towards horses	4.43	1.52-12.86	0.007
	receiving oats more than 2 times a day	3.40	0.89-13.03	0.070
	presence of an aggressive neighbour	–	–	n.s.

Odds ratios (OR) and confidence intervals (CI) of the significant risk factors for stereotypic behaviours included in the final mixed-effect multivariate logistic regression. The non-significant (n.s.) factors have not been included in the final multivariate model, although they were preselected by the univariate analysis.

2.4. Discussion

Prevalence of stereotypic behaviour in Hungarian riding schools was not different from those of other countries (McBride and Long, 2001; Christie et al., 2006). However, factors associated with stereotypic behaviour revealed by our analysis are in apparent contrast with previous epidemiological surveys.

It is not surprising that many risk factors reported by others were not identified in our study, because management practices in the studied nine riding schools were very similar and lacked most of the risk factors identified by previous studies, and other factors, like behaviour of the neighbouring horses, have not been included in previous surveys.

Only two management conditions emerged as risk factors for stereotypic behaviour. First, receiving concentrates more than twice a day increased the odds of wood-chewing and stereotypic behaviour in general, in accordance with previous studies (McGreevy et al., 1995b; Waters et al., 2002; Bachmann et al., 2003a). Second, having more than two riders increased the odds of weaving, but diminished the odds of box-walking. The number of riders as a possible risk factor has not been studied previously, and such a bi-directional effect is difficult to explain, as box-walking was reported to coincide with weaving (Mills et al., 2002). In a previous study, Ninomiya et al. (2007) found that contact with unfamiliar riders increased the incidence of weaving, however, they provided no data on the number of riders. We can only speculate that horses ridden by many riders are more stressed (increasing the odds of weaving) but also receive more exercise, thus reducing the odds of box-walking. Disentangling the possible effects of number of riders, amount of exercise and riding by strangers needs further investigations.

All other risk factors, such as aggression toward horses or presence of a neighbour performing aggression or some kind of stereotypy, are behavioural variables. The nature of the relation between aggression and stereotypies is poorly understood. Dominant horses are usually more aggressive, and according to the findings of Waters et al. (2002), foals of dominant mares are more likely to develop abnormal behaviour, perhaps because they are sensitive to even slight restrictions. Others believe, however, that both stereotypies and aggression are a common consequence of frustration (Mills and Nankervis, 1999).

Presence of an aggressive or stereotypic neighbour increased the odds of cribbiting/wind-sucking and weaving, a finding not reported previously (**Figure 7.**). The possibility that stereotypic horses in proximity to each other experience the same stressor (waiting for food distribution, or to be driven out of the stable) can be ruled out, because the position of stereotypic horses was random in relation to distance to the gate, windows or ventilation of the stable. Caretakers reported that positioning of newly arrived horses was determined by the availability of free space, and stereotypic horses were not deliberately put close to each other in the paddock.

Epidemiological studies are not designed to reveal causal relations; therefore we should be careful to interpret our findings. It is possible that factors not present at the time of the survey are responsible for the development of stereotypies in some individuals. Some existing factors could have been overlooked. For example, we have data on the daily frequency of feeding roughage; however, it is not known how long roughage was available after it was provided. Nevertheless, such possible sampling biases do not explain significant associations between the behaviour of neighbours.

The effect of neighbours has been reported on other species with stereotypic behaviour (in bank voles, *Clethrionomys glareolus*: Cooper and Nicol, 1994; in laboratory mice: Garner et al., 2004; or in Orange-winged Amazon parrots, *Amazona amazonica*: Garner et al., 2006). In horses, copying or observational learning as a mechanism of developing stereotypies is unlikely (Lindberg et al., 1999); however, social facilitation or stimulus enhancement cannot be ruled out. A stereotypic neighbour spends less time eating and resting than normal horses (McGreevy et al., 2001). As stereotypies are more frequent in “disturbed” environment, or when general activity of the horses is great (Cooper et al., 2000), constant movement during weaving, or sound production during wind-sucking by stereotypic horses can make sensitive individuals restless and induce stereotypic behaviour. There is only anecdotal evidence for a possible role of stimulus enhancement. Cooper et al. (2000) noted that horses showed interest in areas cribbed by stereotypic neighbours. Horses who observed cribbing were not seen cribbing themselves; however, contact of horses with no stereotypy with individuals performing stereotypic behaviour was limited to 5–10 days, a short period for the emergence of abnormal stereotypy.

The precise nature of the possible effect of neighbours on stereotypic behaviour should be studied experimentally before concrete suggestions can be given for practical management. However, as the majority of managers believe that stereotypies are not just inherited but also learned from other horses (McBride and Long, 2001) we cannot avoid the responsibility to make some general remarks. On the basis of our epidemiological studies we cannot state that neighbours have a causal effect on stereotypic behaviour, our aim in presenting these findings was to draw attention to the possibility of such effects so that other researchers would include this variable in their surveys. Even if such effects are substantiated by other studies, isolating stereotypic horses is bad management because social deprivation enhances stress and attenuates stereotypic behaviour (Bagshaw et al., 1994). Careful monitoring of the horses for early signs of enhanced displacement behaviour is important to prevent the establishment of stereotypies. Horses susceptible to developing stereotypies might be moved away from stress agents, including stereotypic neighbours.



Figure 7. *Horses performing crib-biting behaviour in community*
(Photo: Krisztina Nagy)

Chapter 3.

Tree-based methods as an alternative to logistic regression in revealing risk factors of crib-biting in horses

3.1. Introduction

Classification methods revealing risk factors of a certain disease provide not just a better understanding of the disease, but are also useful to classify individuals into risk groups with some certainty. Logistic regression is a commonly used statistical method for finding risk factors which is a standard method for predicting a dichotomous dependent variable. A logistic regression model is a linear regression equation in which the response variable is the log odds (Hosmer and Lemeshow, 2004).

The tree-based methods, such as the classification and regression tree (CART) and the conditional inference tree analysis use a form of binary recursive partitioning. If the outcome variable is measured on a continuous scale, the method is called regression tree while in case of a categorical outcome variable (like crib-biting in the present study) it is called classification tree. Tree-based methods split the sample step by step into smaller and smaller groups according to a mathematical condition. There are several variants of tree-based methods with different splitting criteria. For example, one of the oldest tree classification methods, the CHAID technique uses an F test if the dependent variable is continuous and χ^2 if the variable is categorical to decide which group to split (Kass, 1980).

Out of the several criterion functions, Gini-index is the most often used (Kurt et al., 2008). The basic idea is to consider all possible splits and choose the best predictor and the best split to maximize 'purity', i.e. homogeneity of the child nodes. Variables might be selected repeatedly on different levels of the tree, also with different thresholds (Breiman et al., 1984). After an initial large tree is built, pruning is done to remove any overfitting to the training data. An automatic pruning method is the cost-complexity pruning based on cross-validation. It reduces the number of branches of the tree (variables selected as risk

factors) and minimises the percentage misclassified at the same time. A plot of percentage misclassified against the number of terminal nodes helps determine the optimal tree-size.

Recursive fitting procedures have been reported to have two fundamental problems: overfitting and a selection bias towards covariates with many possible splits or missing values. While pruning procedures are able to solve the overfitting problem, the variable selection bias still seriously affects the interpretability of tree-structured regression models.

Conditional inference tree is a rather new tree-based method, which estimates a regression relationship by binary recursive partitioning in a conditional inference framework. Therefore, it selects variables in an unbiased way. A statistically motivated stopping criterion (e.g. c_{quad} -type test statistics) is used and the partitions induced by this recursive partitioning algorithm are not affected by overfitting. Partitions obtained from conditional inference trees have been reported to be generally closer to the true data partition compared to partitions obtained from an exhaustive search procedure with pruning (Hothorn et al., 2006).

Tree-based methods has been widely used in computer sciences, and it is also getting more popular in life sciences; e.g. in human health care (Worth and Cronin, 2003; Ho et al., 2004; Kurt et al., 2008), medical decision making (Harper, 2005), or psychiatry (Thomas et al., 2005); as well as in the field of ecology (Low et al., 2006) or animal behaviour (Kubinyi et al., 2009). However, application of tree-based methods in veterinary sciences is not so prevalent. In this study we would like to show an example how CART and conditional inference trees can be applied in predicting risk factors of stereotypic behaviour in horses. To illustrate the effectiveness of tree-based methods and to compare them to logistic regression, the data set on risk factors of crib-biting in horses presented in Chapter 3. was re-analyzed.

3.2. Methods

3.2.1. Data

Data of horses (for details see 3.2.1) used for non-competition purposes ($N=126$) was analysed by using three statistical approaches: logistic regression, CART and conditional inference tree methods.

3.2.2. Classification techniques

3.2.2.1. Logistic regression

Logistic regression model was built as given in 3.2.3.

3.2.2.2. Classification tree and conditional inference tree

The brief description of the applied binary recursive partitioning algorithm, omitting the mathematical details, is as follows. The method starts with one single group, the whole sample. In the first step an explanatory variable and a threshold is selected and the sample is split into two groups: one in which the value of the selected explanatory variable is over the selected threshold, and the other in which it is below the threshold. That variable and threshold is selected that leads to the split with the most “pure”, i.e., most homogeneous groups with respect to the outcome variable. In each subsequent step a group, an explanatory variable, and a threshold is selected, and the selected group is split into two, based on the selected variable and threshold. The criterion defining the homogeneity of groups, and so driving the whole partitioning process, is called the split criterion, or splitting function. There are several split criteria, of which we applied the most often used one, the so-called Gini index. The process results in a tree-like structure of groups, also called nodes, in which each node has two “child nodes”. Terminal nodes, also called branches of the tree, define the classification of subjects.

Classification tree was built by splitting each node until its child nodes contained less than 3 observations. Gini index was used as splitting function and 10-fold cross-validation was applied to evaluate performance of the classification. After the initial large tree has been constructed, pruning was performed to reduce the size of the tree. Tenfold cross-validation means that the following procedure is repeated ten times: a 10% random sample is selected from the data, the model is fitted to the remaining 90%, and prediction is made for the selected 10% from the fitted model. Classification performance is calculated by pooling classification results of the ten replications.

Conditional inference trees were constructed with c_{quad} -type test statistics and $\alpha=0.10$, with and without simple Bonferroni correction. Each split needed to send at least 1% of the observations into each of the two child nodes.

To compare the three statistical approaches we examined prediction accuracy via the indices of sensitivity (correctly classified stereotypic horses) and specificity (correctly classified non stereotypic horses) of the models. All analyses were carried out using the R 2.7.2. Statistical Software (17). CART analysis and conditional inference trees are implemented in the *rpart* and *party* add-on packages to the R system for statistical computing.

3.3. Results

3.3.1. Risk factors

3.3.1.1. Logistic regression

Eight variables were selected by the univariate GLM into the logistic regression model, but the final model contained only 3 variables (**Table 2.**). Horses that were kept in the neighbourhood of another crib-biting or weaving horse were more likely to show crib-biting themselves, with odds being 7 and 21 times greater respectively. Horses with aggressive behaviour towards other horses had 11 times greater risk of performing crib-biting than non-aggressive horses. The logistic regression model classified horses without stereotypic behaviour more accurately (specificity: 99 %) than crib-biting horses (sensitivity: 40 %).

3.3.1.2. Classification and regression tree

Figure 8. shows the variables selected by the CART model before (**Figure 8a.**) and after (**Figure 8b.**) pruning. The first ramification point represents the first risk factor selected by the procedure. It tells us that the prevalence of crib-biting behaviour is higher amongst horses that have a stereotypic (weaving) neighbour compared to those who do not have any.

The left route represents altogether $2+1+0=3$ horses without and $0+0+2=2$ horses with crib-biting behaviour, that is, 60 percent of those non-competition horses that had stereotypic (weaving) neighbour showed crib-biting behaviour. In this route the next ramification point, i.e., the next predictive factor found by the procedure was presence of a crib-biting neighbour. On the other hand, horses that did not have a weaving neighbour were further divided by the model according to whether they show aggression behaviour or not.

Horses that did not have a weaving neighbour, but were showing aggression towards other horses seemed to have higher prevalence of crib-biting behaviour than those horses that were classified as not aggressive towards other horses by the owners. In other words, 19 percent ($3+1+1+0=5$ out of $5+1+4+17=27$) of those horses that were lacking of a stereotypic neighbour but had aggressive tendencies towards other horses showed crib-biting behaviour.

To improve homogeneity of the child nodes, two more risk factors were selected by the final pruned tree model (**Figure 8b**). Out of those horses that showed aggression towards other horses, crib-biting behaviour seemed to be more prevalent amongst horses housed in stalls (tethered with a rope in the stall, with restricted free movement), or housed in boxes but showing 'escape behaviour' (door opening or knot-untying). The remaining crib biting horses were amongst horses that had no weaving neighbour and showed no aggression towards other horses.

Classification tree method (after pruning) classified 97% of horses without stereotypic behaviour and 60 % of crib-biting horses correctly.

3.3.1.3. Conditional inference tree

Building a tree without any correction included more variables and nodes than the tree built using Bonferroni correction (**Figure 9a**). Similarly to the pruned classification tree, the conditional inference tree with Bonferroni correction contained 4 risk factors and 6 nodes (**Figure 9b**). The interpretation of this tree is similar to that of the CART model. Conditional inference tree with Bonferroni correction classified 97% of horses without stereotypic behaviour and 60 % of crib-biting horses correctly.

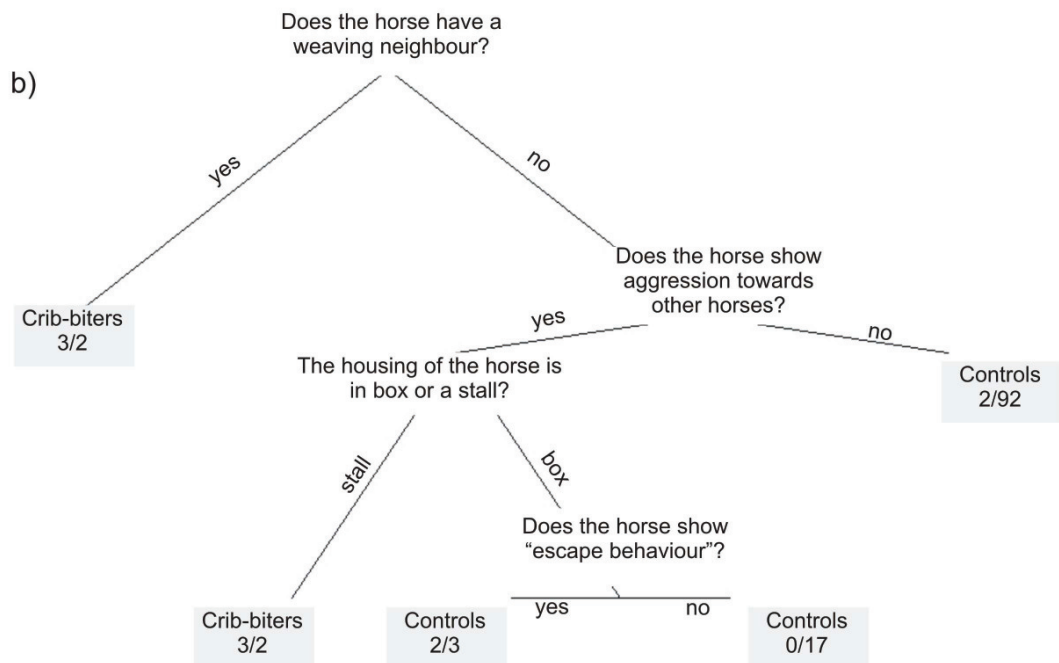
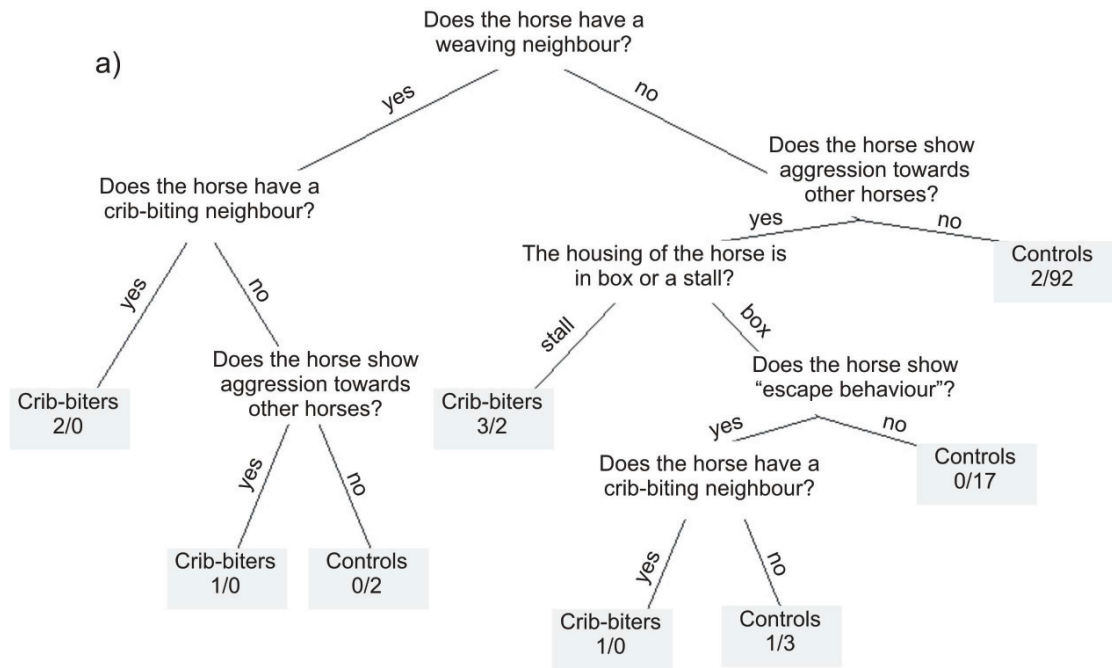


Figure 8. Classification tree before (a) and after (b) pruning.

The ramifications of the tree represent the risk factors. Terminal nodes are categorised as crib-biters or controls (non-stereotypic) according to the number of crib-biting and control horses observed in the sample at that node (the numbers displayed at each terminal node).

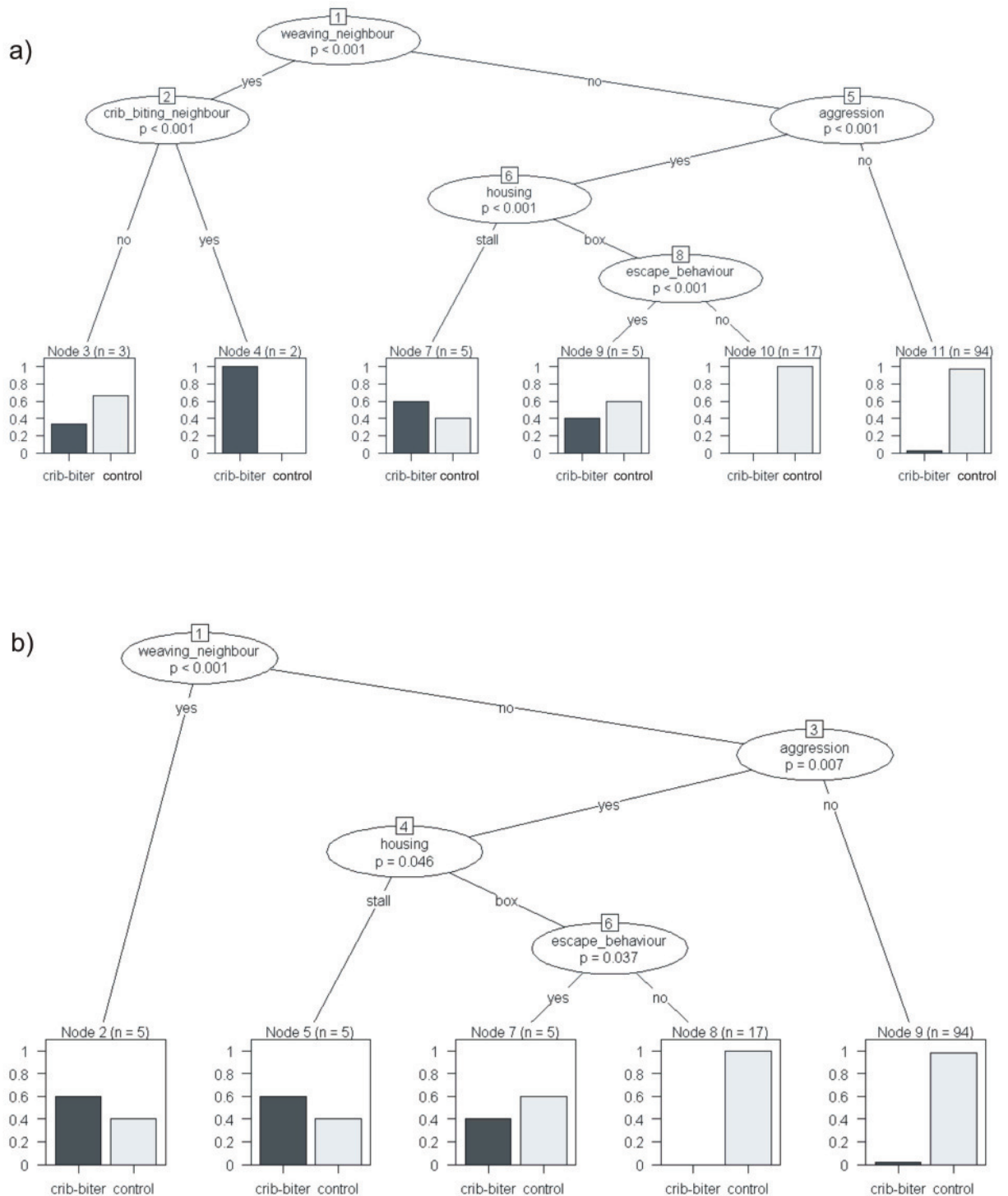


Figure 9. Conditional inference tree without (a) and with (b) correction.

Risk factors selected by the algorithm are symbolised as ramification points of the conditional inference tree along with the uncorrected (a) or corrected (b) p-values. In (a) nodes 3, 4, 7, 9, 10 and 11; in (b) nodes 2, 5, 7, 8 and 9 are the terminal nodes. Bar plots visualise the probability of crib-biting behaviour and the number of observations within each subgroups.

3.4. Discussion

Analyses revealed similar risk factors in all 3 models. Contrary to the findings of Hothorn et al. (2006), we found no difference regarding tree structure or predicting accuracy between classification tree and conditional inference tree methods. Berzal et al. (2003) also reported that different splitting criteria had no significant impact on the accuracy of the classifier, and no single splitting criterion proving to be universally better than the rest.

Risk factors revealed by logistic regression were less in quantity compared to the number of risk factors selected by CART and conditional inference tree methods, but all three methods found the two main risk factors reported previously by Nagy et al. (2008): presence of a weaving neighbour and aggression towards horses. According to tree-based methods, it seems like that the influence of crib-biting neighbours on crib-biting behaviour in horses may manifest itself only in special circumstances, which is also suggested by Albright et al. (2009). Other risk factors selected by the tree-based methods are also in accordance with previous findings. Tethering as a method of managing horses is unsatisfactory from many points of view (National joint Equine Welfare Committee and the R.S.P.C.A.), and our results also suggest that if stable environment has a restrictive nature from a locomotor perspective (horses housed in stalls and tethered with a rope), horses show crib-biting behaviour more likely.

Some risk factors reported by others were not identified in our study, e.g. the effect of breed. This could be due to the small variability of breeds in our study (Nagy et al., 2008). Most horses were Hungarian half-breeds, while the number of thoroughbreds, the breed most likely to be affected by stereotypies (Albright et al., 2009), was as few as six. However, other studies also reported no difference between breeds with respect to stereotypic behaviour (Parker et al., 2008a). Some other factors often mentioned in association with crib-biting behaviour, such as gastrointestinal discomfort (Waters et al., 2002) or basal ganglia dysfunction (McBride and Hemmings, 2009) were not examined in this study.

Compared to the logistic regression, tree-based methods, due to their hierarchical nature, are able to demonstrate factors that are risk factors only under special conditions. Note that the same factor can appear twice or more in the same tree (like crib-biting neighbour in **Figure 8a**), and may have a slightly different role at each appearance,

depending on the context, that is, the preceding factors located above it. This feature allows for detecting non-linear relationships and interactions between the factors.

Due to this flexibility, the tree method helps in better understanding of the problem under study. In our case it also resulted in better prediction accuracy than the logistic regression. Specifically, prediction accuracy of crib-biting horses (sensitivity) was much better by the tree-based methods than by the logistic regression, however, specificity were only slightly lower. This is in accordance with previous findings (Worth and Cronin, 2003; Ho et al., 2004; Thomas et al., 2005).

Tree-based methods do not have strict applicability conditions like the logistic regression, work well with complex datasets, are less influenced by the multicollinearity of the variables, and handle the missing values and low prevalence easily. Their output, the tree diagram, shows the probability of the occurrence of the events and vividly illustrates the structure of the risk factors and their complex interactions, which would be difficult or even impossible to model by logistic regression. Therefore, it makes the findings easier to interpret, even to those with less statistical background (Harper, 2005; Thomas et al., 2005; Kurt et al., 2008).

In summary, we can conclude that tree based methods (either CART or conditional inference tree) are useful tools in finding risk factors, or even for data mining, alone or together with logistic regression method.

Chapter 4.

Differences in temperament traits between crib-biting and control horses

4.1. Introduction

Recent studies have suggested that crib-biting in horses is associated with learning disabilities and with reduced stress tolerance. Studies on reaction of crib-biting horses to short-term stressors are limited. Minero et al. (1999) found that crib-biting horses showed less reactivity while restrained with a lip-twitch. However, when horses were exposed suddenly to the rapid inflation of a balloon, crib-biters tended to react stronger to the stimulus (Minero et al., 1999). Bachmann et al. (2003) found that crib-biters had lower basal vagal and higher sympathetic tone than control horses, resulting in less flexible physiological reactivity when facing a stressor.

It is also a relevant question whether individual ‘personality’ differences play a role in the phenomenon. Personality is often defined as an individual’s distinctive pattern of behaviour that is consistent across time and situations. Animal personality studies, especially in dogs and horses, have become very popular in the last decade (Jones and Gosling, 2005; Lloyd et al., 2007), but differences in equine temperament traits among crib-biting and control horses are not well examined.

Temperament traits can be measured directly by behavioural tests (Le Scolan et al., 1997; Visser et al., 2002, 2003; Lansade et al., 2008) or by questionnaire surveys, using the subjective ratings of the rider or handler who is familiar with the horse (Morris et al., 2002; Momozowa et al., 2005; Lloyd et al., 2007; McGrogan et al., 2008). Advantages of the questionnaire are that it is based on long-term observations of the raters and in a relatively short time several traits can be assessed. The efficacy of questionnaire-based assessment has already been demonstrated in a variety of species. Most animal species have shown personality traits similar to the main human factors (Gosling, 2001).

To reveal dimensions of horse personality, Morris et al. (2002) used a specially modified version of the most commonly used human personality questionnaire (Costa and

McCrae, 1992) based on a five-factor model ('Openness', 'Conscientiousness', 'Extraversion', 'Agreeableness' and 'Neuroticism'). Three of the five factors ('Neuroticism', 'Extraversion', and 'Conscientiousness') had good internal consistency and inter-rater reliability but raters found it hard to apply 'Agreeableness' and 'Openness' to horses. Lloyd et al. (2007) used the rating method of Stevenson-Hinde et al. (1980) to assess the personality of horses and found six main factors that were labelled 'Anxiousness' (which was similar to that of 'neuroticism'), 'Excitability', 'Dominance' (similar to 'Agreeableness'), 'Protection', 'Sociability' (shares common items with 'Extraversion') and 'Inquisitiveness' (relates to 'Openness'). McGrogan et al. (2008) used questionnaire items based on natural language of horse owners to assess the factor structure of horse personality and found three main factors which resembled to the human 'Neuroticism', 'Extraversion' and 'Agreeableness'.

Momozowa et al. (2005) found three main factors which were termed as 'Anxiety', 'Affability' and 'Trainability'. The 'Anxiety' factor is similar to 'Neuroticism' and is based on items reflecting 'Nervousness', 'Excitability', 'Panic', 'Inconsistent emotionality', 'Vigilance', 'Skittishness', and 'Timidity'. 'Affability' contains items as 'Friendliness toward people', 'Cooperation', 'Docility' and 'Friendliness toward horses' and shares similarities to 'Agreeableness'. 'Trainability' is based on 'Concentration', 'Trainability', 'Memory', and 'Perseverance'. With this questionnaire, 'Trainability' could be measured separately from 'Anxiety'. Previous studies did not measure 'Trainability' per se (Morris et al., 2002; Lloyd et al., 2007), or revealed a factor which contained items related to 'Trainability', 'Dominance' and 'Neuroticism' as well ('Extraversion' factor of McGrogan et al., 2008)

To answer the questions of this study, the personality questionnaire of Momozawa et al. (2005) was chosen since it allowed comparisons of 'Anxiety', 'Trainability' and 'Affability' traits between crib-biting and control horses and its reliability and validity has been previously shown. These factors were conserved in two separate surveys representing true temperamental traits. All three temperament traits found by Momozawa et al. (2005) had sufficient internal consistency, and horses evaluated as highly anxious by the raters show greater increase in heart rate and defecate more often during exposure to novel stimuli than other horses (Momozowa et al.; 2003).

4.2. Methods

4.2.1. Animals

Fifty control and 50 crib-biting horses were used in this study. All crib-biting horses had performed stereotypic behaviour for more than 6 months. Horses stayed at different riding schools. Where possible, control horses were selected from the same establishment. Horses were matched as far as possible for breed and management routine as well. All horses were fed oats and hay twice a day, turned out to pasture daily and were ridden regularly.

Mean age (\pm S.E.) was 9.8 ± 0.5 for control and 9.3 ± 0.7 for crib-biting horses. Groups did not differ regarding age ($t_{88}=-0.607$, $p=0.546$), breed ($\chi^2_3=3.717$, $p=0.294$), gender ($\chi^2_2=2.691$, $p=0.260$), usage ($\chi^2_1=1.973$, $p=0.160$) or training level ($\chi^2_1=0.703$, $p=0.402$, **Table 3.**)

Table 3. *Number of horses in each level of the independent factors*

Independent factors	Levels of the factor	Number of control horses	Number of crib-biting horses
Breed	Thoroughbred	3	3
	Hungarian Halfbred	28	20
	Hungarian Sport Horse	14	16
	Other	5	11
	Gender	Stallion	5
Usage	Gelding	21	28
	Mare	24	16
	Leisure	31	23
Training level	Competition	19	27
	Basic	20	15
	Upper	30	35

4.2.2. Personality questionnaire

Horse owners were asked to complete a previously validated equine temperament questionnaire developed by Momozawa et al. (2005) who compared two separate horse groups which consisted of Thoroughbreds of the same age being kept under similar environments and trained as racehorses in 2002 and 2003. For each horse, 3 caretakers completed the questionnaire. In both surveys, 5 factors were extracted, which accounted for 71.4 and 75.5 % of the common variance, respectively. They found sufficient internal consistency in the responses to reliably evaluate ‘Anxiety’, ‘Trainability’ and ‘Affability’

of the equine temperament. Considering that none of the horses in this study had been trained as racehorses, the ‘Gate entrance’ item was excluded from the original questionnaire; therefore 19 questions remained in the present survey. For each question item, a description was added according to Momozawa et al. (2005). The responses were on a scale of 1 to 5, with the latter being the highest rank for a given item (**Table 4**).

4.2.3. Statistical analysis

4.2.3.1. Factor analysis

To condense the items of the personality questionnaire, principal component analysis (PCA) was used with Varimax rotation with Eigenvalue>1. The number of extracted factors was decided after visual inspection, using the rules of the Scree test. Factor scores were calculated by using the Regression method (Reise et al., 2000).

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.765 indicating that the data was suitable for structure detection. Average communalities were 0.65 ± 0.11 (S.D.).

4.2.3.2. Comparison of the factor structures

Factors extracted from the present survey were compared to the factor structures reported by Momozawa et al. (2005). The component scores for each horse were calculated two times. First, using PCA as described above. Second, we calculated scores according to the horse-personality model created by Momozawa et al. (2005). An individual’s score for each component was calculated by the individual’s rating on an item summed over all trait items. Pearson’s correlation coefficients for ‘Anxiety’, ‘Trainability’ and ‘Affability’ factor scores of the present survey and that of Momozawa et al. (2005) were then calculated (**Table 4**).

Table 4. *Questionnaire items with description and the factor loadings of each questionnaire item*

Item	Description (this horse tend to ...)	Scale (1 ↔ 5)	Factor1	Factor2	Factor3	Factor4	Factor5
Nervousness	become nervous about insects, noises, etc.	Calm Nervous		0.569			
Concentration	be trainable and undisturbed by the environment	Poor Excellent	0.593	-0.361			
Self-reliance	be at ease if left alone away from the herd	Restless At ease		-0.447			
Trainability	be trained easily and promptly	Poor Excellent	0.895				
Excitability	get excited easily	Not excitable Excitable		---			-0.346
Friendliness toward people	be never aggressive or fearful	Unfriendly Friendly			0.780		
Curiosity	be interested in novel objects and approach them	Rarely Frequently					0.424
Memory	memorize what it learned or was trained	Poor Excellent	0.748				
Panic	get excited to an abnormal extent	Rarely Frequently		0.660			
Cooperation	be cooperative with caretaker when handled	Rarely Frequently	0.588		<u>0.374</u>		
Inconsistent emotionality	be unpredictable from day to day	Consistent Inconsistent		---			0.710
Stubbornness	be obstinate once it resists a command	Obedient Stubborn	-0.496				0.366
Docility	be docile in general	Active Docile	0.770		---		
Vigilance	be vigilant about surroundings	Rarely Frequently		<u>0.382</u>			0.459
Perseverance	be patient with various stimuli	Impatient Patient	---	-0.568	0.551		
Friendliness toward horses	interact with other horses in a friendly manner	Unfriendly Friendly			0.552		
Competitiveness	be dominant in agonistic encounters with other horses	Subordinate Dominant					0.755
Skittishness	get surprise easily	Not skittish Skittish		0.866			
Timidity	be timid in a novel environment	Audacious Timid		0.621			

Question items are listed according to the order in the actual questionnaire sheet. Bold font represents that the item's absolute value is more than 0.4, which indicates that the item belongs to the given factor. Empty cells indicate that the absolute value of the factor loading is below 0.3. Lines symbolise items that belong to a certain factor according to the validated questionnaire survey (Momozawa et al., 2005).

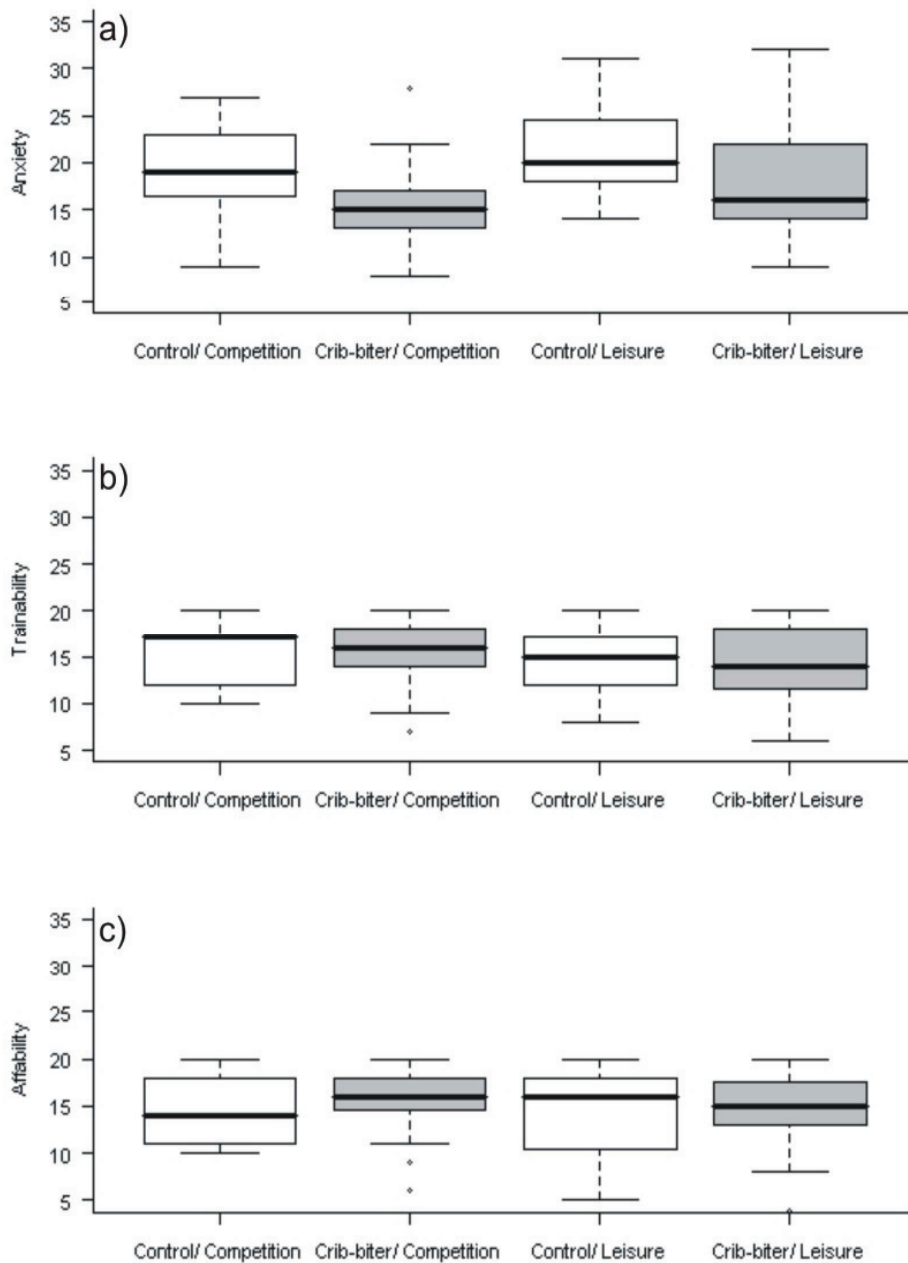


Figure 10. Boxplot of 'Anxiety' (a), 'Trainability' (b) and 'Affability' (c) in control and crib-biting horses used for competition or leisure purposes

4.2.3.3. Differences between groups

Differences in the obtained factor scores among control and crib-biting horses were tested by using general linear models, where ‘Anxiety’, ‘Trainability’ and ‘Affability’ served as dependent factor in the separate models and the effect of group (control or crib-biting horses), age, breed, gender, usage, training level of the horses and their possible interactions were the independent factors. The factor scores for ‘Anxiety’, ‘Trainability’ and ‘Affability’ were calculated by adding the scores of the items belonging to each factor according to Momozawa et al. (2005). Diagnostic plots of the residuals and standardized residuals were used to check the normality and variance homogeneity assumptions of the models. The distribution of the error terms proved to be normal. All analyses were carried out using the R 2.7.2. Statistical Software (R Development Core Team, 2007). The significance level was set at $p < 0.05$ whereas an effect was considered a *trend* when p was between 0.05 and 0.10.

4.3. Results

4.3.1. Comparison of the factor structures

In the present study, items were grouped into 5 factors that accounted for 65.1 % of the common variance. Factor 1 consisted of 3 out of the 4 items listed as ‘Trainability’ by Momozawa et al. (2005) and of 3 extra items associated with trainability: ‘Cooperation’, ‘Stubbornness’, and ‘Docility’. In the 2003 survey of Momozawa et al. (2005) ‘Docility’ also belonged to ‘Trainability’ but not in the 2002 survey. Factor 2 consisted of 4 out of the 7 items listed as ‘Anxiety’ by Momozawa et al. (2005) and of 2 extra items: ‘Self-reliance’ and ‘Perseverance’. Both extra items belonged to ‘Anxiety’ in one of the two surveys of Momozawa et al. (2005). Factor 3 consisted of 2 out of the 4 items listed as ‘Affability’ by Momozawa et al. (2005) and of 1 extra item: ‘Perseverance’.

Pearson’s correlation coefficients between the factor score of the extracted factors of this study and the factor scores of temperament traits calculated according to the factor structure reported by Momozawa (2005) were 0.870 for ‘Anxiety’ ($t_{98}=17.479$, $p<0.001$), 0.824 for ‘Trainability’ ($t_{98}=14.386$, $p<0.001$) and 0.757 for ‘Affability’ ($t_{98}=11.482$, $p<0.001$).

4.3.2. Differences between groups

Crib-biting horses had lower level of ‘Anxiety’ as compared to control horses ($p<0.001$), and the usage of the horse (leisure or competition) had significant effect as well ($p=0.032$). Among both crib-biting and control horses, those used for competition had lower level of ‘Anxiety’ than the ones used for leisure purposes. No differences were found among crib-biting and control horses regarding ‘Trainability’ or ‘Affability’ ($p=0.823$ and 0.543 , respectively), but on ‘Trainability’, the usage of the horse had an effect (**Figure 10.**). There was a trend for competition horses to have higher level of ‘Trainability’ compared to leisure horses ($p=0.068$). No other factors contributed significantly to the variance of the examined temperament.

However, regarding ‘Trainability’, the confounding effect of training level and breed can not be ruled out, since competition horses had higher training level compared to horses used for leisure purposes ($\chi^2_1=23.811$, $p<0.001$) and most of the Hungarian Sport Horses were used for competition ($\chi^2_3=12.993$, $p=0.005$).

4.4. Discussion

The number of the extracted factors of this study explained the common variance less than that reported previously by Momozawa et al. (2005), most probably due to the less homogenous sample. The factor structure that had been conserved in the two separate horse groups studied by Momozawa et al. (2005) and the first 3 extracted factors of the present study showed high consistency regarding the items belonging to each factor and showed high correlation as well. Thus, we can conclude that the survey validated by Momozawa et al. (2005) has been proven to be valid in our study, too.

4.4.1 ‘Anxiety’

Interestingly, crib-biting horses used either for competition or leisure purposes have been given significantly lower scores for items belonging to ‘Anxiety’ than control horses.

It has been suggested that horses being more reactive have higher risk for developing stereotypies. Thoroughbreds are considered to be more reactive and more susceptible to the effects of confinement than other breeds and have been reported to have a high prevalence of crib-biting. However, in this study, only 6 horses were Thoroughbreds, and the lower level of ‘Anxiety’ in crib-biting horses can not account for the effect of breed or discipline.

Both crib-biting and control competition horses were reported by the owners to have lower 'Nervousness' as compared to leisure horses, which could be explained by the higher training level within this group. Competition horses are more often exposed to novel environment and to frightening stimuli (e.g. colourful obstacles) than leisure horses and therefore might have also become more habituated to these types of stimuli. These horses usually have higher training level than horses used for leisure purposes and the same might be true for their riders. It is also possible that more trainable horses are favoured for competition purposes, and also the rider of a competition horse may have more experience defusing nervous behaviour in horses.

Owners' judgement on lower 'Anxiety' level in crib-biting horses does not necessary imply that these horses are 'calmer' than the control horses. Less reactivity to threatening situations also can be caused by apathetic or ambivalent behaviour tendencies. Apathetic or ambivalent behaviour is typical for a passive coping strategy (Koolhaas et al., 1999; Horváth et al., 2007; Koolhaas, 2008).

Human patients with obsessive-compulsive disorder, sharing many similarities with animal stereotypic behaviour, (Mason and Rushen, 2006) have an increased risk for depression, which may manifest behaviourally as a lack of motivation to counteract stressful stimuli (Overbeek et al., 2002; Wu et al., 2006). Similarly, crib-biting horses often seem to respond with ambivalent behaviour when challenged (Minero et al., 1999). The preference of using a passive coping strategy might explain the lower level of 'Anxiety' in crib-biting horses.

4.4.2. 'Trainability'

Experimental studies published so far on equine cognitive function have addressed behaviour, learning and conceptualization processes at a moderately basic cognitive level as compared to studies in other species, and have relied almost exclusively on primary positive reinforcement regimes (Murphy and Arkins, 2007). Studies on the learning performance of crib-biting horses also applied learning tasks using positive reinforcement (Hausberger et al. 2007, Hemmings et al., 2007, Parker et al., 2008; Parker et al., 2009).

Yet, the majority of horse trainers use negative reinforcement more often than primary positive reinforcement in their training procedures. Employing two such different approaches may complicate interpretation and may lead to difficulties in assessing the trainability of the horse.

Earlier research on equine learning often have not had a direct application to training methods commonly applied in the horse industry (McCall, 1990; Sappington et al., 1997; Murphy and Arkins, 2007). This might be the answer why crib-biting horses had not differed significantly from control horses regarding 'Trainability'.

There was a trend for competition horses to obtain higher scores for items belonging to 'Trainability', which can be a result of their higher training level compared to horses used for leisure purposes. It is also possible that more trainable horses are favoured for competition purposes, but the causal relationships are not clear, either. The skill of the riders who made the judgment on the scores may have an influence as well.

4.4.3. 'Affability'

Reactions of horses to interactions with humans are mostly the result of the interplay between their own temperament, the temperament and skills of the human and the experience acquired with humans. Studies show that deficits in the management conditions (housing, feeding, possibilities for social contact and training methods) may lead to relational problems between horses and humans (Hausberger et al., 2008). Little knowledge is available on the relationship between aggression and stereotypic behaviour, although both may be a common consequence of frustration (Mills and Nankervis, 1999). Stereotypies are suggested to arise most likely in an environment which is suboptimal for the horse's welfare. However, stereotypic behaviour may persist even after changes have been made to improve the environment (Mason and Rushen, 2006). In this study, there had not been any differences among crib-biting and control horses regarding management conditions, and their 'Affability' also did not differ.



Figure 11. *A crib-biting horse in a show-jumping competition*
(Photo: Ildikó Gál)

4.4.4. Conclusion

Significant differences have been found between the personality of crib-biting and control horses regarding ‘Anxiety’ but not in ‘Trainability’ or ‘Affability’. It seems that impaired learning abilities of crib-biting horses observed in the laboratory tests do not predict learning deficiencies in training (**Figure 11.**). We suggest that the virtual calmness and lower nervousness of the crib-biting horses might be due to the passive coping style of these animals. Further studies are needed to detect and understand the differences on the behavioural and personality traits of stereotypic and control horses.

Chapter 5.

The effect of a feeding stress-test on the behaviour and heart rate variability of crib-biting horses (with or without inhibition)

5.1. Introduction

Stereotypies may develop to cope with frustration, however, they often persist even after environmental stress is reduced thus fixed abnormal stereotypic behaviour itself may impose stress to the animal. Conversely, if stereotypies are maintained as coping mechanisms, animals may show signs of stress in a frustrating situation if execution of the abnormal behaviour is blocked by prevention methods.

The aims of the present study were to develop a crib-biting triggering stress test and with the help of that stress-test to assess the success-rate of the prevention of crib-biting by the modified Forssell's procedure compared to the collar treatment and to measure stress-related behavioural and physiological variables in order to evaluate the horses' 'quality of life' following treatment as well.

We hypothesised that horses prevented from performing crib-biting may either persist with the stereotypy in a modified form, or may show behavioural and/or physiological indicators of distress.

5.2. Materials and methods

5.2.1. Animals and housing

Between 2001 and 2008 twenty-four horses were operated with the modified Forssell's procedure at the Hungarian Clinic for Large Animals (Szent István University, Faculty of Veterinary Science) to prevent crib-biting. Twenty horses were available for more than 6

months postoperative follow-up evaluation, from which 13 were included in a behaviour test as well.

A self-control experimental design was impossible to conduct, since most of the horses had arrived to the clinic for the surgery wearing collar. Removal of the collar for pre-treatment evaluation would have not been the right solution as horses might have performed crib-biting behaviour more vigorously than usual, as the result of the post-inhibitory rebound (McGreevy and Nicol, 1998b). And even those horses that arrived without collar might not have performed stereotypic behaviour in the same level as usual since novel environment can reduce the occurrence of stereotypic behaviour and some horses might have even stop performing it for a while (McBride and Cuddeford, 2001).

Therefore, we tested 52 horses of four groups: control, crib-biting, collar treated and surgically treated groups. Data of 12 horses were excluded from the analysis. Heart rate data of 7 individuals were not reliable or missing because of technical problems. Horses younger than 4 years of age (3 cases) were excluded from the analysis because they exhibited high stress reaction when tied with rope therefore baseline values could not be established. Data of one mare were not analysed because the attention of that horse was distracted by her foal staying in the vicinity. One horse in the control group exhibited oral stereotypies (licking the wall throughout the test) and was therefore not included in the analysis.

Sample size of the four experimental groups decreased to 9 horses in the control group without stereotypic behaviour, 10 crib-biting horses (crib-biting group) performing stereotypic behaviour since 1-2 years (N=4) or more than two years (N=6), 10 crib-biting horses that had been wearing cribbing collar continuously for at least six months (N=1), between one and two years (N=7) or more than two years (N=2) before the experiment (collar treated group), and 11 horses operated with the modified Forssell's procedure at least six months (N=3), between one and two years (N=3) or more than two years (N=5) prior to the experiment (surgically treated group).

Horses had different owners and stayed at different riding schools. Behavioural tests were conducted in the familiar home environment of the horses.

5.2.2. Experimental design

Behavioural and heart rate variables of horses were assessed in a crib-biting triggering stress-test developed by Nagy and Bodó (2009b). The stressor was a modified version of the arousal-inducing stimulus applied by Bachmann et al. (2003b). The test was introduced 1.5-2 hours following the morning or noon feeding of concentrates. During the whole test the box door was open. Five minutes before the test the horses were tighten to their box with a rope long enough so they could reach the ground (**Figure 12.**).



Figure 12. *Stress-test consists of placing a feeding-bowl filled with oats out of the reach of the horse, from which tidbits (5 g oats) were given in every 2 minutes, altogether 3 times. (Photo: Krisztina Nagy)*

The test lasted for 20 minutes and responses were evaluated in 9 periods (**Table 5.**). Baseline was established in the first 5 minutes without any stimulus. After that, the experimenter was walking up and down in front of the box and was making noise with a bowl filled with oats (feeding-bowl) to direct attention to the bowl. Seven minutes after the start the feeding-bowl was placed in front but out of the reach of the horse. The feeding-bowl stayed there for eight minutes, meanwhile tidbits were given to the horses (~5 grams oats was taken from the feeding-bowl into the feed bin with delivery duration of ~10 sec) three times. Two minutes after the 3rd tidbit the bowl was removed, and for five minutes no stimulus was presented, however, the horses stayed tied up. Behaviour was videotaped and heart rate of horses were recorded continuously throughout the test (**Figure 13.**).

Table 5. *An overview of the test periods of the feeding stress-test*

Test period	1	2	3	4	5	6	7	8	9
Duration (minutes)	2.5	2.5	2	2	2	2	2	2.5	2.5
Description	No stimuli	Experimenter is making noise with a feeding-bowl filled with oats			Feeding-bowl is placed in front but out of the reach of the horse	Tidbits (5 grams oats) are given in the beginning of these periods. At the end of the 7 th period the feeding-bowl is removed.		No stimuli	



Figure 13. *Video-recording of the behaviour during test (left)*
(Photos: Krisztina Nagy)

5.2.3. Behaviour

Mutually exclusive behavioural elements were defined and recorded continuously with precision of one second (continuous sampling method) by replaying and when necessary slowing the video record. Elements were assigned to 4 major behaviour categories.

‘Oral activities’: repetitive oral activities without overt nutritional function; such as tongue flicking (tip of tongue is briefly extended), biting or grasping stable fittings or repeated licking of stable fittings, as well as crib-biting or wind-sucking.

Specific forms of oral stereotypy (e.g. wood-chewing, crib-biting, wall-licking) are sometimes difficult to consistently differentiate (Cooper et al., 2005), and horses treated by collar or surgery are not always able to perform the whole behavioural sequence of crib-biting. These horses may e.g. try seizing a projection and arching the neck but fail to emit the grunting sound. Consequently, behaviour elements listed as oral activities include not just the terminal grunting of this stereotypy, which is in focus of inhibition, but other behaviour elements as well, which are considered to be associated with crib-biting behaviour – e.g. crib-biters tend to lick fixed objects before wind sucking (McGreevy et al. 1995a).

‘Motor activities’: vocalisation, snorting, kicking, pawing, head tossing, head-circling, nodding or manipulation of the rope.

Some of these behaviour elements may initially arise as anticipatory activities, but later may become a conditioned response to feeding cues (Cooper et al., 2005) and are thought to indicate arousal (Weeks and Beck, 1996; Bachmann et al., 2003b).

‘Feeding-related behaviour’: bedding directed activities; standing with muzzle in the feed bin; or muzzle closer than 10 cm to the bedding. Every individual consumed the tidbits rapidly and actual feeding was excluded from this category.

‘Immobility’: standing still and not doing any of the above listed behavioural categories.

Reactions to unexpected stimuli which might have disturbed the horse (e.g. dog barking) and the 10 second periods of tidbit deliveries were marked as distraction and were left out from analysis.

For each test period, the time spent with behavioural categories was calculated. Interobserver agreement for the four behavioural categories was assessed by means of parallel coding of 20% of the total sample by two independent observers. The intraclass correlation coefficients are 0.99 for oral activities, 0.97 for motor activities, 0.87 for feeding related behaviour, and 0.93 for immobility.

5.2.4. Heart rate and heart rate variability

Heart rate (HR) and heart rate variability (HRV) were chosen as physiological indicators of 'emotional' stress (Bachmann et al., 2003b; Rietmann et al., 2004). Beat-to-beat (R-R) intervals were recorded by Polar® Equine S810i heart rate monitor. Prior to HRV analysis, R-R interval data were pre-processed for excluding artefacts by automatic error correction (moderate filter power with minimum protection zone set at 6 bmp/min) of the Polar® HorseTrainer 3.0 software (Polar Electro Oy, Finland). The smoothness priors method was used for detrending of the R-R series ('HRV-analysis software', Niskanen et al., 2004). Average heart rate in each period was compared to baseline value obtained as the average heart rate in the first 5 minutes of the test.

One HRV variable was chosen for analysis: the ratio of the low-frequency component (LF) and the high-frequency component (HF) of the HRV (LF/HF ratio). The low-frequency component corresponds to vasomotor waves, whereas the high-frequency component to respiratory acts. LF/HF ratio is considered a good indicator of the cardiac sympatho-vagal balance representing a measure of stress level. All analyses were carried out with a threshold set at 0.05-0.15 Hz for LF and 0.15-0.4 for HF (Rietmann et al., 2004).

The R-R segments of each test periods were selected and analysed. The beginning and the end of the test periods were time-matched with the video analysis of the behaviour (e.g. 5th period started when the first tidbit was delivered and finished when the 2nd tidbit was given). R-R segments corresponding to 'distraction' were left out from the HRV analysis (**Figure 14.**).

5.2.5. Questionnaire survey

The owners were asked questions of a predetermined survey sheet and responses were recorded by the experimenter. Questions were about the age, breed, gender, current use (sport or leisure), access to pasture and amount of exercise per week, current feeding program as well as details of the crib-biting behaviour of the horse (history of crib-biting; and usually what time of the day; how; on which type of cribbing surface the horse performs crib-biting behaviour) as well as severity of the stereotypy as judged by the owner.

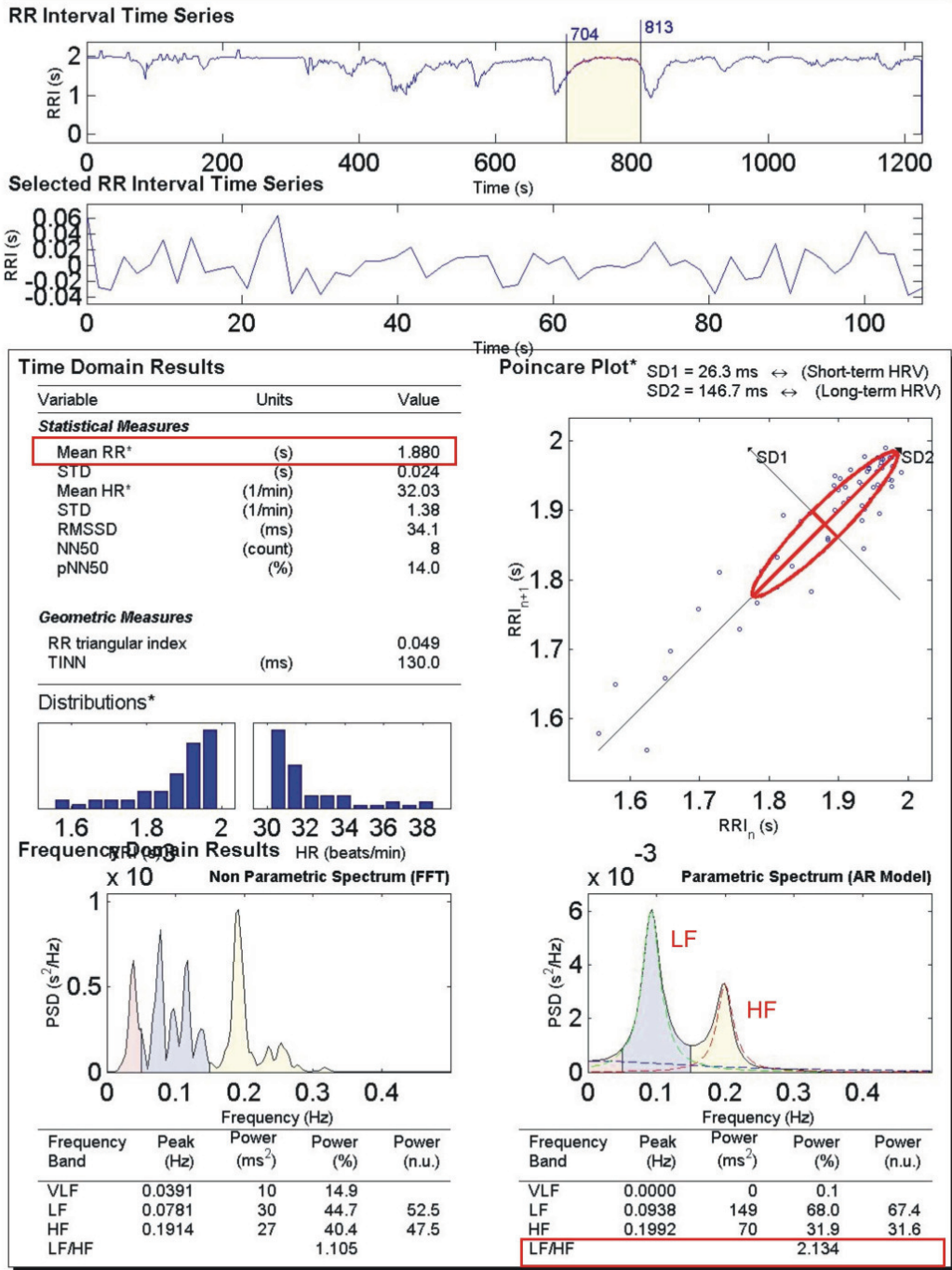
Opinions about the modified Forssell's procedure were collected from owners whose horses were treated by the surgical method ($N=20$). Questions addressed the decisions

about why this treatment has been chosen, methods used for prevention before surgery, postoperative complications, satisfaction with the cosmetic appearance, changes in behaviour/ temperament of the horse after surgery, presence of crib-biting behaviour after surgery, whether there were any changes made regarding housing and management conditions after the surgery, owner's satisfaction with the treatment and whether the owner's would recommend it to others. Individual data of crib-biting horses treated by modified Forssell's procedure are summarised in **Table 6**.

Table 6. Individual data of crib-biting horses treated by modified Forssell's procedure

Age (years), Gender, Breed, Usage	Crib-biting behaviour				
	Type	History (years)	Intensity	After surgery	During stress-test (% of 20 minutes)
5, S, HS, Sp	CB	>2	Severe	continued as before	50.1%
5, G, Q, H	CB	1-2	Severe	improved	27.0%
7,S,WB,H	CHIN	1-2	Moderate	improved	6.7%
6,G,HS,Sp	CB	> 2	Severe	stopped	3.5%
7, M, THB, Sp	CB	> 2	Severe	stopped	1.8%
5, M, Q, H	CB	1-2	Moderate	stopped	1.5%
11, G, HS, Sp	CB	> 2	Severe	stopped	1.3%
4, M, WB, H	CB	<1	Moderate	stopped	0%
4, G, HS, Sp	CB	<1	Moderate	stopped	0%
5, G, WB, Sp	CB	> 2	Severe	stopped	0%
5, M, HS, Sp	WS	1-2	Moderate	continued as before	0%
7, G, WB, Sp	CB	<1	Moderate	stopped	0%
6,S,HS,Sp	WS	1-2	Moderate	improved	0%
4, S,HS, Sp	CB	1-2	Moderate	stopped	Not tested
5, G, WB, Sp	CB	1-2	Severe	stopped	Not tested
5,M,HS,H	CB	> 2	Severe	continued as before	Not tested
7,M,HS,Sp	CB	> 2	Severe	stopped	Not tested
7,M,WB, Sp	CB	> 2	Severe	continued as before	Not tested
9, G, WB, Sp	CB	1-2	Severe	stopped	Not tested
12, M, WB, H	CB	> 2	Moderate	stopped	Not tested

G: gelding, M: mare, S: stallion/ HS: Hungarian sport horse, WB: warmblood, THB: thoroughbred, Q: quarter horse/ H: hobby, Sp: sport/ CB: crib-biting in traditional way, CHIN: using chin instead of incisors, WS: wind-sucking without grasping fixed objects. Not tested: no behaviour test, only questionnaire survey



23-Jan-2008 - HRV Analysis Software v1.1 SP1

*Results are calculated from the non-detrended selected RRI signal.

The Biomedical Signal Analysis Group
Department of Applied Physics
University of Kuopio, Finland

Figure 14. A typical output of the HRV-analysis software, which is freely downloadable

The analysed R-R segments were time-matched with the video analysis of the behaviour. The red framed parameters of heart rate (R-R) and the heart rate variability (LF/F ratio) were calculated for every test period. (Niskanen et al., 2004)

5.2.6. Statistical analysis

All analyses were carried out by R 2.7.2. Statistical Software (R Development Core Team, 2007). The significance level was set at $p < 0.05$ and an effect was considered a trend when p was between 0.05 and 0.10. For hypothesis testing, general linear mixed model was fit to the data (Pinheiro and Bates, 2000) with random effects for each horse and fixed effects for the different groups, test periods and their interaction. The given HR, HRV and behavioural parameters served as response variables in the different models. Square root transformation of frequencies of behaviour categories was applied to satisfy the normality and variance homogeneity assumptions of the models. Due to the large inter-individual variability in the absolute levels of HR and HRV, changes in HR and HRV for a given horse in the test periods were calculated by subtracting the baseline values (average rate of the 1st and 2nd test periods). Baseline values for HR and HRV were included as covariates into the fitted models. Because of multiple comparisons p -values were corrected according to Tukey-Kramer.

5.3. Results

5.3.1. Animals

Groups did not differ significantly regarding age, breed, gender, housing and management conditions or the usage of the horse (Fisher's tests, $p > 0.100$, in all cases). The pre-treatment proportion of moderate and severe crib-biters did not differ significantly between collar treated, surgically treated crib-biting groups (Fisher's test, $p = 0.413$). Altogether 9 stereotypic horses were categorised by the owners as moderate and 22 as severe crib-biter.

5.3.2. Reliability of the questionnaire survey

History and severity of crib-biting as evaluated by the owners were compared to crib-biting elicited by the test. The prevalence of severe crib-biting were significantly lower among horses that had a history of this abnormal behaviour for less than 1 year (Fisher's test, $p < 0.001$), used only one or two types of cribbing surface (Fisher's test, $p = 0.049$), or performed crib-biting usually following consumption of concentrates rather than continuously throughout the whole day (Fisher's test, $p < 0.001$).

The stress-test successfully triggered crib-biting in all non-prevented crib-biters and to a certain degree in some of the collar or surgically treated horses. Even among control horses oral activities, e.g. grasping, could be observed occasionally.

Horses that were categorised by the owners as moderate crib-biters spent $3\pm 1\%$ (mean \pm S.E.) of the total time with oral activities whereas horses categorised as severe crib-biters performed it significantly longer, $16\pm 4\%$ ($t_{25} = -3.329$, $p=0.003$).

5.3.3. Owner's opinion about the modified Forssell's procedure

Most owners had chosen the surgery to be able to sell the horse for a better price. Usual secondary reasons were prevention of colic and prevention of other horses learning crib-biting. Only 7 out of 20 owners have chosen surgery because collar-treatment had not been successful or it caused skin trauma. Three owners had not even tried out collar treatment before surgery. Two owners have chosen surgery because they had found crib-biting irritating.

All horses returned to their previous usage within 2 months after surgery. Three horses had complications: 1 needed tracheotomy after surgery and was later also operated with laryngeal hemiplegia, 2 had wound healing difficulties; one of these had recrudescant swelling at the laryngeal area. In 17 cases, final cosmetic result was regarded as excellent and only one horse, operated at two years of age, got a swan-like neck. One horse was found harder to collect in canter. Two horses were reported to become calmer and 3 horses were reported to gain weight after surgery.

According to owners' judgement, 13 of the 20 operated horses showed no crib-biting behaviour after surgery, 3 horses showed improvement and 4 horses continued to crib-bite as before. None of the horses developed other types of stereotypy, although one has learnt crib-biting in a modified form after surgery (on vertical surface, by rotating the neck). The success of the operation did not depend on how severe or moderate crib-biter the horse had been (Fisher's-test, $p=0.999$), or on whether the horse performed the stereotypy more or less than one year (Fisher's-test, $p=0.521$). Breed ($p=0.394$), age ($p=0.999$) and gender ($p=0.165$) non-significant factors either, but type of crib-biting had a significant effect (Fisher's-test, $p=0.031$). All 3 horses performing a morphological variation of crib-biting continued to crib-bite in a greater or smaller degree after surgery. Only 3 owners tried to make environmental changes after prevention.

All owners attributed the outcome solely to the surgery. All horse-owners except one would recommend to others the surgery, even if their horse had complications or continued crib-biting after surgery.

5.3.4. Behaviour

Groups did not differ significantly regarding the baseline values (average of 1st and 2nd test periods) of the percentage of time spent with any of the behavioural categories. Immobility increased, motor activity decreased in the 3rd test period, when the bowl with oats was presented.

5.3.4.1. Oral activities

Throughout the test, crib-biting horses performed significantly more oral activities than controls ($p < 0.001$), whereas levels of the two inhibited groups were in between them. Values of collar and surgically treated horses did not differ from each other ($p = 0.305$). Control horses differed significantly from collar treated ($p = 0.016$) but not from surgically treated horses ($p = 0.520$). The frequency of oral activities increased remarkably after the first tidbit was presented (5th test period), especially in crib-biting horses (**Table 7, Figure 15a**). Tongue flicking was observed in 3 collar-treated horses.

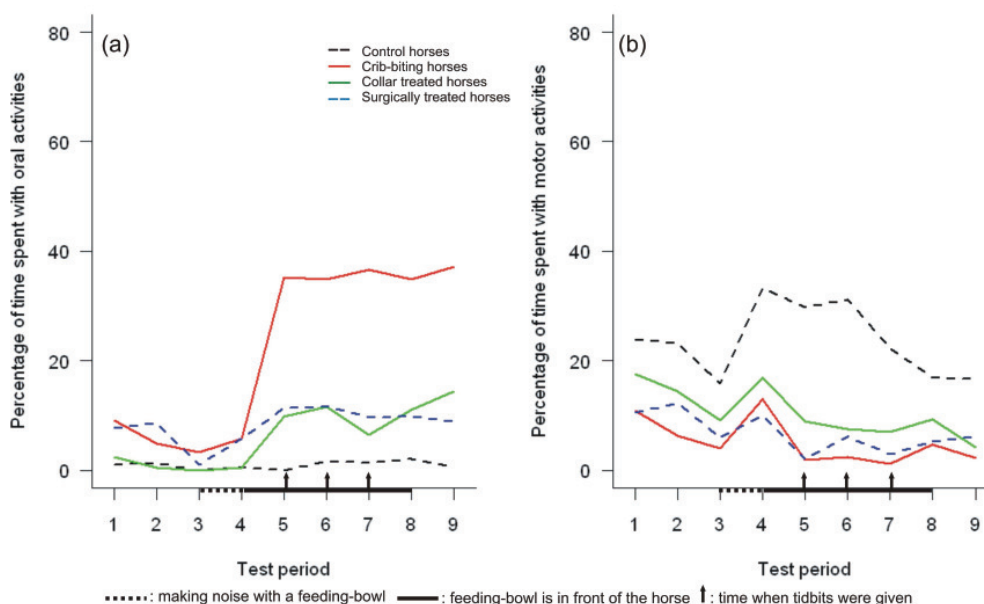


Figure 15. Mean percentage of time spent with oral (a) and motor (b) activities during the test periods.

5.3.4.2. Motor activities

Control horses spent significantly more time with motor activities compared to crib-biting ($p=0.003$), or surgically treated horses ($p=0.011$). There was a trend for difference between control and collar treated horses as well ($p=0.064$). Crib-biting, collar treated and surgically treated horses did not differ from each other. Placing the bowl in front of the horses elicited high motor activities, especially in control horses (**Table 7, Figure 15b**).

5.3.4.3. Feeding-related behaviour

Frequency of feeding-related behaviour sharply declined when pre-feeding activities were imitated by the experimenter, and increased above base-line levels following tidbit presentation. Controls exhibited somewhat lower feeding-related activity, however, differences among groups were not significant (**Table 7, Figure 16a**).

5.3.4.4. Immobility

Throughout the test, crib-biting horses spent significantly less time with immobility compared to control horses ($p=0.047$), however, none of the other comparisons reached level of significance (**Table 7, Figure 16b**).

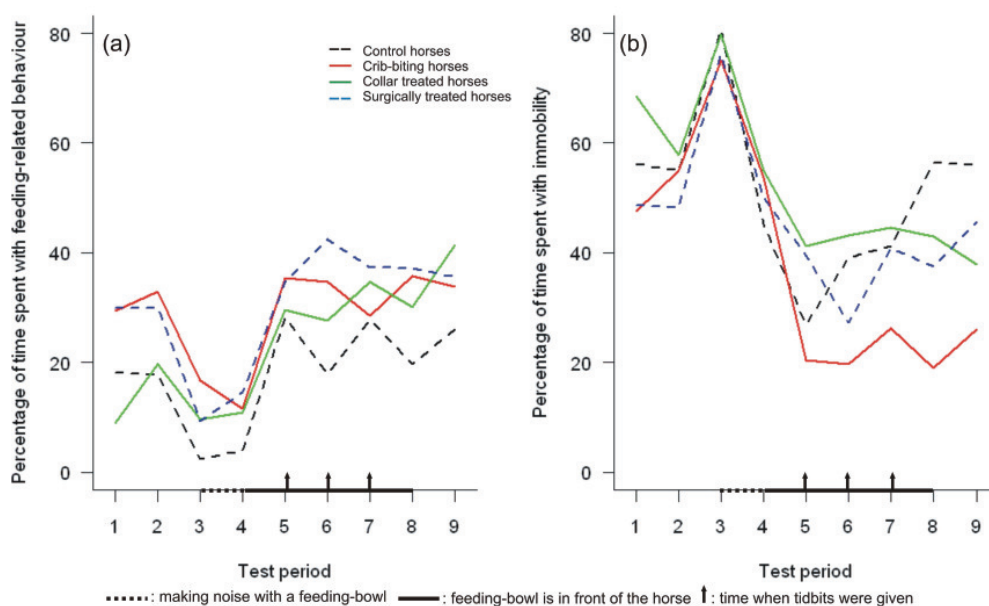


Figure 16. Mean percentage of time spent with feeding-related behaviour (a) and immobility (b) during the test periods.

5.3.5. Heart rate and heart rate variability

Baseline HR value (first 5 minutes of test) were similar in the four groups ($F_3=0.757$, $p=0.526$). Heart rate of most horses increased during pre-feeding imitation. However, changes in heart rate did not differ among groups (**Table 8, Figure 17a**).

Regarding the baseline value of LF/HF ratio there was a trend for differences among groups ($F_3=2.858$, $p=0.051$). Throughout the stress-test the changes in LF/HF ratio differed significantly among groups (**Table 7, Figure 17b**). The time dependence of LF/HF ratio of crib-biting horses differed significantly from collar treated ($p=0.004$) and surgically treated horses ($p < 0.001$) but did not differ from control horses ($p=0.989$). Control horses differed significantly from collar treated ($p=0.014$) and surgically treated horses ($p = 0.004$). Collar and surgically treated horses did not differ from each other ($p=0.986$).

Table 7. Test statistics and significance level of the treatment, test period and their interaction in the case of the given behavioural categories, heart rate (HR) and LF/HF ratio according to the final significant general linear mixed models.

	Treatment		Test period		Interaction of treatment and test period	
	$F_{3,36}$ value	p -value	$F_{1,316}$ value	p -value	$F_{3,316}$ value	p -value
Oral activities (% of time)	4.649	0.008	69.784	<0.001	16.144	<0.001
Motor activities (% of time)	5.648	0.003	7.731	0.006	n.s.	n.s.
Feeding-related behaviour (% of time)	n.s.	n.s.	60.416	<0.001	n.s.	n.s.
Immobility (% of time)	2.998	0.043	32.745	<0.001	2.666	0.048
Changes in HR (bpm)	n.s.	n.s.	6.185	0.013	n.s.	n.s.
Changes in LF/HF ratio (n.u.)	3.953	0.016	n.s.	n.s.	6.605	<0.001

bpm: beat per minutes, n.u.: normalised units, n.s.: not significant.

Table 8. Mean baseline values (\pm standard error) of heart rate (HR) and heart rate variability (LF/HF ratio) during 1st and 2nd test period; and the significance level of their comparison (using general linear models).

	Control horses (N=9)	Crib-biting horses (N=10)	Collar treated horses (N=10)	Surgically treated horses (N=11)
HR (bpm)	36.3 (0.8)	36.1 (1.7)	38.8 (2.4)	38.5 (0.8)
LF/HF ratio (n.u.)	3.54 (0.37)	3.58 (0.57)	2.35 (0.16)	2.43 (0.32)

bpm: beat per minutes, n.u.: normalised units.

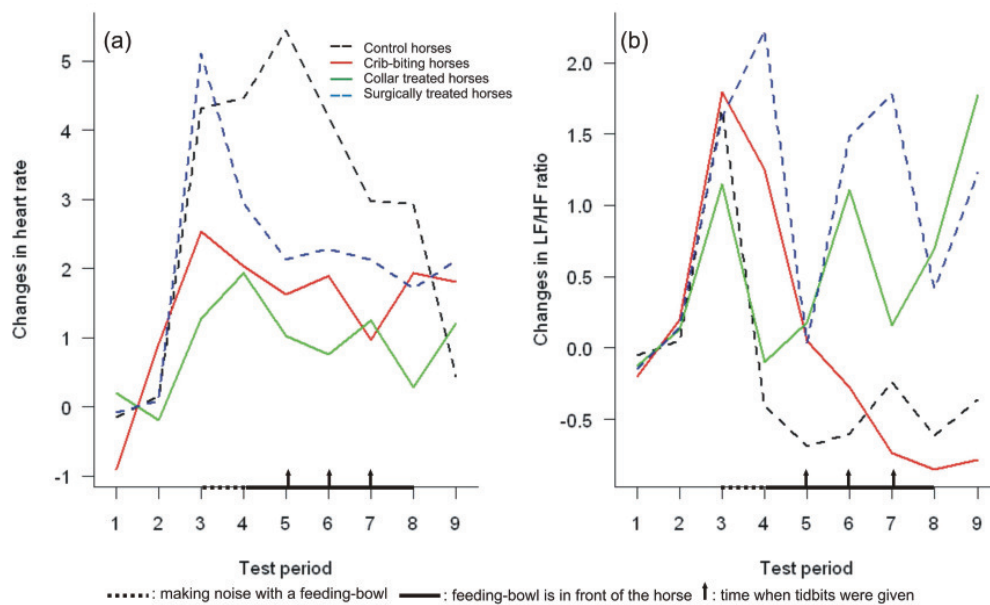


Figure 17. Changes in mean heart rate (a) and LF/HF ratio (b) interpreted as the sympatho-vagal balance or stress level of horses during the test periods.

5.4. Discussion

5.4.1. Crib-biting triggering stress-test

With the help of the reported stress-test crib-biting was successfully triggered. The significant relationships found between the intensity and the history of crib-biting behaviour suggests that the owner's assessment on the horse's crib-biting status (severe or moderate crib-biter) was valid. The significant relationship found between crib-biting intensity and the total time spent with *oral activities* throughout the test proves the reported stress-test is useful for answering the questions of this study.

Unfortunately, a self-control experimental design was impossible to conduct, since most of the horses had arrived to the clinic for the surgery wearing collar. Removal of the collar for pre-treatment evaluation would have not been the right solution as horses might have performed crib-biting behaviour more vigorously than usual, as the result of the post-inhibitory rebound (McGreevy and Nicol, 1998b). And those horses that arrived without collar might not have performed stereotypic behaviour in the same level as usual since novel environment can reduce the occurrence of stereotypic behaviour (McBride and Cuddeford, 2001). Therefore, the experimental design used in this study might not have been appropriate to evaluate the treatment of the individual animals (since horses were not tested before prevention), but it can be considered as a relevant tool for evaluating the effect of the given treatments and the differences among control, crib-biting, collar and surgically treated horses (Mason and Rushen, 2006; Christiansen and Forkman, 2007).

The stressor in this study was a modified version of the arousal-inducing procedure introduced by Bachmann et al. (2003b). They also presented a food bucket without allowing the horses to feed. That test induced a significant reaction in heart rate as well as arousal behaviour in both crib-biting and control horses, but no significant change in cortisol plasma concentration was observed. Moreover, crib-biting behaviour was also not triggered by that stimulus.

Therefore, in this present study, in order to increase arousal level of horses, an anticipation part was added before presenting the food-stimulus, in which pre-feeding cues were given to the horse (Cooper et al., 2005). Instead of a food-bucket used by Bachmann et al (2003b), a feeding-bowl was used allowing horses to see the oats better. Furthermore, the box door stayed open as horses were tighten to their box with the rope, so they could almost reach the feeding-bowl. In addition, to trigger crib-biting and oral behaviour more

efficiently, tidbits of oats were given to the horses 3 times (McGreevy et al. 1995a). By this method, triggering stereotypic behaviour was successful in all examined crib-biting horses. Crib-biting behaviour were more frequent in severe than in moderate crib-biters, and even normal horses spent some time with oral activities (e.g. grasping). Modifications may have contributed individually or in combination to the high success-rate of triggering oral behaviour and crib-biting within this test. Most probably tidbits from oats had a significant impact. The bases of the relationship between feeding of concentrates and crib-biting behaviour are being widely investigated, although the exact mechanism is not well known (see also 1.2.1).

According to our knowledge, crib-biting triggering stress-test has not been reported before in the literature. The test we used did not require any special paraphernalia and triggered crib-biting behaviour in a relatively short period of time in most crib-biters. The test also gives reliable and valid information on the severity of crib-biting. Therefore, it might be considered as a useful tool in both research and field-veterinarian practice, e.g. during pre-purchase veterinary examination.

5.4.2. Owner's opinion about the modified Forssell's procedure

Most common causes for choosing surgery were property destruction and prevention of colic, not the owner's annoyance, as previously reported by Turner et al. (1984) and not because collar treatment was unsuccessful. Contrary to the previous suggestions (Hakansson et al., 1992; Turner et al., 1984), but similarly to the findings of Schofield and Mulville (1998) the initial frequency of crib-biting, age of the horse and duration of stereotypic behaviour before surgery were not correlated significantly with the outcome of surgery. However, owners should be warned before choosing surgical treatment, that success-rate of surgery might be less among crib-biters performing a morphological variation of this stereotypy (e.g. horses using chin instead of the incisors, or solely wind-sucking).

According to owners' judgment, 65% of the operated horses showed no crib-biting behaviour and 15% improved after surgery. The success-rate of modified Forssell's procedure reported in this study falls within the range (good result 30-100 %, improved: 0-31 %) reported by previous studies (Turner et al., 1984; Hakansson et al., 1992; Schofield and Mulville, 1998; Delacalle et al., 2002). Certain questions, like owner's satisfaction

with the procedure, can not be used to make welfare assessments, as the answers were not overlapping with the success of prevention. Other questions may reflect to some aspects of the horse's well-being: e.g. some horses became calmer or gained weight after surgical treatment and cosmetic appearance was also satisfying in most cases. As suggested by Turner et al. (1984), the possibility of getting laryngeal hemiplegia as a complication of the modified Forssell's procedure can not be ruled out, although further studies are needed to prove it.

5.4.3. *The stress coping strategy of horses*

Before the stress-stimulus no significant differences were found either in behaviour or in heart rate and heart rate variability among the four groups. The presentation of the food stimulus evoked significant changes both in behaviour HR or HRV in different groups. After an initial increase, the HRV (LF/HF ratio) of both control and crib-biting horses returned to the basal value, which indicates a successful coping in both groups. Control horses were usually trying to reach the feeding-bowl while present (pawing, head-tossing etc.) and were standing still afterwards, while crib-biting horses did not really make efforts to reach the feeding-bowl, spent less time with immobility in favour of performing *oral activities* (crib-biting) throughout the entire test, even after the removal of the stressor/feeding-bowl (**Figure 18.**). Both control and crib-biting horses can be labelled as active or proactive copers as these horses adopted a fight (obtain the food) or flight response (perform crib-biting) to the stress-challenge. In previous studies the evidence for a general coping function of crib-biting has been described as weak (see also 1.3). Our results support the hypothesis that crib-biting may serve as a "stress-reducing" mechanism.

Collar and surgically treated horses could not use crib-biting behaviour as a coping style considering that both prevention method inhibited crib-biting successfully. At the same time, similarly to crib-biting and contrary to control horses, prevented horses did not have the impetus to try acting upon the stressor (obtain the feeding-bowl). The HRV of the two treated groups remained elevated and showed large oscillations throughout the test, which shows they had not found a successful coping behaviour. As coping strategies are believed to be consistent over time and across situations (Koolhaas et al., 1999; Koolhaas, 2008), our results might indicate, that horses prevented from crib-biting may experience difficulties in stress-adaptation.

If an animal can neither escape from nor remove an aversive stimulus, it is not adaptive to repeat these coping strategies over and over again. As an alternative, the animal may conserve energy and wait for a spontaneous change in the aversive situation (Weichsler, 1995; Koolhaas et al., 1999; Koolhaas, 2008). It has been also noted that animals can transfer this strategy from one aversive situation to another, which means animals may fail or even not try to escape or remove aversive stimulus even if escape/removal is possible. Therefore, the behaviour of collar and surgically treated horses might be interpreted as ambivalent or apathetic behaviour (Horváth et al., 2007), or even as ‘learned helplessness’ (Weichsler, 1995). Not being able to cope with stressors for long period of time may cause permanent changes in physiological variables, and may lead to adaptation diseases (Koolhaas, 2008).



Figure 18. *Coping styles differed markedly amongst groups. Normal horses were usually trying to reach the food-bucket while present and were standing still afterwards (left), whereas the other three groups (crib-biting horses and horses prevented from crib-biting by collar or surgery) had not really made efforts to reach the bucket, spent less time with resting, and performed or tried crib-biting (right). (Photos: Krisztina Nagy)*

5.4.4. Conclusions

Crib-biting is probably the most detrimental abnormal stereotypy in horses, prevention of its development is very important. Once fixed, crib-biting is difficult to eliminate by behaviour therapy, therefore several methods to inhibit its performance have been worked out. However, if crib-biting is a strategy to cope with stress, the effects of inhibition should be carefully estimated.

In the present study we found that crib-biting horses spent more time with oral activities, primarily with cribbing than controls or inhibited horses, whereas their stress level as indicated by heart rate variability were indistinguishable from controls and significantly lower than that of the inhibited groups. Overall our results suggest that performance of oral stereotypies in a stress situation successfully diminishes stress, while inhibition of such stereotypy elevates it. Thus crib-biting may be a true coping strategy. However, further research is needed to investigate whether the coping effect is truly causal in the development and persistence of crib-biting or it is merely a beneficial side effect (Mason and Rushen, 2006).

Considering that treatment by collar or surgery had not resulted in any significant behavioural or physiological differences, the superiority of the modified Forssell's procedure compared to collar treatment might be questionable. Using collar may cause severe pain and often skin trauma to the horse (McBride and Cuddeford, 2001). The observed tongue flicking might indicate pain, too. On the other hand, success of surgery might be also compromised by complications (**Figure 19.**). The welfare of the animal might be threatened by both prevention methods. Since both prevention methods may significantly increase distress, the treatment in itself, without changing the motivation of the horse to perform the replacement behaviour seems to be unsatisfactory and insufficient. Mason et al. (2007) emphasise that improving welfare (e.g. with environmental enrichment) should be as important as reducing the abnormal stereotypic behaviour.



Figure 19. *Complications due to the prevention methods: development of swan-like neck resulted from the modified Forssell's operation (left) and tongue flicking behaviour in horses treated with anti-cribbing collar (right).* (Photos: Krisztina Nagy)

Chapter 6.

Final conclusions

In terms of management and welfare of horses, understanding the cause and function of stereotypies is important. During the last decade more investigations have been made to unravel causal factors in order to understand *why* animals perform stereotypic behaviours rather than simply describing *what* the animal is doing. Thus, the practical desire to prevent these behaviours from occurring wherever possible can be based upon a greater understanding than previously.

Preventing highly motivated behaviours may often cause stress. Physical prevention of crib-biting may be therefore suboptimal for the horse from a welfare perspective. However, the importance of reducing the incidence of crib-biting is supported by its undesirable consequences. Mason et al. (2007) emphasise that improving welfare (e.g. with environmental enrichment) should be as important as reducing the abnormal stereotypic behaviour.

Early life experiences are critical in stereotypy predisposition, and weaning experience is extremely important in this regard. **Figure 2.** summarise and symbolise the relationship among considered causal factors, and may provide additional strategies for the treatment of crib-biting. Offering low energy forage in higher quantities along with daily access to paddock after weaning may reduce the risk of developing stereotypies in this critical period (Waters et al., 2002, Parker et al., 2008a). Once stereotypy is established, dopaminergic mesoaccumbens activity leading to high motivational states can be dampened pharmacologically (e.g. through various antagonist administration) or non-pharmacologically (e.g. through acupuncture). The enhanced activation of the basal ganglia can be also avoided with the removal of the pre-feeding cues or even the highly palatable food itself (e.g. through applying a more prolonged, ad libitum feeding strategy, Nagy and Bodó, 2009).

It gives hope, that other stereotypies of the horse (e.g. weaving) can be successfully decreased by environmental enrichment, through increasing visual access to conspecifics or even by the use of a mirror or a picture (Cooper et al., 2000; McAfee et al., 2002; Mills and Davenport, 2002; Mills and Riezebos, 2005, **Figure 20.**).

Therefore, a behavioural therapy, as the combined use of prevention, improvement of husbandry along with a potential medical treatment (pharmacological or non-pharmacological, e.g. acupuncture) together, could be taken into consideration as future studies in order to enhance the effectiveness of the recently available treatment methods of crib-biting in horses (Whittal et al., 2005; McBride et al., 2009).

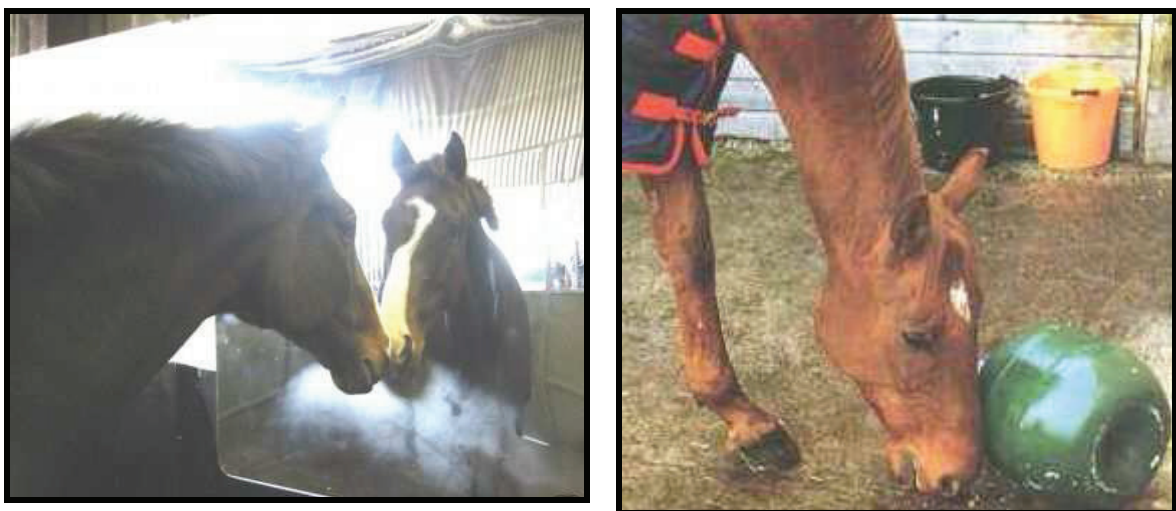


Figure 20. *Environmental enrichments for weaving and crib-biting: horse with a stable-mirror (left) and with a feeding ball (right). (Photo left: www.hanleyshorses.com, and right: www.link.vet.ed.ac.uk)*

Chapter 7.

New scientific results

1. I showed empirically for the first time in the literature that exposure to a stereotypic neighbour has significant effect on the odds of horses performing stereotypic behaviour.
2. I demonstrated that tree-based classification methods are useful tools in finding risk factors, or even for data mining in veterinarian science, alone or together with logistic regression method
3. I compared the temperament traits of crib-biting and control horses with a previously validated personality questionnaire. I showed that the passive coping strategy of crib-biters is responsible for the lower level of ‘Nervousness’ in crib-biting horses compared to control horses. No differences were found regarding ‘Trainability’ or ‘Affability’. With that I demonstrated that the previously reported impaired learning of stereotypic horses does not affect the horse’s performance or trainability in a negative way.
4. I developed a crib-biting provoking stress-test, which does not require any special paraphernalia and trigger crib-biting behaviour successfully in a relatively short period of time. The test also gives reliable information about the severity of crib-biting, therefore it is a useful tool in both research and field-veterinarian practice.
5. With the help of the developed crib-biting provoking stress-test I compared the stress-coping abilities between control and crib-biting horses (with or without inhibition). Inhibition of crib-biting behaviour either by collar or modified Forssell’s surgical procedure decreased crib-biting to a similar extent. No significant behavioural or physiological differences were found between the two prevention methods. I showed that performance of oral stereotypies in a stress situation successfully diminishes stress, while inhibition of such stereotypy elevates it. Thus crib-biting has proven to be a true coping strategy. Therefore, inhibition without changing the motivation of the horse to perform the replacement behaviour is unsatisfactory and insufficient.

Chapter 8.

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Chapter 9.

Publications

10.1. Publications related to the dissertation

1. Full-text papers published in peer-reviewed journals in English

1.1. **Nagy, K.**, Schrott, A., Kabai, P. Possible influence of neighbours on stereotypic behaviour in horses. *Appl. Anim. Behav. Sci.* 2008. 111, 321–328. (IF: 1.823)

1.2. **Nagy, K.**, Reiczigel, J., Harnos, A., Schrott, A., Kabai, P. Classification tree or conditional inference tree as an alternative to logistic regression in predicting crib-biting behaviour in horses. *J.Equine Vet. Sci.* accepted for publication (IF: 0.515)

1.3. **Nagy, K.**, Bárdos, Gy., Bánszky, N., Bodó, G. Differences in temperament traits between crib-biting and control horses. *Appl. Anim. Behav. Sci.* submitted for publication (IF: 1.823)

1.4. **Nagy, K.**, Bodó, G., Bárdos, Gy., Harnos, A., Kabai, P. The effect of a feeding stress-test on the behaviour and heart rate variability of crib-biting horses (with or without inhibition). *Appl. Anim. Behav. Sci.* submitted for publication (IF: 1.823)

2. Full-text papers published in peer-reviewed journals in Hungarian

2.1. **Nagy, K.**, Bodó, G. A megtámasztásos levegőnyelés és gyógykezelésének új lehetőségei. *Magy. Állatorv. Lapja* 2009. 131. 8-17. (IF: 0.104)

2.2. **Nagy, K.**, Bodó, G. Levegőnyelést provokáló stressz-teszt lovak számára. *Magy. Állatorv. Lapja* accepted for publication (IF: 0.104)

3. *Papers in conference proceedings*

Nagy, K., A lovak rendellenes sztereotip viselkedésének előfordulási gyakorisága magyarországi lovardákban, Poszter. XII. Lógyógyászati Kongresszus, Budapest, (poster, abstract) 2004

Nagy, K., Schrott A, Kabai P. Lehet-e tanult a lovak sztereotip viselkedése? IX. Magyar Etológiai Konferencia, Göd, (oral presentation, abstract) 2004.

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Csörgő T., Harnos A., Kovács Sz., **Nagy K.** A klímaváltozás hatásainak vizsgálata hosszútávú madárgyűrűzési adatsorok elemzésével. *Természetvédelmi Közlemények* , *in press*

Kovács Sz., Csörgő T., Harnos A., **Nagy K.**, Reiczigel, J. A kerti poszáta (*Sylvia borin*) vonulási fenológiájának változásai Ócsán 1984-2007. között. *Természetvédelmi Közlemények* , *in press*

Chapter 10.

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