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EFFECT OF EARLY ENVIRONMENT ON STRESS-COPING STRATEGIES OF FOALS

By

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ABSTRACT

Effect of early environment on stress-coping strategies of foals

By

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The mare plays the most important role in rearing a foal and the permanent separation of mare and foal during weaning is stressful for both. In rodent literature, it has been shown that high levels of maternal care can facilitate coping with a stressor during early stages in life. We tested whether repeated 10-min separations of mare and foal would lead to an immediate and prolonged increase in maternal care. Furthermore, we assessed the effect of these short-term separations on weaning stress using behavioral and physiological markers. Short-term separations caused an immediate increase in tactile and non-tactile interactions between mare and foal ($P < 0.05$), but we could not identify these effects after 4 to 6 days. In addition, the immediate increase in maternal care by repeated separations did not reduce the response to weaning as measured by heart rate, salivary cortisol, and behavioral indicators. We did find that fillies walked longer, lay down less, and vocalized more than colts ($P < 0.05$) and that the separations complicated the weaning process for fillies more than for colts as indicated by the difference in frequency of defecating ($P < 0.05$). These data show that, despite an immediate increase in maternal care, repeated short-term separations did not appear to facilitate weaning.

In loving memory of my grandparents

Palmyre Moons-Devijver (° July 15, 1921 – † February 16, 2000)
and
Victor Moons (° April 14, 1915 – † March 13, 2001)

Through your unconditional love for us and for each other,
You showed me there is a time to fight and a time to let go.
I will never forget...

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CHAPTER I: GENERAL INTRODUCTION AND OBJECTIVES

Rearing foals to strong, healthy, and competitive animals is the goal of all commercial horse farms. Loss of foals, or foals showing behavioral problems and physical blemishes, results in an economic cost, since much was invested in breeding a quality mare to a quality stallion, in carrying out foaling, and in providing the optimal environment for the foal to develop. Many factors influence the development of foals, but the most important influence in a foal's neonatal life is the dam. She provides the foal with initial immunity, protection and nourishment throughout the first months after birth. The bond between mare and foal is strong and studies have shown that separations of short duration of mare and foal are stress-causing events.

Under feral conditions, mares do not wean their foals until they are about to give birth again. However, in managed horse farms, the sooner a foal can be weaned, the sooner both mare and foal can be prepared for routine activities. Weaning results in maternal deprivation, often social isolation, and a sudden transition in the source of nutrients. It's been shown using behavioral and physiological indicators that weaning is stressful for mares and foals. Because the stress of weaning puts foals at risk for decreased immunity, reduced growth, and injury, researchers have investigated ways to reduce the response to stress, i.e., facilitate weaning. Proposed mechanisms include paired-stall versus individual-stall weaning, gradual versus abrupt weaning, and group-pasture versus individual-stall weaning.

Another potential mechanism to reduce stress at weaning is to make use of the natural bond that exists between mare and foal. In rodents, it has been shown that intensive early maternal care can help rat pups in adulthood adapt better to stressors by modulating the hypothalamus-pituitary-adrenal gland axis. Certain horse experts argued that maternal care in horses can be intensified by short-term separations or by a threat to the foal. If separations of short duration indeed lead to more intensive maternal care, this mechanism may aid foals in coping with stressors such as weaning later on in life.

To assess stress, scientists have used markers such as concentrations of fecal corticosteroid metabolites and urinary, plasma, serum, and salivary cortisol concentrations. Serum and plasma cortisol concentrations to date are the most commonly used markers, but collection of the samples involves invasive techniques that in themselves are stressful for the foals. On the other hand, salivary cortisol measurements are minimally invasive and are highly correlated to serum and plasma cortisol concentrations in different species, including horses.

The overall hypothesis for this Master's project was that increased maternal care early in life will facilitate weaning of foals.

Following hypotheses were tested during the study:

1. Short-term separations cause an immediate increase in mare-foal interactions and maternal care. (Chapter III)

2. Foals that have undergone repeated short-term separations from their dam regain homeostasis faster after weaning than foals that have never been separated from their mother. (Chapter IV)

The objectives of this Master's project were to:

1. Behaviorally assess the immediate and prolonged effects on mare-foal interactions of short-term separations and to evaluate the response of foals to subsequent exposures of isolation. (Chapter III)

2. Investigate the behavioral and physiological responses of foals to weaning stress and compare the responses of foals exposed to early short-term separations with foals that had never been separated from their dam. (Chapter IV)

Chapters from this thesis have been or will be submitted to scientific journals. All chapters were formatted according to the guidelines of *Animal Behaviour*.

CHAPTER II: LITERATURE REVIEW

Maternal Care

Development of mare-foal bond after parturition

Horses and other ungulates are precocial mammals, meaning that the foal can sustain its own thermoregulation, has an adequate sense of smell and vision when it is born, and develops its sensory and motor systems much faster compared to altricial mammals such as rodents (Kendrick et al. 1997). Nonetheless, the dam provides the foal with vital nourishment, protection and initial colostral antibodies. The neonatal foal depends on maternal care to survive the first months after birth. Maternal behavior can be stimulated in the dam by separation or a threat to the foal (Crowell-Davis & Houpt 1986). The relationship between mare and foal changes over time as the young animal gains nutritional and psychological independence.

Less than half an hour after birth, mare and foal begin to show interest in one another. Initially the standing foal follows any large moving object, but McCall (1991) observed that after the second hour postpartum, the foal is less attracted by any random, large, moving object and becomes more attentive to its dam. As the mare recovers from parturition, she licks the foal and fetal membranes. The purpose of this interaction is not so much to clean the foal, but rather to establish a sense of familiarity by gaining olfactory information (Beaver 1981). After licking, the dam appears able to distinguish her foal from others and formation of the mare-foal bond has been initiated. Visual and olfactory cues seem to be the main factors involved in the bonding process. Crowell-Davis and Houpt (1986) reviewed that primary socialization appears to be established within 2 h of parturition. The strong selective individual bond is of significance when mare and foal are out on pasture with other horses because it ensures that the foal only nurses with its own dam and that the mare protects her foal from other herd members (Kendrick et al. 1997). Separation of mare and foal following the bonding period results in signs of disorientation in the foal and extreme distress in the mare.

The role of sensory systems in mare-foal social recognition was investigated using a variety of scenarios (Wolksi et al. 1980). Foal and mare were separated for 10-20 min and then allowed to choose between the relative and an alien horse. Vocal cues appeared to be very important when members of a mare-foal dyad were attempting to locate one another. However, in the tests both mares and foals responded more to the animal with the highest vocalization rate, whether that was the relative or not (Wolksi et al. 1980). Finally, overt olfactory behaviors, such as sniffing, were noted only when mare and foal were in close proximity. After parturition mare and foal are in close proximity and it is mainly visual and olfactory cues that initiate the mare-foal bonding process.

Elements of maternal care in horses

Different aspects of maternal care in horses show that mare and foal form a strong bond with one another, which is then disrupted at the time of weaning. The aspects of maternal care are proximity, nursing behavior, aggression, play, mutual grooming, and communication.

Proximity

A close proximity, the distance between the mare and her foal, is characteristic of the Equidae (Crowell-Davis & Houpt 1986). Mares also show a recumbency response (Barber & Crowell-Davis 1994) and this supports the assumption that close proximity reflects the vigilance of the mare towards the foal. The recumbency response is defined as follows: "when her foal lies down, the mare stops engaging in any other activity and stays near her young, by instinct on alert for predators, even though there may be no such dangers anywhere near the property" (Anonymous 1994). Whether the mare stands close to her foal as described in the recumbency response or lies down next to it, there is very little physical contact between them (Crowell-Davis 1985). As the foal grows older, resting time, nursing time, and proximity decrease and plateau at about 24 weeks of age.

Foals in general are never more than 5 m apart from their mother during the first day after birth (Barber & Crowell-Davis 1994; Crowell-Davis 1985). Barber and Crowell-Davis (1994) studied maternal behavior of 10 Belgian mares on pasture. They reported that, during the first day post-partum, mare and upright foal were within 1 m of each other for at least 95% of the time. In contrast to what we could expect, given the recumbency response, which should draw the mare closer to her foal, this percentage was lower for recumbent foals, namely 85%. Thus, mares did not spend as much time in close proximity with foals that laid down compared to foals that were upright. This rather surprising comparison possibly results from a change in behavior such as that observed by Houpt (1992). When the foal laid down, the mare either stood over or grazed around it in circles. When the foal was upright, the mare grazed more in a straight line and the foal followed the mare, causing the distance to be shorter than when the foal was lying down. The distinction in behavior of the mare toward upright and recumbent foals remained constant throughout the development of the foal. For upright foals, the proximity to the mare gradually decreased with age. The distance between recumbent foals and their dams increased with age, but from 3 weeks after birth until approximately 21 weeks, proximity remained constant (Houpt 1992).

The spatial relationship between mare and foal is mainly due to the mare's behavior when the foal is recumbent and due to mare as well as foal behavior when the foal is standing. Smith-Funk and Crowell-Davis (1992) reported that, during the first days after birth, foals laid down regardless of whether the mare was upright or recumbent. With increasing age, foals mimicked the resting pattern of the mare, often walking towards her and lying down beside her. While standing, a foal usually follows the mare as she grazes, thereby maintaining close proximity.

Nursing

Definition

A considerable amount of variation exists between nursing bout definitions across scientific publications. Throughout consecutive studies more elements have been included, such as pre-suck nuzzle, pre-suck pause, intra-suck nuzzle, and intra-suck pause (Barber & Crowell-Davis 1994). Appendix A shows an overview of how nursing bout definitions developed over 10 years.

Function

Nursing serves multiple functions: to comfort the foal (Crowell-Davis 1985), to nourish it, and, especially during the first day of life, to passively transfer immunity from mother to offspring. Antibodies (Immunoglobulins, Ig) are concentrated in the mare's milk during the last week of pregnancy (Naylor & Bell 1985). The initial Ig concentrations in colostrum, however, drop to 15% between 4 and 8 h after the first nursing (Naylor & Bell 1985). In order to maximize the transfer of passive immunity from mare to foal, it is therefore important that the foal nurses as soon as possible after birth, preferably within 8 h.

In the normal nursing position, the foal stands in reverse parallel next to the mare (McCall 1991). Crowell-Davis (1985) reported that the first nursing bout takes place within 30 to 120 min after birth. Very often, a foal bunts (bobs the head up and down against the mare's udder) before or during nursing. "The function of this bunting is to stimulate release of oxytocin from the neurohypophysis, which in turn controls milk ejection in the udder" (Carson & Wood-Gush 1983a). Successful nursing is dependent on the foal's ability to find the udder and on the mare's willingness to stand still during the initial nursing attempts. Because of the inexperience of primiparous mares, or because mares with tender udders experience pain during the first nursing, foals of these dams may take longer to nurse successfully after birth (McCall 1991). Nursing is also an expression of the bond that has been established between mare and foal. As the foal ages, it becomes less dependent on the dam's milk (Carson and Wood-Gush 1983a) and joins the mare in eating more solid food such as hay and pellets (Crowell-Davis 1985), but it will continue to nurse until weaning.

Nursing behavior

In general, the foal initiates a nursing bout (Anonymous 1994). When the mare does initiate, she first ceases other activities. She then either watches the foal and vocalizes to it to come closer, or she approaches it herself. If the foal is immediately next to the mare, it positions itself in a reverse parallel orientation next to the mare and places its muzzle medial to the mare's pelvic limb. When approaching from a distance, the foal initiates nursing by approaching the mare and extending head and neck while bobbing the head up and down. Another indicator of nursing intention is "crossing the bow". The foal approaches its mother, crosses under her neck and then moves caudally down to the udder of the mare (Crowell-Davis 1985). This 'crossing the bow' actively blocks the forward movement of the mare, therefore facilitating nursing (Barber & Crowell-Davis 1994).

Crowell-Davis (1985) reported that if Welsh pony mares continued walking after their foals initiated nursing, the foals either aggressed against them, continued circling after 'crossing the bow', ceased attempting to nurse, or nursed anyway. These types of reactions to a resisting mare were also reported for Thoroughbred foals (Carson & Wood-Gush 1983a). When a mare prevented her young from nursing, the foal repeated the pattern of 'crossing the bow' to make the dam stand still. If unsuccessful in its efforts to nurse, the foal also pawed the ground and occasionally kicked. If a dam was lying down, sometimes the foal pawed near the udder until the mare stood up.

Nursing bout frequency and duration

Different studies report variation in nursing bout frequency. For Belgian horses, Barber and Crowell-Davis (1994) reported an average of seven successful nursing bouts per hour during the first day after birth. The rate declined by 50% during the first month. The duration of nursing bouts ranged from 78 to 84 sec. For draft mares and their mule foals, the nursing rate on day one was approximately 14 times per hour (Smith-Funk & Crowell-Davis 1992). Nursing frequency decreased steadily, from a maximum of eight bouts per hour after the first week to two bouts per hour during the seventeenth week after birth. Both successful and unsuccessful bouts followed the overall trend.

Bout termination

Crowell-Davis (1986) reported that the duration of nursing bouts varies with individual foals and remains constant for a given foal regardless of age. Bout terminations by the mare were very infrequent during the first day after birth in Belgian draft horses (Barber & Crowell-Davis 1994). This may be a mechanism to insure sufficient ingestion of Ig-rich colostrum. Barber and Crowell-Davis (1994) recorded that mares terminated bouts less often than foals. When a mare terminated a bout, she usually walked away; overt aggression was rare.

Aggression

Aggression of a mare towards her foal arises only rarely during the foal's first month, and is almost always nursing-related (Anonymous 1994). The mare's udder may be sore from being full and initial nursing may cause discomfort. Upon nursing, the pressure in the udder decreases and the mare allows the foal to suckle. Normally a foal is not harmed by the mother's aggression (Crowell-Davis & Houpt 1986) and aggression is potentially a way to regulate expression of the mare-foal bond.

In addition, at any time when a foal bunts the udder too hard or bites the teat, the mare may intervene with some form of aggression. Crowell-Davis (1985) recorded eight different forms of aggression during nursing: laying back of ears, threat to bite, bite, threat to kick, kick, squeal, bunt, and tail swish. Smith-Funk and Crowell-Davis (1992) added a ninth category: smacking, which was described as "ears being laid back, the head turned toward the foal and the mare's mouth opening abruptly, resulting in a loud smacking sound". Barber and Crowell-Davis (1994) suggested that peak aggression occurs at a time during the foal's development when a nutritional transition to grain and forage is about to occur. Maternal aggression possibly encourages that change by reinforcing grazing through resistance to nursing attempts by the foal, potentially leading up to a natural process of weaning.

Foals exhibit a variety of reactions to maternal aggression (Crowell-Davis 1985). The most common one is no discernable response. The second most likely reaction is a short pause while removing the muzzle from the udder, followed by continued nursing.

The third most common reaction is the foal aggressing back at the mare, kicking her or threatening to kick. For Belgian draft horses, no discernable response was exhibited by foals in 66% of all aggressive incidents (Barber & Crowell-Davis 1994). Reciprocating aggression (always a kick in response to a bite) towards the mare occurred in 14% of the aggressions. No response by the foal was recorded in 100% of the cases of laying back the ears by the mare and in 93% of the threats to bite. Welsh pony mares aggressed against their foal during nursing, and this trend increased gradually until the foal was about 13 to 16 weeks old and it leveled off until weaning (Crowell-Davis 1985).

Other interactions

Play

The first kind of play exhibited by the foal is solitary play, when the foal explores its environment for example, by mouthing parts of the stall, or the mare's tail, ears and halter. Playful behavior also includes running and bucking near the mother (Anonymous 1994). When mare and foal are on pasture, playing can also be galloping away from the mare in what appears to be thrill-seeking behavior (Houpt 1992). During the second and third month of a foal's life, a foal kept on pasture with other foals learns to play with herd mates (Anonymous 1994). Three major types of play are exhibited: 1) general motor play involving running/bucking, 2) interactive play, and 3) object manipulation. Colts seem to engage more in interactive play whereas fillies do more running and bucking (Crowell-Davis 1986).

Allogrooming

Allogrooming or mutual grooming is defined as reciprocal coat care in which the partners nuzzle and chew on one another's coats (Boyd & Houpt 1994). In herds it is suggested that grooming actually occurs according to a dominance hierarchy, where the lower ranking animal may or may not be denied access to a mutual grooming session (Moons 1999). Foals mutual-groom preferentially with other foals rather than with their mothers when together in a herd (Crowell-Davis 1986). Fillies groom about twice as often as colts do. In addition, allogrooming with other foals peaks at about the third month of life, and then steadily declines (Crowell-Davis 1986). However, within a stall setting where mare and foal are by themselves, allogrooming can provide an important indication of the mare-foal attachment.

Communication

Communication can be divided into three major types: auditory, visual, and olfactory. Olfactory communication, which occurs through pheromones, has the advantage that it works even when the signaler is not present. Visual cues are used in particular for communication over relatively short distances, while auditory cues are effective over long and short distances (Houpt 1992). When a foal is out a mare's field of vision, the mare looks around to find the foal and communicates with the foal vocally. The vocal repertoire consists of the whinny (or neigh), snort, nicker, and squeal. The whinny is a typical separation call when horses are out of sight of one another. This rarely happens under managed conditions as mare and foal are either kept in a stall or on a pasture where they have visual contact with one another at all times. The snort can be an attempt to clear the nostrils, but it can also indicate increased anxiety in a restrained or frightened horse. The squeal is heard when agonistic interactions occur, and it is often difficult to tell if the aggressor or victim is vocalizing (Houpt 1992).

Factors affecting maternal care

As demonstrated previously through the different components of maternal care, mare and foal form a close attachment. This attachment and more specifically maternal care is affected by several factors: breed, parity, individual variation, environment and foal age. Dwyer and Lawrence (1998) showed that when comparing maternal care in two breeds of sheep, Scottish Blackface and Suffolk ewes, the Scottish Blackface breed showed higher levels of maternal care as measured in time spent grooming lambs and cooperation during sucking attempts. In another study, Dwyer and Lawrence (2000) showed that parity had an effect on the occurrence of rejection behaviors (withdrawal, aggression, lack of cooperation with lamb sucking attempts). Primiparous ewes for both Scottish Blackface and Suffolk showed more rejection behaviors towards their lambs compared to ewes that had had at least one lamb prior to the study. Dwyer and Lawrence (2000) also found that, regardless of parity and breed, individual ewes were consistent in their expression of maternal care across parities. Barber and Crowell-Davis (1994) reported casual observations done on Belgian draft horses where individual variation in maternal care among mares occurred regardless of parity state.

A third factor that influences maternal care is the neonatal environment and manipulation thereof. Liu at al. (1997) showed that when rat pups were handled during the first 10 days after birth, the dams showed an increase in maternal care as measured by the occurrence of licking and grooming. The dams of handled rat pups showed 155 ± 21 occurrences of licking and grooming as opposed to dams of non-handled pups who displayed 78 ± 25 occurrences. In horses, Crowell-Davis and Houpt (1986) argued that if the environment of the foal is manipulated through a threat (such as the presence of a dog) or short-term separations from the dam, maternal care is stimulated.

Finally, age is another factor that influences maternal care. First of all, as the foal grows older, it spends less time in close proximity with the dam (Tyler 1972). While observing New Forest ponies, Tyler (1972) found that during the first week of life, the foal spent 94 % of its time within 5 m of the mare. By week 20, this percentage decreased to 52 % and by week 32, only 20% of the foal's time was spent within 5 m of the dam. Nursing-related aggression displayed by Welsh pony mares increased from approximately 0.1 ± 0.03 aggressions per nursing bout during the first month to 0.3 ± 0.06 aggressions during the sixth month after birth (Crowell-Davis 1985). The association between increase in age and nursing-related aggressions is supported by the decrease in the amount of time a foal spends nursing (Carson and Wood-Gush 1983a). During the first week of life, Thoroughbred foals nursed for a total of 710 ± 50 sec per hour, whereas by the 24th week, total duration of nursing time per hour was 80 ± 15 sec. In addition to the decrease of time spent nursing, aging foals start to adapt to the mare's diet by increasing ingestion of available grass, hay, and concentrate (Crowell-Davis 1985). At 1 week of age, foals were feeding (nursing excluded) 8 ± 2 % of their time whereas by week 21, this percentage had increased to 46 ± 4 % of the total time budget. Thus, as the foal ages, there are changes in the mare-foal relationship and maternal care.

Stress Response

Stress

To assess all the events occurring during a stress response, we need to clearly define "stress". Stressors can be physical (e.g. injury or pain), psychological (e.g. fear) and/or psychosocial (e.g. modification of social interactions) in nature (Mason 1975). Different definitions of stress have been developed as the science of animal behavior has evolved. Most of the definitions for stress rely on the physiological changes within an animal with less emphasis on changes in behavior as part of the stress response (Friend 1991). In 1956, stress was defined as "the nonspecific response of the body to any demand" (Selye 1956). In addition, the author used the term "eustress" for a beneficial or pleasant type of stress and applied "distress" as the more serious, harmful type of stress. Later, stress was defined as "an environmental effect on an individual which overtaxes its control systems and reduces its fitness or appears likely to do so" (Broom & Johnson 1993). More recently, Moberg (2000) defined stress as "the biological response elicited when an individual perceives a threat to its homeostasis". Homeostasis according to Broom and Johnson (1993) is the "relatively steady state of a body variable, which is maintained by means of physiological or behavioral regulation". Maintaining homeostasis in body fluids and tissues is essential for an animal's survival. Whether the threat is real or not is of no importance according to this definition. An animal 'perceiving' a certain stimulus as threatening to its homeostasis will show a stress response.

Similar to the different types of stressors, there are different types of responses to stress. Moberg (2000) describes four different kinds of responses to stress and they all shift available energy and resources away from maintenance processes to ensure the animal is prepared for "fight or flight". The behavioral response is biologically the most economical response. The animal can attempt to escape the threat simply by moving away from the stressor. The second most important line of defense is the autonomic nervous system response. Here, a number of biological systems are affected during stress, resulting in changes in heart rate, blood pressure and gastrointestinal activity. The biological effects are of relatively short duration. This is not the case for the third type of response, the neuroendocrine response, which is explained below in the section "Physiogical response to stress". The neuroendocrine response is responsible for profoundly altering biological functions, potentially impairing the welfare of an animal. The fourth response is the immune response, resulting in depression of lymphocytes and other defense mechanisms in the immune system. However, it is debatable that reduction of immune competence during stress is a mechanism of defense.

All of the above-mentioned defense mechanisms constitute coping strategies when an animal is faced with a stressor. Coping is defined as "having control of mental and bodily stability" (Fraser & Broom 1990). More specifically, in his review of factors affecting physiological coping systems during development, Plotsky (2000) defines coping more extensively as the "utilization of available strategies and resources to successfully reduce the impact of a stressor or to meet daily environmental challenges". Moberg (2000) explained why sometimes stress is harmless and why it sometimes leads to serious consequences. An animal possesses a certain amount of resources to perform all the essential biological functions. The risk of stress is "the shift in biological functions away from non-stress functions or activities in order for the animal to cope with the stressor". When an animal is exposed to a prolonged stressor, too many resources are shifted away from the non-stress functions. It is possible that the impairment of those biological functions poses a threat to an animal's well being.

Behavioral response to stress

The behavioral response to stress can be assessed by the systematic techniques of behavioral observation. Behaviors displayed by the animals of interest are recorded on check sheets or computer files and quantified according to intensity, frequencies and duration. Variations in these measurements can indicate a disturbance in the animal (Broom and Johnson, 1993).

The natural behavior of animals that are not used to close human contact can be confounded by the presence of the observer (Martin & Bateson 1999). Even if the animal does not shy from humans, its behavior may still be altered in more subtle yet significant ways. In order to obtain reliable data, the animals need to be desensitized to human presence, or the observer should resort to the use of cameras, one-way screens or camouflaged hides. Most of these solutions, however, still allow the animal to detect olfactory or auditory cues indicating the presence of the observer. Critical evaluation of the confounding factors of the study should not be neglected when reporting on results.

Behavioral responses to stress can be categorized according to the duration of the stressor(s). Investigators have described acute and chronic stress very precisely (Moberg 1985). When an animal is exposed to one stressor for a relatively short period of time, this is usually referred to as acute stress. Chronic stress results from consecutive acute stressors, regardless of whether those stressors are identical.

Behavioral responses to acute stress occur when an animal experiences short-term pain or fear, or when it is frustrated (Friend 1991). Acute pain or fear can be recognized through observation of the animal's posture. The animal makes itself look smaller by crouching and it may even sweat or tremble. Frustrated animals that are prevented from performing certain behaviors may substitute the prohibited behavior for another. Pawing, vocalizing, defecating and overall increase in activity are other behavioral responses to acute stress (Hoffman 1995).

When responding to chronic stress, animals may initially show fierce resistance to the stressors imposed on them as if responding to acute stress, but after a certain period of time, the animals often adapt. Adaptation may indicate that the animals responded so profoundly to the acute stress that the resources to fight for prolonged periods of time are depleted. When animals cannot avoid the chronic stress, such as repeated exposure to electric shock (Seligman & Maier 1967), learned helplessness can set in. In this case, the drive to avoid the stimulus decreases to the point where the animal is apathetic. More commonly, the animals show an intensification of drive-motivated behaviors as a response to chronic stress. For example, a study on calves showed that animals, deprived of space through confinement, were more motivated to show locomotor behavior during an open field test (Dellmeier et al. 1985). Similarly, when comparing distance traveled in an open field test between horses on pasture, horses stalled with tactile access to neighbors, and horses stalled in total isolation, researchers found that stalled horses traveled the furthest when compared to horses housed on pasture (Mal et al. 1989). Another way of coping with stress occurs in the form of stereotypies, which are invariant and repetitive behaviors that seemingly have no function (Nicol 1999). Stereotypies can be considered displacement behaviors, but they result from chronic instead of acute stress. They arise in captive animals faced with unsolvable problems (Mason 1991).

Physiological response to stress

Anatomy of hypothalamus-pituitary-adrenal gland axis

The physiological response to stress is mediated through the hypothalamus-pituitary-adrenal gland axis (HPA-axis). Because we wish to give a complete overview of the HPA-axis and its hormonal regulation, this section and the next summarize the anatomy and hormonal regulation as reported in a book chapter written by Matteri et al. (2000). Because it is important to understand the underlying mechanisms of a stress response, we feel summarizing the review by Matteri et al. (2000) is justified. The hypothalamus is a bilaterally symmetric region on the floor of the brain, above the anterior pituitary gland. From top to bottom, the hypothalamus extends from the floor of the third ventricle to the median eminence, where the pituitary stalk is formed. From the hypothalamus, there are two kinds of neurons traveling towards the pituitary. The axon terminals of the parvicellular neurons release hormones into a capillary network, the hypophyseal portal vasculature, that traverses the pituitary stalk from median eminence to anterior pituitary gland. The hormones released by these axons regulate the function of the anterior pituitary gland. The second type of neurons consists of magnocellular neurons and they extend directly through the pituitary stalk into the posterior pituitary gland. The neurons produce vasopressin and oxytocin, which are released from the posterior pituitary gland.

The pituitary gland consists of three regions. The anterior lobe secretes growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, and β-endorphin. The posterior lobe of the pituitary gland acts as a storage site for oxytocin and vasopressin (VP, also known as antidiuretic hormone). The third region is the intermediate lobe and its function is species specific.

The adrenal gland consists of two regions: an inner part, medulla, and a surrounding capsule, the cortex. The medulla releases catecholamines (epinephrine, norepinephrine, and dopamine) into the general bloodstream. The cortex consists of three layers: the zona glomerulosa (secretes mineralocorticoids such as aldosterone), the zona reticularis, and the zona fasciculata. The latter two layers are responsible for production of androgens and glucocorticoids (GC, e.g. cortisol).

HPA-axis hormonal regulation

Activation of the HPA-axis can occur through stressors, circadian drives, and humoral influences (Plotsky 2000). In what follows, we summarize a review by Matteri et al.

(2000) for the same reasons as stated in the previous paragraph. During a stress response, the sympathetic nervous system is activated and stimulates catecholamines production from the adrenal medulla. The catecholamines influence the HPA axis at many concentrations, including stimulation of neurohormone release from the hypothalamus and ACTH release from the pituitary gland.

In addition, corticotropin-releasing hormone (CRH) is released by the hypothalamus and this hormone reaches the pituitary through local transport. Corticotropin-releasing hormone and VP work independently as well as in concert to cause ACTH release from the pituitary. Two other factors have been reported to induce ACTH secretion: epinephrine and oxytocin. In the pituitary, ACTH is released into the general bloodstream that reaches the adrenal gland. Consequently, the adrenal cortex produces GC that travel to various regions in the body through the general circulation.

Glucocorticoids play a role in the process of gluconeogenesis: they stimulate the liver to convert fat and protein to intermediate metabolites. These metabolites are then transformed to glucose, a source of energy. In addition, GC have a potentiating effect on the synthesis of epinephrine. It should be noted that having constant, moderate concentrations of GC in the body is necessary to maintain homeostasis. However, when animals are faced with chronic elevations in GC, they are faced with a substantial challenge. This situation can lead to protein catabolism, hyperglycemia, immune suppression, and susceptibility to infection. It is because of these potential deleterious actions that an important function of the steroids is to curtail the HPA response to stress.

This is effected through negative feedback inhibition. In this process, high concentrations of GC in blood inhibit further HPA response at the brain and pituitary level.

Effects of maternal care on the HPA-axis

Before and after parturition, the HPA-axis can be modulated during the development of an animal. External signals interact with the central nervous system to shape the way the HPA-axis responds to stressors and this may be beneficial or not. Factors taking advantage of this neuroplasticity by translating outside stimuli into internal neuronal changes are cyclic adenosine monophosphate and neurotrophic factor cascades (Plotsky 2000). The exact termination of the critical period for plasticity of the HPA-axis is species-dependent and is not yet determined in many cases.

The mother-infant relationship is thought to be an important factor in the way the HPA-axis develops (Plotsky 2000). The primary care giver in general is a hidden regulator of the development of different regulatory systems in a newborn (Hofer 1994). The dam-offspring "security of attachment", introduced in the late 1970's, states that both mare and foal develop the boundaries of their relationship together and they can rely on a certain amount of predictability when interacting with each other (Bowlby 1977). This implies that if the care of the mother towards the infant is inadequate, the development of the offspring is impaired (Liu et al. 1997). This impairment in an animal can lead to a skewed perception and evaluation of the immediate environment, thereby affecting stress coping strategies.

A number of studies in rodents have investigated the effect of early-life stress on the coping strategies of developing and adult animals. The stressor can increase or decrease HPA-axis sensitivity, depending on breed genetics, time of exposure, and type of stressor. The breed effect was explored in a study where two strains of rats (congenital learned helplessness, cLH and congenital non-learned helplessness, cNLH) were exposed to cold stress and maternal deprivation stress on day 7 or day 21 post-partum, researchers found that the ACTH release after adult stress in cLH rats was much higher than that in cNLH rats (King & Edwards 1999). Conversely, adrenal responsiveness was much lower in cLH rats. These findings indicated a dissociation of the pituitary and the adrenal gland and dysfunctional stress response in cLH rats. This study showed that breed had an effect the responsiveness of the HPA-axis.

The effect of time of exposure was explored during an experiment where rats were exposed daily between day 2 and 14 of age to 180 min of maternal separation and handling (HMS180) versus 15 min separation (HMS15) or no separation (AFR) (Plotsky & Meaney 1993). The animals were weaned on day 22 and housed per 3 in a cage. The first group showed hyper-responsiveness to psychological/emotional stressors when compared to the 15-min separation group or the control. This hyper-responsiveness remained visible throughout the animals' lives. In the same study, maternal care seemed to be disrupted more for the HMS180 rats than for the HMS15 or AFR. Compared to control animals (AFR and HMS180), neonatal rats that were handled for relatively short periods of time showed a higher expression of corticosterone receptor proteins in the hippocampus, an area in the brain associated with the mediation of cognitive control

(Toates 2000). Corticosterone is the rodent equivalent of cortisol. This in turn results in an enhanced sensitivity of the negative feedback system regulating corticosteroid release because more GC can bind on the hypothalamus. The rat pups that had been subjected to 'severe stress' (HMS180) showed HPA-hyper-responsiveness later on in life, as indicated by e.g., anxiety-like behavior, compared to the ones subjected to 'mild stress' (HMS15).

Changes in maternal care, rather than the effect of separation itself, most likely mediates the effects of post-handling stress as described above. When rat pups were reunited with their dam after a separation and handling session, the amount of licking and grooming displayed by the mother towards the offspring increased and the differences with the control group remained stable over the 10-day testing period. (Liu et al. 1997). The same researchers subsequently investigated the individual differences in intensity of maternal care in relation to stress-coping strategies of the pups later on in life. The pups that received high quality maternal care ($n = 9$) actually showed a reduced response to the stressors compared to pups that received low frequencies of licking, grooming, and arched-back nursing. Plasma corticosterone concentrations immediately after restraint stress was 1711.0 ± 55.2 nmol/l for low maternal care pups as opposed to 1186.8 ± 82.8 nmol/l for pups that received high maternal care ($P < 0.05$). Two hours post-stressor, the difference was still present ($P < 0.05$) with corticosterone concentration in low maternal care pups being 1021.2 ± 69 nmol/l and 496.8 ± 27.6 nmol/l in high maternal care pups. The greater the frequency of licking and grooming during infancy, the lower the HPA-response to stress was in adulthood. Offspring of females showing high maternal care had a greater suppression of plasma ACTH after corticosterone treatment when compared to the low maternal care counterparts (Liu et al. 1997). In addition, the glucocorticoid receptor mRNA expression in the hippocampus of the high-maternal care dams was higher and correlated with the frequency of licking and grooming compared to low-maternal care dams. Thus, the animals that received the most maternal care seemed to have the most sensitive negative feedback mechanism regulating the HPA-axis response. In addition, individual variations in maternal care and increased maternal care post-handling sessions during early stages of life had the same dampening effect on the response to restraint stress as adults. Therefore, the researches concluded that it was the increase in maternal care after separation that accounted for the stress resistance of pups in adulthood and not as much the separation and handling procedure itself. This effect of maternal care on responsiveness of the HPA-axis of rat pups was confirmed in another study where fearfulness to novelty in rat pups was examined (Caldji et al. 1998). Offspring of mothers that showed high frequencies of licking and grooming showed substantially reduced behavioral fearfulness in response to novelty when compared to offspring of low-maternal care dams.

Salivary Cortisol

When an animal perceives a certain stimulus as threatening, a stress response occurs, resulting in the release of GC. Cortisol, the common GC in humans, pigs, and horses is largely bound to serum proteins, corticosteroid-binding globulin (CBG), and albumin (Vining et al. 1983). During social stress (introduction of adult horses and foals into a new herd), the binding capacity of these CBG has been shown to decrease (Alexander & Irvine 1998). So in addition to neogenesis of cortisol from the adrenal gland, cortisol bound to proteins is released. The bound fraction of cortisol is biologically inactive whereas the smaller, free fraction is available to produce physiological effects (Kirschbaum & Hellhammer 1989; Umeda et al. 1981). The unbound cortisol fraction in non-stressed human serum is about 1 to 15% of the total amount of cortisol and is reflected by the salivary cortisol concentrations (Vining et al. 1983). Circulating unbound cortisol passes readily through the parotid membrane. Vining et al. (1983) found that human salivary cortisol concentrations are one third lower than the serum unbound-cortisol concentrations, which they attributed to conversion of cortisol to a metabolite, cortisone, when passing through the parotid membrane. This difference was also reported in another study where salivary cortisol concentrations were found to be 30% lower than those of serum unbound cortisol (Umeda et al. 1981). When assessing salivary cortisol, flow rate of saliva did not influence cortisol concentrations (Hubert & de Jong-Meyer 1989).

Salivary cortisol is a minimally invasive measure in humans to assess adrenal gland corticosteroid output during a stress response compared to blood cortisol. One major advantage to taking saliva samples is that we avoid the stress of venipuncture. For blood sampling, animals must undergo capture, restraint and venipuncture all at the same time and this procedure in itself is a potential stressor (Blackshaw & Blackshaw 1989). Taking salivary cortisol samples requires much less restraint. Saliva sample collection for cortisol analysis has been demonstrated to be successful in guinea pigs (Fenske 1996), tree shrews (Ohl et al. 1999), squirrel monkeys (Fuchs et al. 1997), dogs (Beerda et al. 1996; Beerda et al. 1998), sheep (Fell et al. 1985), pigs (Parrott & Misson 1989; Parrott et al. 1989; Geverink et al. 1999), calves (Fell & Shutt 1986), and horses (van der Kolk et al. 2001). Blackshaw and Blackshaw (1989) did encounter some difficulties when assessing correlation between salivary and plasma cortisol concentrations in pigs. Salivary secretion in pigs is discontinuous and normally restricted to feeding. Another study showed that when using a specialized saliva collection device (oral diffusion sink), continuous assessment of salivary cortisol during transport in pigs was possible (Schonreiter et al. 1999).

Blood sampling in horses creates the same difficulties as demonstrated for other species (Lebelt et al. 1996). Recent studies investigated the correlation between saliva and plasma cortisol during ACTH challenge and transportation in horses (Warren et al. 2001). They found the correlation in horses ranged between 0.61 and 0.87 for the ACTH administration experiment. Similarly, Lebelt et al. (1996) found a correlation coefficient of 0.83 and Van der Kolk et al.(2001) found $r = 0.93$. Salivary cortisol concentrations averaged 6.6 ± 1.3 % of the plasma cortisol concentrations in the study by Warren et al. (2001). This value is high in comparison to a study by Lebelt et al. (1996) where salivary cortisol concentrations were 3 to 5 % of plasma total concentrations and in the study by Van der Kolk et al. (2001) this percentage was 1.20 ± 0.37 %. As in other species, horses show a circadian pattern in salivary cortisol concentrations with highest concentrations found during the morning hours (2.76 nmol/l ± 1.05) and the lowest concentrations found during the afternoon (1.73 nmol ± 0.65; Lebelt et al. 1996). Van der Kolk et al. (2001) did not find a significant diurnal variation for salivary cortisol, but plasma total cortisol concentrations did show a circadian pattern. In contrast, in another study morning concentrations of plasma total cortisol measured 251 ± 115 nmol/l and differed from evening cortisol concentrations, which measured 142 ± 53 nmol/l. Lebelt et al. (1996) also showed that there were great inter-and intra-individual differences in daytime profile.

Heart rate

The relationship between behavior and heart rate has long been established and researchers have measured heart rate to evaluate the stress response. Catecholamines, norepinephrine and epinephrine, released in higher concentrations by the adrenal gland during stress than when compared to non-stress situations, enhance cardiac concentration, heart and skeletal muscle blood flow, blood glucose concentration, lypolysis, and oxygen consumption (Thornton 1985). In addition, catecholamines in humans have been associated with emotionality of a stress response as measured by the response during participation in a video game. (Cleroux et al. 1985; Niezgoda & Tischner 1995). Niezgoda and Tischner (1995) found that 5 min into the separation of mare and foal, concentrations of catecholamines peaked. A limitation of the use of heart rate measurements to assess a stress response is that, concomitant with the catecholamines increase, an increase in activity and thus an increase in metabolic rate lead to an increase in heart rate (Broom & Johnson 1993). Heart rate shows a linear increase with increasing exercise level (Lowe 1990; Persson & Ullberg 1974), but exercise also increases release of cortisol (Rose & Hodgson 1994). It is therefore important to take the level of activity of an animal into account when evaluating cardiac changes to an external stimulus that is a potential stressor.

For resting foals, the average heart rate at 8 to10 weeks of age is 60 to 79 beats min^{-1} whereas for 6-month old foals, this pulse rate is 60 to 71 beats min^{-1} (Lowe 1990). A decrease in heart rate in Camargue horses has been associated with positive social contact, i.e., grooming at the back of the neck, which appeared to be the preferred grooming site (Feh & de Mazieres 1993). The measurements of heart rate for that particular study were taken with a stethoscope. An important limitation to the use of a stethoscope is that one can only measure heart rate when the animal is in a state allowing manipulation. However, when intense stress occurs, the behavioral response might be of such magnitude that handling by humans is impossible. In addition, studies have reported that approach of humans can cause elevated heart rate in animals, such as sheep (Baldock & Sibly 1990). This increase is even greater when a dog is involved in the approach test.

The development of telemetrical heart rate monitoring devices provided the solution for continuous non-invasive heart rate monitoring under a variety of circumstances. Three major types of heart rate meters have been used to investigate heart rate in horses: Equistat (EQB, Unionville, PA), Hippocard (Isler Bioengineering, Zurich, Switzerland), and an adaptation of Polar (Polar CIC Inc, Port Washington, NY) equipment used for monitoring heart rate during exercise in humans. All of these heart rate meters require electrodes being placed on the heart girth of the horse and each meter has a data-storing unit attached to or placed near the electrodes. The Equistat monitor has successfully been used in a study investigating the influence of a lactate-reducing supplement, betaine, on untrained and trained horses exercising to fatigue (Warren et al. 1999). Other studies investigating the relationship between exercise and heart rate have used the Hippocard monitoring device to take accurate heart rate measures (Evans et al. 1993; Snow et al. 1992). Geverink et al. (1999) conducted a study investigating the effect of shot biopsy on heart rate in slaughter pigs. The experimenters used Polar Sport Tester consisting of an electrode belt with built-in transmitter and a wristwatch receiver. The data collected in the wristwatch was downloaded via a Polar interface to a PC. Very little research on horses has been done recording heart rate using Polar technology. Rivera al. (1999) investigated behavioral and physiological responses (including heart rate) to initial training to compare stalled versus pastured horses. Polar Vantage NV receivers were used and the transmitters with electrodes were held in place by a custom-made belt.

Response to psychosocial stress

Psychosocial stress refers to stress caused by a disturbance in social interactions or by the social interactions themselves. Isolation is an example of a psychosocial stressor (Asterita 1985). The physiological responses to isolation have been studied in depth. When sheep were isolated from the flock, a rapid rise in cortisol output was measured with the highest values occurring 15 min after isolation (Parrott et al. 1988). The study also showed that cortisol concentration decreased after 30 min and was elevated again 90 min after isolation. There was no significant increase in prolactin, a hormone used as an indicator of chronic stress in sheep, following treatment.

Although they are also herd animals, horses often undergo social isolation due to standard operating farm protocol (SOP) or during routine husbandry procedures. Changes in behavior and physiology of horses subjected to acute isolation stress has been reported

(Bagshaw et al. 1994). Walking-activity was higher for animals in isolation than for the mares that had visual contact with conspecifics. Whinnying, blowing (defined as "high-pitched 'whoosh' sound produced as the horse exhales through the nose while standing alert"), and defecating increased with isolation as well. Since only two mares pawed throughout the experiment, no effects of isolation on this behavior could be reported.

Mares and foals are often separated during routine veterinary procedures such as foal-heat breeding, which usually occurs around 10 days after foaling (Harper 1999). In light of the strong mare-foal bond described earlier in this chapter, considerable stress can be associated with these separations. Niezgoda and Tischner (1995) investigated physiological responses to separations, such as changes in plasma catecholamines (epinephrine and norepinephrine) and cortisol when separating 10 Polish pony mares and foals for 10 min on day 5, 20 and 50 post-parturition (Niezgoda & Tischner 1995). The strongest physiological response was observed during the first separation at 5 days of age. Epinephrine concentrations showed a maximum increase (foals: 1.21 ± 0.1 to 8.3 ± 0.7 nmol/l, mares: 0.98 ± 0.1 to 6.9 ± 0.9 nmol/l; $P < 0.01$) after 5 min, as did norepinephrine levels (foals: 1.99 ± 0.03 to 6.9 ± 0.3 nmol/l, mares: 1.98 ± 0.06 to 5.7 ± 0.5 nmol/l; $P < 0.05$), and cortisol concentrations increased significantly after 10 min (foals: 133.5 ± 15 to 181.2 ± 15 nmol/l, mares: 116.6 ± 5.5 to 164.2 ± 11.0 nmol/l; $P < 0.01$). Concentrations of hormones were lower in subsequent isolations, especially in mares ($P < 0.01$-0.05). In addition, conflicting results were seen in response to the separations on day 20 and 50. On day 20, only foals showed a significant increase in cortisol. On day 50, a significant increase was observed in mares but not in foals. Previous research has not looked at the behavioral effect of short-term separations on mare-foal interactions and neither have reports on foal behavior during and after the separations been published.

Weaning

Introduction

As described above, mare and foal form a strong bond and this is expressed in several behaviors associated with maternal care. Weaning disrupts this attachment between dam and offspring. At weaning, a foal becomes independent of its mother for food, protection and social interaction. In the wild, mares wean their foals gradually, shortly before the birth of a new foal, 1 or 2 years after the weanling was born (Crowell-Davis 1986). However, in managed herds weaning is controlled by the breeder and occurs between 3 and 6 months. Weaning causes an increase in general motor activity and vocalizations in several species such as red deer calves (Pollard et al. 1992), pigs (Varley 1995), and horses (Crowell-Davis 1986). Casual observations, like those reported by Houpt et al. (1984), show that the foal neighs, attempts to rejoin its dam and can show loss of appetite. In addition, foals may attempt to escape and injure themselves.

The earlier the foal is weaned, the sooner the mare can be used for spring, summer, and fall riding and show activities (Kohler 1972). In a two-year study, Kohler investigated the effect of early weaning (at 30 days) as compared to weaning at 6 months of age on average daily weight gain, "bloom scores" (body condition scores) and several management procedures. They found that although early-weaned foals did not gain weight as fast during the first 12 weeks, they were comparable in weight to foals weaned at 6 months. The early-weaned foals did have better body condition scores because of less exposure to the elements and they underwent the management procedures after weaning more readily because they had been handled more. Weaning tended to make foals, especially those weaned at 30 days, more sensitive to noise, rapid movements, and other factors disturbing their environment (Kohler 1972).

Under unmanaged conditions, weaning is considered to be a gradual process and elicits little reaction from the foal (Tyler 1972), but current management schemes require that a foal is weaned earlier and the response to weaning can be quite extensive behaviorally and physiologically (McCall et al. 1985; McCall et al. 1987; Hoffman et al. 1995, Houpt et al. 1984). Besides the time at which a foal is weaned, there are other factors influencing the way a foal copes with the stress of weaning. Foals that had access to concentrate (Hoffman et al. 1995) or creep feed (McCall et al. 1985) as well as pasture grazing and hay tended to cope better with weaning than foals that had access to pasture grazing and hay. The effect of diet on the way mares cope with stress has also been investigated (Holland et al. 1997). The researchers found that serum cortisol concentrations after weaning were lower for mares on a sugar and starch concentrate in comparison to mares fed a fat and fiber concentrate, but both groups showed initial depression of lymphocytes. Thus, although both groups were affected by weaning, the animals that were fed the sugar and starch concentrate diet showed a lesser physiological response during weaning.

Weaning methods

Abrupt versus gradual weaning

Abrupt weaning involves the sudden, total separation of mare and foal. The foal can no longer see the mare, although auditory contact may still be possible. Gradual weaning involves the separation of mare and foal by a fence, but the animals can maintain visual, tactile and auditory contact for a while. A study on 21 Quarter Horse foals compared the behavioral (McCall et al. 1985) and physiological (McCall et al. 1987) responses to abrupt weaning versus gradual weaning. Gradually-weaned mares and foals were allowed fence-line contact for 7 days and were subsequently moved out of auditory, olfactory, and visual contact. Foals in the abrupt weaning method spent more time walking and less time standing still. They also laid down sternally more often than laterally, indicating they were less relaxed. Abrupt weaning led to a higher frequency of vocalizations compared to gradual weaning. This is most likely because mare and foal were still in contact during the first 7 days of gradual weaning. Other behaviors displayed by the weaned foals were aggression to other foals and pawing. The adrenal response to weaning varied greatly between animals, but the abruptly weaned animals showed a significant positive adrenal response to weaning whereas the gradually weaned animals did not. From these results we can conclude that gradual weaning causes a lesser stress response than abrupt weaning.

Stall versus pasture weaning

Foals are often weaned individually in stalls, but the alternative of weaning foals in a group out on pasture has encouraged researchers to compare stressfulness of both scenarios (Heleski et al. In Press). In their study, Heleski et al. (In Press) weaned 6 Quarter Horse foals in individual box stalls and 6 were weaned in groups of 3 in a paddock. Paddock-weaned foals engaged in a greater variety of activities (provided by the opportunity to graze and engage in social interactions) and showed a lesser number of aberrant behaviors, such as licking, biting, or kicking the stall/shed wall. Based on the reduced occurrence of aberrant behaviors, it appeared that foals in the paddock-weaned situation were coping better with weaning than their stall-weaned counterparts. Conversely, in a study comparing the effects of indoor housing versus housing outdoor after weaning of red deer calves, showed that the indoor housed calves had a better weight gain, showed less pacing, and ate more (Pollard et al. 1992). Although the indoor-housed animals seemed to cope better with weaning, it should be noted that these calves were kept in groups whereas the stalled foals in the study by Heleski et al. (In Press) were housed individually. That red deer calves were able to form social relations with their 'herd members', may explain the decrease of the weaning impact.

Weaning foals individually or in pairs

It has been argued that the presence of conspecifics can decrease the stressfulness of weaning. However, contradicting evidence for this finding has been presented. When weaning eight ponies individually in stalls and comparing their behaviors to 14 ponies weaned in pairs, researchers found that there were no differences between plasma cortisol concentrations due to weaning or treatment (Houpt et al. 1984). In contrast, the foals did vocalize (neigh) more after weaning than before and they were also more active. The only difference between individual and paired weanlings was that individually housed foals vocalized more after weaning. The researchers concluded that weaning foals in pairs seemed less stressful than when weaning foals individual. However, a more recent study showed that paired foals, although there was a tendency to be less vocal, displayed significant aggression towards one another (Hoffman et al. 1995). It should be noted that the occurrence of aggression may be related to the relationships, if any, existing between the foals prior to weaning. Though it is not clearly stated, the publications of both Houpt et al. (1984) and Hoffman et al. (1995) suggest that the foals had spent one or more months together on pasture prior to weaning. There was a tendency for the serum cortisol concentrations prior to ACTH challenge to be higher in paired foals than single-housed foals. In addition, the physiological response of individually housed foals to an ACTH-challenge was less than the response of paired foals. The researchers concluded that there was little or no real advantage to weaning foals in pairs. Companionship during weaning may not have an effect unless there is enough space within a confined area to avoid aggression, or unless the companion behaves in a similar way as the dam of which a foal was deprived. In the red deer study by Pollard et al. (1992), the presence of dry hinds in the groups of weaned calves seemed to have beneficial effects on the stress coping strategies of the calves. Though it was not indicated as such by the authors, the possibility exists that the hinds behaved in ways similar to the mother that the calves were deprived of.

Another study investigating the effect of paired versus individual weaning in horses showed no physiological differences between the two treatments (Malinowski et al. 1990). Cortisol concentrations were higher in weaned animals when compared to control animals, but no difference was found between foals weaned individually and weaned in pairs. Cell-mediated immunity, as measured by lymphocyte proliferation response, was lower in foals weaned in pairs compared to foals weaned individually. Thus, according to the physiological indicators, weaning foals individually was preferred over weaning foals in pairs.

CHAPTER III: IMMEDIATE AND PROLONGED EFFECTS OF SHORT-TERM SEPARATIONS ON MARE-FOAL INTERACTIONS

Abstract

Routine husbandry procedures often cause farm managers to separate a mare from her unweaned foal for short periods of time. Research has shown that these are stress-causing events for mare and foal. However, the separations in themselves may lead to intensified mare-foal interactions and increased maternal care displayed by the mare. Exposure to increased maternal care in rodent pups has been shown to dampen stress responses in the animals as adults. In this study we investigated the immediate and prolonged effects of short-term separations on mare-foal interactions. We hypothesized that short-term separations increase the occurrence of mare-foal interactions. As expected, immediately after a separation, both mare and foal initiated more contact behaviors compared to pre-separation (foal: 1.1 ± 0.6 to 4.1 ± 1.0 times per 30 min, $P = 0.0105$; mare: 0.3 ± 0.3 to 1.7 ± 0.7 times per 30 min, $P = 0.0087$) and were more attentive to one another (foal: 1.5 ± 0.7 to 3.9 ± 1.0, $P = 0.0050$; mare: 1.5 ± 0.5 to 3.1 ± 0.7, $P = 0.066$). They also stayed closer to one another ($P = 0.00090$). Fillies tended to remain closer to their dams than colts did ($P = 0.061$). However, prolonged effects of the separations could not be identified by behaviorally evaluating mare-foal interactions 4 to 6 days post-separation. Based on studies done in rodents, we predict that the effect of the immediate increase in maternal care post-separation becomes apparent when submitting foals to a stressor.

Introduction

When carrying out routine husbandry procedures for mares and their foals, horse farm managers can allow the foal to accompany the mare while she undergoes the procedures, or leave the foal in the stall by itself. Both options have obvious advantages. When leaving the foal in the stall, handlers do not need to monitor it to make sure it does not escape, stay behind, or injure itself while running back and forth. However, if the foal is left in the stall and the mare loses visual contact, the mare-foal bond is disrupted, which in turn agitates the mare and makes her more difficult to handle. In addition, other animals within hearing range of the separated pair may become aroused because of vocal interaction between dam and foal. For the farm workers, the environment becomes quite unpleasant to work in and, moreover, the commotion can impact other animals (personal observation).

Casual observations such as those mentioned above have led scientists to investigate how short-term separations impact mare and foal. A study looking into the behavioral responses of mares and their foals to weekly five-minute separations from birth until 12 weeks of age found that animals neighed more while alone (Houpt et al. 1981). A maximal vocal response of the foals was found at four weeks of age. Mares were overall more active during the separation compared to when their foal was near them. Foals were not more active during the first week, but they did show increased activity during the separation from week 2 until week 12. The results showed that the age of the foal plays an important role in how much short-term separations affect foals. Separation from young foals affected mares more than when they were separated from an older foal whereas foals less than four weeks old did not seem as affected by the separations compared to when they were older (Houpt et al. 1981).

Other researchers investigated physiological responses to separations, such as changes in plasma catecholamines (epinephrine and norepinephrine) and cortisol when separating 10 Polish pony mares and foals for 10 min on day 5, 20 and 50 post-parturition (Niezgoda & Tischner 1995). The strongest physiological response was observed during the first separation at 5 days of age. Epinephrine concentrations showed a maximum increase (foals: 1.21 ± 0.1 to 8.3 ± 0.7 nmol/l, mares: 0.98 ± 0.1 to 6.9 ± 0.9 nmol/l; $P < 0.01$) after 5 min, as did norepinephrine levels (foals: 1.99 ± 0.03 to 6.9 ± 0.3 nmol/l, mares: 1.98 ± 0.06 to 5.7 ± 0.5 nmol/l; $P < 0.05$), and cortisol concentrations increased significantly after 10 min (foals: 133.5 ± 15 to 181.2 ± 15 nmol/l, mares: 116.6 ± 5.5 to 164.2 ± 11.0 nmol/l; $P < 0.01$). Concentrations of hormones were lower in subsequent isolations, especially in mares ($P < 0.01$-0.05). In addition, conflicting results were seen in response to the separations on day 20 and 50. On day 20, only foals showed a significant increase in cortisol. On day 50, a significant increase was observed in mares but not in foals.

Separations of short duration, applied by many horse farms during routine management and veterinary procedures, are stress-causing events. However, horse experts have argued in the past that a threat to the foal or short-term separations of mare and foal can stimulate maternal care in horses (Crowell-Davis & Houpt 1986). Consequently, the natural variation in maternal care that potentially exists between horses could be decreased as the levels of maternal care increase. Intensive maternal care at early stages in life has been found to dampen stress responses during adulthood in rodents (Liu et al. 1997). Having received maternal care from a mother who displayed high licking and grooming and who facilitated nursing by standing in an arched back position, rat pups exposed to restraint stress as adults showed a smaller increase in ACTH and corticosterone concentrations, which was also of shorter duration.

As foals on farms are exposed to multiple stressors, ranging from weaning to halter and riding training, we deemed research looking into the effects of short-term separations on maternal care and mare-foal interactions in general was warranted. The objective of this study was to investigate how mare-foal interactions were impacted immediately after separations. In addition, we evaluated how the mare-foal relationship evolved throughout the period in which separations were performed by comparing mare-foal interactions in a separation group with mare-foal interactions in a control group where mare and foal were never separated. We hypothesized that interactions between mare and foal would increase after separation and that this effect would be apparent during all six separations. Furthermore, we hypothesized that, by observing control and separation group mare-foal interactions, we would find that separations increased mare-foal interactions in the separation group as compared to a control group.

Materials and Methods

Animals

This research was conducted at MSU Merillat Equine Center from January through October 2000. A total of 10 mare-foal pairs were studied (Appendix C). The animals were divided into two groups according to a random design blocked by foal gender. Each group consisted of three fillies and two colts. The ten foals were sired by three different stallions as indicated in Appendix C. Each group represented a treatment. There was a control group (n=5) in which mare and foal were never separated from one another and a separation group (n=5) where mare and foal underwent six 10 min separations performed every other week from the time the foals were 2 weeks old until 3 months of age. The protocol was designed and some decisions (10 min separation) were based on our current understanding of foal physiology and behavior, whereas others (separations every other two weeks) were based on farm logistics. The MSU All University Committee on Animal Use and Care reviewed and approved the experimental protocols described in this chapter.

Housing and feeding

From January through April 2000, mares were individually housed in Barn A in 13.38 m^2 box stalls. The stalls were bedded with wood shavings and had a combination feeder in one corner. The mares had ad libitum access to water via an automated drinker. Prior to foaling, mares were moved to one of two 26.76 m^2 foaling stalls that were bedded with straw. By May 2000, all mares had foaled and the animals were moved to the climate controlled Barn B. All mares were fed twice daily according to NRC recommendations (National Research Council, 1989). The morning feeding occurred between 08:00 and 10:00 and the afternoon feeding, between 15:00 and 17:00.

Video recording and data collection

A Panasonic CCTV WV-BP334 video camera was installed out of the animals' reach on the wall between two stalls housing the study subjects. All cameras were connected to a Panasonic WV-CM146 field switcher monitor. In addition to the live observations, we recorded the animals on VHS tape using a Panasonic AG-6740 VCR. Depending on which dyad would be subjected to the separation, we turned the camera towards the stall of interest (Fig. 1 and 2).

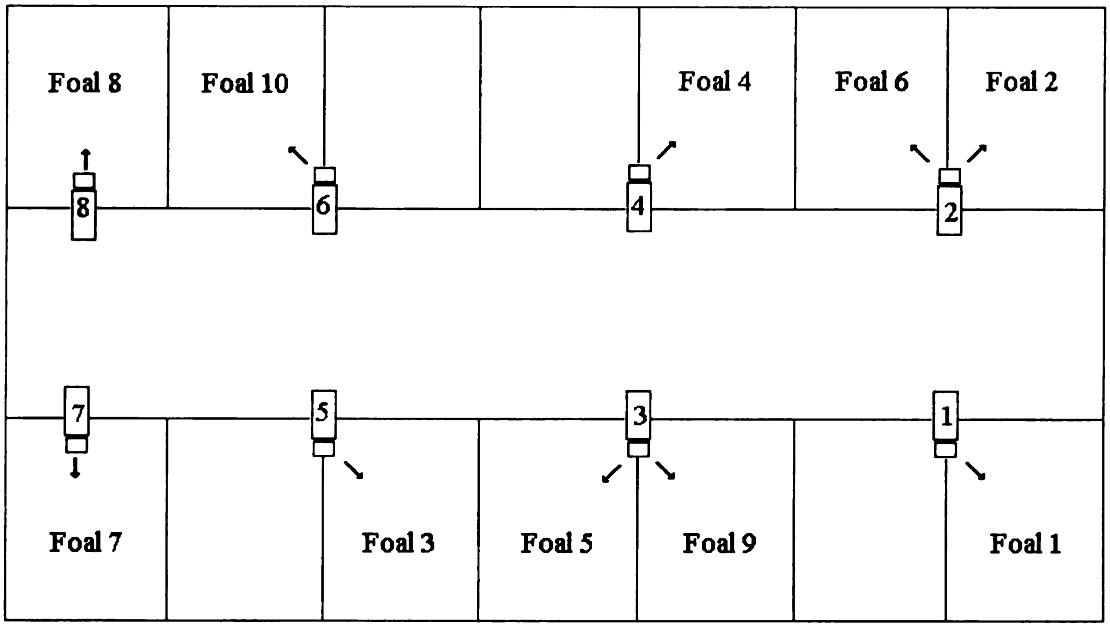

Figure 1. Scheme of part of MSU Merillat Equine Center Barn A, not drawn to scale. Each rectangle represents a stall. The numbered boxes are cameras and the arrows indicate which stalls were covered by each camera.

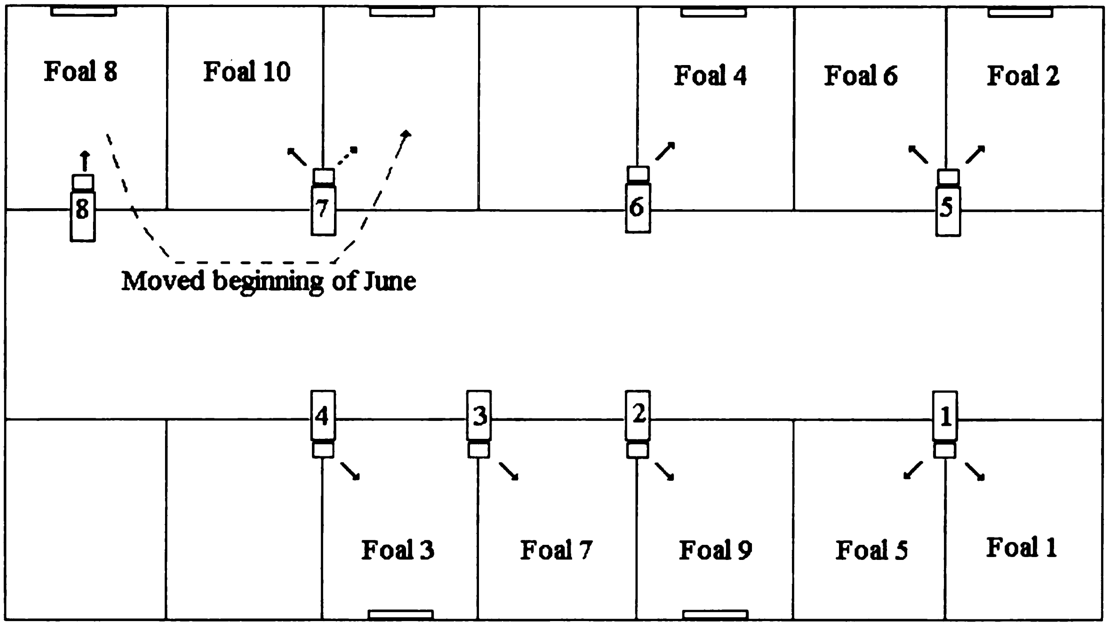

Figure 2. Scheme of part of MSU Merillat Equine Center Barn B, not drawn to scale. After May 5, 2000 all the animals on the project were moved to this barn. Merillat management assigned foals to stalls according to availability of Dutch doors that could be opened to allow airflow.

Eight cameras were used, which is the maximum number of cameras from which the field switcher could collect data before sending them to the time lapse VCR. The data were recorded onto VHS tapes either in 18 h or 24 h time mode. We used the same equipment for the post-separation observations when we evaluated mare-foal interactions. The tapes were decoded on-site using a Psion Workabout (Psion, PLC, England) equipped with The Observer™ behavioral software (version 3.0, Noldus Information Technology Inc., Sterling, Virginia) or in the behavioral laboratory using personal computers with the same software. The equipment was moved when the animals were relocated from Barn A to Barn B.

Short-term separations

Each mare-foal dyad in the test group was subjected to six 10 min separations performed every other week, starting when the foal was approximately 2 weeks old until 12 weeks of age. Separations were performed during the first part of the week. An undergraduate student working on the farm entered the stall, haltered the mare, and led her out of the stall while making sure the foal stayed inside. The foal could not see the mare, but we were unable to take the mare out of the foal's hearing range when both animals vocalized during the separation. After 10 min, the handler reunited mare and foal.

For each separation we recorded behaviors 30 min before and after separation as well as during the 10 min of separation. To evaluate the immediate effect of separations, each mare-foal pair served as its own control as we compared mare-foal interactions before and after separation. The complete ethogram used is described in Appendix E. Because of

interference of the Panasonic field switcher with the time-lapse recording, we were unable to reliably record durations for the behavioral categories of walking, trotting, cantering, and nursing during separation trials and post-separation evaluations.
It should be noted that only “eating”, “urinating”, “defecating”, “vocalizing”, “investigating”, “pawing”, and “looking forward” were discussed for the 10-minute separations. We chose these behaviors on the basis of their occurrence and relevance in demonstrating a stress response (Hoffman 1995; Houpt 1984; Heleski, in Press). For the pre- and post-separation evaluation we discussed a different set of behaviors. Analysis of behaviors performed by the mare consisted of “contact” (including “nuzzling” and “allogrooming” events), “watch foal”, and “aggression” (including “laying back ears”, “tail swish”, “bite threat”, “bite”, “kick threat”, and “kick”). Analyzed behaviors displayed by the foal towards the mare were “contact”, “watch mare”, and “aggression”.

We also visually assessed the distance between mare and foal every 5 min for the observation period pre- and post- separation on a scale of “a” to “c”; “a” denoted the distance when the anterior part of mare and foal was closer than 1/3 the length of the stall; “c” was scored when the animals were in opposite corners of the stall; “b” denoted any distance between “a” and “c”. To analyze these data, we replaced the initial codes for the distance between mares and foals by numbers (“a”=3, “b”=2, “c”=1). For the data collection prior to and after separation, we collected seven data points per 30 min of observation time. The values replacing the letter scores were averaged and these ‘proximity scores’ were analyzed for differences between pre- and post-separation. We weighed the number of proximity scores we used per average.

The data we collected represented counted values, so we square root transformed the data to normalize the distribution. For the 10-min separations, we used the repeated measure of trial in mixed model procedures of SAS. Trials refer to the two-weekly separation, which is inter-related with the age of the foal, as indicated by the graphs in the results section. The variables used in the model for the 10-minute separation were foal, gender, and trial. To analyze the effect of the separations on mare-foal interactions we used period (before and after separation) in mixed model procedures of SAS. The model for the data analysis included gender and period as main effects and the two-way interaction between them. Significant main and interaction effects were analyzed using multiple comparisons t-test. We used a Bonferroni adjustment to correct for the number of comparisons.

Evaluations of interactions initiated by foal and mare 4-6 days after separation

We evaluated mare-foal interactions at the end of every week following a separation, allowing 4 to 6 days between separation and assessment of maternal care. In this part of the experiment, we compared mare-foal interactions in the separation group (n=5) with mare-foal interactions in an actual control group (n=5). Although we used 12 h, 18 h, or 24 h time-lapse mode to record, the videotapes were analyzed in 12 h mode. This provided a better transition between frames and reduced observation time. The observation schedule (test animals and corresponding control animals) listed in Appendix D was such that no more than five pairs of animals were observed within one week. We divided the observation day into four two-hour blocks equally distributed between 08:30 and 18:00 (08:30-10:30; 10:50-12:50; 13:30-15:30; 15:50-17:50). We used continuous recording and focal sampling to record behaviors. Every 5 min we recorded behaviors for a different mother-foal pair and continued this rotation for two hours. At the start of each 5 min observation session we visually assessed the distance between mare and foal according to the scale described previously. For a given mare-foal pair we collected 100 min of data per post-separation observation day. The complete ethogram we used is described in Appendix E, but the behaviors we discussed were limited to the mare-foal interactions recorded on the day of separation, which are described previously.

The behaviors recorded represented count data, so we transformed the data using a square root transformation to normalize the distribution. The model for the post-separation evaluation included treatment, trial, period, and their two- and three-way interactions. It should be noted that the class factor "period" for post-separation evaluations represents one of four time blocks of the observation day during which data were collected. Significant factors from the mixed model were further investigated using multiple comparisons t-test with Bonferroni adjustment. When no difference was found between control and separation group, data were combined for further analysis. The means reported in this study are lsmeans ± SEM.

To analyze proximity, a similar procedure was used as that for separations and pre- and post-separation observations (see *Short-term separations*), but per observation block we had a maximum of 5 proximity values, which we averaged to obtain the proximity score.

Results

Foal behavior during short-term separations

The frequency of “investigating” ($F_{5,15}$=3.94, P = 0.018) and “looking forward” ($F_{5,15}$=3.05, P = 0.043) were influenced by the main effect of trial (Fig. 3, 4). However, frequencies of “eating” ($F_{5,15}$=0.42, P = 0.83), “urinating” ($F_{5,15}$=0.92, P = 0.49), “vocalizing” ($F_{5,15}$=0.15, P = 0.98), and “pawing” ($F_{5,15}$=1.70, P = 0.20) did not differ between subsequent trials.

In Fig. 3, we show that the first trial, when foals were 2 weeks old, has significantly lower investigating frequencies than the fifth trial, at 10 weeks of age. Frequencies of investigating at 4 weeks of age are significantly lower when compared to 8, 10, and 12 weeks ($P < 0.050$). In addition, at 4 weeks of age, investigative behavior tended to have lower frequencies than at 6 weeks as well ($P < 0.10$). Finally, at 6 weeks, less investigative behavior was displayed when compared to 10 weeks of age ($P < 0.050$).

Figure 4 shows that the frequencies of looking forward for trial 3 (6 weeks of age) and 4 (8 weeks of age) are lower than for trial 1 (2 weeks), 5 (10 weeks), and 6 (12 weeks). It should be noted that probability for the difference between trial 1 and 4 is P = 0.063.

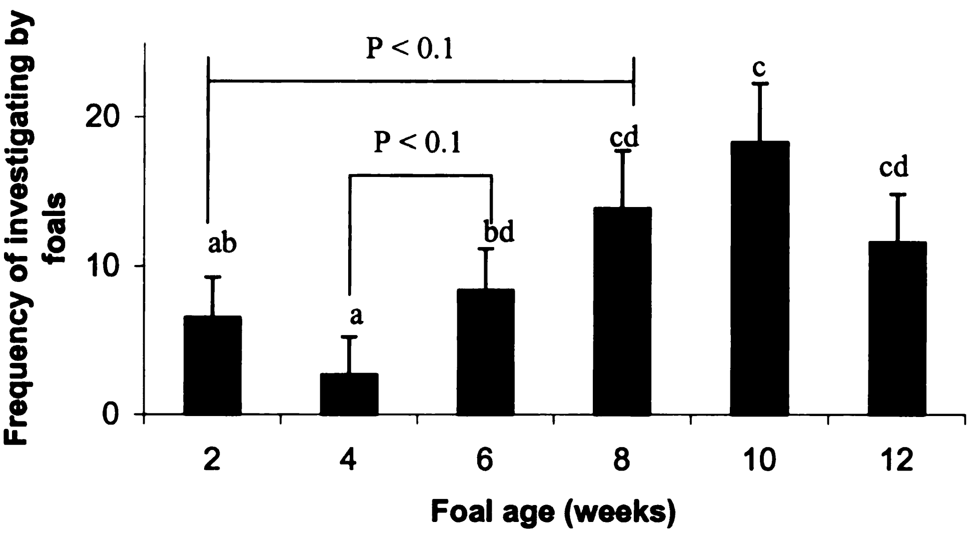

Figure 3. Mean frequency ± SEM of investigating by foals during 10-min separation for subsequent trials. (N=5, P < 0.05 except where indicated). Differences are indicated by the letters (a,b,c,d).

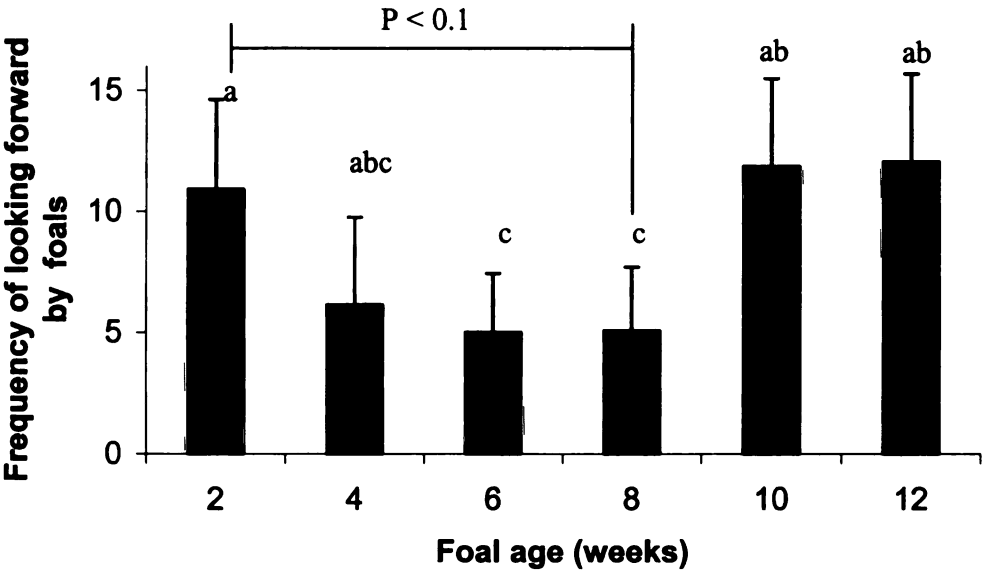

Figure 4. Mean frequency ± SEM of looking forward by foals during 10-min separation for subsequent trials. (N=5, P < 0.05 except where indicated). Differences are indicated by the letters (a,b,c).

Frequency of vocalizations did not prove to be significantly different between subsequent trials (Fig. 5). During the separations, frequency of vocalizations ranged between 18.2 ± 14.2 and 31.00 ± 12.2 vocalizations over 10 min. Table 1 shows the mean frequency ± SEM for eating, urinating, and pawing per trial.

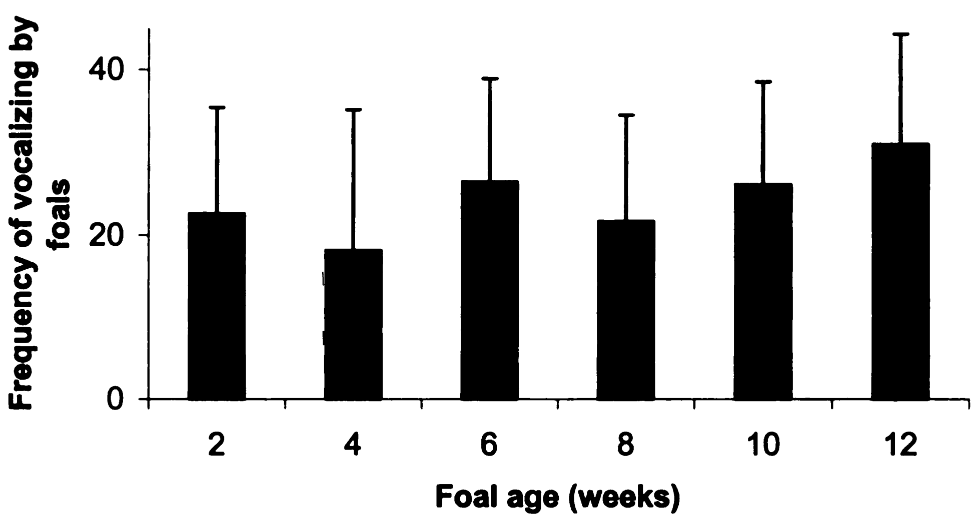

Figure 5. Mean frequency ± SEM of vocalizing by foals during 10-min separation for subsequent trials. (N=5, NS).

Foal gender did not affect the frequencies of "eating" ($F_{1,3}$=0.22, P = 0.67), "urinating" ($F_{1,3}$=0.14, P = 0.73), "vocalizing" ($F_{1,3}$=0.81, P = 0.43), "investigating" ($F_{1,3}$=0.20, P = 0.68), "pawing" ($F_{1,3}$=1.09, P = 0.37), and "looking forward" ($F_{1,3}$=1.23, P = 0.35) during short-term separations.

Table 1. Frequency ± SEM (per 10 min) for foals of "eating", "pawing", and "urinating" per foal age. No significant differences were revealed by the square root transformed analysis.

	Eat (P = 0.83)	**Paw (P = 0.20)**	**Urinate (P = 0.50)**
2 weeks	0.13 ± 0.25	0.13 ± 0.17	0.13 ± 0.16
4 weeks	0.16 ± 0.37	1.20 ± 0.80	0.0044 ± 0.0350
6 weeks	0.092 ± 0.190	0.00035 ± 0.00800	0.035 ± 0.077
8 weeks	0.020 ± 0.098	0.00061 ± 0.01200	0.058 ± 0.110
10 weeks	0.25 ± 0.32	0.15 ± 0.17	0.035 ± 0.077
12 weeks	0.42 ± 0.41	0.22 ± 0.20	0.00018 ± 0.00550

Proximity before and after short-term separations

The statistical analysis revealed that proximity was affected by the main effect of period (period = data collected 30 min before or after separation), i.e., the distance between mare and foal was different during pre- and post-separation observations ($F_{1,37}$=13.08, P = 0.00090). In addition, there was a tendency for an effect of gender ($F_{1,37}$=3.74, P = 0.061). The interaction between these two main effects was not significant (F1,37=1.59, P = 0.22). As indicated previously, proximity scores were analyzed with 3 being the closest proximity and 1 the furthest. The overall proximity score for fillies was 2.70 ± 0.05 and for colts 2.61 ± 0.04, indicating that both sexes stayed fairly close to their dams at all times. Before separation, the overall proximity score was 2.51 ± 0.05 and after separation, the score increased to 2.80 ± 0.06. The effect of the separation on proximity is represented in Fig. 6. After the separation, foals remained closer to their dam than before.

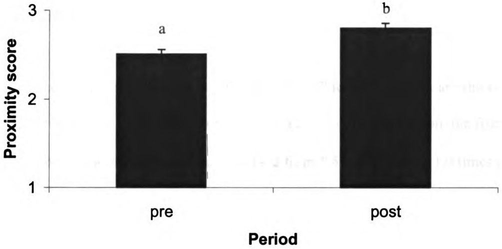

Figure 6. Mean proximity score ± SEM between mares and foals for pre- and post-separation observation periods. (N=5, P < 0.05). Differences are indicated by the letters (a,b).

Interactions displayed by the foal before and after separation

In all cases, the foal initiated nursing immediately following 10 min of separation. Of the four interactions displayed by the foal, "aggression" was the only one that did not differ during pre- and post-separation observation blocks. In fact, aggressions from the foal directed towards the mare were extremely rare; only 6 occurrences were recorded. Prior to the separation, foals aggressed 0.0085 ± 0.0130 times during 30 min whereas after the separation, this was 0.0089 ± 0.0130 times.

Frequencies of "watching mare" and "contact" for both periods are shown in Fig. 7 and 8, respectively. The figure shows that, as a result of the separation, the frequency with which foals watched their dams increased from 1.5 ± 0.7 to 3.9 ± 1.0 times per 30 min ($F_{1,42}$=8.76, P = 0.0050). The same pattern was found for the interaction in which physical contact occurred that was initiated by the foal. The frequency increased from 1.1 ± 0.6 to 4.1 ± 1.0 times per 30 min ($F_{1,42}$=7.19, P = 0.011).

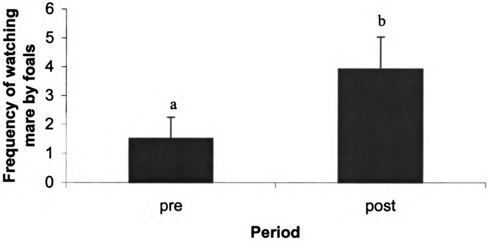

Figure 7. Mean frequency ± SEM of foals watching mare during 30 min for pre- and post-separation observation periods (N=5, $P < 0.05$). Differences are indicated by the letters (a,b).

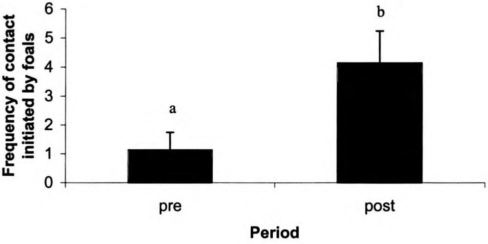

Figure 8. Mean frequency ± SEM of contact initiated by foals during 30 min for pre- and post-separation observation period. (N=5, $P < 0.05$). Differences are indicated by the letters (a,b).

Interactions displayed by the mare before and after separation

Gender of foal did not affect the frequencies of any of the mare-initiated interactions investigated. Aggression was the only behavior that was not affected by the separations ($F_{1,42}$=0.13, P = 0.72). This indicates that the increased contact initiated by the foal did not lead to increased rejection by the mare, but instead was tolerated. In fact, the frequency of contact behavior directed towards the foal increased after the separations ($F_{1,42}$=7.58, P = 0.0087), as shown in Fig. 9. Per 30 min, frequency of contact before the separation was 0.3 ± 0.3 and after separation 1.7 ± 0.7 times.

Following the separations, mares tended to watch their foals more (F1,42=3.56, P = 0.066), which is represented in Fig. 10. Frequency per 30 min increased from 1.5 ± 0.5 times before separation to 3.11 ± 0.72 times after separation.

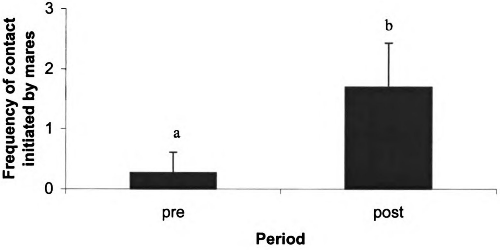

Figure 9. Mean frequency ± SEM of contact behaviors during 30 min displayed by the mare towards the foal during pre- and post-separation observation periods. (N=5, $P < 0.05$). Differences are indicated by the letters (a,b).

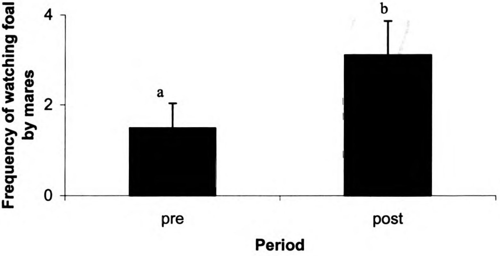

Figure 10. Mean frequency ± SEM of watching foal during 30 min displayed by the mare pre- and post-separations observations. (N=5, $P < 0.05$). Differences are indicated by the letters (a,b).

Proximity during evaluations 4-6 days post-separation

Proximity did not change over subsequent trials ($F_{5,164}$=1.16, P = 0.33). Similarly, there was no effect of separation on the distance between foals and their dam ($F_{1,164}$=1.77, P = 0.19). We did find an effect of gender on proximity, as shown in Fig. 11 ($F_{1,164}$=3.92, P = 0.049). The average proximity score for fillies was 2.63 ± 0.04 whereas for colts this was 2.50 ± 0.05.

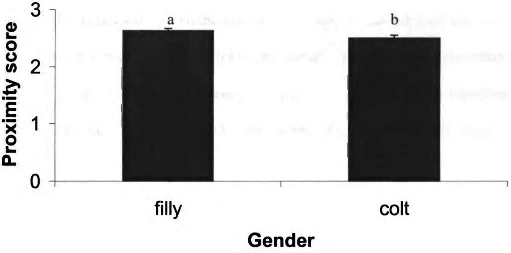

Figure 11. Mean proximity scores ± SEM between mares and foals for both genders during post-separation evaluations. (N=10, P < 0.05). Differences are indicated by the letters (a,b).

Evaluations of interactions initiated by the foal 4-6 days post-separation

Out of all the behaviors we investigated, the occurrence of aggression was too low to reliably detect any differences (7 total occurrences), so we removed this interaction from the analysis. Regarding the frequency of "watching mare", there was a significant day by treatment (treatment = control versus separation) interaction ($F_{5,150}$=2.37, P = 0.042). However, none of the trials showed a significant treatment difference. When comparing trials within treatment we found that, for foals exposed to short-term separations at week 4 of age, they had a higher frequency of "watching mare" than at 2 or 6 weeks. Frequencies of "watching mare" between 2 and 6 weeks of age did not significantly differ.

The frequency with which the foal initiated contact with the mare was not affected by treatment ($F_{1,8}$=0.21, P = 0.66). We did see a trend regarding the effect of observation period. Figure 12 illustrates how frequencies of contact were significantly lower during the last observation block when compared to the first or third block.

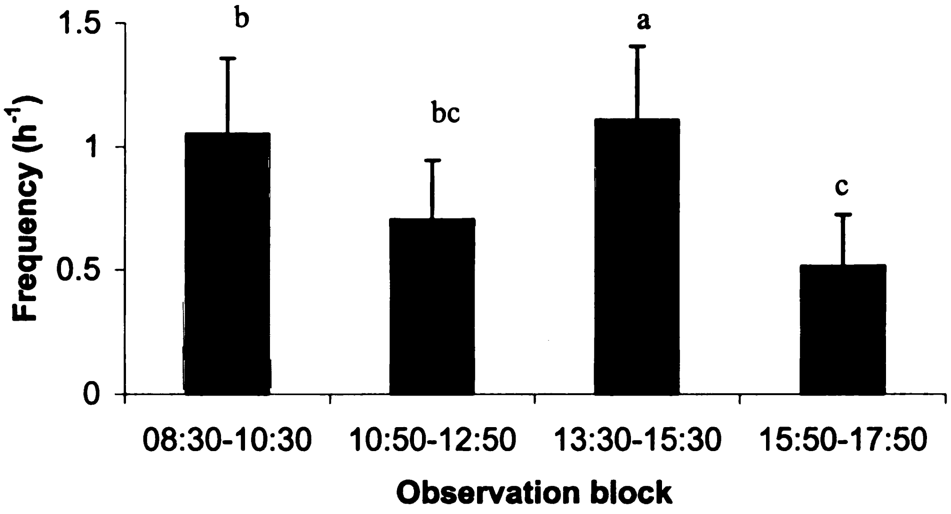

Figure 12. Mean frequency ± SEM of contact initiated by the foal per observation block during post-separation evaluations. (N=10, $P < 0.05$). Differences are indicated by the letters (a,b).

Evaluations of interactions initiated by the mare 4-6 days post-separation

The frequency with which mares watched their foals was not affected by any of the factors in the model. Figure 13 shows the mean frequency per trial.

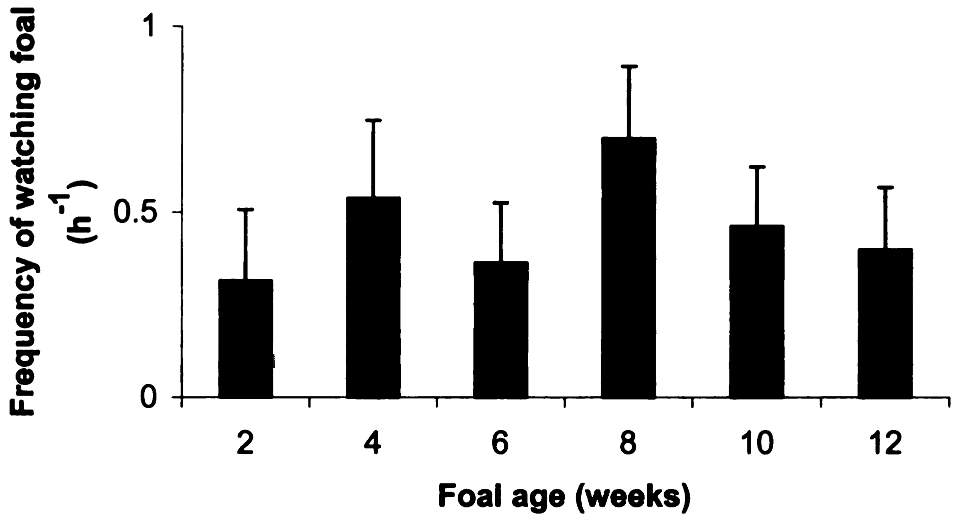

Figure 13. Mean frequency ± SEM of watching foal displayed by mares, per trial, during post-separation evaluation. (N=10, NS).

The frequency with which the mare directed contact behaviors towards the foals depended on the time of observation ($F_{3,135}$=6.88, P = 0.0002). Figure 14 shows the difference in frequency between the different periods. Most of the contact by the mare was initiated during the third observation block, i.e., early afternoon.

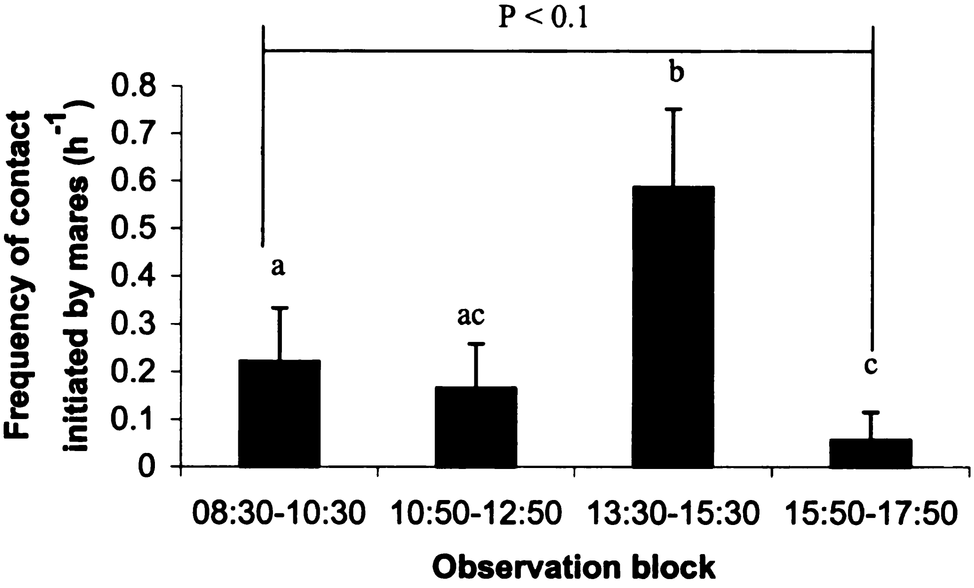

Figure 14. Mean frequency ± SEM of contact directed to the foal by the mare per observation period during post-separation evaluations. (N=10, $P < 0.05$ except where indicated). Differences are indicated by the letters (a,b,c).

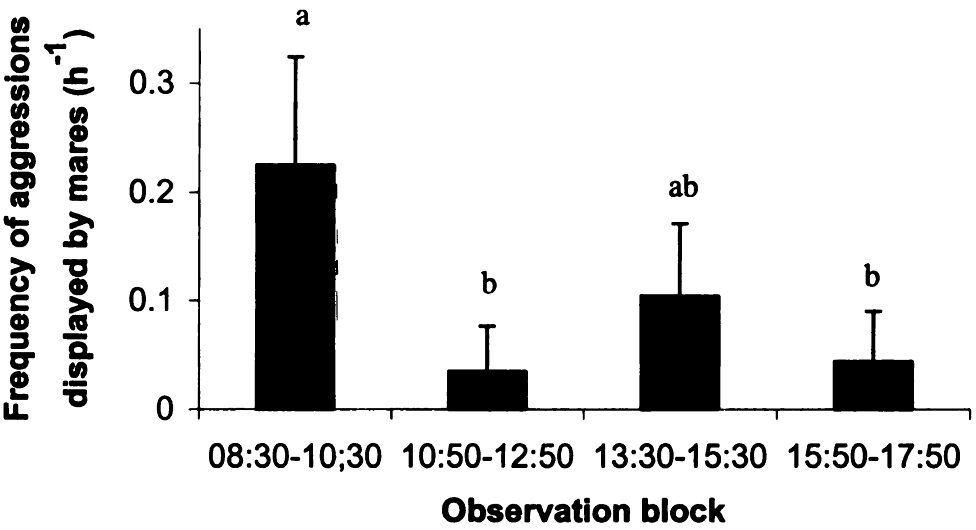

Figure 15. Mean frequency ± SEM of aggressions directed towards the foal by the mare per observation block during post-separation evaluations. (N=10, $P < 0.05$). Differences are indicated by the letters (a,b).

Aggression occurred during specific time blocks throughout the day ($F_{3,135}$=3.36, P =0.021). Figure 15 represents the main effect of observation period on the frequency of aggressions by the mare directed towards the foal. The graph shows that aggression was significantly higher during the first observation block when compared to block 2 and 4. No difference was found between blocks 2, 3, and 4.

There was a significant treatment by trial interaction for the frequency of aggression ($F_{5,135}$=2.41, P = 0.039). The frequency of aggression for the separated group at age 8 weeks was 0.3 ± 0.1 occurrences per 30 min. This was significantly different from the frequency at 6 weeks (0.024 ± 0.042; t_{135}=-2.57, P = 0.011) and from the frequency at week 8 within the control group (0.003 ± 0.015; t_{135}=2.56, P =0.011).

Discussion and conclusion

This study set out to investigate immediate and prolonged effects of 10-min separations on mare-foal interactions. Supporting our selection of the duration for the separation, we mention work by Niezgoda and Tischner (1995). When investigating stressfulness of short-term separations in mares and foals, they showed that a significant increase in catecholamines occurred 5 min into the separation. In addition, their results showed that an increase in cortisol in the foal occurred 10 min into the separation. We predicted that 10 min of separation would be sufficient for the changes in cortisol to be biologically relevant in our study. Barber and Crowell-Davis (1994) reported 9 nursing bouts per hour during the first week after parturition and about 4 bouts per hour at one month of age.

Draft mule foals nursed 14 times per hour during the first week and nursed twice an hour by week 17 according to Smith-Funk and Crowell-Davis (1992). Given the high nursing-bout frequency in foals during the first weeks of life, we predicted that 10-min separations would not cause nutritional deprivation.

Repeated separations caused changes in the foals' response to short-term isolation. The frequency of investigating was low during the first two trials (two and four weeks of age) and then increased during subsequent trials. Houpt (1981) reported that foals were least affected by the separations during the first month of life. This is in agreement with the results we found for the frequency with which foals performed investigative behavior in our study. We speculate that "Investigating" is displayed because the foal attempts to gather olfactory cues from the mare during her absence. Casual observations support this hypothesis in that foals were observed to frequently investigate and paw in areas where the dam urinated or defecated. However, pawing as well as urinating, vocalizing and eating, did not show an effect of repeated exposures.

In contrast, the frequency of looking forward was high at first, then decreased until trial 4 (eight weeks of age) and subsequently showed equally high concentrations during trial 5 (10 weeks of age) and 6 (12 weeks of age). By recording this behavior we assume that the foal establishes a link between the direction the mare disappeared in and the direction it needs to go to find the mare. When comparing the different frequencies per trial, we may be observing a habituation effect. The low frequencies during the second, third, and fourth trial are in part due to the fact that the foal is engaged in other behaviors such as investigating. However, for the fifth and sixth trial, at 10 and 12 weeks of age, the frequencies for both investigating and looking forward increase, contradicting our previous statement regarding a habituation to separations. Because increase in activity is related to an increase in the response to stress, we propose that the last two separations (at 10 and 12 weeks of age) were more stressful for the foals. This result is based on behavioral indicators and supports the finding by Houpt et al. (1981) that at a young age foals are not as affected by the separations as compared to when they are older. Future research may be useful to investigate whether physiological indicators correspond with the behavioral results we presented.

However, studies using physiological markers of stress found that foals show the strongest response to maternal deprivation during early stages in life (Niezgoda & Tischner 1995). Cortisol and catecholamines concentrations for foals showed the biggest increase at 5 days of age and smaller responses were seen on day 20 and 50. More specifically, on day 20, only foals showed a significant increase in cortisol whereas on day 50, a significant increase was observed in mares but not in foals. Based on previous findings by Cleroux et al (1985), Niezgoda and Tischner (1995) also argued that the decreasing ratio of epinephrine over norepinephrine indicated repeated separations were less emotional in nature. However, no behavioral evidence supporting this finding was found in our study. There is a significant difference in protocol between our study and that conducted by Niezgoda and Tischner (1995). The Polish pony mares were separated from their foals on three occasions (day 5, 20, and 50) whereas in our study mares were separated six times, every other week, starting at approximately day 14. Niezgoda and

Tischner saw a decrease in response to maternal separation, but our study did not reveal such a trend. We propose that there is a critical period prior to 14 days of age, and thus earlier than the 30-day period as argued by Houpt et al (1981) in which the foal is most sensitive to separations. It is then during this critical period that the actual modification of the HPA-axis occurs, leading to a decrease in response to stress during subsequent separations. Supporting this prediction, we mention the study by Houpt et al. (1981) where mares and foals were separated for 5 minutes every week between 1 and 12 weeks of age. Though the response of foals to separations did not peak until 3 to 4 weeks of age, results showed a steady decline in vocalizations and overall activity towards 12 weeks of age.

It should be noted that we could not make statements in our study regarding overall activity (duration of stand, walk, trot, and nursing as measured by "muzzle at udder"). As stated above, we could not reliably record these data, and our conclusions therefore are based on behaviors (walking, standing, and trotting excluded) previously shown to increase during a stress response (Houpt et al. 1984; Hoffman et al. 1995).

When mare and foal were reunited, the foal immediately nursed, supporting the theory that nursing serves to comfort as well as nourish the foal (Crowell-Davis 1985). Though we did have information on frequency with which foals performed the "muzzle at udder" behavior, casual observations suggested it is important that the distinction is made between nursing attempts and actual suckling, based on the duration of "muzzle at udder". Relatively speaking, one nursing bout consists of a few short "muzzle at udder" sessions and one or two longer ones. Though milk ingestion was not measured, it is likely that the longer "muzzle at udder" occurrences represent successful nursing. Unfortunately, we could not reliably record the true duration of each "muzzle at udder" occurrence in this study.

When comparing mare-foal interactions immediately before and after separations, we find that the occurrence of aggressions was not influenced by the separations. Moreover, we found an increase in the frequency with which mare and foal watched each other and with which they initiated contact. Thus, mares show greater interaction with their foals following separation than before and this interaction is reciprocal. In addition, proximity scores immediately after separations were significantly higher than before, again indicating that the short-term separations intensified mare-foal interaction. We do acknowledge that the foals were limited in the distance they could move away from the mare due to stall confinement. However, we attribute the increase in mare-foal interactions to the immediate effect of short-term separations. This argument is supported by findings in rodents. After rat pups were handled by humans for a short period of time, dams would show increased concentrations of licking and grooming of pups and arched-back nursing (Liu et al. 1997).

In addition, fillies tended to stay closer to their dam compared to colts during the separation trials as well as during post-separation evaluations. We did not see any effects of gender on other behaviors we investigated. Very little evidence exists that maternal care is directed differently towards colts and fillies (Crowell-Davis 1986). However, this significant difference in proximity, despite the limitation to move away from the mare due to stall walls, suggests that fillies are more closely associated with their dam than are colts. Fillies engage in mutual grooming behavior twice as much as colts do and prefer to groom with other fillies and their dam (Crowell-Davis 1986). Crowell-Davis (1986) also argued that herd dynamics have shown that mares will stay within a herd, regardless of the presence of a stallion. Therefore, the close association between mare and filly as reported in our study and the higher frequency of mutual grooming by fillies potentially serves to reinforce the female-female bond.

Analyzing data from the evaluations 4-6 days post-separation of separation group mare-foal pairs and their age-matched controls, we did not find a clear effect of treatment (short-term separations) on mare-foal interactions. The frequency with which mares watched their foals was not affected by whether or not mares had been separated from their foals previously. Similarly, no effect of treatment was found on the occurrence of contact, but where contact initiated by foals occurred mostly during early morning and afternoon, contact initiated by mares occurred the most during early afternoon. In fact, aggressive behaviors displayed by the mare were noticeably higher during the early morning than compared to observations later in the day, indicating that the interactions from the foal were not welcomed at that time. As early morning observations coincided with pre-feeding and sometimes feeding time, this increased aggression may be associated with the anticipation of feeding. It should be noted that aggressions by the mare were also increased during the early afternoon, but friendly contact initiated by the mare was higher at this time as well. It is possible that the aggression displayed during this time block served to restrict contact-interactions initiated by the foal.

Thus, our data differs from results reported from research on rodents in that we saw an immediate increase in maternal care due to social isolation, but this increase disappeared soon after the separation. We propose that the explanation lies in the evolutionary biology. Rodents are altricial mammals, born naked with no means to sustain essential processes such as thermoregulation and digestion, and developing more slowly than precocial mammals, such as the horse (Kendrick et al. 1997). Evolutionary, horses are a follower species and it is imperative foals get up and move with the herd within a few hours after birth.

In sheep for example, two breeds of the same species can differ in the intensity of maternal care (Dwyer & Lawrence 1999; Dwyer & Lawrence 2000). Since maternal styles can vary between breeds within the same species, we assume that differences exist between species. Compared to horses, a different intensity of maternal care is required for the rearing of young rodents. Rat mothers give constant care through physical contact (licking, grooming, nursing) and only leave their pups sporadically for 15 to 25 minutes (Plotsky & Meaney 1993). In addition, immediate care is given upon return to the nest. In contrast, mares do not often let the foal get out sight and are rarely in physical contact with their foals, showing less nursing and allogrooming bouts (Crowell-Davis 1985). Thus, although maternal care increased immediately after separation similar to findings in rodents when dam and pup were reunited, the increase in maternal care was not sustained.

This could be an expression of the fundamental differences in the regulation of maternal behavior between horses and rodents.

In conclusion, in agreement with our prediction, we found that interactions between mares and foals intensified immediately after separations. Contrary to the second part of our hypothesis, we did not see a prolonged effect on mare-foal interactions based on mare-foal interactions of separated versus control animals 4-6 days post-separation. Based on rodent literature, future research needs to look into the impact of a stressor on both groups of foals at an older age to investigate whether the immediate increase in maternal care following a separation leads to a dampened stress response later on in life. Chapter V describes the study we conducted investigating how the foals from the separation group responded to weaning when compared to the foals from the control group.

CHAPTER IV: EFFECT OF SHORT-TERM SEPARATIONS ON WEANING STRESS IN FOALS

Abstract

Weaning is a stress-causing event that may have prolonged effects on foal welfare. Based on results found in rodents, we anticipated that intensive maternal care during early stages in life aid foals to cope with stressors, such as weaning, later on in life. We previously demonstrated that maternal care is intensified immediately after short-term separations. To verify whether these separations had a long-lasting effect, this study investigated physiological (salivary cortisol and heart rate) and behavioral markers of weaning stress in foals that have undergone six short-term separations from their dam compared to control foals. Measures taken during weaning indicated that separations of mare and foal at an early age did not affect heart rate (P = 0.85), the duration of udder-directed behaviors -"muzzle at udder"- (P = 0.46), standing (P = 0.43), walking (P = 0.22), or the frequency of vocalizing (P = 0.66), urinating (P = 0.27), eating (P = 0.12), drinking (P = 0.87), looking forward (P = 0.96), investigating (P = 0.0.49), and pawing (0.90). However, based on sustained elevation of salivary cortisol during the post-weaning day we concluded that the separation group was impacted longer by the weaning process (separation pre-weaning: 1.82 ± 0.55 nmol/l, post: 5.61 ± 1.13 nmol/l; control pre: 2.75 ± 0.83 nmol/l, post: 3.43 ± 1.37 nmol/l) (P = 0.066). Furthermore, fillies in the test group defecated more than fillies in the control group (0.8 ± 0.5 h^{-1} versus 0.1 ± 0.2 h^{-1}; t_{61}=2.34, P = 0.023) whereas for colts the reverse was observed (0.093 ± 0.15 h^{-1} versus 0.55 ± 0.36 h^{-1}; t_{61}=-2.05, P=0.05). We also saw a main gender difference in the

reaction to weaning in that fillies walked longer (P = 0.039) than colts and laid down less (P = 0.019). Finally, fillies vocalized more on the post-weaning day than colts did (18.6 ± 5.6 and 7.9 ± 4.1 vocalizations h^{-1}, respectively, P = 0.057). Thus, we did not find that increased maternal care through short-term separations facilitated weaning. However, our findings indicate that fillies in general are more affected by the weaning process and that short-term separations may complicate weaning for fillies more so than for colts.

Introduction

Weaning in foals results in maternal deprivation, often social isolation, and a sudden transition in the source of nutrients. The stressfulness of weaning has been demonstrated using behavioral indicators, such as increase in general motor activity and vocalizations (Houpt et al. 1984) (Hoffman et al. 1995). Physiological measurements showed an increase in plasma cortisol concentration and a subsequent decrease in cell-mediated immunity (Malinowski et al. 1990). Although the stress response may facilitate the adaptation of an animal to its environment, chronic stress causes damage to the immune system, as well as a decrease in growth and reproduction (Malinowski et al. 1990; Compton 1987; Welsh et al. 1979).

The impact of weaning can be modulated by affecting timing of weaning and the type of environment in which weaning occurs. When allowed fence line contact during gradual weaning, foals are able to maintain visual contact with the mare for a certain period of time before the complete separation occurs (McCall et al. 1985; McCall et al. 1987). In the abrupt weaning method, foals are immediately and without further contact separated from their dams. McCall et al. (1985) found that foals that were abruptly weaned spent more time walking (118.8 min versus 27.5 min) and less time standing still (162.5 min versus 272.5 min) compared to foals that had time to habituate to the separation from their dam. The suddenly weaned foals also had a higher frequency of vocalizations compared to gradually weaned foals (93.0 versus 16.8 vocalizations in the first hour and 39.8 versus 2.3 vocalizations in the fifth hour post-weaning) and elevated cortisol concentrations (McCall et al. 1987, 153.18 ± 21.53 nmol/l versus 56.58 ± 21.53 nmol/l). Thus, by prolonging the weaning process through fence line contact, the impact of weaning can be reduced.

Research on social enrichment of the weaning environment reports conflicting results. Houpt et al. (1984) concluded that paired foals were less impacted by weaning than individually weaned foals as measured by a decrease in vocalizations (30.9 ± 7.2 vocalizations per 15 min for animals weaned individually versus 14 ± 3.7 vocalizations per 15 min). However, Hoffman et al. (1995) found that foals weaned in pairs displayed more aggression towards each other. In addition, the increase in serum cortisol in response to an adrenocorticotropic hormone (ACTH) challenge was less in foals weaned in pairs (165.6 ± 11.04 nmol/l) than in individually weaned foals (190.44 ± 11.04 nmol/l), indicating depletion of adrenal reserves in foals weaned in pairs. A study by Malinowski et al. (1990) found that foals weaned in pairs showed a decrease in lymphocyte proliferation, representing a decrease in immune response associated with paired weaning. Thus, contrary to what Houpt et al. (1984) concluded, it is beneficial to wean foals without social enrichment when weaning in a stall. A major contributing factor to this result is potentially the confinement, which limits the ability of animals to move away when aggression is displayed against them. In fact, research has shown that weaning groups of animals on pasture, i.e., combining social enrichment and environmental enrichment, is preferred over weaning in an isolated, simple environment such as a stall. Comparing six individually stall-weaned versus six pasture-weaned foals (two groups of three foals), Heleski et al. (in press) found that paddock-weaned, group-housed animals could engage in a broader range of activities, provided by social (e.g., mutual grooming) and environmental enrichment (e.g., grazing), and showed less aberrant behaviours as measured by e.g., sniffing, licking, and kicking the stall/shed walls.

From the aforementioned results, we conclude that the response to weaning can be modified by several factors. The mother-infant relationship is thought to be an important factor in the way the HPA-axis develops (Plotsky 2000). The dam-offspring "security of attachment", introduced in the late 1970's, states that both mare and foal develop the boundaries of their relationship together and they can rely on a certain amount of predictability when interacting with each other (Bowlby 1977). This implies that if the care of the mother towards the infant is inadequate, the development of the offspring is impaired (Liu et al. 1997). This impairment in an animal can lead to a skewed perception and evaluation of the immediate environment, thereby affecting stress coping strategies. As shown in rodents, the amount of maternal care received during early stages in the life has a long-term effect on stress coping strategies of the animals as adults (Liu et al. 1997). It has been shown that rat pups who received intensive maternal care characterized by high licking and grooming, and arched-back nursing responded less intensely and for a shorter period of time to restraint stress as adults when compared to rat pups that received low maternal care characterized by low licking and grooming, and arched-back nursing. We predicted that, by reinforcing the natural mare-foal bond, we could facilitate weaning in foals. Maternal care in horses can potentially be stimulated by a threat to the foal or by a short-term separation (Crowell-Davis & Houpt 1986).

In chapter III, we demonstrated that mare-foal interactions do intensify immediately after a separation of short duration. Contrary to what we stated in our hypothesis, we were unable to show, through post-separation evaluations of test and control group animals, that the increase in maternal care was still apparent a few days after separation. In research performed on rodents, researchers showed that high levels of maternal care had an effect on rats as adults when a stressor was applied. To compliment findings in chapter III, we needed to apply a stressor on the foals of the separation group and of the control group to investigate whether the animals in each treatment respond differently. To measure concentrations of a common marker of stress, i.e., cortisol, blood or saliva are commonly used. When a foal is weaned, taking blood samples becomes more challenging and this implies a greater risk for animal and handler. The use of saliva to measure cortisol in horses has been validated previously (Lebelt et al. 1996; van der Kolk et al. 2001; Warren et al. 2001) and we prefer this less invasive method.

The objective of this study was to investigate behavioral and physiological markers of weaning stress for foals that had undergone short-term separations versus control foals that had never been separated from their mare prior to weaning. We hypothesized that foals exposed to short-term separations early in life would regain homeostasis faster after weaning when compared to foals that were never separated from their mother.

Materials and methods

Animals and treatment prior to weaning

This research was conducted at MSU Merillat Equine Center from January through October 2000. A total of 10 mare-foal pairs were studied (Appendix C). The animals were divided into two groups blocked by gender. Each consisted of three fillies and two colts. Each group represented a treatment. In the control group, mare and foal were never separated prior to weaning. In the separation group, every two weeks, starting when the foals were 2 weeks old until 3 months of age, the mares in the were separated from their foals for 10 min. Each mare-foal pair in the test group was separated six times (Chapter III). The MSU All University Committee on Animal Use and Care reviewed and approved the experimental protocols described in this chapter.

Housing and feeding

From January through April 2000, mares were individually housed in Barn A in 13.38 m^2 box stalls. The stalls were bedded with wood shavings and had a combination feeder in one corner. The mares had ad libitum access to water via an automated drinker. By May 2000, all mares had foaled and the animals were moved to the climate controlled Barn B. The climate-controlled environment was ensured by the availability of fans in the barn. In addition, during the day, mares and foals remained together on pasture from the time foals were approximately one month old. All mares were fed twice daily. The morning feeding occurred between 08:00 and 10:00 and the afternoon feeding between 15:00 and 17:00. Horses were fed according to NRC recommendations (Council 1989).

Weaning protocol

Weaning in test and control groups occurred when the foals were 6 ± 0.5 months old. During the weaning trials we evaluated mare-foal interactions and observed the physiological and behavioral response of foals to weaning.

One weaning trial consisted of three days: pre-weaning, weaning, and post-weaning data collection day. The experimental timeline is described in Appendix G. Per weaning trial we weaned a foal from the control group with a foal of similar age from the test group. Foals were weaned in individual box stalls measuring 20.90 m^2 and were deprived of visual contact with one another.

The choice of weaning stalls was based on their location relative to the breeding room. To place and check heart rate monitors or to take saliva samples from the foal for cortisol measurements, the mare was led out (and the foal would follow) to the breeding room where mare and foal were placed in a metal chute with which the animals were familiar. After weaning, when the mare was no longer present, the stall door was opened and two to three people directed the foal towards the chute. Some foals were extremely difficult to handle after weaning and required a lead rope across the withers to prevent the animal from jumping out of the chute.

During each weaning trial, we collected saliva samples for cortisol analysis, measured heart rate and collected behavioral data as described below.

Salivary cortisol

Sampling

For saliva sampling, an in-house constructed saliva collection device (SCD) (figure 16) was used. On average 17.5 cm^2 of Tygon® tubing (R-3603, Norton Performance Plastics Corporation, Akron, OH 44309) was used and 8 to 13 holes were cut in the tube. Gauze was cut into separate pieces, rolled and, by using a metal wire, inserted into the tubes. At both ends of the tube, a suspender clip was attached which in turn was connected to a swivel-head clip using elastic ribbon that was adapted in length to comfortably fit the foal's head (figure 17). The swivel-head clips were attached to the halter and the tube was placed inside the foal's mouth. The animal immediately began to chew, thereby stimulating saliva production. After collection, the gauze was removed from the tube, folded in half and placed in 15 ml polypropylene tubes. The cap was placed on the tubes to keep the gauze in place and the samples were stored on ice.

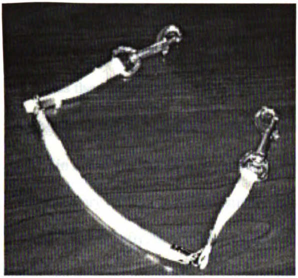

Figure 16: Saliva collection device to collect saliva from foals. Gauze is placed in the tubing with holes to provide the foal with a substrate to chew and to collect produced saliva. The elastic band allows for adaptation of the device to different foal head sizes.

Figure 17: Saliva collection device in place in a foal's mouth. The position of the device depended on the foal's ability to manipulate the tube to parts of the mouth where it might chew through the tubing.

Analysis

The tubes holding the saliva-soaked gauze were centrifuged in a clinical centrifuge model CL (International Equipment Company, Needham, Massachusetts) at 4 104 x g. The saliva was then transferred to cryovials and placed in liquid nitrogen at -195 °C as this was logistically more feasible than finding storage at –30 °C. To quantify the amount of cortisol in the saliva, we used an Active Cortisol EIA (for saliva) kit prepared to analyze human salivary cortisol by Diagnostic Systems Laboratory (DSL-10-67100, Webster, Texas 77598). Briefly, in a 96-well microtiter plate coated with goat anti-rabbit globulin serum, we pipetted in duplicate 50 µl of sample, 100 µl of enzyme conjugate solution and 100 µl rabbit anti-cortisol serum. After incubation for 45 min on a plate shaker, we washed the wells five times with buffered saline wash solution and added 100 µl tetramethylbenzidine chromogen solution. After another 15 to 20 min incubation time, we pipetted 100 µl 0.2 M sulfuric acid into the wells to stop the color reaction. The absorbance of the wells was read at 450 nm. From previous research we found that foal saliva has a much lower cortisol concentration than human saliva; we modified the standards so they ranged from 0 to 4 µg/dl or 0 to 110.4 nmol/l. We plated the kit's quality controls (QC 1 = 0.2 µg/dl and QC 2 = 2.0 µg/dl or 5.52 nmol/l and 55.2 nmol/l) in the wells following the standards and again in the last 4 wells of the plate. The samples were plated after the first set of quality controls. We then included eight wells of pooled foal saliva collected from different foals at the MSU Horse Research and Teaching Center. Four wells contained saliva collected during the morning (QC 3 and QC 4) and another four wells contained saliva collected during the afternoon (QC 5 and QC 6). We assayed one plate per day for 4 days and used the quality controls to determine inter-assay variability.

After log-transformation of the data, the factors of day (pre-weaning, weaning, and post-weaning data collection day) and time (morning: 07:00-09:00, noon: 11:00-13:00, afternoon: 15:00-17:00) were used as repeated measures within mixed model procedures of SAS with foal nested within treatment in the subject statement. The fixed effects in the model consisted of gender, treatment, day, and time whereas the random effect was trial (n=5). Significant results were further investigated using a multiple comparisons t-test. When no difference was found between separation and control group, data were combined for further analysis. The results are represented as lsmeans ± SEM, back transformed to the observed scale.

Heart rate

Techniques

To measure heart rate, we used a Polar Vantage XL telemetric system (Polar Electro Inc., Port Washington, NY 11050, USA). A transmitter was connected to a custom made elastic belt with a plastic clip. We wet the foal's coat where the transmitter would be on the heart girth, and applied Meditrace conductivity gel (Graphic Controls, Buffalo, NY 14240). A receiver watch was secured to the elastic belt and programmed to record the beats per minute every 15 sec. Up to 8 h of data could be stored on one receiver and the receiver was replaced either after 4 or 8 h of data recording, or after the receiver had accidentally stopped recording. Heart rate was recorded from 06:00 until 18:00 during three consecutive weaning trial days.

Analysis

Per two hours of heart rate monitoring, a maximum of 480 measurements were recorded. Because the heart rate monitors did not always stay attached to the foal or because the transmission was hindered due to a loose belt, some measurements were lost or invalid. We averaged the data available per two hours of observation and in the analysis we weighted the number of values used to obtain each average. We opted to use the mean instead of the median because we predicted that the few peaks that occurred would not significantly influence the average of all the observations and the mean gives us easily interpretable results.

After log-transformation of the data, the factors of day (pre-weaning, weaning, and post-weaning data collection day) and time (morning: 07:00-09:00, noon: 11:00-13:00, afternoon: 15:00-17:00) were used as repeated measures within mixed model procedures of SAS with foal nested within treatment in the subject statement. The fixed effects in the model consisted of gender, treatment, day, and time whereas the random effect was trial (n=5). Significant results were further investigated using a multiple comparisons t-test. When no difference was found between separation and control group, data were combined for further analysis. The results are represented as lsmeans ± SEM, back transformed to the observed scale.

Behavior

Techniques

Per stall, a Panasonic CCTV WV-BP334 video camera was installed out of the animals' reach. Both cameras were connected to a Panasonic WV-CM146 field switcher monitor. In addition to the live observations, we recorded the animals on VHS tape using a Panasonic AG-6740 VCR. In addition, an Optimus Unidirectional Condenser microphone was placed in each weaning stall and the audio data were also recorded using the VCR.

Each day of the weaning trial, the foals were observed during three two-hour observation blocks (07:00 to 09:00; 11:00 to 13:00; 15:00 to 17:00). On the weaning day, the foals were weaned at approximately 07:00. The weaning procedure was identical to the short-term separation protocol as described in chapter IV, except that the mare was not brought back to the stall after 10 min. Instead, the handler took her to a pasture approximately 400 m away from the breeding barn. The 'Dutch doors' were shut prior to weaning. The ethogram with the behaviors observed during the weaning trial is described in Appendix F. The behaviors retained for analysis included: duration of "muzzle at udder" (on pre-weaning day), stand, walk, and lie down, as well as the frequency of vocalize, defecate, urinate, eat, drink, look forward, investigate, and paw. It should be noted that nursing behavior was included in "muzzle at udder". We assumed that mare-foal interactions are reflected in whether or not a mare allows her foal to nuzzle the udder, with or without actual ingestion of milk. The location of our observation station made it impossible to reliably detect sucking, and thus our definition of a nursing bout is closest to the definition by Crowell-Davis based on observations in 1979 (see Appendix

A). For the same reason, no distinction will be made between successful and unsuccessful nursing bouts. Smith-Funk (1992) described successful nursing bouts as including pre-nurse nuzzling, sucking, intra-bout pauses and intra-bout nuzzling. Unsuccessful nursing bouts, according to the same author, did not include sucking, which again would have been impossible to reliably detect in our experimental setup.

Analysis

Day (pre-weaning, weaning, and post-weaning data collection day) and time (morning: 07:00-09:00, noon: 11:00-13:00, afternoon: 15:00-17:00) were used as repeated measures within mixed model procedures of SAS with foal nested within treatment in the subject statement. The fixed effects in the model consisted of gender, treatment, day, and time whereas the random effect was trial (n=5). Significant results were further investigated using multiple comparisons t-test. Frequencies obtained were regarded as count data and square root transformations were performed prior to analysis. When no difference was found between separation and control group, data were combined for further analysis. The results are represented as lsmeans ± SEM, back transformed to the observed scale.

Results

Salivary cortisol

A total of four plates were assayed with respective within-assay coefficients of variation of 9.2 %, 3.7 %, 3.2 %, and 3.7 %. Between-assay variation coefficient of variation was 9.8 %. Salivary cortisol concentrations ranged from 0.17 to 101.02 nmol/l. However, after testing for outliers, the range of salivary cortisol concentration varied between 0.17

and 45.13 nmol/l. The results did not reveal a main effect of treatment (ANOVA, $F_{1,66}$=0.04, P = 0.89). There was a significant day by time interaction (ANOVA, $F_{6,66}$=7.67, P < 0.0001), as shown in Fig. 18.

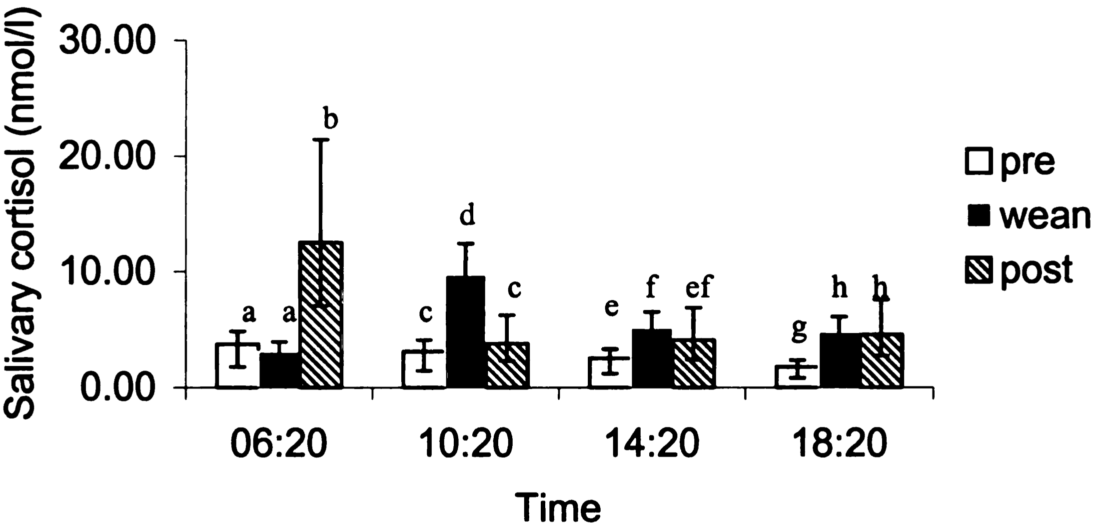

Figure 18. Mean salivary cortisol concentration ± SEM per sampling time for each weaning trial day (N=10, P < 0.05). Differences as indicated by the letters (a,b; c,d; e,f; g,h) are reported within time blocks.

The cortisol concentration at 10:20 was higher on the weaning day than when compared to pre- (t_{66}=-5.75, $P < 0.0001$) or post-weaning day (t_{66}=3.04, $P = 0.0034$). Similarly, for 14:20 and 18:20, pre-weaning cortisol concentrations were significantly lower when compared to weaning day concentrations (14:20: t_{66}=-3.36, $P = 0.0013$; 18:20: t_{66}=-4.71, $P < 0.0001$). Pre-weaning and post-weaning concentrations were significantly different for the 18:20 sample (t_{66}=-2.65, $P = 0.010$), but not for the 14:20 measurement (t_{66}=-1.45, $P = 0.15$).

In addition, we investigated the presence of diurnal variation during the pre-weaning day as represented in the day by time interaction. Only one significant difference was found in the cortisol concentrations for sampling at different time points. The 6:20 sample showed a significantly higher concentration than the 18:20 sample (3.71 ± 1.54 nmol/l versus 1.77 ± 0.77 nmol/l; t_{66}=2.21, $P = 0.030$). Another trend was found when comparing the cortisol concentrations from the 10:20 (3.10 ± 1.33 nmol/l) and 18:20 sample (t_{66}=1.81, $P = 0.075$). The cortisol concentrations at 06:20 for pre-weaning and weaning day are not significantly different (t_{66}=0.71, $P = 0.48$). This is to be expected, as weaning on weaning day did not occur until 07:00. All samples taken after weaning show higher cortisol concentrations when compared to the pre-weaning sample on the weaning day, though for the 18:20 sample, this approaches significance (10:20 t_{66}=-5.00, $P < 0.0001$; 14:20 t_{66}=-2.08, $P = 0.041$; 18:20 t_{66}=-1.91, 0.061). The cortisol concentration at 10:20 is higher than those at 14:20 (t_{66}=2.82, $P = 0.0064$) and 18:20

(t_{66}=3.00, P = 0.0039). Finally, concentrations of cortisol did not differ between 14:20 and 18:20 (t_{66}=0.17, P = 0.87). On the day post-weaning, we found a high cortisol concentration at 06:20 (12.51 ± 7.21 nmol/l), which was significantly higher than the concentration at 10:20 (t_{66}=2.32, P = 0.024), 14:20 (t_{66}=2.03, P = 0.046), and tended to be higher than 18:20 (t_{66}=1.87, P = 0.066). No differences were found between other time blocks during the post-weaning day.

Heart Rate

Heart rate during the pre-weaning day averaged 59.60 ± 3.40 BPM. This average increased on the weaning day to 78.00 ± 4.31 BPM and decreased to 71.37 ± 4.51 during the post-weaning data collection day. The main effect of treatment was not significant (ANOVA, $F_{1,56}$ = 0.03, P = 0.85) and no other interactions involving treatment were found to be significant. Similar to the salivary cortisol results, there was a significant day by time interaction (ANOVA, $F_{4,56}$ = 6.79, P = 0.0002), as shown in Fig. 19.

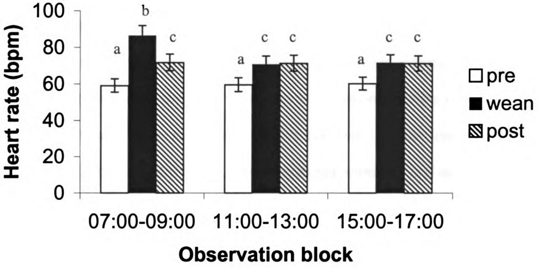

Figure 19. Mean heart rate ± SEM per observation block for each weaning trial day. (N=10, P < 0.05). Differences are indicated by the letters (a,b,c).

The mean heart rate did not differ between weaning and post-weaning days except for the first time block (t_{56}=3.86, P = 0.0003). Weaning caused a significant increase in mean heart rate for each time block when comparing pre-weaning to weaning heart rate (07:00-09:00 t_{56}=-8.12, P < 0.0001; 11:00-13:00 t_{56}=-3.51, P = 0.0009; 15:00-17:00 t_{56}=-4.36, P < 0.0001). Similarly, pre-weaning and post-weaning heart rate concentrations were different for all time blocks rate (07:00-09:00 t_{56}=-3.89, P = 0.0003; 11:00-13:00 t_{56}=-3.75, P = 0.0004; 15:00-17:00 t_{56}=-4.15, P = 0.0001).

Behavioral results

Muzzle at udder

Because we couldn't report on nursing in chapter IV, duration of nursing (muzzle at udder) was analyzed during the pre-weaning day to test for pre-weaning treatment differences. At time 07:00 on the weaning day the mare was removed from the stall. The separations did not cause a difference in the time during which the foal touched the mare's udder (test: 1.76 ± 0.82 min, control: 2.59 ± 0.82 min, $F_{1,16}$=0.58, P = 0.46). The other main effects and two-way interactions investigated were not found to be significant.

Stand

The duration of standing over the course of an hour on the pre-weaning day ranged from 2.85 to 60 min (Mean 47.49 ± 3.08 min) whereas during the weaning and post-weaning day, the values ranged from 22.19 to 60 min (Mean weaning: 46.04 ± 4.00 min, Mean post-weaning: 41.27 ± 1.84 min). No effect of treatment was found on the time during which foals were standing (test: 43.50 ±2.76 min; control: 46.62 ± 2.86, $F_{1,66}$=0.62, P = 0.43). There was a trend for a gender by day interaction ($F_{2,66}$=2.77, P = 0.070): fillies stood longer on day 1 when compared to day 2 (t_{66}=1.98, P = 0.0515) and 3 (t_{66}=3.14, P = 0.0025).

Walk

The duration of walking over the course of an hour on the pre-weaning day ranged from 0.61 to 37.10 min (Mean: 3.00 ± 1.11 min). After weaning, the duration of walking ranged from 0.42 to 58.60 min (Mean weaning day: 9.01 ± 1.91 min, Mean post-weaning day: 14.73 ± 1.62 min). No main effect of treatment was found for the duration of walking ($F_{1,66}$=1.49, P = 0.23). However, a main effect of day was present, indicating the effect of weaning ($F_{2,66}$=16.03, $P < 0.0001$). Figure 20 shows the main effect of day. All days proved to be significantly different from one another, with increasing duration between consecutive days.

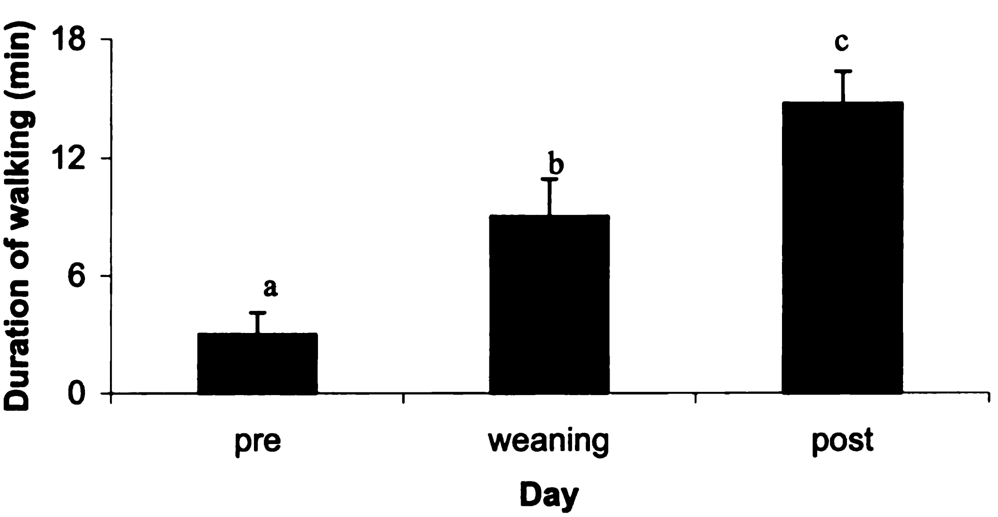

Figure 20. Mean duration ± SEM of walking per weaning trial day. (N=10, $P < 0.05$). Differences are indicated by the letters (a,b,c).

The interaction between gender and time also showed to be significant (F2,66=3.42, P = 0.039) and is represented in Fig. 21.

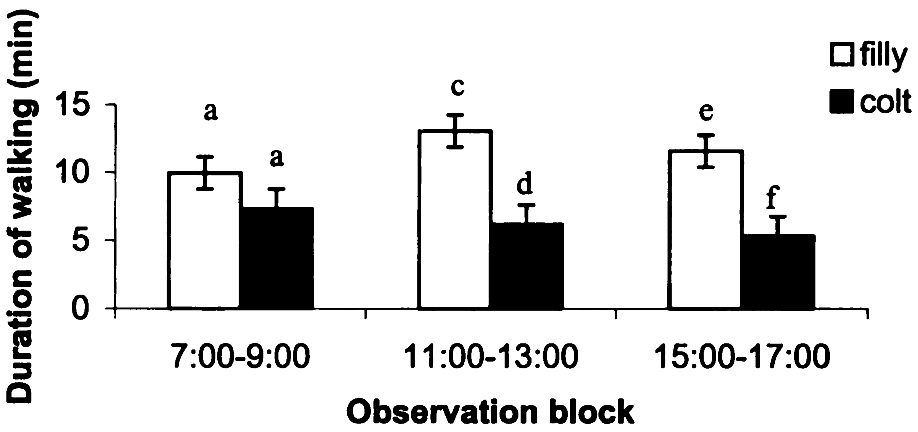

Figure 21. Mean duration ± SEM of walking per observation block for each gender. (N=10, P < 0.05). Differences as indicated by the letters (a,b; c,d; e,f) are reported within observation block.

There is no difference for the first time block between genders (fillies: 9.96 ± 1.19 min, colts: 7.33 ± 1.44 min, P = 0.15), but for both subsequent time blocks, fillies walk more than colts do (fillies: 13.07 ± 1.19 min, colts: 6.18 ± 1.44 min, P = 0.0003 and fillies: 11.59 ± 1.19 min, colts: 5.33 ± 1.44 min, P = 0.0010, respectively).

Lying down

During the day before weaning, foals laid down between 0 and 20.33 min (Mean: 8.07 ± 2.05 min). During the day of weaning, the foals laid down between 0 and 42.75 min per hour (Mean: 0.31 ± 0.67 min). The range on the post-weaning day was 0 to 32.35 min per hour (Mean: 2.30 ± 1.29 min). The short-term separations showed a tendency to increase the time during which foals laid down ($F_{1,66}$=3.44, P = 0.068). However, there was a dominating day by treatment interaction ($F_{2,66}$=8.24, P = 0.0006), which showed that only during the pre-weaning day there was a significant higher duration for the test group when compared to the control animals (t_{66}=3.47, P = 0.0009). This interaction is represented in Fig. 22.

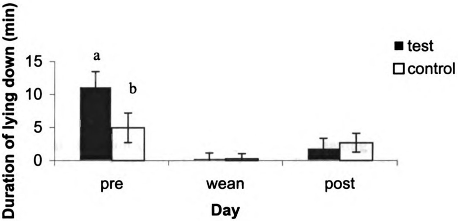

Figure 22. Mean duration ± SEM of lying down per weaning trial day for each treatment group. (N=10, P < 0.05). Differences, as indicated by the letters (a,b) are reported within day.

In addition, there was a difference between genders when looking at the duration of lying down per day ($F_{2,66}=4.22$, $P = 0.019$), Fig. 23.

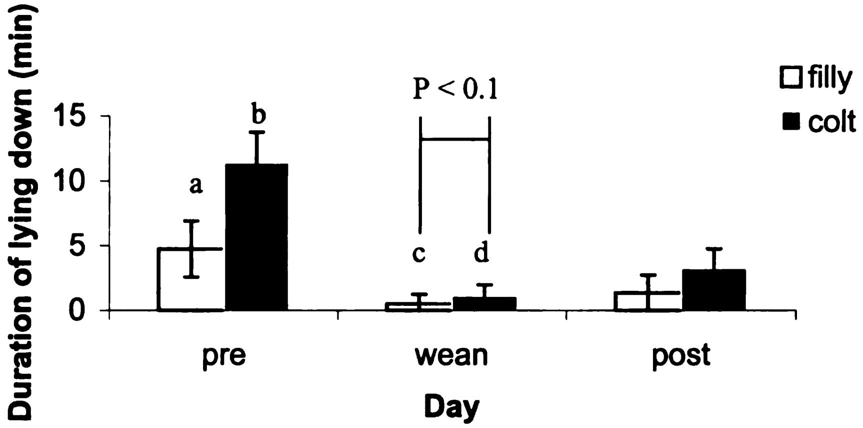

Figure 23. Mean duration ± SEM of lying down per weaning trial day for each gender. (N=10, $P < 0.05$, except where indicated). Differences, as indicated by the letters (a,b and c,d) are reported within day.

As the graph shows, fillies lie down less during the pre-weaning day and tend to lie down less on the weaning day as well. No difference between genders was found on the post-weaning day.

Vocalizing

The range of vocalizations on pre-weaning day was 0 to 7 vocalizations per hour (Mean: 0.040 ± 0.11 h^{-1}). On the weaning day the number of vocalizations per hour ranged from 2 to 181 (Mean: 36.15 ± 7.99 h^{-1}) and during the post-weaning day the number ranged from 0 to 63 vocalizations per hour (Mean: 12.68 ± 4.08 h^{-1}). The main effect of treatment did not affect the frequency of vocalizations ($F_{1,61}$=0.19, P = 0.66). We did find a trend in the gender by treatment interaction ($F_{1,61}$=2.89, P = 0.094) and after investigation we found that within the control group, fillies vocalized more than colts (t_{61}=2.58, P = 0.012). There was also a gender by day interaction (F2,61=4.08, P = 0.022) indicating that fillies tended to vocalize more than colts in particular on the post-weaning day (t_{61}=1.94, P = 0.057). Finally, there was a significant day by time interaction ($F_{4,61}$=4.50, P = 0.0030), which is represented in Fig. 24.

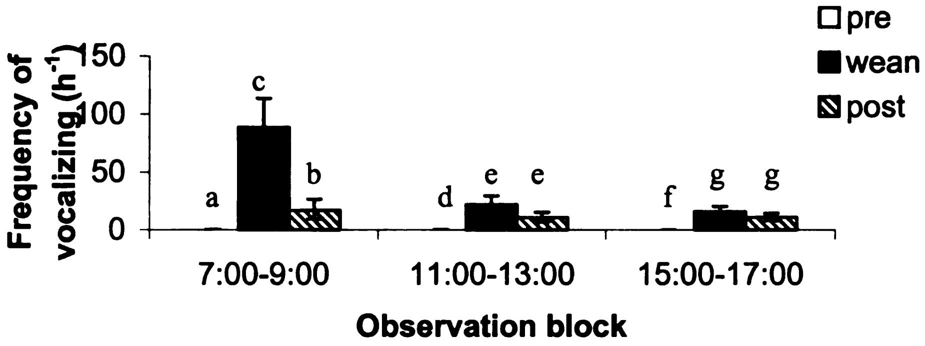

Figure 24. Mean frequency ± SEM of vocalizations per time block for each weaning trial day. (N=10, P < 0.05). Differences, as indicated by the letters (a,b,c; d,e; f,g) are reported within observation block.

There were significantly more vocalizations on the weaning day than when compared to pre- and post-weaning days for all time blocks. Results from the first observation block (07:00-09:00) showed the only weaning versus post-weaning difference of all the time blocks.

Defecating

The frequency of defecating ranged from 0 to 1 per hour on the pre-weaning day (Mean: 0.09 ± 0.13 h^{-1}). For the weaning day, defecating frequency ranged from 0 to 6 (Mean: 0.56 ± 0.33 h^{-1}). For the post-weaning day, defecating frequency ranged from 0 to 3 defecations per hour (Mean: 0.46 ± 0.32 h^{-1}). There was a significant gender by treatment interaction ($F_{1,61}$=7.87, P = 0.0067): For fillies, the test group animals defecated more often than the control animals (t_{61}=2.34, P = 0.023), whereas for colts, the reverse was true (t_{61}=-2.05, P = 0.0045). In addition, within the test group, fillies defecated more than colts (t_{61} = 2.33, P = 0.023), but in the control group fillies defecated less than colts (t_{61} = -2.13, P = 0.037). The results also showed a significant day by time interaction, as represented in Fig. 25.

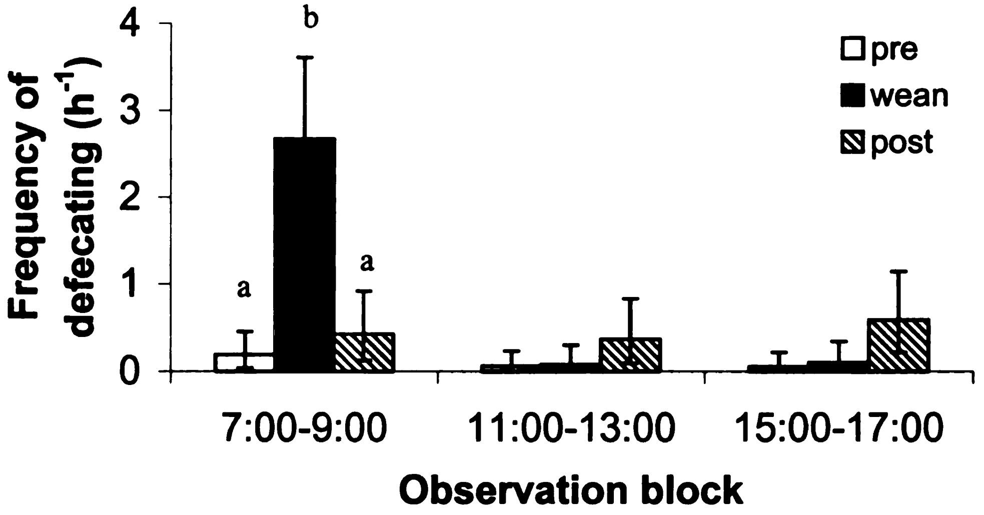

Figure 25. Mean frequency ± SEM of defecation per observation block for each weaning trial day. (N=10, P < 0.05). Differences, as indicated by the letters (a,b) are reported within observation blocks.

Urinating

The range of urinating frequency on pre-weaning day is 0 to 5 per hour (Mean: 0.18 ± 0.12 h^{-1}). During the weaning day, the frequency ranged between 0 and 4 (Mean: 0.65 ± 0.30 h^{-1}) and for the post-weaning day, the frequency ranged from 0 to 3 per hour (Mean: 0.43 ± 0.19). There was no significant main effect of treatment ($F_{1,66}$=1.26, P = 0.27). No other interactions involving treatment were significant. The main effect of time did show significance ($F_{2,66}$=4.25, P = 0.018). The highest frequency of urinating occurred during 15:00-17:00 and this number was significantly higher than when compared to 07:00-09:00 (t_{66}=2.75, P = 0.0077) and to 11:00-13:00 (t_{66}=2.21, P = 0.030).

Eating and Drinking

We did not collect data on the duration of time spent eating and drinking. Hence, the frequencies of eating and drinking, as indicated by the definitions in Appendix F, represent the number of visits to the feeder or drinker. Data on nursing, as recorded by "muzzle at udder" on the pre-weaning day, was collected separately from eating and drinking data. Frequency of eating on the day prior to weaning ranged from 0 to 26 per hour (Mean: 3.36 ± 1.81 h^{-1}). The frequency on the weaning day ranged between 0 and 69 (Mean: 10.15 ± 3.19 h^{-1}) whereas for the post-weaning observations, the count ranged between 1 and 57 (Mean: 18.95 ± 3.59 h^{-1}). For drinking, the ranges for the frequency were 0 to 10 for pre-weaning (Mean: 0.48 ± 0.27 h^{-1}), 0 to 7 for weaning (Mean: 0.39 ± 0.25 h^{-1}), and between 1 and 8 for post-weaning observations (Mean: 2.95 ± 0.54 h^{-1}). Both behaviors showed a main effect of day, but no effect of treatment (Eat: $F_{1,66}$=2.52, P = 0.12; Drink: $F_{1,66}$=0.03, P = 0.87). The main effect of day is represented in Fig. 26. For both behaviors, a significant main effect of time was also present for both behaviors and this is represented in Fig. 27.

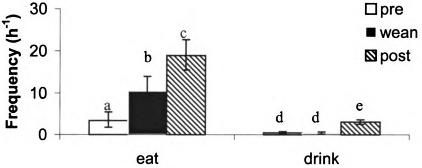

Figure 26. Mean frequency ± SEM of eating and drinking for each weaning trial day. (N=10, P < 0.05). Differences, as indicated by the letters (a,b,c and d,e) are reported within each behavior.

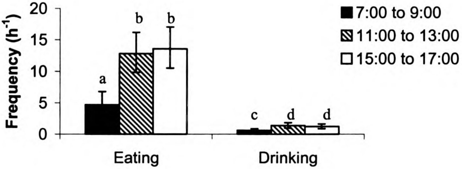

Figure 27. Mean frequency ± SEM of eating and drinking per observation block. (N=10, P < 0.05). Differences, as indicated by the letters (a,b and c,d), are reported within each behavior.

Finally, for drinking there was a significant main effect of gender ($F_{1,66}$=8.94, P = 0.0039), which is represented in Fig. 28. Overall, fillies drink more often than colts do (t_{66}=2.99, P = 0.0039).

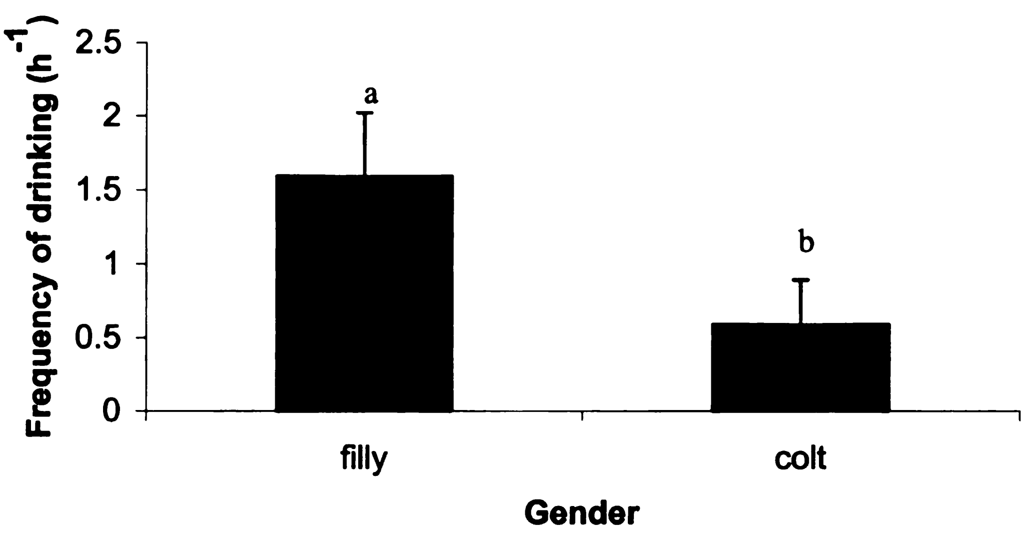

Figure 28. Mean frequency ± SEM of drinking for each gender. (N=10, P < 0.05). Differences are indicated by the letters (a,b).

Looking forward

The frequency for looking forward ranged from 0 to 26 per hour on the day before weaning (Mean: 5.91 ±1.76 h^{-1}). During weaning day, this range was 0 to 118 (Mean: 29.81 ± 6.25 h^{-1}) and during post-weaning observations, the frequencies ranged between 7 and 81 per hour (Mean: 32.31 ± 4.96 h^{-1}). Overall, separations did not affect the frequency of looking forward ($F_{1,61}=0.00$, $P = 0.96$), but there was an interaction between gender and treatment ($F_{2,61}=17.93$, $P < 0.0001$) as represented in Fig. 29.

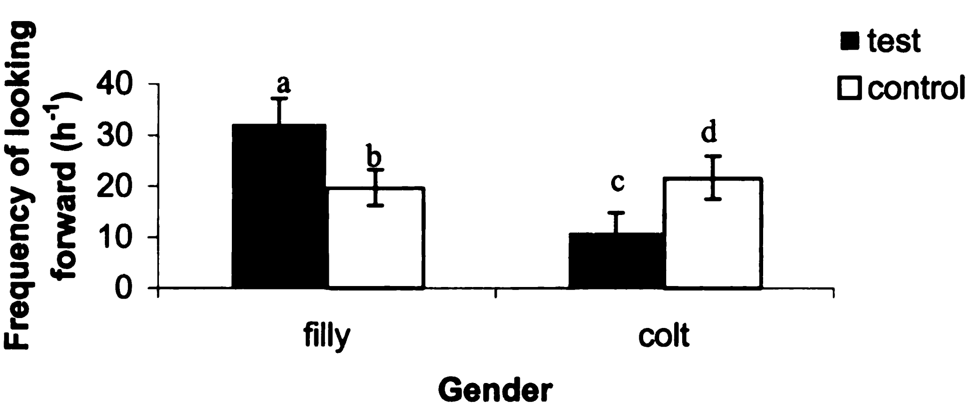

Figure 29. Mean frequency ± SEM of looking forward per gender for each treatment group. (N=10, P < 0.05). Differences, as indicated by the letters (a,b and c,d), are reported within gender.

Test group fillies look forward more frequently than the control animals, but colts that have been separated look forward less than the animals in the control group. In addition to the significant gender by treatment interaction, we found a significant day by time interaction ($F_{4,61}=3.77$, $P = 0.0083$). This interaction is represented in Fig. 30.

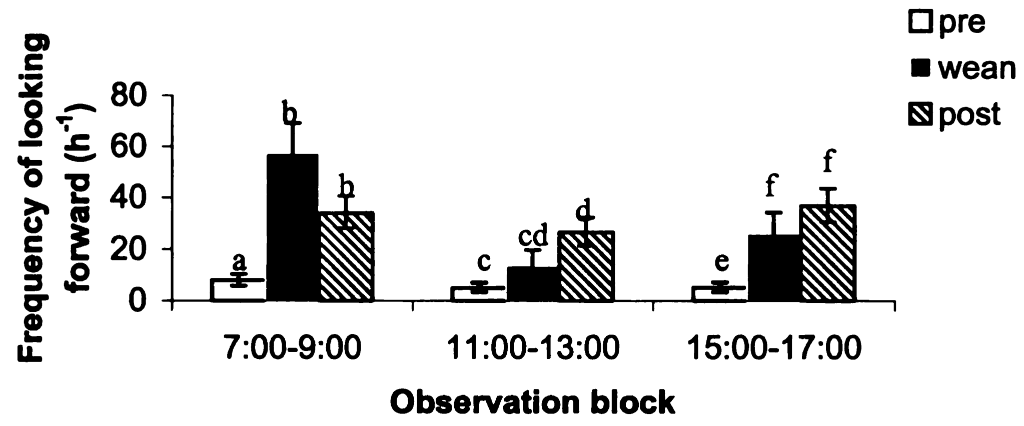

Figure 30. Mean frequency ± SEM of looking forward per observation block for each weaning trial day. (N=10, $P < 0.05$). Differences, as indicated by the letters (a,b; c,d; e,f), are reported within observation blocks.

Weaning caused a significant increase in the frequency of looking forward for the first and third time block between pre-weaning and weaning day. Weaning and post-weaning days did not show significant differences for all three time blocks.

Investigating

The frequency-range for investigating on the pre-weaning day was 0 to 35 (Mean: 11.03 ± 2.32 h^{-1}). During the weaning day, the frequencies ranged between 1 and 78 per hour (Mean: 23.33 ± 3.98 h^{-1}), whereas for the post-weaning day, values ranged from 2 to 49 per hour (Mean: 18.95 ± 2.79 h^{-1}). No significant effect of treatment was found for the frequency of investigating ($F_{1,61}=0.56$, $P = 0.46$). As for other events, a significant day by time interaction was present ($F_{4,61}=10.02$, $P < 0.0001$), see Fig. 31.

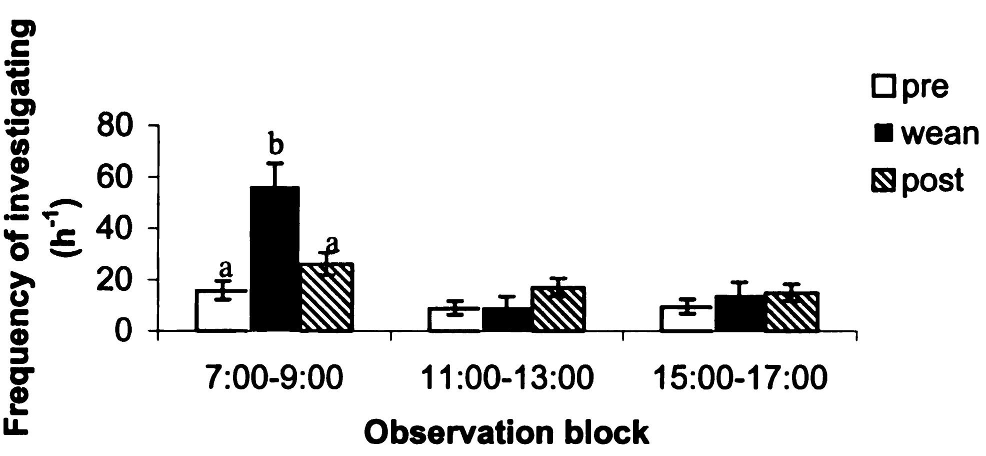

Figure 31. Mean frequency ± SEM of investigating per observation block for each weaning trial day. (N=10, P < 0.05). Differences, as indicated by the letters (a,b), are reported within each observation block.

Weaning caused a significant increase in the frequency of investigating for the first time block compared to pre- and post-weaning values. Pre- and post-weaning concentrations were not significantly different.

Pawing

Pawing frequencies ranged between 0 and 40 occurrences per hour for the pre-weaning observations (Mean:1.41 ± 1.15 h^{-1}), whereas for weaning observations values ranged between 0 and 279 per hour (Mean: 19.40 ± 6.22 h^{-1}) and for post-weaning frequencies between 0 and 254 (Mean: 5.17 ± 5.00 h^{-1}). Short-term separations had no effect on the frequency of pawing ($F_{1,61}$=0.02, P = 0.98). Fig. 32 shows a significant day by time interaction $F_{4,61}$=10.93, P < 0.0001) for the frequency of pawing.

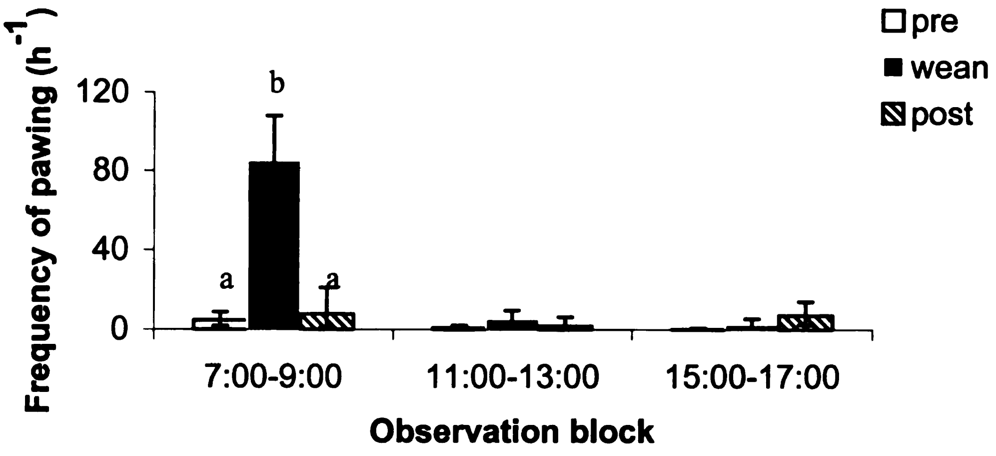

Figure 32. Mean frequency ± SEM of pawing per observation block for each weaning trial day. (N=10, $P < 0.05$). Differences, as indicated by the letters (a,b), are reported within each observation block.

Similar to results found for investigating, during the first observation block following weaning, pawing increased from pre-weaning to weaning day (4.71 ± 3.48 h^{-1} versus 85.20 ± 21.46 h^{-1}, t_{61}=-6.02, $P < 0.0001$) and decreased on post-weaning day (7.77 ± 10.07 h^{-1}, t_{61}=2.77, $P = 0.0075$) to a frequency not significantly different from pre-weaning values (t_{61}=-0.30, $P = 0.76$).

Discussion and Conclusion

Salivary cortisol

Cortisol concentrations showed a significant increase due to weaning and this is in agreement with other studies who investigated changes in plasma or serum total cortisol (Malinowski et al. 1990, McCall et al. 1987), except for a preliminary study conducted by Houpt et al. (1984). No changes in plasma cortisol were found after weaning, but it should be noted that the researchers relied on four jugular blood samples (0 h, 6 h, 24 h, and 1 week post-weaning). Due to the circadian pattern, only the 0 h and 24h should be compared, but nonetheless, confounding factors associated with blood sampling may have masked the effect of weaning.

In addition, we did not find a gender difference in the salivary cortisol response to weaning. These results are in agreement with Hoffman et al. (1995) who, despite a pre-ACTH gender difference in plasma cortisol (fillies having higher concentrations than colts), did not see a difference between colts and fillies in the adrenal response to an ACTH-challenge.

When investigating diurnal variation on salivary cortisol, we found that during the pre-weaning day the 6:20 and the 18:20 sample showed different cortisol concentrations, and that concentrations at 10:20 and 18:20 tended to differ as well. This result is in agreement with Lebelt et al. (1996) who reported that the highest concentrations of salivary cortisol were found in the morning and that the lowest concentrations occurred during late afternoon. Similar results were reported in our validation study described in chapter III. Though results were similar, Van der Kolk et al. (2001) did not find a significant difference in cortisol concentrations between 06:00 and 18:00. Lebelt et al. (1996) indicated that minimal environmental disturbance may cause a disruption in the circadian pattern of cortisol in horses. Our results support this finding in that on the weaning day, cortisol concentrations remained elevated after weaning compared to the pre-weaning 06:20 sample. In addition, the concentration of cortisol measured at 06:20 on the post-weaning data collection day was remarkably higher than the concentrations at 10:20, 14:20 and 18:20. We speculate that this high cortisol concentration is due to two factors. First of all, only 5 samples out of 10 (2 from separation group, 3 from control group) could be used for the analysis and second, we measured high concentration of cortisol for one animal at 06:20 on the post-weaning day. This caused the average to shift towards higher values, but the standard error indicates that the actual average can be as low as 5.3 nmol/l. Because this one influential value was not considered to be a statistical outlier, we chose not to remove it from the analysis.

Heart rate

Our results show that average heart rate was fairly constant between time blocks within days, the only exception being the first observation block on the weaning day. Overall, average heart rate increased from pre-weaning to weaning day. The immediate effect of weaning became apparent in the decrease in heart rate from weaning to post-weaning day during the first observation block, whereas for subsequent time blocks, weaning and post-weaning concentrations did not differ. Niezgoda and Tischner (1995) found a maximal increase in catecholamines 5 min after separation of mare from foal and this may explain the increase in heart rate caused by weaning. However, the concentration of activity increased as well due to weaning and it is possible to attribute the increase in heart rate to behavioral changes. To the best of our knowledge, no other research has measured heart rate in foals during the weaning process.

Behavior

The duration of "muzzle at udder" between test group and control group animals did not differ. Duration for the test group animals was 1.76 ± 0.82 and for control animals 2.59 ± 0.82. Nursing bouts in our study were longer than those found in Belgian mares (Barber & Crowell-Davis 1994). We believe that the discrepancy is due to the difference in the definition of start and end of nursing bouts, which does not allow us to compare duration of nursing bouts. However, in agreement with the nursing definition of Barber and Crowell-Davis (1994) we saw that each nursing bout usually consisted of several "muzzle at udder" occurrences of short duration (< 10 sec) and one or two longer ones. Casual observations suggested that the mare controlled the duration of the attempts and that perseverance by the foal led to longer, successful nursing bouts. We believe further research focusing on the behaviors displayed by the mare during nursing bouts is warranted, as these behaviors control nursing by the foal and therefore may allow us to create an index assessing the mare-foal relationship.

Weaning had an effect on the frequencies and durations of most behaviors investigated. We observed an increase in the frequency of vocalizing, defecating, eating, drinking, looking forward, investigating, and pawing. Weaning did not cause an effect in the frequency of urinating, contrary to findings in the study by Houpt et al. (1984). We did see an increase in the duration of walking, but not of standing. Finally, we found a decrease in the duration with which foals laid down on weaning and post-weaning day when compared to pre-weaning durations.

Houpt et al. (1984) found an increase in vocalizations with an average of 20 ± 4 during the first 15 min after weaning. In our study, during the first two hours, foals vocalized 88.5 ± 0.086 times per hour. This is approximately 22.13 vocalizations per 15 min, if we assume vocalizations during the first two hours are randomly distributed. Similarly, McCall (1985) found a total of 93 vocalizations during the first hour after weaning. During the fifth hour post-weaning, this frequency decreased to 39.8 vocalizations. The corresponding time block during our study is the 11:00 to 13:00 where on average foals vocalized 21.84 ± 7.04 times per hour. This is somewhat lower than the findings by McCall (1985), but it should be noted that the frequency in our study is derived from a total of one-hour observation time per foal during a two-hour observation block and it is possible that there is a significant difference in frequency of vocalizations between the fifth and sixth hour post-weaning.

In addition to the frequency of vocalizing, we found an increase in the duration of walking on subsequent weaning days and an increase in defecation. Houpt et al. (1984) found similar results, but in addition they found an increase in frequency of urination. The latter finding was not seen in our study, perhaps due to the low occurrence of urinating in general.

Eating frequency increased on subsequent days and the frequency of drinking was increased during the post-weaning day. The latter could be associated with the increase in locomotor activity caused by weaning. It should also be noted that casual observations indicated that during the pre-weaning day, foals followed the mare's eating pattern. If the mare was eating, the foal joined. But after weaning, the pattern changed and foals made an increased number of short, seemingly random visits to the feeder. We propose that this change to an erratic feeding pattern caused an increase in eating frequency, but we emphasize that this does not inherently imply an increased food intake. Hoffman et al. (1995) did find an increase in time spent eating between 0 to 24 h and 48 to 72 h after weaning, but we do not have the data to support this finding. The fact that nutritional gain through nursing was possible during the pre-weaning day may affect the amount of hay and concentrate foals ingested on this day. Consequently, as nursing was no longer possible during weaning and post-weaning day, we would expect foals to ingest more hay and concentrate. However, the observation that feeders still contained feed at the time of the next feeding leads us to speculate that the immediate impact of weaning was such that foals did not compensate for the loss of nutrients, previously acquired through nursing.

The increase in frequencies of looking forward, investigating, and pawing were most apparent during the first observation block during the weaning day. Investigating and pawing are behaviors that are interrelated as they often occurred almost simultaneously in 'exploration bouts'. The frequency of looking forward increased with weaning and we attribute this to foals observing humans or animals moving up and down the aisles between the stalls.

Hoffman et al. (1995) found that 24 to 48 h post-weaning, foals vocalized less and spent more time standing when compared to 0 to 24 h. In addition, all behaviors changed over time indicating foals were adapting to the weaning process. In our study, the duration of standing did not change with weaning and the duration for walking increased on the subsequent weaning day, indicating that adaptation may not have occurred at the end of the weaning trial. In addition, the duration of lying down did not increase significantly during the post-weaning day again suggesting that activity levels were indeed still higher than during the pre-weaning day. Frequencies of vocalization and defecation did decrease but vocalization rate had not returned to pre-weaning day levels.

No effect was found for the frequencies or durations of any behaviors based on separated versus control foals, except for defecation. Fillies in the test group showed higher frequency of defecating than fillies in the control group. Contrary, colts in the test group defecated less than colts in the control group. As increase in defecation is a behavioral indicator of stress, this could indicate that short-term separations complicate fillies' stress coping strategies during weaning, but that they also facilitate weaning for colts. Houpt et al. (1984) found a numerical, though statistically not significant increase in frequency of vocalization for foals that had been separated from their dam a few times prior to weaning. This observation and the knowledge that vocalizations are a good indicator for assessing weaning stress support our prediction that short-term separations prior to weaning complicate adaptation to weaning.

A potential explanation for the finding that separations had no effect on weaning stress in foals, apart from the small sample size used, is that the duration of our post-weaning study was limited by logistic factors. McCall (1987) found that pre-weaning cortisol concentrations were significantly lower than concentrations measured 2 days post-weaning, whereas no difference was found when compared to cortisol measured 9 days post-weaning. Thus, the impact of weaning appears to extend beyond 36 hours after weaning and it is possible that the immediate intense response to weaning masked treatment differences. Continuing observations for a longer period of time could have revealed such differences.

From our results, it seems that gender plays an important role in the display of certain behaviors during stressful events. We do not have data to support findings by Hoffman et al. (1995) that colts spend more time eating. However, in agreement with results from the study by McCall et al. (1985) we did find that fillies have a higher level of activity and lie down less compared to colts. Potentially associated with the higher level of activity in fillies is the fact that they drink more than colts do. In our study we also showed that fillies vocalized more during the post-weaning day than colts did, indicating that fillies may be more affected by the weaning process. In addition, within the test group: fillies defecated more than colts, but for the control group the reverse was true. The same differences exist in the frequency of looking forward. This suggests that separations aid colts in adapting to weaning stress whereas for fillies short-term separations during early stages in life may not be beneficial.

In conclusion, we did not find a difference based on separated versus control foals. However, despite a small sample size, we did find gender-related differences in the response to weaning. Fillies seemed more affected by weaning stress than colts, especially the fillies in the separation group. On the other hand, colts in the separation group tended to have a less profound and long lasting impact of weaning. We argue that these findings warrant further investigation into gender-related reactions to stressors.

CHAPTER V: GENERAL DISCUSSION

This study assessed the immediate and prolonged impact of short-term separations on maternal care through behavioral indicators. In addition, we combined physiological (salivary cortisol and heart rate) and behavioral measures to investigate the effect of increased maternal care by short-term separations on weaning stress in foals.

When weaning occurred at MSU Merillat Equine Center, heart rate levels of foals increased from 59.086 ± 3.66 BPM on the day before weaning to 86.47 ± 6.032 BPM on the day of weaning during the first observation block. It should be noted that using mean heart rate measurements provides a rough estimate compared to when one compares certain bursts of activities with corresponding heart rate data. Heart rate can increase because of low oxygen concentrations in the blood stream, as a result of increased activity, or because of an increase in catecholamines associated with the psychological response to maternal deprivation. Investigating whether increase in heart rate is related to increase in activity or increase in catecholamines is important. If it seems that high and low periods of heart rate correspond to similar bursts of activity, underlying physiological factors may be responsible for the variations in heart rate. In our study we did not have enough detail to the data to perform such an analysis. We propose future research be done investigating the correlation between activity levels and heart rate during psychosocial stressors during precisely timed trials.

Proximity scores during separation trials and post-separation evaluations were on average greater than 2, with a score of 3 indicating greatest proximity and 1 smallest. Thus, foals stayed close to their mothers at all times. However, because of the confinement of the stall, the distance between mare and foal was limited. The overall finding was that fillies stayed closer to their mothers than colts. This may be an indication of a closer association between fillies and their dams when compared to colts. Supporting this finding, we saw an effect of gender on several behaviors during the weaning trial suggesting that fillies had a more profound and longer lasting response to weaning than colts. Under feral conditions, there do not seem to be any differences in nursing behavior and both colts and fillies almost always leave the native harem before becoming sexually mature (Tyler 1972; Carson & Wood-Gush 1983b). By being exposed to close maternal contact, fillies may become acquainted with maternal behavior from their mothers. It is likely that this promotes reproductive fitness and insures survival of genes into future generations through successful foalings and rearing of offspring to healthy, competitive animals. In this respect, colts, who do not aid in the rearing of foals would not benefit from a close association with the dam. In fact, the sooner they are independent, the faster they can breed dams and pass on their genes into future generations. It should be noted that scientists have not yet concluded the discussion on how much of maternal care displayed by mares is innate and how much is learned from the dam and other mares, or how much was developed through experience.

Stressors can be physical, psychological, and/or psychosocial in nature (Mason 1975). The psychological impact of weaning has been demonstrated in other studies

(Houpt et al. 1984; Hoffman et al. 1995) and also during short-term separations, stress has been postulated to be emotional in nature (Niezgoda & Tischner 1995). We confirmed that weaning caused an increase in most behaviors investigated and an increase in overall activity of foals. We could not investigate duration of states (e.g., walking) during the separations. Technical difficulties using the Panasonic field switcher and 24- or 12- hour time lapse recording on a VCR resulted in unreliable timing when decoding videotapes. It seems there is a trade-off in the clarity of the tapes and the sequence of frames between the number of cameras and the time-lapse interval. For future research using multiple cameras and time-lapse recording, we recommend to limit the time-lapse recording to six-hour intervals as this still renders a clear image with continuous motion.

Contrary to findings in rodents, early increase in maternal care by short-term separations did not lead to a dampened stress response of the HPA axis in response to stressors later on in life. In fact, separation group animals showed a sustained elevation of cortisol compared to the control group. Ten-minute separations led to immediate increase in maternal care but this effect could no longer be identified 4-6 days post-separation. We conducted this research using a small number of animals and acknowledge that this might have been an influential factor. However, we speculate that there are alternative explanations for the differences between rodents and horses. It is possible that by applying the stressor, i.e., 10-min separations, at 2 weeks of age we missed the window of time in which the HPA axis can be modulated. It is not known whether there is a critical period during the development of foals in which the HPA axis is sensitive to external stimuli. Second, in rodent studies, the response to a stressor was tested when the pups had become mature (Liu et al. 1997). Weaning in our study was performed at 24 ± 2 weeks of age, when foals were still maturing. The anticipated dampened stress response may not have been apparent at weaning because of the different rate of development in rodents and horses. The HPA-axis was still developing and, as a result, weaning in itself may have had a modulating effect on the HPA-axis. To verify this, it would have been beneficial for us to take physiological measures to support behavioral data. In addition, because it is permanent by definition, weaning has a longer lasting impact compared to restraint stress as applied by Liu et al. (1997). We propose the complimentary explanation that the nature and frequency of the test stressor, and the age at which it is applied are important when evaluating the effect of increased maternal care. This prediction could be tested by developing protocols with different types of stressors applied at different ages.

Though we did not find an effect of increase in maternal care by short-term separations, and despite the limitation of a small sample size in our study, we found that a distinction should be made between fillies and colts. Fillies in particular seem to be affected negatively by the separations when investigating the gender by treatment interactions for defecating and looking forward. When observing behavioral indicators, colts showed a lesser response to weaning when they had been exposed to repeated short-term separations from their dam prior to weaning.

In conclusion, repeated short-term separations of mares from their foals resulted in an immediate increase in mare-foal interactions. This difference was no longer apparent after four to six days when comparing the test group mare-foal interactions to those of age-matched controls. To test the longevity of the impact of increased maternal care through separations on maternal care, we investigated weaning stress in foals that had been exposed to short-term separations versus foals that had never been separated from their dam. Contrary to our prediction, certain indicators suggested that separating mares from their foals during early stages in life complicated the adaptation to weaning, especially for fillies. We therefore recommend minimizing the number of times foals are separated from their dam prior to weaning, especially where fillies are concerned.

Appendix A

Overview of nursing bout definitions

1983: "The time foal first nuzzled the mare's udder for at least 3 seconds until the final time the foal removed its head from the medial side of the mare's hind limb" (Crowell-Davis, 1985; based on observations in 1979)

1983: Start of a nursing bout was recorded when the foal actually sucked for the first time. (Crowell-Davis, 1985; based on observations in 1980-1981)

1983: "A period of nursing activity delimited by intervals of non-nursing activity lasting for 27 seconds or longer" (Carson & Wood-Gush, 1983)

1992: Including pre-nurse nuzzling, sucking, intra-bout pauses and intra-bout nuzzling (Smith-Funk & Crowell-Davis, 1992)

1994: Including pre-suck nuzzle, pre-suck pause, suck, intra-suck nuzzle and intra-suck pause (Barber & Crowell-Davis, 1994).

Ingestion of milk -referred to as sucking- was recognized by distinctive characteristics that were difficult to observe when the horses were on pasture, but fairly obvious when they were placed in stalls. Holding the head still, audible sucking sounds, and the movement of fluid down the esophagus were the indicators of sucking (Barber & Crowell-Davis, 1994).

Appendix B

Foal age at first sampling

	Foal #	Date of Birth	Age at first sampling (days)
Group 1	1	04-Apr-00	97
	2	15-Apr-00	86
	3	11-Apr-00	90
	4	18-Apr-00	83
	5	12-Apr-00	89
Group 2	6	17-Apr-00	85
	7	27-Apr-00	75
	8	26-May-00	46
	9	17-May-00	55
	10	18-May-00	54

Appendix C

Treatment group, date of birth, gender, and sire of foals used in the study at MSU Merillat Equine Center

Foal	**Date of birth**	**Gender foal**	**Sire**
1(*)	***January 19***	***Filly***	***S***
2	***February 1***	***Filly***	***Z***
3(*)	***February 14***	***Filly***	***S***
4	***February 4***	***Filly***	***Z***
5(*)	***March 4***	***Colt***	***Z***
6	***March 7***	***Filly***	***Z***
7(*)	***March 24***	***Filly***	***S***
8	***March 14***	***Colt***	***Z***
9(*)	***April 21***	***Colt***	***O***
10	***April 24***	***Colt***	***S***

(*) = test group. Sires are Shadow (S), Zippos (Z), Other (O).

Appendix D

Merillat observation schedule

Legend: underlined = birth
bold = separation
italic = post-separation observation. Though the control animals were not separated, they were observed at ages similar to the test group
Separated: 1-3-5-7-9 Control: 2-4-6-8-10

Jan-00

MON	TUE	WED	THU	FRI	SAT	SUN
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
		1				
24	25	26	27	28	29	30

Feb-00

MON	TUE	WED	THU	FRI	SAT	SUN
	1	2	3	4	5	6
	2			4		
7	8	9	10	11	12	13
14	15	16	17	18	19	20
3		1				*1* *2* *4*
21	22	23	24	25	26	27
28	29					
1						

Mar-00

MON	TUE	WED	THU	FRI	SAT	SUN
		1	2	3	4	5
		3				*1* *2* *3* *4* *5*
6	7	8	9	10	11	12
	6					
13	14	15	16	17	18	19
1	8	3				*1* *2* *3* *4*
20	21	22	23	24	25	26
	3	5		7		*5* *6*
27	28	29	30	31		
1		3				

Apr-00

MON	TUE	WED	THU	FRI	SAT	SUN
					1	2
						1 2 3 4
3	4	5	6	7	8	9
	5					5 6 8
10	11	12	13	14	15	16
1		3	7			1 2 3 4 7
17	18	19	20	21	22	23
	5			9		5 6 8
24	25	26	27	28	29	30
1 10		3	7			1 2 3 4 7

May-00

MON	TUE	WED	THU	FRI	SAT	SUN
1	2	3	4	5	6	7
	5					5 6 8
8	9	10	11	12	13	14
	9	3	7			3 9 7 10
15	16	17	18	19	20	21
	5					5 6 8
22	23	24	25	26	27	28
	9	7				7 9 10
29	30	31				
	5					

Jun-00

MON	TUE	WED	THU	FRI	SAT	SUN
			1	2	3	4
						5 *6* *8*
5	6	7	8	9	10	11
	9	**7**				*7* *9* *10*
12	13	14	15	16	17	18
						8
19	20	21	22	23	24	25
	9	**7**				*7* *9* *10*
26	27	28	29	30		

Jul-00

MON	TUE	WED	THU	FRI	SAT	SUN
					1	2
3	4	5	6	7	8	9
	9					*9* *10*
10	11	12	13	14	15	16
17	18	19	20	21	22	23
	9					*9* *10*
24	25	26	27	28	29	30
31						

Appendix E

Ethogram used for post-separation observations

E denotes an event whereas S denotes a state

Eat (E)	Animal stands by feeder, ingesting either hay or concentrate
Drink (E)	Animal stands by waterer, ingesting water
Stand idle (S)	No forward or backward movements shown and all four legs support weight of the animal. Also removal of foal's muzzle from udder when foal is not lying down or in motion.
Lie Down (S)	No forward or backward movements shown but animal is not supported by its legs. Also removal of muzzle from udder by the foal when foal is not standing idle or in motion.
Walk (S)	Slow, forward motion in a four-beat gait.
Trot (S)	Forward motion, faster than walk, in a two-beat diagonal gait
Lope (S)	Forward motion, faster than walk, in a three-beat diagonal gate
Pawing (E)	Striking motion on the ground with one front leg

Rear (E)	While standing the animal lifts its front legs off the ground, raises upper body off the ground and the body weight is supported by the hind legs. Next, the front legs are placed back on the ground and all four legs support the animal's body
Defecate (E)	Animal raises its tail and excretes feces
Urinate (E)	Animal spreads hind legs a bit further apart than when standing idle, raises tail, points dock back and in a slight squatting position excretes urine
Nuzzle (E)	Animal touches other animal by probing with lips and potentially smelling scent, excluding when foal shows the "muzzle at udder" behavior
Investigate (E)	Animal probing object with lips and potentially smelling scent
Allogroom (E)	Animal uses lips and teeth to touch, nibble and pull on other animal's coat
Self-groom (E)	The animal uses lips and teeth to touch, nibble and pull on own coat

Watch Foal (E)	Mare turns head towards foal or if head is already turned towards foal, mare points her ears towards foal
Watch Mare (E)	Foal turns head towards mare or if head is already turned towards mare, foal points its ears towards mare
Look Forward (E)	Animal is present in front half (closest to the aisle) of stall, and turns its head to look outside of stall or if head is already directed that way, points ears forward.
Muzzle at Udder (S)	Foal is standing next to mare using muzzle to make contact with udder to nurse
Ears back (E)	Animal points ears backward potentially accompanied with a head swing in the direction of another animal
Kick threat (E)	Animal positions hind towards other animal, squats down a bit and points ears back, potentially lifting hind leg, but no striking or physical contact occurs
Kick (E)	Animal positions hind towards other animal, squats down a bit and points ears back, lifts one or both hind legs and then strikes at the other animal

Bite threat (E)	Animal swings head towards other animal and stretches neck, points ears back, opens mouth a bit, potentially bearing teeth, but no physical contact occurs
Bite (E)	Animal swings head towards other animal and stretches neck, points ears back, opens mouth a bit, potentially bearing teeth, and bites
Jump (E)	While in motion or at the start of motion, the animal thrusts its body forward and upward while arching its back and potentially swinging its head or kicking with the hind legs
Mount mare (E)	Foal lifts its front legs off the ground as if rearing, but leans on any part of its mother's body (shoulders, rump, hind…)
Tail swish (E)	Mare swishes its tail left to right in the direction of the foal. This behavior is a direct result of contact initiated by the foal
Lick (E)	Stroking object or other animal with tongue
Cross the bow (E)	Foal approaches the mare from the front, passes underneath her neck and continues towards the stifle as part of a nursing attempt

Appendix F

Behaviors observed during weaning

Eat (E)	Animal stands by feeder, ingesting either hay or concentrate
Drink (E)	Animal stands by waterer, ingesting water
Stand idle (S)	No forward or backward movements shown and all four legs support weight of the animal. Also removal of foal's muzzle from udder when foal is not lying down or in motion.
Lie Down (S)	No forward or backward movements shown but animal is not supported by its legs. Also removal of muzzle from udder by the foal when foal is not standing idle or in motion.
Walk (S)	Slow, forward motion in a four-beat gait.
Trot (S)	Forward motion, faster than walk, in a two-beat diagonal gait
Lope (S)	Forward motion, faster than walk, in a three-beat diagonal gate
Pawing (E)	Striking motion on the ground with one front leg

Rear (E)	While standing the animal lifts its front legs off the ground, raises upper body off the ground and the body weight is supported by the hind legs. Next, the front legs are placed back on the ground and all four legs support the animal's body
Defecate (E)	Animal raises its tail and excretes feces
Urinate (E)	Animal spreads hind legs a bit further apart than when standing idle, raises tail, points dock back and in a slight squatting position excretes urine
Nuzzle (E)	Animal touches other animal by probing with lips and potentially smelling scent
Investigate (E)	Animal probing object with lips and potentially smelling scent
Allogroom (E)	Animal uses lips and teeth to touch, nibble and pull on other animal's coat
Self-groom (E)	Animal uses lips and teeth to touch, nibble and pull on own coat
Vocalize (E)	Production of sound by animal

Watch Foal (E)	Mare turns head towards foal or if head is already turned towards foal, mare points her ears towards foal
Watch Mare (E)	Foal turns head towards mare or if head is already turned towards mare, foal points its ears towards mare
Look Forward (E)	Animal is present in front half (closest to the aisle) of stall, and turns its head to look outside of stall or if head is already directed that way, points ears forward.
Muzzle at Udder (S)	Foal is standing next to mare using muzzle to make contact with udder to nurse
Leave (E)	While in motion, the animal increases the distance between itself and the other animal
Approach (E)	While in motion, the animal decreases the distance between itself and the other animal
Ears back (E)	Animal points ears backward potentially accompanied with a head swing in the direction of another animal

Kick threat (E)	Animal positions hind towards other animal, squats down a bit and points ears back, potentially lifting hind leg, but no striking or physical contact occurs
Kick (E)	Animal positions hind towards other animal, squats down a bit and points ears back, lifts one or both hind legs and then strikes at the other animal
Bite threat (E)	Animal swings head towards other animal and stretches neck, points ears back, opens mouth a bit, potentially bearing teeth, but no physical contact occurs
Bite (E)	Animal swings head towards other animal and stretches neck, points ears back, opens mouth a bit, potentially bearing teeth, and bites
Squeal (E)	A high-pitched vocalization that's given in the event of an agonistic interaction, by aggressor or victim
Jump (E)	While in motion or at the start of motion, the animal thrusts its body forward and upward while arching its back and potentially swinging its head or kicking with the hind legs

Mount mare (E)	Foal lifts its front legs off the ground as if rearing, but leans on any part of its mother's body (shoulders, rump, hind…)
Null (E)	Indicates that there's no change in state by an animal after the other animal lies down
Tail swish (E)	Mare swishes its tail left to right in the direction of the foal. This behavior is a direct result of contact initiated by the foal
Lick (E)	Stroking object or other animal with tongue
Cross the bow (E)	Foal approaches the mare from the front, passes underneath her neck and continues towards the stifle as part of a nursing attempt
Bunt (E)	A forceful thrust of one animal's muzzle into another animal

Events during weaning

Weaning	Handler walks in stall, halters mare and leads her out, leaving foal in the stall by itself. Mare is taken to outside pasture.

Appendix G

Protocol during weaning

Friday:
6:00 Place heart rate monitor + saliva sample

7:00 Start observations

9:00 End observations

10:20 Saliva sample + check HRM

11:00 Start observations

13:00 End observations

14:20 Saliva sample + check HRM

15:00 Start observations

17:00 End observations

18:20 Saliva sample + remove HRM

Saturday

6:00 Place heart rate monitor + saliva sample

7:00 <u>Weaning</u> + Start observations

9:00 End observations

10:20 Saliva sample + check HRM

11:00 Start observations

13:00 End observations

14:20 Saliva sample + check HRM

15:00 Start observations

17:00 End observations

18:20 Saliva sample + remove HRM

Sunday

6:00 Place heart rate monitor + saliva sample

7:00 Start observations

9:00 End observations

10:20 Saliva sample + check HRM

11:00 Start observations

13:00 End observations

14:20 Saliva sample + check HRM

15:00 Start observations

17:00 End observations

18:20 Saliva sample + remove HRM

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