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Stressful Situations in Foals and Young Horses

Belastungssituationen bei Fohlen und Jungpferden

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4. Concluding Discussion

Young horses are exposed to a variety of potentially stressful situations. In the studies included in this thesis we have analyzed the stress response of foals to hot iron branding versus microchip implantation, to different weaning protocols, and the response of young mares to changes in the husbandry system during initial equestrian training. For investigation of the stress load salivary cortisol concentration heart rate, heart rate variability and behavior parameters were analyzed. Based on the parameters investigated, a stress response to all three situations studied could be demonstrated.

In undisturbed horses, salivary cortisol concentrations follow a physiological diurnal rhythm with highest concentrations in the morning and a gradual decline throughout the day (BOTTOMS et al., 1972; HOFFSIS et al., 1970; ZOLOVICK et al., 1996, BECKER-BIRCK et al., 2012a and b). Such a rhythm was evident on control days in all three experiments of our study but was less pronounced in 3-year old mares compared to foals with an age of 6 month or younger. It has been shown previously that even minimal environmental disturbances may disrupt the pattern of circadian cortisol release in horses (IRVINE and ALEXANDER, 1994). This is confirmed by our studies where the diurnal rhythm in cortisol release in response to different stress situations was clearly absent.

The most pronounced cortisol release was found in response to weaning. Peak concentrations approximately doubled the values measured in mares stabled individually for the first time and in foals in response to the short term stimulus of hot iron branding or microchip implantation. The result indicates that weaning is a major stressful event in horses.

In foals either branded or implanted with a microchip the increase in salivary cortisol at first did not appear to be pronounced but the marked decline throughout the day as found on the days before the identification was missing. On the day after the respective event, a circadian rhythm in cortisol release was re-established but shifted to higher values for both, weaned foals and mares stabled individually for the first time, indicating a longer lasting effect beyond the actual day of both events. Corresponding values for the foals that received identification are not available.

An increase in heart rate was found in response to all investigated situations but was of shorter duration than the increase in cortisol release. As could be expected, an elevated heart rate occurs not only in response to mental stress it is also caused by increased physical

activity, e.g., raised locomotion activity in foals just weaned from their mothers, or when horses were exercised. In foals identified either by microchip or by hot iron branding, fixation alone induced a transient increase in heart rate. Hot iron branding and injection of a microchip itself evoked a comparable response in heart rate, but the attribution of the stress response to the individual procedure of identification is difficult because it might be masked by the response related to fixation alone.

Heart rate variability decreased transiently in foals at weaning and in mares individually stabled for the first time, while identification of foals by microchip implantation or branding was not associated with a decrease in HRV. In general, increases in the HRV parameters root mean square of successive RR differences (RMSSD) and standard deviation of RR interval (SDRR) reflect a shift towards parasympathetic dominance while reduced values indicate sympathetic dominance (von BORELL et al., 2007). The short term stimuli caused by identification apparently was not sufficient to cause detectable changes in HRV although more pronounced stimuli such as separation from the mother at weaning or from group mates at individual stabling can elicit them.

Behavior data in our study, support the results obtained from physiological stress parameters. Locomotion has been suggested as an objective measure of behavioral distress in horses (HOUPT and HINTZ, 1983; McCALL et al., 1985). Locomotion and lying behavior in our studies was determined by pedometers measuring locomotion activity, lying time, and temperature (ALT pedometers) with the temperature function not used in this study. These pedometers measure movement activity independent from the gait but allow an accurate quantification of locomotion activity and simultaneous measurements in several animals (ROSE-MEIERHÖFER et al., 2010). Increased locomotion activity in foals at weaning has been reported earlier (HOUPT and HINTZ, 1983; McCALL et al., 1987, HOFFMANN et al., 1995) but was determined by observation only and not with automated systems such as pedometers. In our study, locomotion activity increased for one and two days in foals weaned either simultaneously and consecutively, respectively. This response did not occur in foals weaned in the presence of two familiar but non-related adult mares. Since foals standing still are considered to be less anxious than those moving frequently (HOUPT and HINTZ, 1983; McCALL et al., 1987, HOFFMANN et al., 1995), the obtained results demonstrate the least pronounced stress response in foals weaned in the companionship of familiar mares. The presence of adult horses, thus, seems to have a positive effect and induce a more normal activity in the foals (BOURJADE et al. 2008, HENRY, 2012).

Weaning of foals caused a transient weight loss which confirms other studies (PRICE, 2003; ROGERS, 2004). A distinct loss of weight was found in the weaning study and was most pronounced after simultaneous weaning without adult mares. Less weight loss was found in foals of the other groups. Because the simultaneously weaned foals staying without adult mares, showed highest locomotion activity and a pronounced reduction in feed intake, the most pronounced weight loss in foals of this group is easily explained. This indicates that a weaning method which resembles weaning under natural conditions can reduce weight loss significantly.

Comparable to weaning also the change from group stables to individual boxes caused an initial increase in locomotion activity, but thereafter locomotion activity decreased. The latter might be due to insufficient space for locomotion in single boxes and might, therefore, be another potential stressor in horses. In previous studies determining the stress response in horses exercised without a rider (KINNUNEN et al., 2006) and in inexperienced ridden horses (SCHMIDT et al., 2010b), a higher state of relaxation has been suggested when horses were allowed to move than when requested to stand still.

An exaggerated locomotion behavior has been estimated as a sign of stress but may also be helpful for horses to cope with a stressful situation. This is not possible for horses restricted in movement by the surrounding conditions and can so increase the stress load. Equestrian training in our study reduced but did not totally fulfill the horses' needs to move freely. This is in agreement with a previous study (JØRGENSEN and BØE, 2007).

In the two studies in which horses were separated from their mothers or group mates lying time was reduced to almost zero on the day of the respective event. A few days after weaning, foals of all groups were again lying regularly and not differing from the situation before weaning. In contrast, when comparing the situation before to the one after transfer from a group stable to individual boxes, lying time of the mares changed. They spent more time lying when kept individually than in a group stable. Corresponding results were found for foals that have been weaned either in single boxes or in groups (HELESKI, 2002). Although individual stables reduce the opportunity for social interaction, they might provide the opportunity for the horse to lie down in an undisturbed environment in which they feel safe. Another hypothesis states that stalled horses do lie more often due to boredom than horses in a group with more options for social interaction (HELESKI, 2002). The opportunity of free movement in group stables might have a training effect, with exercising themselves at least to a certain degree.

Although the least pronounced stress response was found in response to branding and microchip implantation, this does not imply that hot-iron branding is an acceptable procedure in horses. Branding caused a necrotizing burn wound and a generalized increase in superficial body temperature over several days, which together are indicative of significant tissue damage. In foals, hot iron branding apparently triggered a reset in thermoregulatory pathways as have been found in humans in the initial stages of postburn hypermetabolism (KELEMEN et al., 1996). This should be taken into account when discussing branding under animal welfare aspects. In addition, while allowing to demonstrate an acute stress response, our study did not measure pain perceived by foals at branding and microchip implantation. Further studies are needed focusing also on non-stress related pathologies as the determination of metabolic and inflammatory changes.

In conclusion, identification and weaning as well as changes in husbandry system from group housing to individual stabling are perceived as stressful in horses. However, the magnitude of the stress response differs between the investigated situations. The most pronounced stress reaction was found in foals in response to weaning indicated by a marked increase in salivary cortisol concentration, behavioral changes and weight loss, especially in simultaneous weaned foals staying without adult horses present after separation. In comparison, the least pronounced stress reaction was found in foals weaned with two familiar, unrelated mares. The loss of the mother may to a certain degree be compensated by the presence of adult and familiar horses. Thus, this method of weaning should be recommended to horse breeders.

Although exposure of young domestic horses to potential stressors cannot totally be avoided, at most times the stress response is only transient and horses habituate rapidly to new situations. However, by adequate management and handling, the stress response can often be minimized thus avoiding a longer-lasting potentially negative experience of the young horse.

5. Summary

Regina Erber (2012)

Stressful situations in foals and young horses

Domestic horses are exposed to a variety of situations that have been suggested as potentially stressful. Some of them already occur when the horses are still young. Three of these potential stressors - identification of approximately 3-month old foals by hot iron branding or microchip, weaning of approximately 6-month old foals, and changes in husbandry system in 3-year old mares - have been investigated in this thesis. Stress responses were assessed by analysis of cortisol release, heart rate, heart rate variability, locomotion activity and behavioral parameters.

In the first study, the response of foals aged between 4 and 19 (10.4 ± 1.4) weeks to branding and microchip implantation was determined. They were either marked by hot iron branding on the right thigh (n=7) or were implanted with a microchip on the left side of the neck (n=7). Salivary cortisol concentrations, heart rate and heart rate variability, behavior, localized alterations at the branding and chipping site and superficial body temperature were studied. Salivary cortisol concentrations increased in foals of both groups and peaked at 60-min after microchip implantation (1.4 ± 0.1 ng/ml) and 30 min after branding (1.8 ± 0.2 ng/ml). Mean heart rate increased from 72 ± 4 and 70 ± 3 beats/min 60 min before branding or microchip implantation, respectively, to 88 ± 6 and 90 ± 8 beats/min directly thereafter. In both groups, mean heart rate already increased when the foals were caught, decreased with ongoing fixation and increased again after branding and microchip implantation (differences over time, $p < 0.001$). Branding and microchip implantation induced a comparable aversive behaviour (branding, score 3.86 ± 0.85; microchip, score 4.00 ± 0.82). Pronounced aversive behavior such as rearing or lashing out with the hind legs was not shown. Neither cortisol release, nor HR or behavior scores differed between groups. Branding, but not microchip implantation, caused a necrotizing burn wound and a generalized increase in superficial body temperature ($P < 0.001$), which together are indicative of significant tissue damage.

In the second study, the stress response of foals to different weaning protocols was investigated. Foals were weaned either simultaneously without adult horses present (group A, n=6), in the presence of two adult females familiar but unrelated to the foals (group B, n=5), or were weaned consecutively by removing two mares per day (group C, n=6). Salivary

cortisol concentration, beat-to-beat (RR) interval, heart rate variability (HRV), behavior, weight and locomotion were measured from 2 days before to 8 days after weaning. Weaning caused a significant cortisol release on weaning day in all groups and also on the day thereafter in group C ($p<0.05$). Peak cortisol concentrations were 3.5 ± 0.6, 2.4 ± 0.2 and 2.5 ± 0.7 ng/ml, respectively, in groups A, B and C. In weanlings of group A, heart rate increased while in groups B and C it remained almost unchanged (group A: 84.6 ± 6.1, group B: 56.2 ±2.1, group C: 60.0±3.1 bpm 30 min after weaning, group A versus groups B and C $p<0.05$). Locomotion determined by ALT-pedometers was most pronounced on weaning day in foals of group A and lowest in group B ($p<0.05$). In addition to increases vocalization on weaning day, also the highest ($p<0.05$) weight loss was found in foals of group A (-8.3±1.6 kg).

In the third study, the stress response of 3-year old Warmblood mares (n=8) to changes in the husbandry during initial equestrian training were determined. Mares were transferred abruptly from a group stable with access to a paddock into individual boxes without paddock, a situation which is often conducted in domestic horses for initial training. During the days in individual boxes mares underwent equestrian training for about 30 min per day except on day 2, where they remained in individual boxes. Salivary cortisol concentration, locomotion activity, heart rate and HRV were determined from 4 days before to 5 days after changing the stable. The circadian rhythm in cortisol existing in mares on the days in the group stable with highest values around 0.6 ng/ml in the morning was disturbed for 1 day when mares were brought into single boxes showing peak cortisol concentrations of 1.8±0.2 ng/ml, 30 min after transfer. Locomotion activity was only transiently elevated in response to separation (83±10 min/5 h) compared the time mares spent in paddock on the days before the transfer (60-70 min/5h). Thereafter, although being ridden, mares kept in individual boxes moved less than during the time in the group stable. On day 0, short-term decreases of the HRV variables SDRR and RMSSD were found immediately after individual stabling while heart rate increased to 103±8 beats/min. The latter returned to near baseline values (66±5 beats/min) within approximately 60 min.

In conclusion, identification and weaning as well as changes in husbandry system from group housing to initial individual stabling are perceived as stressful in horses. However, the magnitude of the stress response differs between the investigated situations. Hot iron branding and microchip implantation induced a transient stress response in foals that did not differ between groups and was less pronounced than the reaction of horses to other anthropogenic stressors. Nevertheless, the increase in salivary cortisol indicates that both procedures are stressful to the animals. In contrast to microchip implantation, branding causes significant

tissue damage and, thus, induces more prolonged alterations in foals than implantation of a microchip.

The most pronounced stress response was found in foals at weaning indicated by a marked increase in salivary cortisol concentration, behavioral changes and weight reduction, especially in foals weaned abruptly staying without adult horses present after separation. In comparison between groups of this study, in foals weaned consecutively, the stress response was less pronounced, but protracted over 2 days while foals weaned with 2 familiar, unrelated mares showed the least pronounced stress reaction. The loss of the mother may to a certain degree be compensated by the presence of adult and familiar horses. Thus, as a result of the study, this method of weaning is recommended for breeders.

Although the stress response to separation during initial training from the group of familiar conspecifics is less pronounced than induced by weaning it has been found to be an additional stressor in young horses. Despite an initial increase in locomotion activity on the day of transfer, mares moved less in single boxes compared to the time in group housing. The latter is thus associated with mares exercising themselves and this effect is reduced or absent, when they are housed individually.

6. Zusammenfassung

Regina Erber (2012)

Belastungssituationen bei Fohlen und Jungpferden

Pferde sind heutzutage verschiedenen Situationen ausgesetzt, die potentielle Belastungen darstellen können. Mit einigen dieser Situationen werden sie schon in jungem Alter konfrontiert. Im Rahmen dieser Doktorarbeit wurden drei dieser potentiellen Stresssituationen näher untersucht: Die Kennzeichnung mittels Heißbrand oder Mikrochip bei etwa 3 Monate alten Fohlen, das Absetzen im Alter von etwa 6 Monaten und die Haltungsumstellung bei dreijährigen Stuten. Die Stressreaktion wurde anhand der Kortisolkonzentration im Speichel, der Herzfrequenz und Herzfrequenzvariabilität, der Bewegungsaktivität und anhand von Verhaltensbeobachtungen analysiert.

In der ersten Studie wurde bei Fohlen im Alter von 4-19 Wochen (10.4±1.4 Wochen) die Reaktion auf die Kennzeichnung gemessen. Die Kennzeichnung erfolgte entweder durch das Setzen eines Heißbrandes auf den rechten Oberschenkel (n=7) oder durch die Injektion eines Mikrochips in die linke Halsseite (n=7). Neben den Stressparametern wurden Hautveränderungen im Bereich der Brand- und Injektionsstellen und die Oberflächentemperatur der Haut erfasst. Nach der Kennzeichnung stieg die Kortisolkonzentration in beiden Gruppen an. Höchstwerte wurden 60 min nach der Mikrochipimplantation (1.4 ± 0.1 ng/ml) and 30 min nach dem Brennen (1.8 ± 0.2 ng/ml) erreicht. Die Herzfrequenz betrug 72 ± 4 und 70 ± 3 Schläge/min 60 min vor dem Brennen bzw. Chippen und stieg auf 88 ± 6 und 90 ± 8 Schläge/min direkt nach der Kennzeichnung an. Bei den Fohlen in beiden Gruppen stieg die Herzfrequenz bereits zu dem Zeitpunkt, als die Fohlen eingefangen wurden, sank während der Fixierung um direkt nach der Kennzeichnung erneut wieder anzusteigen (Unterschiede über die Zeit, $p < 0.001$). In beiden Gruppen fielen die Abwehrreaktionen auf die jeweilige Kennzeichnung in etwa gleich aus (Heißbrand, Score 3.86 ± 0.85; Mikrochip, Score 4.00 ± 0.82). Starke Abwehrreaktionen wie Steigen oder Ausschlagen wurden dabei nicht beobachtet. Weder in den Kortisolkonzentrationen noch bei den Herzfrequenzen und dem Verhalten wurden Unterschiede zwischen den beiden Gruppen sichtbar. Jedoch kam es nach dem Heißbrand und nicht nach der Mikrochipimplantation im Bereich der Brandstelle zu Hautnekrosen und einem systemischen Anstieg der

Körpertemperatur über mehrere Tage ($p < 0.001$), beides sind Indikatoren für eine signifikante Gewebsschädigung.

In der zweiten Studie wurde die Stressreaktion auf unterschiedliche Absetzmethoden untersucht. Die Fohlen wurden entweder abrupt abgesetzt und nach dem Absetzen von der Mutter ohne (Gruppe A, n=6) oder mit vertrauten Begleitstuten (Gruppe B, n=5) gehalten oder graduell abgesetzt (Gruppe C, n=6), wobei an 3 aufeinanderfolgenden Tagen jeweils 2 Mutterstuten aus der Gruppe entfernt wurden. Anhand der Kortisolkonzentration im Speichel, Herzfrequenz und Herzfrequenzvariabilität, Entwicklung des Körpergewichts und der Bewegungsaktivität im Zeitraum von 2 Tagen vor bis 8 Tage nach dem Absetzen wurde die Reaktion der Fohlen auf die Trennung von den Mutterstuten analysiert. Am Absetztag stieg die Kortisolkonzentration nach der Trennung von den Mutterstuten bei allen Fohlen an, die jeweiligen Höchstkonzentrationen betrugen 3.5 ± 0.6, 2.4 ± 0.2 und 2.5 ± 0.7 ng/ml, in Gruppe A, B und C. Außerdem waren am Tag nach dem Absetzen bei den graduell abgesetzten Fohlen (Gruppe C) noch deutlich erhöhte Kortisolkonzentrationen zu verzeichnen ($p<0.05$). Fohlen der Gruppe A hatten nach dem Absetzen eine höhere Herzfrequenz, bei den Fohlen der anderen beiden Gruppen hingegen lagen die Herzfrequenzen auf einem ähnlichen Niveau wie am Tag vor dem Absetzen (Gruppe A: 84.6 ± 6.1, Gruppe B: 56.2 ±2.1, Gruppe: 60.0±3.1 Schläge/min, 30 min nach dem Absetzen, Gruppe A versus Gruppe B und C $p<0.05$). Die mithilfe von ALT-Pedometern gemessene Bewegungsaktivität fiel am Absetztag bei den abrupt abgesetzten Fohlen der Gruppe A am größten, in denen der Gruppe B am geringsten aus ($p<0.05$). Neben der höchsten Frequenz in der Lautgebung (Wiehern) fiel auch der Gewichtsverlust bei den Fohlen der Gruppe A (-8.3±1.6 kg) am stärksten aus ($p<0.05$).

In der dritten Studie wurde die Stressreaktion auf die Haltungsumstellung in der initialen Trainingsphase bei dreijährigen Stuten gemessen. Die Stuten wurden abrupt vom Gruppenlaufstall mit mehrstündigem Auslauf im Paddock in Einzelboxen umgestellt. Dies wird häufig vor dem Beginn des Anreitens durchgeführt. Während der Aufstallung in den Einzelboxen wurden die Stuten täglich für ca. 30 min trainiert, mit Ausnahme von Tag 2, an dem sie in den Boxen blieben. Beginnend von 4 Tagen vor bis 5 Tage nach der Umstellung wurde die Kortisolkonzentration, die Bewegungsaktivität, die Herzfrequenz und die Herzfrequenzvariabilität der Stuten bestimmt. Die Tagesrhythmik der Kortisolfreisetzung mit Höchstwerten um 0.6 ng/ml, wie sie vor der Haltungsumstellung bei den Stuten gefunden

wurde, war am Tag der Verbringung in Einzelboxen unterbrochen. Höchstwerte an diesem Tag lagen 30 min nach der Umstallung bei 1.8±0.2 ng/ml. Im Vergleich zur Bewegungsaktivität im Gruppenlaufstall mit der Möglichkeit zur freien Bewegung im Paddock (60-70min/5h) war diese nach der Haltungsumstellung nur leicht erhöht (83±10min/5h). Obwohl die Stuten geritten wurden, bewegten sie sich an den folgenden Tagen in der Einzelhaltung im Verhältnis zur Gruppenhaltung weniger. Kurz nach der Verbringung in die Einzelboxen am Tag der Umstellung stieg die Herzfrequenz auf 103±8 Schläge/min. Zur gleichen Zeit konnten bei den analysierten Parametern der Herzfrequenzvariabilität, SDRR und RMSSD, verringerte Werte gemessen werden. Die Herzfrequenz sank innerhalb von ca. 60 min wieder auf Basalwerte (66±5 Schläge/min) ab.

Abschließend ist festzustellen, dass die Kennzeichnung, das Absetzen und die initiale Haltungsumstellung vom Gruppenlaufstall in Einzelboxen eine Stressreaktion bei Pferden auslösen. Das Ausmaß der Reaktion unterschied sich jedoch deutlich zwischen den untersuchten Belastungssituationen. Die stärkste Stressreaktion wurde als Antwort auf das Absetzen gemessen und hier v. a. bei den abrupt abgesetzten Fohlen, die nach der Trennung von ihren Mutterstuten ohne vertraute Begleitstuten gehalten wurden. Im Vergleich zwischen den Gruppen fiel die Belastung der graduell abgesetzten Fohlen geringer aus, hielt aber über 2 Tage an, während die Fohlen, bei denen nach dem Absetzen 2 Begleitstuten in der Herde verblieben, am geringsten auf das Absetzen reagierten. Der Verlust der Mutterstute scheint zu einem gewissen Grad durch die Anwesenheit von vertrauten, erwachsenen Stuten kompensierbar. Als Ergebnis der Studie wird diese Absetzmethode für Züchter empfohlen.

Obwohl die Belastung bei der Haltungsumstellung dreijähriger Stuten geringer war als die durch das Absetzen, stellt sie einen zusätzlichen Stressfaktor für junge Pferde dar. Abgesehen von dem kurzzeitigen Anstieg in der Bewegungsaktivität am Tag des Verbringens in die Einzelboxen, bewegten sich die Stuten weniger als zuvor in der Gruppenhaltung. Die Kennzeichnung mittels Heißbrand oder Mikrochip löste eine leichte Stressreaktion aus, die sich aber zwischen beiden Gruppen in der Höhe nicht unterschied und geringer ausfiel als andere vom Menschen ausgehende Belastungen, denen Pferde regelmäßig ausgesetzt werden. Der Anstieg in der Kortisolkonzentration weist trotzdem darauf hin, dass beide Kennzeichnungsverfahren eine Belastung für die Fohlen darstellt. Im Gegensatz zur Mikrochipimplantation führte das Brennen zu einer signifikanten Gewebeschädigung im

Bereich der Brandstelle und zu einer systemischen Hyperthermie über mehrere Tage, d.h. zu pathologischen Veränderungen, die über eine Stressreaktion hinausgehen.