



**UNIVERSIDAD NACIONAL AUTÓNOMA
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**Reconocimiento vocal de la cría por su madre en caballo
doméstico (*Equus caballus*)**

T E S I S

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Reconocimiento vocal de la cría por su madre en caballo doméstico (*Equus caballus*)

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'They love their horses next to their kin.
And not without reason, for the horses of the Riddermark
come from the fields of the North, far from the Shadow, and
their race, as that of their masters, is descended from the free days of old.' - Boromir

'...for now the horse is wild and will let no man handle him.' - Eomer

'noro lim, noro
lim, Asfaloth!' - Glorfindel

The Lord of the Rings- J.R.R.Tolkien

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RESUMEN

El reconocimiento madre-cría es muy importante en muchas especies. Es importante para la madre para asignar los esfuerzos parentales a su descendencia e importante para la cría, ya que esta depende principalmente de la nutrición y la protección que la madre le proporciona. Para las crías recién nacidas de estas especies el intento de amamantarse de una madre ajena puede resultar no solo en el rechazo, sino también en serias lesiones e incluso la muerte

El proceso de reconocimiento entre madres y su descendencia implica el uso de señales visuales, olfatorias y auditivas. Las señales visuales son útiles para el reconocimiento tanto a cortas como a largas distancias, sin embargo, se ve limitado por el número de integrantes y otros posibles factores que interfieran con el campo visual. El olfato apoya el reconocimiento principalmente en rangos cortos de distancia y es utilizado por las madres para una verificación final de la identidad de su descendencia antes de permitir amamantar. Las señales acústicas, por otro lado, son eficientes tanto a cortas como a muy largas distancias, por lo tanto, la comunicación vocal parece ser un factor clave para el reconocimiento madre-cría.

En ungulados, grupo taxonómico al que pertenecen los caballos, existen dos estrategias principales para evadir a los depredadores: esconderse y seguir. Para evadir a los depredadores, las crías que se esconden permanecen ocultas en la vegetación durante las primeras semanas después de su nacimiento y antes de unirse a los grupos sociales; mientras que las crías que siguen a sus madres poco después de haber nacido cuentan con la defensa tanto de la propia madre como del grupo social al que pertenecen. Las crías “seguidoras” usualmente permanecen cerca de sus madres e interactúan a menudo con ellas. En especies “escondedoras”, las hembras memorizan la ubicación aproximada donde se esconde su descendencia y llaman a las crías mientras se acercan al sitio donde se encuentran para iniciar la lactancia. Estas estrategias de evasión del depredador tienen

impacto en la comunicación vocal madre-cría. En especies “escondedoras” el reconocimiento vocal se da de las crías a la madre (unidireccional). Por otro lado, madres y crías de especies “seguidoras” son capaces de reconocerse mutuamente (reconocimiento bidireccional). En estas especies el reconocimiento mutuo es crucial para mantener el contacto madre-cría y evitar cuidado materno mal dirigido. Los caballos son considerados “seguidores”. Los potros desarrollan habilidades motrices rápidamente y comienzan a edad temprana a alejarse de su madre. Los caballos utilizan algunas vocalizaciones para comunicarse entre ellos y se sabe que tanto madres como potros utilizan vocalizaciones extensivas durante situaciones estresantes.

El reconocimiento madre-cría ha sido ampliamente estudiado en especies de ungulados que viven en manadas y en especies que viven en colonias como pinnípedos y en murciélagos y se ha encontrado ya que la comunicación acústica juega una parte importante en el reconocimiento de recién nacidos por sus madres en gran cantidad de especies de mamíferos. Sin embargo y a pesar de la importancia económica, cultural e histórica del caballo en nuestra sociedad, el reconocimiento vocal de la cría por su madre en esta especie es limitado. Por lo tanto, el objetivo de este trabajo fue determinar si existe reconocimiento vocal de las crías de caballo domestico mediante experimentos de reproducción de vocalizaciones inducidas por separación.

Varios estudios han encontrado que los caballos adultos son capaces de discriminar miembros de su grupo social o incluso reconocerlos. El único estudio que existe actualmente sobre reconocimiento madre-cría (Wolski, Houpt et al. 1980) sugiere que las vocalizaciones son importantes en este contexto pero por varias razones no fue capaz de probar el reconocimiento individual.

Justificación.

Se analizará si las madres son capaces de reconocer a su potro basándose en las llamadas de estrés emitidas durante la separación, lo cual es necesario para facilitar activamente la reunión o proveer asistencia en una situación potencialmente peligrosa. Así mismo, los resultados obtenidos a partir de este estudio podrían aportar información relevante que permita implementar nuevas técnicas en el manejo actual del caballo doméstico.

Metodología.

Sitio y animales.

El presente estudio se realizó en las instalaciones de la Unidad de Policía Metropolitana Montada (UPM Montada) de la Ciudad de México, localizada en Av. Guelatao No. 100, Col. Álvaro Obregón, Del. Iztapalapa, CDMX, México. La UPM Montada, cuenta con un total de 690 caballos de diversas edades. En estas instalaciones, los animales nacen y se alojan durante toda su vida, bajo condiciones similares de manejo.

A lo largo de este estudio, todos los animales que participaron fueron tratados bajo el protocolo de manejo de la ASAB/ABS (2016) “Guías para el tratamiento de animales para la investigación en conducta”, el protocolo de manejo del Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, y de acuerdo a la NOM-062-200-1999.

Obtención de vocalizaciones y edición de estímulos.

Para obtener las vocalizaciones de los potros, se realizó una separación de las madres y las crías en la sexta semana postparto. La separación se llevó a cabo como se indica en la **figura 1**.

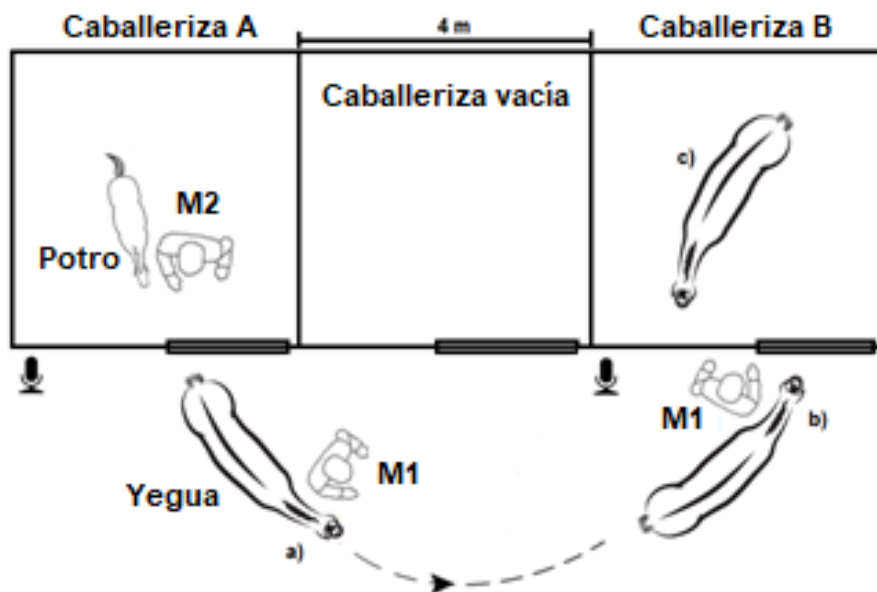


Figura 1. Para la grabación de las vocalizaciones: a. El manejador 1 (M1) extrajo a la yegua de la caballeriza que comparte con su potro (caballeriza A), mientras que el manejador 2 (M2) retuvo al potro en la caballeriza A; b. El manejador 1 situó a la yegua en una caballeriza vacía (caballeriza B); c. Ambos manejadores salieron de las caballerizas. Las vocalizaciones se grabaron con dos micrófonos unidireccionales dirigidos al interior de las caballerizas A y B.

Durante la separación no hubo contacto visual entre los potros y sus madres. La separación tuvo una duración de dos minutos, después de los cuales el manejador 1 regresó a la yegua a la caballeriza A donde se encontraba su potro. Veterinarios y entrenadores estuvieron siempre presentes para detener el procedimiento de ser necesario y todos los manejadores eran conocidos por los caballos.

Durante los dos minutos de separación, las vocalizaciones emitidas por los potros y sus madres fueron grabadas utilizando dos micrófonos unidireccionales (Sennheiser ME66, Wedemark, Germany; rango de frecuencia: 40 – 20 000 Hz) en soportes colocados fuera de los establos, conectados a una grabadora (Tascam DR-40, Montebello CA, USA; 96 kHz / 24 bit).

Reproducción del estímulo.

La edición del estímulo se llevó a cabo en Audacity 1.2.6, y se guardó en formato lossless AIFF. Cada estímulo comenzó con cinco minutos de silencio, seguido por 2 minutos de vocalizaciones del mismo individuo, una cada 2 segundos y finalizó con otros dos minutos de silencio.

Para cada sujeto de prueba se reprodujo el estímulo auditivo de de su propia cría, y de una cría ajena de manera aleatoria. Las reproducciones se llevaron a cabo al menos uno, pero no más de tres días después de ser grabadas las vocalizaciones. La presión de sonido de cada estímulo fue normalizada a 60 dB medidos desde 1m de distancia con un metro de presión sonora (General DSM402SD, New York, NY USA). La reproducción del estímulo fue presentada con un altavoz inalámbrico (SoundLink® Mini, Bose Inc., Framingham MA, USA) conectado a un iPod® 5th generation (Apple Inc. Cupertino CA, USA) colocado en una de las esquinas de la caballeriza de prueba.

Respuestas fisiológicas y conductuales.

Las respuestas conductuales se evaluaron utilizando grabaciones de video por medio de una cámara GoPro HERO 4 y los cambios fisiológicos fueron monitoreados mediante un sensor de frecuencia cardiaca Polar Equine V800 Science (Polar USA). Se midió la latencia, frecuencia y duración de las conductas descritas en la **tabla 1**.

Tabla 1. Conductas observadas durante la presentación del estímulo.

Variable conductual	Descripción
Contacto corporal	El cuerpo de la yegua está en contacto directo con el cuerpo del potro (o a 25 cm de distancia).
Estado alerta	Postura estática, cuello elevado y cabeza orientada hacia el objeto o animal de interés. Las orejas se mantienen estrictamente en posición vertical y apuntando hacia Adelante. Los nostrilos pueden estar ligeramente o muy dilatados.
Vocalizar	Emitir un relincho o resoplido.
Mirar al potro	La yegua gira su Cabeza en dirección a su potro.

La variabilidad de la frecuencia cardiaca (HRV por sus siglas en inglés) registrada por el Polar Equine V800 Science (Polar USA) se analizaró mediante el software Kubios Las variables relacionadas con HRV que se analizaron se muestran en la **tabla 2**.

Tabla 2. Variables de HRV analizadas y su descripción. SNS: Sistema Nervioso Simpático, SNP: Sistema Nervioso Parasimpático, AF: Alta frecuencia, BF: Baja frecuencia.

Variable	Descripción	Importancia fisiológica	Unit
Mean RR	Media de los intervalos RR consecutivos.	Representa la media de todos los intervalos RR	ms
StdRR	Desviación estándar de los Intervalos RR	Indicador de la variabilidad de los datos, reflejan la variabilidad a largo plazo de la actividad cardiaca bajo influencia tanto del SNS y de SNP.	ms

RMSSD	La raíz cuadrada de los cuadrados de los promedios de los intervalos RR consecutivos	Representa la variación a corto plazo de los intervalos RR y es útil para evaluar la influencia de la actividad vagal.	mss
FFT-LFHF	Transformada rápida de Fourier	Estima la influencia del SNP (AF) y el SNS (BF) y constituye un indicador del equilibrio simpto-vagal.	s ² /Hz

Resultados

Respuestas conductuales de las yeguas

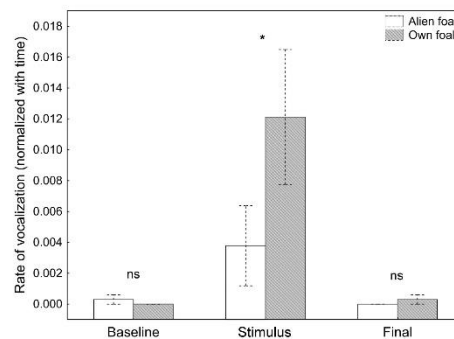


Figura 2. Tasa de vocalización (normalizada con el tiempo \pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. Los asteriscos marcan diferencias significativas en la prueba post-hoc. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

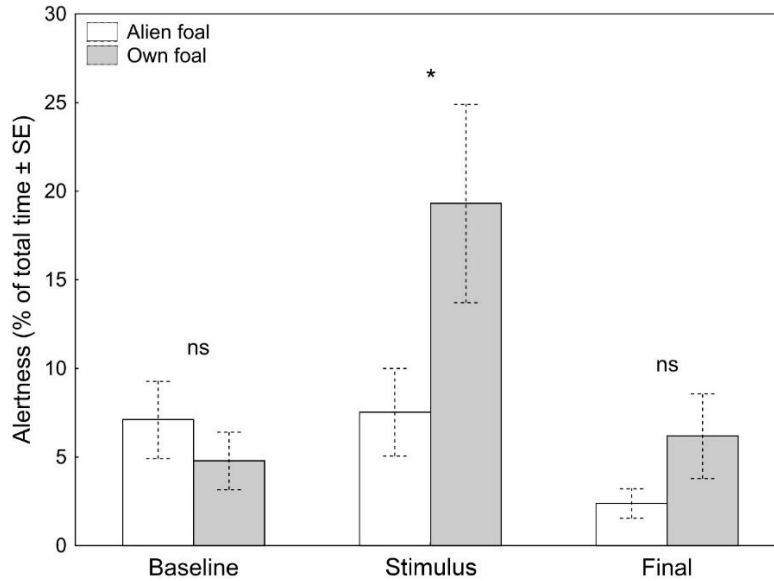


Figura 3. Porcentaje del tiempo total de la madre en estado alerta (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. Los asteriscos marcan diferencias significativas en la prueba post-hoc. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

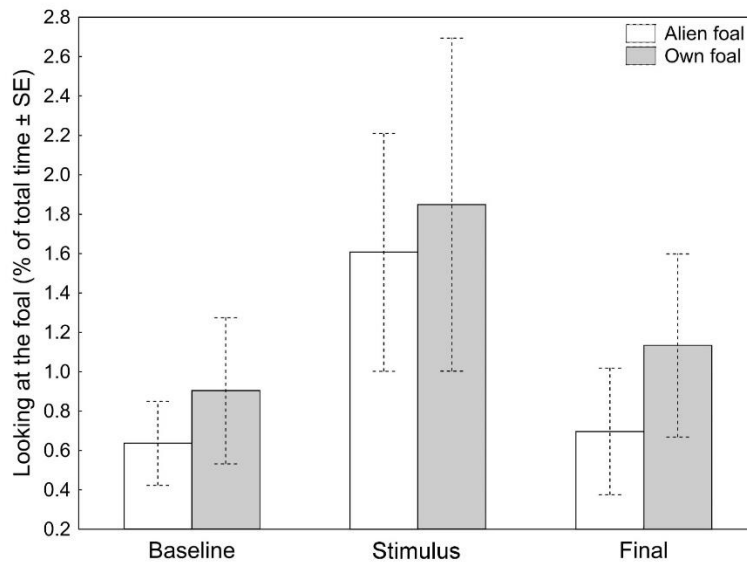


Figura 4. Porcentaje de tiempo que las yeguas destinaron a mirar a su potro (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

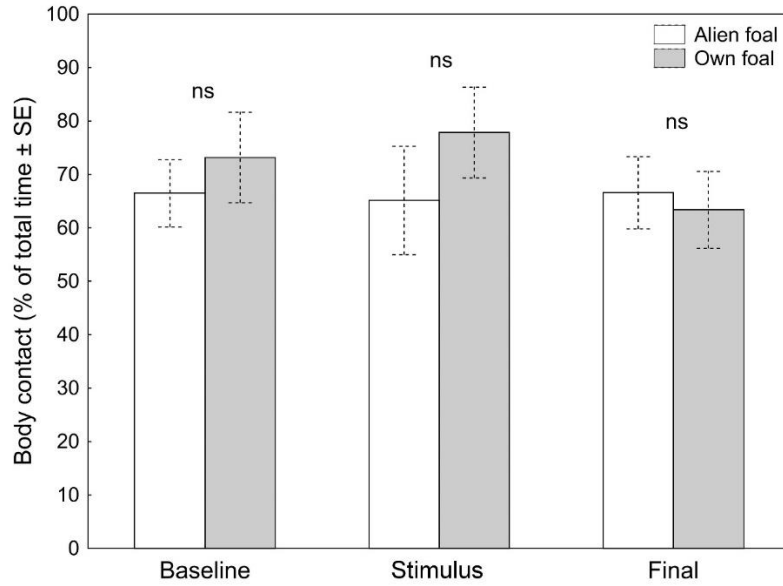


Figura 5. Porcentaje de tiempo en que las madres mantuvieron contacto con su potro (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

Respuestas fisiológicas de las yeguas

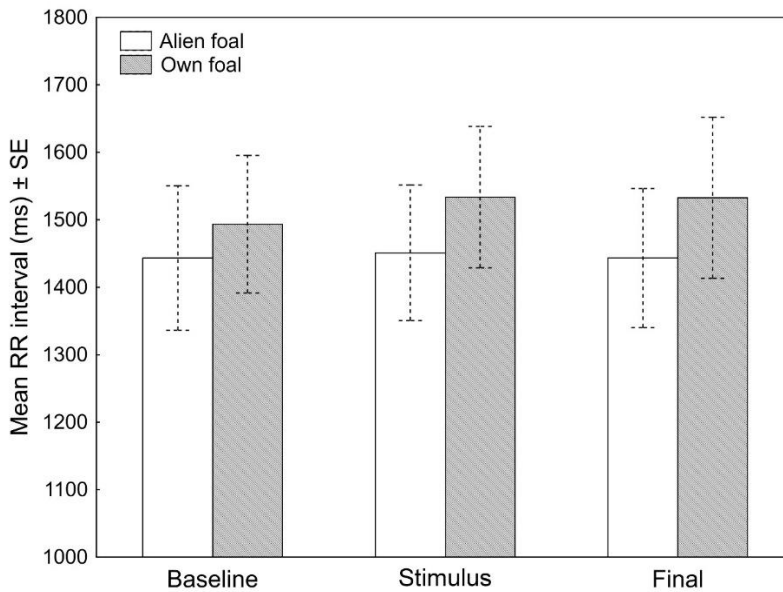


Figura 6. Media de los intervalos RR de las yeguas (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

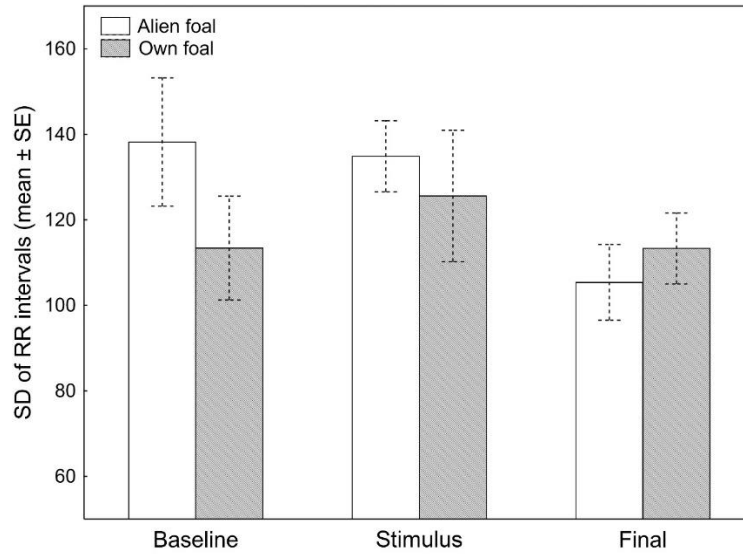


Figura 7. StdRR de las yeguas (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

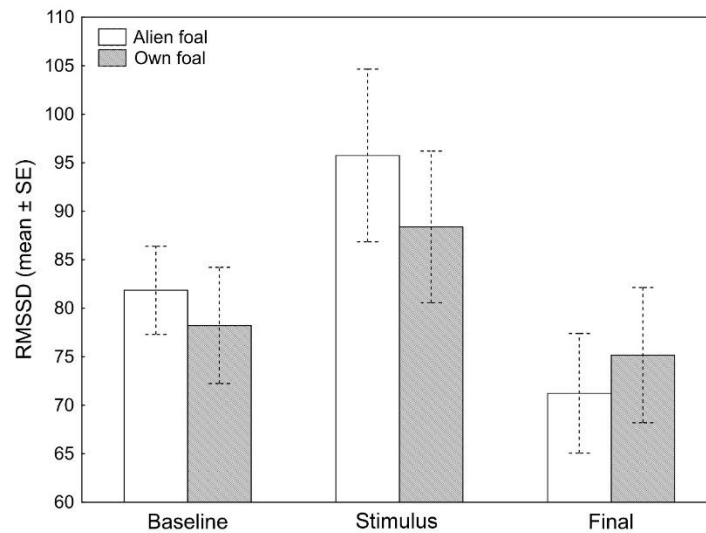


Figura 8. Valores medios de RMSSD de las yeguas (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno.

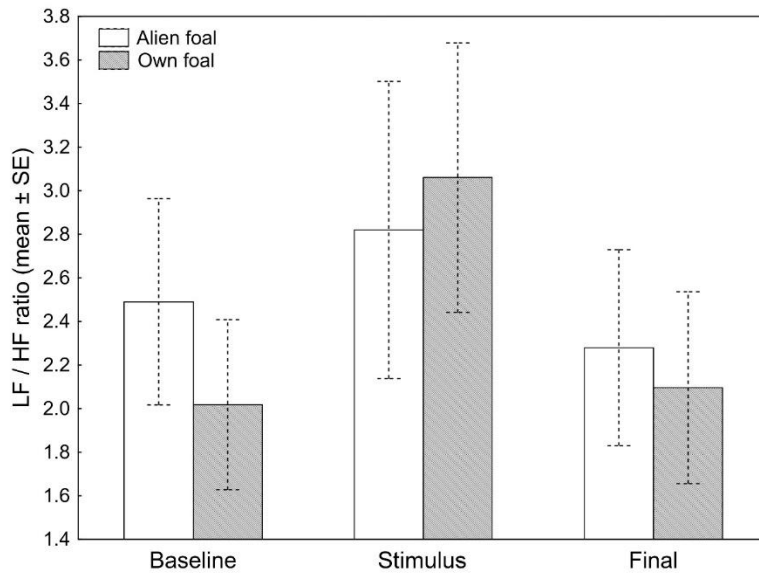


Figura 9. Valores medios de la Tasa AF/BF de las yeguas (\pm ES) durante la reproducción de los llamados de separación del potro ajeno y el propio potro. El gráfico gris pertenece al propio potro y el gráfico blanco pertenece al potro ajeno (\pm

A pesar de que los parámetros fisiológicos de Variabilidad de Frecuencia Cardíaca no mostraron diferencias significativas, las variables conductuales: estado alerta y vocalizar, sí mostraron diferencias significativas. Las yeguas vocalizaron con mayor frecuencia y se mantuvieron en estado alerta durante más tiempo durante la reproducción de los llamados de separación de su propio potro en comparación con los llamados de separación del potro ajeno. Estos resultados sugieren que las yeguas son capaces de reconocer a su potro basándose únicamente en sus vocalizaciones.

ABSTRACT

In animals, recognition can take place from the species to the individual level. Individual recognition refers to a subset of discrimination when one individual identifies another according to its distinctive characteristics. Depending on the context in which an individual's identity is learned it can be used to recognize e.g. a parent, young, sibling, mate or rival. Parent-offspring recognition is common in species providing directed parental care, which is especially likely to occur in organisms that breed in large, high-density colonies with synchronous breeding. Even though parent-offspring recognition can be based on almost all sensory modalities, or even spatial memory, it has been found that vocalization plays an important role in a variety of mammals.

Despite the status and commercial importance of horses, knowledge related to mother-young vocal recognition is limited. Under natural conditions, horses are considered as follower strategists –contrary to other domestic ungulates–, the mare and her foal soon after birth leave the foaling site to join with the herd. This suggests a strong selection pressure on both parties to develop a bidirectional vocal recognition.

The aim of this study was to determine whether mares are able to recognize their own foal based only on vocal cues. To assess this, first, we collected vocalizations (separation calls) from the foals during a short separation from their mother. Then we performed played back experiments with eleven mothers and measured their behavioral and physiological response when they were presented with the own foal or an alien foal vocalization. Although physiological results indicated no difference between the response to the alien and the own foal, the behavioral results showed mares responded more strongly to the separation calls of their own foal than to an alien one, suggesting that mares can recognize their young purely based on auditory cues.

INTRODUCTION

Observational and experimental evidence has shown that birds (Aubin, Jouventin, & Hildebrand, 2000), fish (Hoejesjoe, Johnsson, Petersson, & Jaervi, 1998), mammals (Sayigh et al., 1998), reptiles (Olsson, 1994) and even invertebrates (Sheehan & Tibbetts, 2011) regularly show differential behavior toward particular conspecifics. Individuals in many animal species are able to discriminate their kin with whom they have matured or familiar individuals with whom they interact regularly (Cheney & Seyfarth, 1999; Fletcher & Michener, 1987; Gamboa, Reeve, & Holmes, 1991; Hepper, 1986). The capacity to distinguish between individuals is a sophisticated cognitive ability, adaptive to most aspects of social behavior, such as the maintenance of social groups, creation of dominance hierarchies, territorial defense, cooperative breeding, monogamous pairing, mate or parent-offspring recognition (Hurst et al., 2001; Tibbetts & Dale, 2007).

Individual recognition refers to a subset of discrimination when an individual (receiver) identifies another (signaler) according to its distinctive characteristics (Dale, Lank, & Reeve, 2001; Gheusi, Bluthé, Goodall, & Dantzer, 1994). During individual recognition, the receiver learns specific cues of another individual and associates them with other information (i.e. neighboring, familiarity, social status). Later, the receiver can use these cues to identify the individual. Depending on the context in which an individual's identity is learned, individual recognition can be used to discriminate e.g. a mate, offspring, sibling or rival. Individual recognition allows to avoid wasting time and energy during social interactions, as it may reduce the chance of expensive fights among territorial neighbors during resource defense, or allow mates, kin or parents and offspring to find each other where familiar individuals intermingle with unfamiliar ones and even to avoid inbreeding (Lambrechts & Dhondt, 1995). Individual recognition is frequently considered a complex form of communication, so there has been extensive interest in the diversity and sophistication of receiver behavior in many different taxa (Mateo, 2004, 2006). Although behavioral research does not specifically address the cognitive mechanisms underlying

recognition behavior, a few studies suggest that animals might form relatively complex representations of each other (Tibbetts & Dale, 2007).

Emergence of identity signals is favored by natural selection when there is the possibility of costly confusion between the signaler and another individual. Thus far, it seems that high-density, colonial breeding, dominance hierarchies and other forms of complex social interactions favor the occurrence of identity signaling especially when there are repeated social interactions between individuals with differing roles in the group (Tibbetts, 2004; Tibbetts & Dale, 2007). In these contexts, perceptual and cognitive capacities for the recognition of group membership are needed (Krueger & Flauger, 2011).

Individual recognition process requires flexible learning and memory, therefore it has the potential to dramatically increase cognitive demands (Sheehan & Tibbetts, 2011). Thereby, during individual recognition, the receivers forms internal representations of the other individual's characteristics, so called "templates", and recognition occurs when the perceived cue matches the template (Sherman, Reeve, & Pfenning, 2003). For all recognition processes, specific templates should be learned. Some templates are learned in early life (imprinted), and other templates have to be updated when their characteristics change over time (Krueger & Flauger, 2011).

Mother-offspring recognition

In many birds and mammals, parents and offspring develop the ability to recognize each other (T. Halliday, 1983), which provides mutual fitness benefits for both parties (Trivers, 1974). For parents, offspring recognition prevents misdirected parental care, limits their expenditure of energy, and ensures their reproductive success (Hamilton, 1964). In mammals where lactation is the most expensive aspect of parental care, which can nearly triple the mother's caloric requirements (Gittleman, Thompson, & Thompson, 1988) mothers often display selective maternal care to their own offspring. This decreases maternal energetic expenditure and increases the fitness of breeders (Trivers, 1972). For young, recognition of the parents can be also essential to their survival since usually they're the only ones who are willing to provide protection and even an attempt to suckle from an alien mother may result

not only in rejection but also in serious injury or even death (Harcourt, 1992; Trillmich, 1981; Wolski, Houpt, & Aronson, 1980). Other potential benefits associated with mother-offspring recognition include decreased risk of inbreeding and decreased inter and intraspecific brood parasitism (Tibbetts & Dale, 2007).

Parent-offspring recognition is necessary to elicit well-directed parental care and is especially likely to occur in organisms that reproduce in large, high-density colonies with synchronous breeding and thus have reduced recognition potential based on positional information (Tibbetts & Dale, 2007). Well known examples include pinnipeds (Trillmich, 1981) and bats (Balcombe & McCracken, 1992), and it also occurs in domestic animals like sheep (*Ovis aries*, Searby & Jouventin, 2003), cows (*Bos taurus*, Padilla De La Torre, Briefer, Ochocki, McElligott, & Reader, 2016) and goats (*Capra hircus*, Briefer & McElligott, 2011). The sensory background and the degree of recognition (i.e. bi or unidirectional) can vary between species and may depend on ecological constraints (Charrier, Mathevon, & Jouventin, 2003).

Sensory modalities in mother-offspring recognition processes

The recognition processes may involve sensory modalities such as vision, olfaction, touch and audition. The relative involvement of these modalities in parent-offspring recognition differs among species (Halpin, 1991) and are mostly based on the distance range of the recognition process. Visual and acoustic cues are useful over long distances whereas olfaction is mostly used in close-range recognition. Previous studies showed that olfactory cues allow recognition at short distances and permit the mother to check the young's identity before allowing suckling. Such selective nursing were reduced in pregnant ewes (*Ovis aries*) that were made anosmic before delivery (Baldwin & Shillito, 1974; Morgan, Boundy, Arnold, & Lindsay, 1975; Poindron & Carrick, 1976). Further studies have demonstrated the importance of both vision and audition in mother-offspring recognition at longer distances (Ferreira et al., 2000; Lindsay & Fletcher, 1968; Poindron & Carrick, 1976; Terrazas et al., 1999). However, the efficiency of visual cues might be reduced by distance and by the potential gathering into larger groups. Because acoustic signals are efficient over long and

short distances, this type of cues appear to be fundamental for most mammals in the recognition process (Searby & Jouventin, 2003). It has been found that recognition based on acoustic signals play an important part in the recognition by newborn of their mother in a variety of mammals, e.g. bats (Balcombe & McCracken, 1992), cows (Barfield, Tang-Martinez, & Trainer, 1994), goats (Briefer & McElligott, 2011), seals (Charrier et al., 2003; Le Boeuf, Whiting, & Gantt, 1973) and sheep (Sèbe, Nowak, & Poindron, 2007).

Hider and follower strategies in ungulates

Two main strategies in maternal care and infant behavior have evolved in ungulates for avoiding predators during the first weeks of life: following and hiding (Lent, 1974). In follower species the newborn becomes mobile and starts to accompany the mother within hours or - even - minutes. The young mostly rely on fleeing and maternal or group defense to avoid predation. These species usually have highly developed social systems and inhabit open or rugged terrain with low vegetation. In hider species the young stay isolated, relatively stationary, even with cryptic coloration. They usually do not move and stay silent to avoid detection by predators. They are active only when the mother returns for nursing. The hider characteristics decreases as the young grows, as they remain active for longer periods with each maternal visit. Hiders often live in habitats of dense, high vegetative profile (Carl & Robbins, 1988; Fisher, Blomberg, & Owens, 2002).

These strategies result in differences in mother-offspring interactions, and thus different selection pressures are acting on the recognition processes. Although not being fully demonstrated, it seems that in hider species the offspring has low level of call individuality leading only to unidirectional recognition of the mothers by the offspring while followers usually show high offspring call individuality and mutual recognition (Blank & Yang, 2017; Briefer & McElligott, 2011; Padilla De La Torre et al., 2016; Torriani, Vannoni, & McElligott, 2006).

Due to the importance of vocal cues in mother-offspring recognition, several studies on this subject had been made in many species of ungulates. Torriani *et al* (2006), through

playback experiments showed that fawns of the fallow deer (*Dama dama*), a hider species, can distinguish the calls of the own mothers from alien ones, but mothers could not discriminate their own and alien fawn calls. Briefer and McElligott (2011) found in goats, also a hider species, that despite their main hypothesis (unidirectional vocal recognition) there is sometimes mutual recognition between mothers and their kids although they do not rule out the possibility that this might be only a consequence of domestication. The study of Searby and Jouventin (2003) showed that ewes and their lambs - sheep, a follower species - can recognize each other based solely on their calls. Also Sébe and Nowak (2007) found that early vocal recognition between the parties play an important role for the maintenance of mother–young contact in the same species.

The domestic horse

The domestic horse (*Equus caballus*) belongs to the order Perissodactyla (odd-toed ungulates). Their original social organization is hard to describe precisely because of the effect of thousand years of domestication. Even feral horses are all descendants of once domesticated specimens and mostly living in areas different from their original distribution. It is certain though that they are highly social animals, living in relatively stable social units, called bands, family groups or harems which are typical of female defense polygyny (Klingel, 1972). Band is used for the discrete social groups, while the herd is a localized population consisting normally of one or more bands as well as solitary individuals.

Even though the spatial organization of different feral horse populations is remarkably similar throughout the world, band structure of unmanaged populations varies widely. Bands consist of several mares (1–26) and their offspring until 2–3 years of age, accompanied by one or more stallions that defend the band from other stallions year-round (Linklater, 2000). Up to half of the bands contain more than one and as many as five stallions. These males are not necessarily related but have a strongly hierarchical relationship which determines their relative proximity and access to the group of mares. Subordinate stallions contribute disproportionately to defending the mares in the band but copulate less often with them than the dominant stallion (Berger, 1986; Feh, 1999; Franke Stevens, 1990;

Linklater, 1998; Miller, 1981). Surplus stallions usually gather in bachelor bands (Berger, 1977). A scheme of the social organization of horses as shown by Waring (2003) can be seen on **Figure 1**.

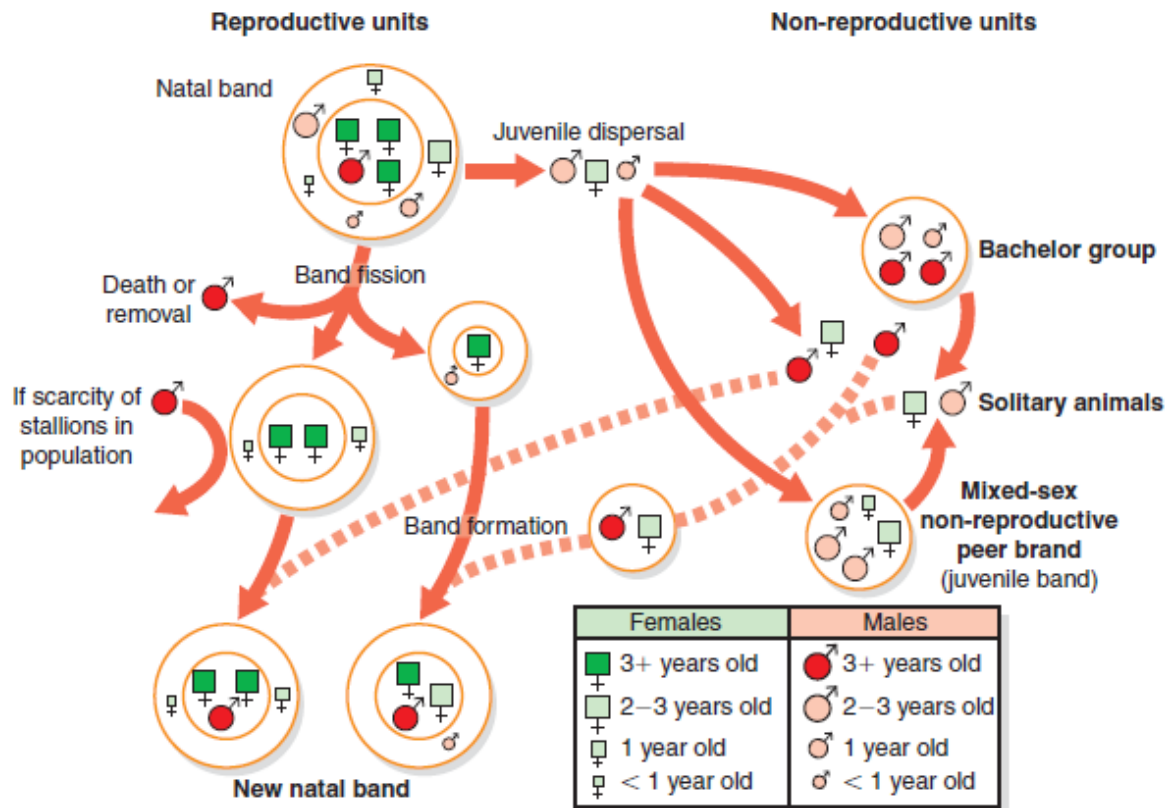


Figure 1. Social organization patterns exhibited by free-roaming horses (Waring, 2003).

The family groups make occasional encounters (e.g. at home range borders or at water source) with neighboring groups, like other harems or bachelor bands (Waring, 2003). Such complex social system requires that horses memorize social experiences, distinguish between familiar and unfamiliar individuals and even identify familiar horses and recognize their social status (Krueger & Heinze, 2008; Péron, Ward, & Burman, 2014; Proops, McComb, & Reby, 2009). Horses exhibit excellent long term memory of memberships of their own group (Waring, 2003) and their behavior can change depending upon whether a conspecific

is known or unknown, raising the likely conclusion that they are able to distinguish between familiar group members and strangers (Krueger & Flauger, 2011; Rubenstein & Hack, 1992).

Besides their vocal repertoire (Lemasson, Boutin, Boivin, Blois-Heulin, & Hausberger, 2009) (**Table 1**), horses also display a range of visual signals (e.g. facial mimics, body-tail-ear postures). This can be due to the fact that horses often communicate without using vocalization, presumably because they are social prey animals that must organize themselves as a group member without attracting predators (Lemasson et al., 2009; McGreevy, 2004). Several perceptual methods have been observed for social recognition in horses. The horse has a well-developed sense of hearing and is capable to perceive sounds at frequencies above those perceived by man (31 Hz – 17.6 kHz). The hearing range of the horse is between 55 Hz and 33.5 kHz, with the best sensitivity range of 1-16 kHz with the lowest threshold of 7 dB (Heffner & Heffner, 1983). Therefore, it is understandable that they use auditory cues to stay in contact with each other (Feh, 2005; Feist, Mccullough, & Dean, 1976; Kiley, 1972; Lemasson et al., 2009; Rubenstein & Hack, 1992). However, horses can also distinguish individuals among their group mates by the smell of their feces and discriminate stimuli derived from body odor samples of unfamiliar conspecifics (Krueger & Flauger, 2011; Péron, Ward, & Burman, 2014). Recognition ability of adult horses, on the basis of auditory cues has already been shown, revealing that whinny calls carry social information about the caller's sex, body size, and identity, also imply the use of social memory (Lemasson et al., 2009). Auditory recognition in conjunction with visual cues has been used to demonstrate cross modal individual recognition in horses (Proops et al., 2009)

Table 1. Vocal repertoire of horses. Modified from Yeon (2012).

Vocalization	Behavioral context	Duration (ms)	Amplitude	Fundamental frequency (Hz)
Whinny (neigh)	Greeting or separation call to maintain or regain contact with affiliates or offspring, anticipation of a pleasant or unpleasant event, disturbance, distress/frustration, curiosity about an event, seeking another horse	500–2800	m-h	400-2000
Nicker	Prior to feeding, stallion’s sexual interest in mare, mare to foal (expressing concern)	200–1700	m-l	100-150
Squeal	Aggressive interaction (e.g., between stallions), sexual behavior (mare’s protest to stallion’s attention), olfactory investigation, prancing, acute pain	100–1700	m-h	150-250
Scream	More serious aggression		h	
Groan	Discomfort, parturition, conflict situations, suffering, physical effort, pain relaxation	100–1700	l	
Blow	Olfactory investigation, arousal as part of an exploratory sniffing	340–1300	m	
Snore	Prior to alarm blow, labored breathing, recumbent sleep	300–1800	l-m	
Snort	Play situations	490–1310	m-h	
Roar	Extreme arousal to mare by stallion, confident greeting, anticipation of pleasant or unpleasant event, frustration	300–1500	m-h	50-100

l: low, m: medium, h: high

Foal development

Horses are seasonal breeders with ovulatory activity being related to long days. The breeding season on the northern hemisphere is usually between May and October but it can be shorter at higher latitudes. The average length of the estrous cycle is about 22 days with 5–7 days. The postpartum estrus typically occurs six to nine days after foaling. Horses are predominately monovulatory. The length of pregnancy is approximately eleven months, however, environmental factors, such as season and nutrition, interacting with other factors e.g. the sex of the foal can affect the duration of pregnancy (Waring, 2003).

After a normal parturition, mares will stand still for 15 to 20 minutes while begin to nuzzle and lick the foal. This period, referred to as the “critical period” is an important time for establishing the mare-foal bond. The licking and cleaning behavior, which usually starts at the head, serves to stimulate the foal while also drying it. The cleaning is probably also part of the initial bonding process and typically accompanied by nickers and a thorough visual and olfactory examination of the foal by the mare (McGreevy, 2004). During the “critical period” the mother rapidly learns to distinguish her foal and usually this is accomplished in the first one or two hours postpartum (Waring, 2003). Once the mare has developed an attachment with the foal, it is difficult to get her to accept any other neonate (Tyler, 1972; Waring, 2003).

The foal may stand as soon as 30 minutes, usually after several failed attempts. As soon as it is steady on its feet, often within an hour after birth, the foal will start looking for its mother’s udder. The reciprocal mare-to-foal bond assures adequate parental investment to maintain close protection of the foal, to prevent spatial separation, and to provide for the needs of the foal (Waring, 2003). Such protectiveness is important since lost or orphaned foals are often attacked by other horses (Feist et al., 1976).

As the foals grow older the frequency and duration of the suckling sessions decreases, while they may leave their mother’s side temporarily to seek age-mates for play and mutual grooming (Waring, 2003). By five to seven months, over 75 percent of their nutrients may come from non-milk sources. Weaning usually occurs in the few weeks or days before the mare is to foal again. For example, in Camargue horses, most mares gave birth once a year,

and the foal was weaned 15 weeks before the arrival of its sibling, at the age of 7 to 8 months. With the subsequent parturition and arrival of a new foal, the mare's attention and social activity shift abruptly to the neonate (Waring, 2003). The mother starts to reject the sucking attempts of her offspring in relation to the date of birth of her next foal. (Feh, 2005). After weaning, the mare and her offspring maintain some degree of companionship that may last into adulthood or only until the offspring becomes sexually mature or departs from the original group (Tyler, 1972).

Non-offspring nursing is very rare in horses. Mares are typically intolerant or even behave aggressively with alien foals and other mares that approach her or their own young (Feist et al., 1976; Tyler, 1972). Previous young and strange foals are normally rejected by bite threats or kick feigning, but if the intruder persists, the mare is apt to kick or bite. Adoption or swapping of offspring are also uncommon or it is induced by human intervention in domestic horses (Tyler, 1972; Waring, 2003).

Heart rate variability

Heart rate variability (HRV) is the change in the time intervals between adjacent heartbeats (Shaffer, McCraty, & Zerr, 2014). Measurement of HRV is a non-invasive technique that can be used to investigate the function of the autonomic nervous system, especially the balance between sympathetic and vagal activity or sympathovagal balance (von Borell et al., 2007). Sympathovagal balance refers to a reciprocal functional relationship (Parati, Saul, Di Rienzo, & Mancina, 1995) implying that when one of the two components of the autonomic outflow (parasympathetic and sympathetic) is excited, the other is inhibited (Sleight & Bernardi, 1998).

Compared to the simple heart rate (HR) analysis, HRV analysis allows a much more accurate and detailed determination of the functional regulatory characteristics of the autonomic nervous system (Cerutti, Bianchi, & Mainardi, 1995).

HRV has been applied increasingly in veterinary and behavioral research to investigate changes in sympathovagal balance related to pathological conditions (Nolan et al., 1998; Pomfrett, Glover, Bollen, & Pollard, 2004), stress (Jong et al., 2000), management

practices (Francis, Diorio, Plotsky, & Meaney, 2002), training regimes (Cottin et al., 2005) as well as temperament and emotional states (Désiré, Veissier, Després, & Boissy, 2004; Visser et al., 2002) in a number of farm and companion animal species (von Borell et al., 2007).

OBJECTIVE

The objective of this study is to investigate the vocal recognition of the offspring by the mother in domestic horse (*Equus caballus*) through measures of physiological and behavioral responses of the mothers to own and alien foals' separation calls during playback experiments.

RESEARCH HYPOTHESIS

If mares are capable of vocally recognizing their own foals, then we will find differences in the physiological and behavioral variables during the presentation of the stimulus of the own foal compared with the stimulus of the alien foal.

METHODS

Location and conditions

The research experimental part was conducted at the Metropolitan Mounted Police Unit's (MMPU) facilities in Mexico City (Guelatao Avenue 100, Col. Alvaro Obregón, Del. Iztapalapa, Mexico City, Mexico). All horses were bred and housed under the same conditions and under constant veterinary supervision. The MMPU has a Reproduction and Maternity Unit with 40 stalls (4m x 4m each) for the mares and their foals. The stalls are bedded with straw and wood shavings and cleaned regularly. The foals remain in a single stall with their mothers until six months of age, after which the foal is weaned and introduced into the herd of youngsters.

Horses are fed twice a day with hay, alfalfa and commercial oat-based horse feed and have access to water *ad libitum*. All horses of Reproduction and Maternity Unit have access to a semi-open area (0.5 hectare) once a day, with a minimum of three hours depending on the weather. Here they can run, exercise, interact and establish bonds with other mares and their foals.

Ethics note

Throughout the study, animals were treated according to the ASAB/ABS (2016) Guidelines for the treatment of animals in behavioral research, and the guidelines of the Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, and according to the National Guide for the Production, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062-200-1999).

Animals

Eleven mares of different ages and their foals participated in this study in 2016, between September 6 and November 25. Ten of the mares were multiparous and one was primiparous

(Table 2). All foals born without complications and were developing properly according to the veterinary team of the MMPU. None of the animals showed any signs of illness or injury during the tests.

Table 2. Approximate age and number of foal of each tested mare.

Mare ID	Age	Parity
K26	7	2
K29	7	1
J11	8	2
J13	8	2
J52	8	2
I28	9	3
G26	11	6
F26	12	6
D14	14	8
D22	14	7
C3	15	7

Recording of vocalizations

To obtain the foal's vocalizations we separated the mares and their young for a short period when the foals were six weeks \pm three days old. The separation was performed by two experienced handlers whose were familiar to the horses. Handler 1 led only the mare out from the stall where she was housed with her foal (Stall A) into an empty one (Stall B) while Handler 2 held back the foal in Stall A. Then both experimenters left the stalls. There was always an empty stall between the Stall A and B (**Figure 2**). During the separation, foals and mares didn't have any visual contact to each other. The separation lasted 2 minutes. Then Handler 1 led back the mare into Stall A next to her foal. Veterinarians and trainers were always present in case of any complication.

During the two minutes of separation, vocalizations emitted by the foals and the mares were recorded with two unidirectional microphones (Sennheiser ME66, Wedemark, Germany; frequency range: 40 – 20 000 Hz) directed toward the stalls from the outside at an approximate distance of 1.5 meters and connected to a sound recorder (Tascam DR-40, Montebello CA, USA; 96 kHz / 24 bit), hence two channels of vocalizations were recorded at the same time. Recordings were saved in lossless WAV format and the stimulus was saved in lossless AIFF format.

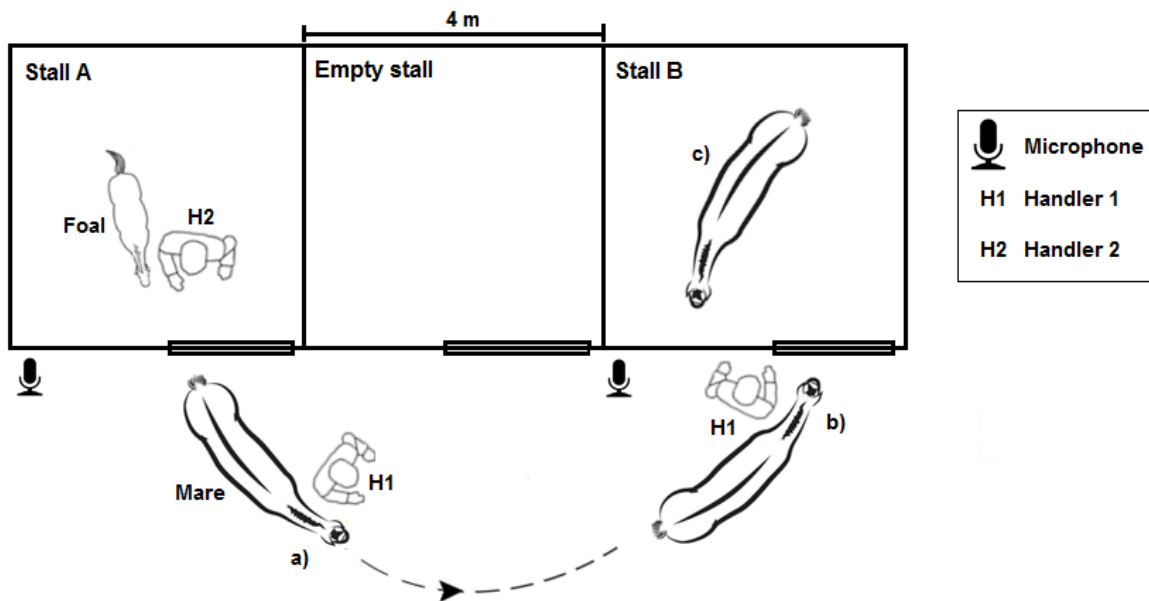


Figure 2. For the recording of vocalizations: **a.** Handler 1 led the mare out of the stall where she lived with her foal (Stall A) while Handler 2 held the foal in Stall A; **b.** Handler 1 led the mare into an empty stall (Stall B); **c.** Both experimenters left the stalls. Vocalizations were recorded with two unidirectional microphones directed toward the stalls

Stimulus edition

Stimulus edition was made in Audacity 2.1.2. First, the channel containing the foals' vocalizations were separated from the mares', then all the vocalizations that were emitted by the foals during the separation were isolated from the obtained audio file (**Figure 3/l**).

To control for the differences in the vocalization rate between foals, at least six clear, isolated vocalizations were randomly selected from each foal. All playbacks started with an audio marker (440 Hz "Beep") – in order to reliably register the start of the playback in the

video recording – and was followed by 300 seconds of silence (**Baseline**). After that, a sequence of the previously selected individual vocalizations (settled in a randomized order) from the same foal was added; individual calls were separated by five seconds of silence (**Stimulus**). Each vocalization was never repeated more than three times. The part of the playback with vocalizations lasted two minutes and contained 14 ± 2 individual calls. After that another 300 seconds of silence followed (**Final**) and then ended with a similar audio marker to the initial one (**Figure 3/II**).

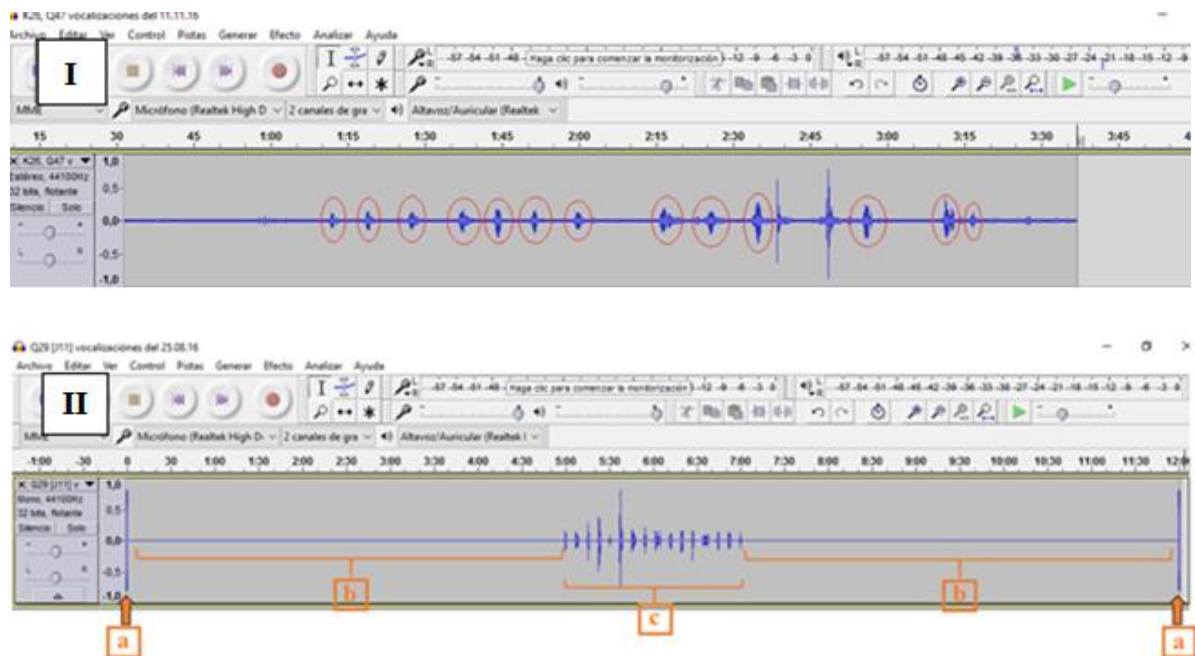


Figure 3. I. Example of the original audio file obtained during two minutes of mother-young separation. Individual vocalizations of foals are indicated with red circles, II. Playback structure seen from Audacity 2.1.2., a: one second marker (“beep”) at the beginning and the end of the playback, b: five minutes of silence (Baseline and Final), c: two minutes of vocalizations with five seconds of silence between each other (Stimulus).

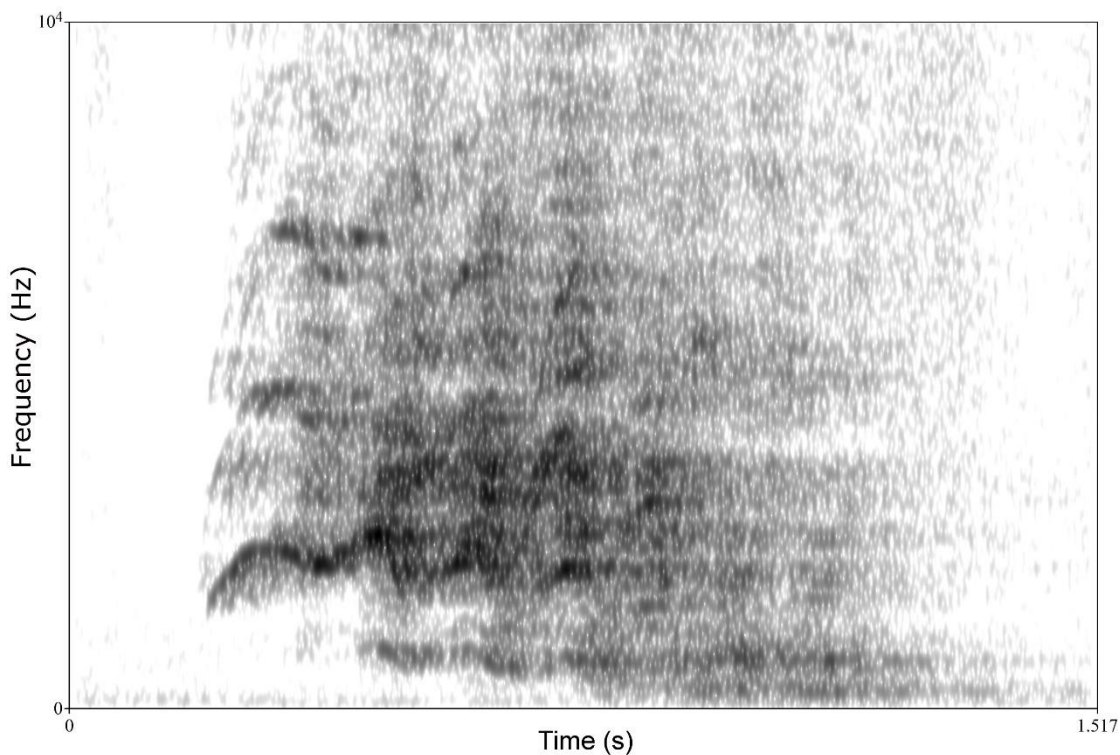


Figure 4. Spectrogram of a foal's individual vocalization. Frequency range 0–10 000 Hz, window length 0.01 s, dynamic range 50 dB.

Playback procedure

All testing took place between 10:00 to 14:00 in the mare's own stalls. Since mares become extremely agitated when separated from their foals at this age we performed the tests with the young staying with their mothers. Previous to the playback tests, we removed as much food as possible from the stalls.

Playbacks were performed between one to seven days after the vocalizations were recorded. Applying tests at 6 weeks postpartum ensured that mothers and foals had the opportunity and the time to learn each-other's calls before the tests. Sound pressure of each stimulus was normalized to 70 dB measured from one meter with a sound pressure meter (General DSM402SD, New York, NY USA) which corresponds to the average loudness measured 1 m from the mouth of a whinnying horse (Lemasson et al., 2009). Stimulus was reproduced through a wireless speaker (SoundLink® Mini, Bose Inc., Framingham MA, USA) connected to an iPod® 5th generation (Apple Inc. Cupertino CA, USA) placed outside of the stall, next to the access door.

Each mother was subjected to two types of playbacks: an **own foal** stimulus (with vocalizations recorded from its own foal – condition: own) and an **alien foal** stimulus (vocalizations recorded from a same sex, same age alien foal, condition: alien). All mares were tested with a different alien foal’s stimulus and the two playbacks were presented on the same day in a balanced order. The time between the two tests was at least one hour. Only one own and one alien playback per mare was performed to avoid habituation to the test. For this study, we considered the playback of the own foal’s calls as a stressful event for the tested mothers since the vocalizations were recorded during a period of mother-foal separation which can cause considerable distress to the mares (Pond, Darre, Scheifele, & Browning, 2010). Since our experimental design did not involve unknown enclosures or handling techniques for the horses, any potential stress unrelated to the playback of the stimulus was kept in minimum.

Behavioral and physiological recordings

In order to register the behavioral response of the mares to the playbacks all tests were recorded with a GoPro HERO 4 camera placed in a corner out of the stall (**Figure 5**). For the physiological changes heart rate variability measurements were carried out with a Polar Equine V800 Science (Polar USA) heart monitor. It was attached to the precordium area with an elastic band around the thoracic perimeter of the mare. The band was always wet with water and the electrode area was smeared with water-based ultrasound gel to improve the contact between the skin and the device (**Figure 6**).

Once the heart rate monitor and the camera were placed, the animals were left to habituate to the equipment for ten minutes before the playbacks began. During the playback, the experimenters, the handlers and the trainers who were present for security reasons stayed in approximately 20 meters away from the experimental area without directly facing the horses allowing stress free sampling.



Figure 5. Camera placed in a corner out of the stall to record behavioral responses. Stimulus was reproduced through a wireless speaker placed outside of the test stall, near the access door.



Figure 6. Heart monitor (in red circle) attached to the height of the precordium area with an elastic band around the thoracic perimeter of the mare.

Data analysis: Behavioral variables

Frequency, latency and duration of four behavioral variables (**Table 3**) were quantified with Solomon Coder software (Peter, 2015). These behaviors were selected based on literature on horse behavior (Waring, 2003; McGreevy, 2004) and by the obtained ethogram from two pilot tests. The number or durations of the behavioral variables produced during the experiment was normalized by the total duration of the part (Baseline = 300 s, Stimulus = 120 s, Final = 300 s) for comparability (see also below).

Table 3. Behavioral variables and their description

Behavioral variable	Description
Body contact	Mare's body is touching (or 25 cm near) to their foal's body (duration)
Alertness	Rigid stance with neck elevated and head oriented toward the object or animal of focus. The ears are held stiffly upright and forward and the nostrils may be slightly dilated (duration, McDonnell & Haviland, 1995).
Vocalization	To whinny or nicker (rate, Yeon, 2012).
Look at the foal	The mare turns her head in the direction of her foal (duration)

Data analysis: Heart Rate variability (HRV)

Heart rate analysis were carried out in this study in order to asses the emotional state of the mares when presented with the vocalizations of their own foal and vocalizations of an alien one. Four variables of HRV were analyzed from the measurements obtained with the Polar V800heart frequency monitor and visualized through Kubios HRV software (**Table 4**). Data from three animals had to be excluded from the heart rate variability analysis due to malfunctions of the equipment during at least one of the playbacks.

Table 4. HRV analyzed variables and their description. SNS: sympathetic nervous system, PNS: parasympathetic nervous system, HF: high frequency, LF: low frequency.

Variable	Description	Physiological value	Unit
Mean RR	Mean of RR (beat-to-beat) intervals	Represents the mean of all the RR intervals.	ms
StdRR	Standard deviation of RR (beat-to-beat) intervals	Indicator of the variability of the data, reflecting the long-term variability of the cardiac activity under influence by both SNS and PNS.	ms
RMSSD	Root mean square of successive beat-to-beat interval differences	Represents the short-term variation of RR intervals and it is useful to evaluate the vagal activity influence.	mss
FFT-LFHF	Fast Fourier Transformation: Frequency analysis with superior (HF) and inferior limit (LF)	Estimates the vagal (HF) and sympathetic influence (LF) and constitutes an indicator of sympathovagal equilibrium. An increase in the LF/HF ratio suggest a SNS predominance	s ² /Hz

Data treatment and statistical analysis

Because the Baseline, Stimulus and Final parts of the tests were not the same length (5-2-5 minutes) every variable of the behavioral measurements were normalized to represent the percent of total time (durations) or rate (frequencies) in that section. Non of the behavioral variables were normally distributed therefore were analyzed with Generalized Linear Mixed Models (GLMM) with Poisson error distribution with the 'log' link and with mothers' identity as a random factor. Models included two fixed factors: condition with two level (own or alien), part of the test with three levels (Baseline, Stimulus, Final) and their interaction.

All measured HRV variables were normally distributed hence were analyzed with General Linear Mixed Models (LMM) with mothers' identity as a random factor. Models included two fixed factors: condition with two level (own or alien) and part of the test with three levels (Baseline, Stimulus, Final) and their interaction.

All statistical analyses were done using the program R, version 3.3.1 (R Core Team, 2014), and all linear models were performed using the package *lme4* (Bates, Maechler, Bolker, & Walker, 2015). *P* values were extracted using Wald Chi-square (type II) tests. Post hoc comparisons following LMMs and GLMMs when both fixed factors had significant effects were made by pair-wise comparisons with Wilcoxon matched-pair tests.

RESULTS

Behavioral response of the mother

Rate of vocalization

Both the condition, the part of the test and their interaction had a significant effect on the vocalization rate of the mares (GLMM condition $\chi^2 = 149.7$, $p < 0.001$, part $\chi^2 = 2214$, $p < 0.001$, condition*part $\chi^2 = 410.5$, $p < 0.001$). In the Baseline and Final part of the tests we found no differences, but mares responded to their own foal's calls significantly higher frequency during the Stimulus (Figure 7).

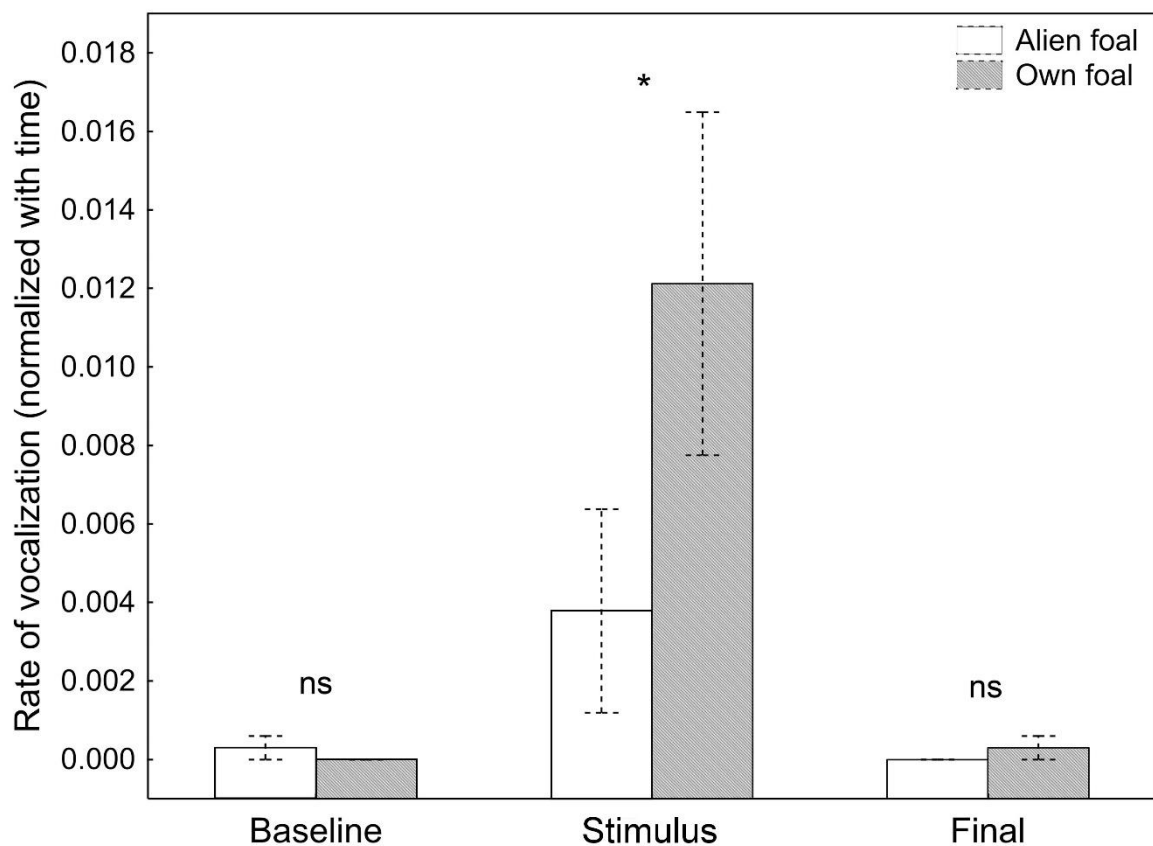


Figure 7. Rate of vocalization (normalized with time, \pm SE) during the playback of alien foal and own foal's separation calls. Asterisk mark significant differences in post-hoc comparisons.

Duration of alertness

Both conditions (alien or own foal's voice), the part of the test (Baseline, Stimulus, Final) and their interaction had a significant effect on the duration the mares showed alert behavior (GLMM condition $\chi^2 = 35.83$, $p < 0.001$, part $\chi^2 = 109.8$ $p < 0.001$, condition*part $\chi^2 = 40.64$, $p < 0.001$). In the Baseline and Final part of the tests we found no differences, but mares were alert significantly longer when presented with their own foal's calls (**Figure 8**).

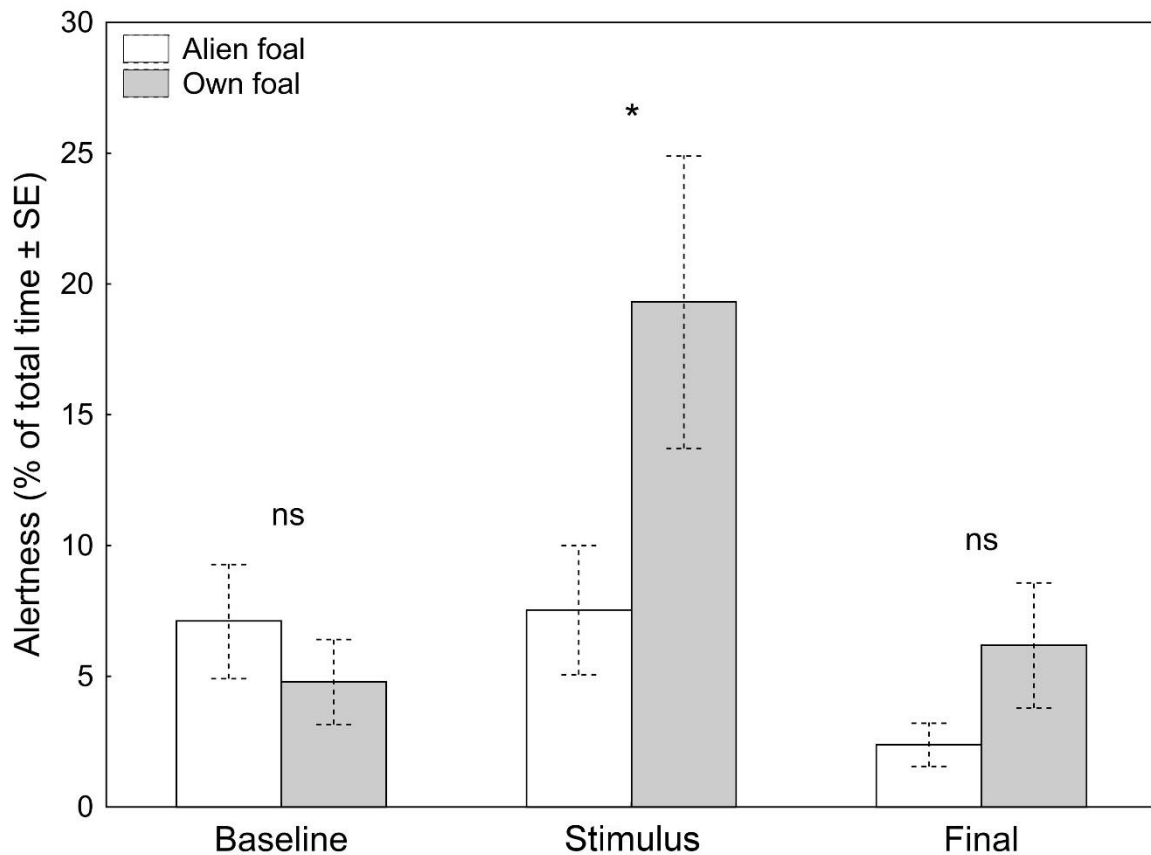


Figure 8. Percentage of total time of the mare in alert position (\pm SE) during the playback of alien foal and own foal's separation calls. Asterisk mark significant differences in post-hoc comparisons.

Looking at the foal

Only the part of the test had significant effect on the time mares looked at their foal (GLMM condition $\chi^2 = 1.18$, $p = 0.27$, part $\chi^2 = 11.29$, $p < 0.01$, condition*part $\chi^2 = 1.15$, $p = 0.56$). When hearing the playback, mares looked significantly more toward their young compared to the Baseline and Final part but there was no difference based on the type of the call (Figure 9).

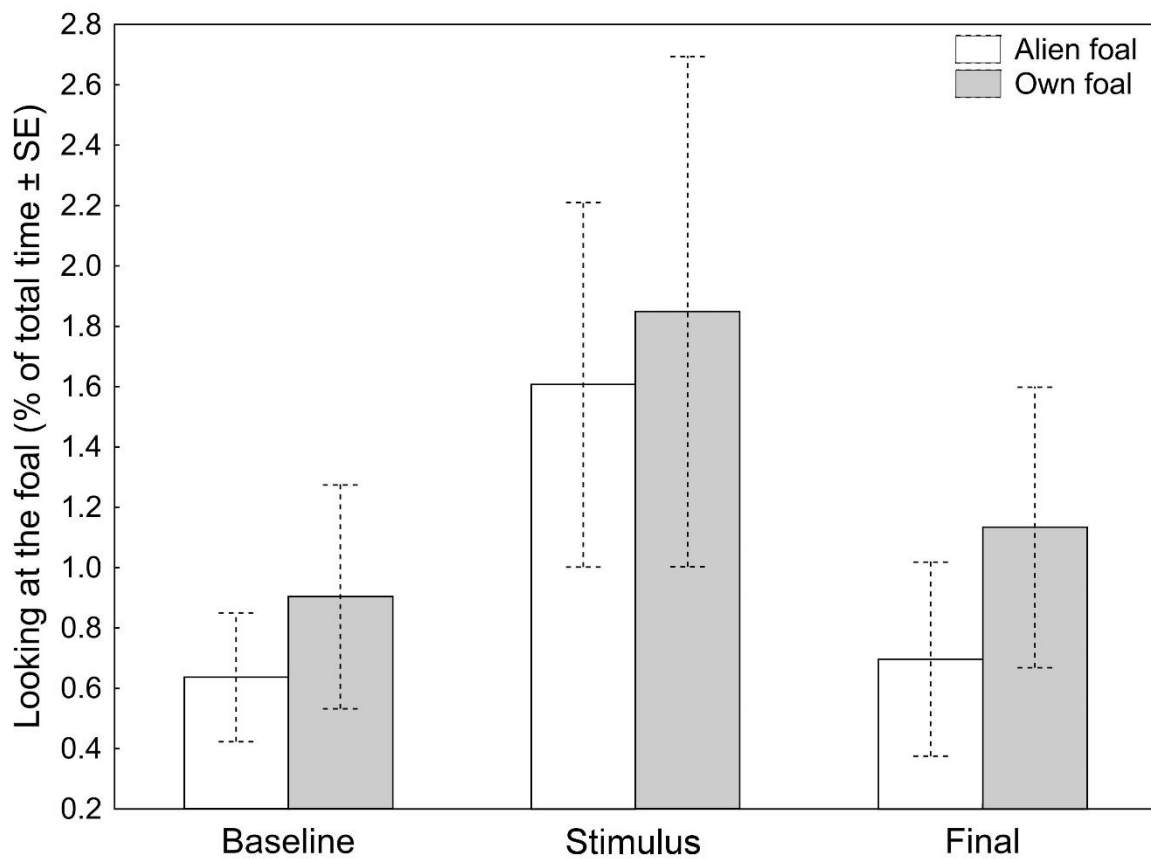


Figure 9. Percentage of time mares spent looking at their foal (\pm SE) during the playback of alien foal and own foal's separation calls.

Maintaining body contact

Although both conditions, part of the test and their interaction had a significant effect on the time the mares were in physical contact with their foals (GLMM condition $\chi^2 = 7.02$, $p < 0.01$, part $\chi^2 = 7.42$, $p < 0.05$, condition*part $\chi^2 = 9.68$, $p < 0.01$) post-hoc tests revealed no significant differences. This might be due to very slight but consistent effects which are too small for the Wilcoxon-test to reveal (Figure 10).

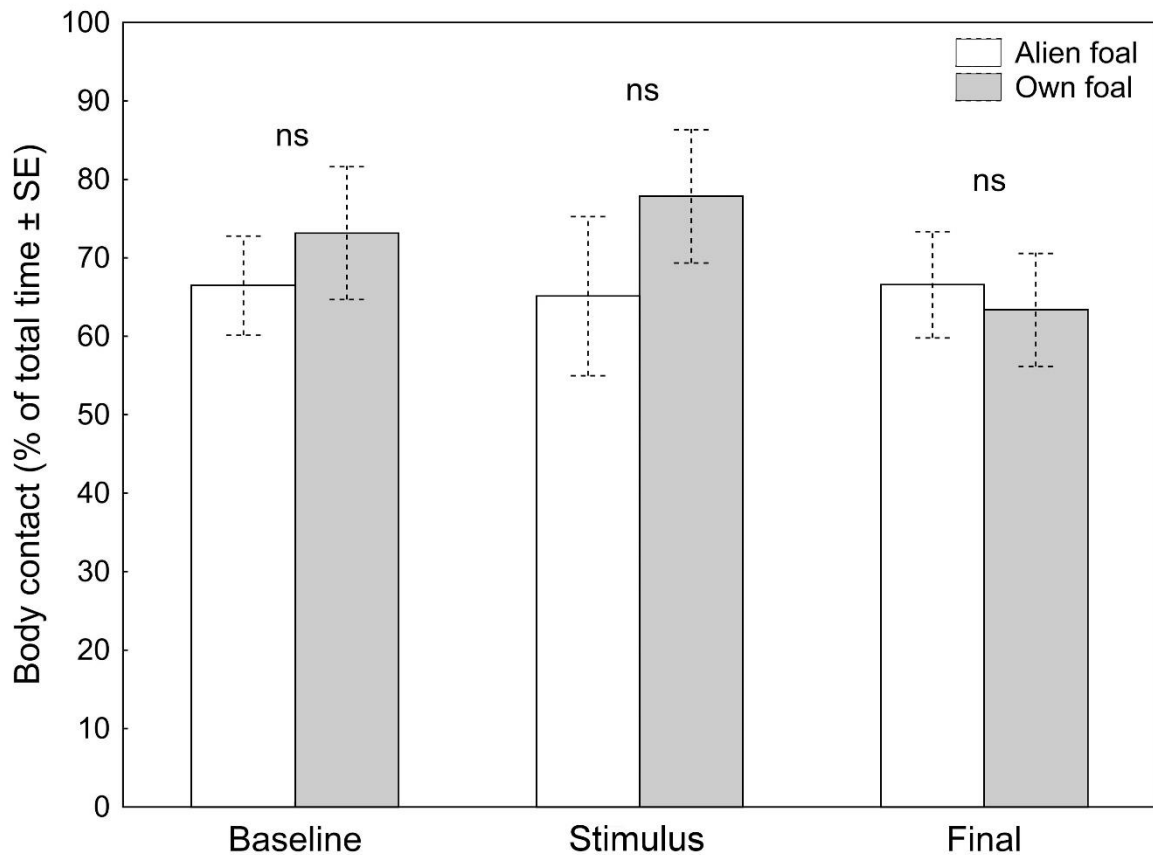


Figure 10. Percentage of time mares spent in contact with their foal (\pm SE) during the playback of alien foal and own foal's separation calls.

Physiological response of the mother: Heart Rate Variability Variables

Mean RR interval

Only the condition had significant effect on the mean RR interval of the mares during the tests (GLMM condition $\chi^2 = 6.47$, $p < 0.01$, part $\chi^2 = 0.51$, $p = 0.77$, condition*part $\chi^2 = 0.35$, $p = 0.84$). Which means that we could not find any effect of the stimuli on this variable (**Figure 11**). In both part mean RR intervals were higher in the own foal condition which might be due to stochastic error of the sample size (3 mares were excluded, see methods).

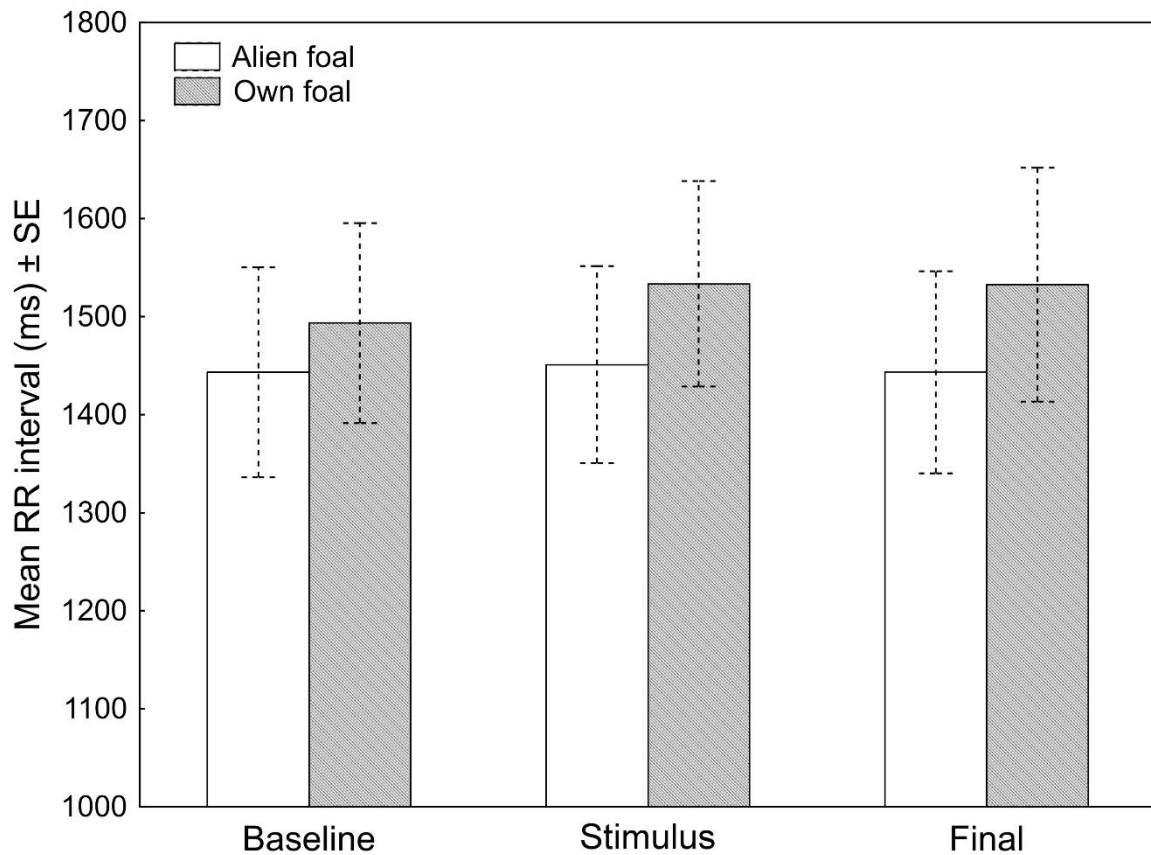


Figure 11. Mean RR intervals of the mares (\pm SE) during the playback of alien foal and own foal's separation calls.

Standard deviation of RR intervals

Any effect was found neither of part of the test nor of condition (GLMM condition $\chi^2 = 0.25$, $p = 0.62$, part $\chi^2 = 4.70$, $p = 0.09$, condition*part $\chi^2 = 4.03$, $p = 0.13$) in the standard deviation of the means of RR intervals (StdRR, **Figure 12**).

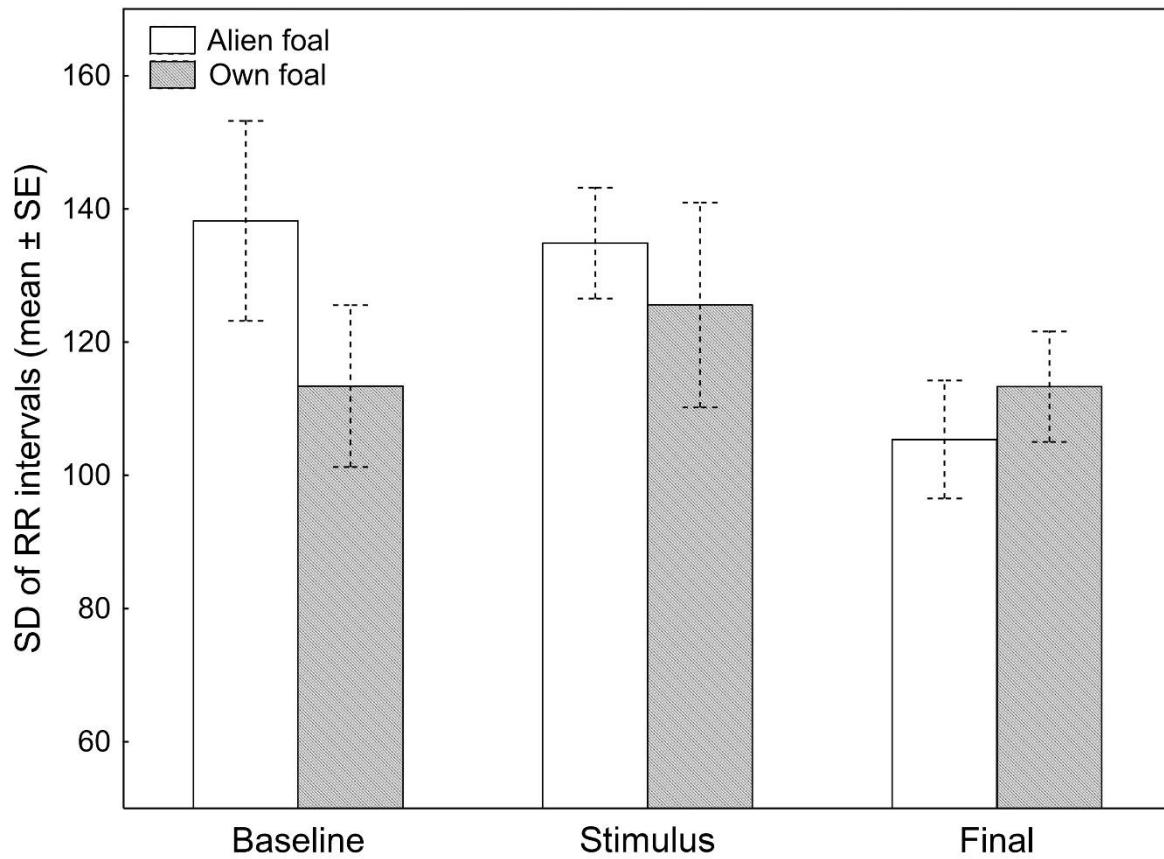


Figure 12. StdRR of the mares (\pm SE) during the playback of alien foal and own foal's separation calls.

RMSSD

We found significant effect of the part on the RMSSD (root mean square of successive beat-to-beat interval differences) but not the condition (GLMM condition $\chi^2 = 0.23$, $p = 0.62$, part $\chi^2 = 10.33$, $p < 0.01$, condition*part $\chi^2 = 0.94$, $p = 0.62$). Which means that mares responded to the vocal stimuli regardless of it was from an alien or own foal (**Figure 13**). As this HRV parameter appears to be different during the presentation of the vocal stimuli, independent whether it was from its own foal or an alien foal, the RMSSD seems to be a good indicator of stress or attention in horses but do not reflect different responses based on the type of stimuli, at least not with sample size this low.

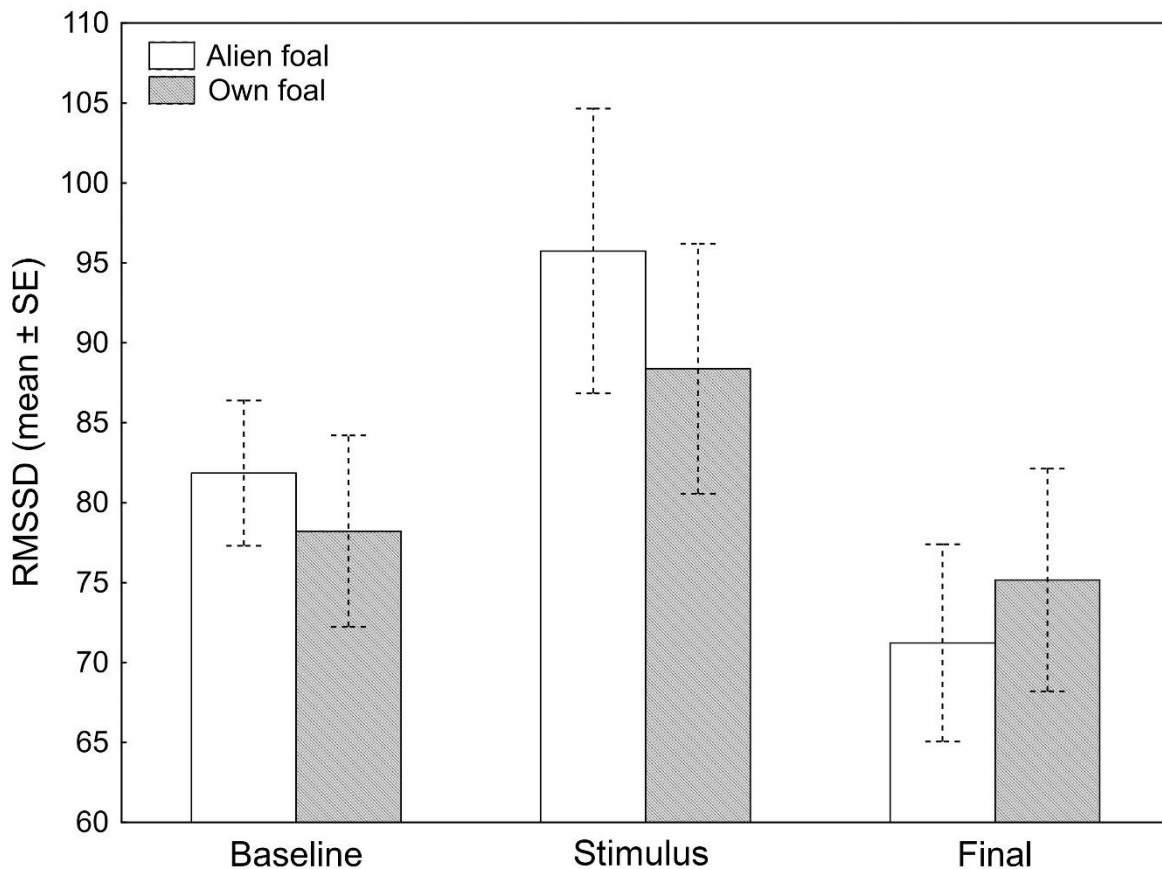


Figure 13. RMSSD mean values (\pm SE) during the playback of alien foal and own foal's separation calls.

Low frequency / High Frequency

None of the factors had significant effect on the LF/HF ratio of the mares during the tests (GLMM condition $\chi^2 = 0.16$, $p = 0.68$, part $\chi^2 = 3.96$, $p = 0.14$, condition*part $\chi^2 = 0.72$, $p = 0.69$). We found that the LF/HF ratio measurements were especially afflicted with measurement errors resulting in high variance (**Figure 14**).

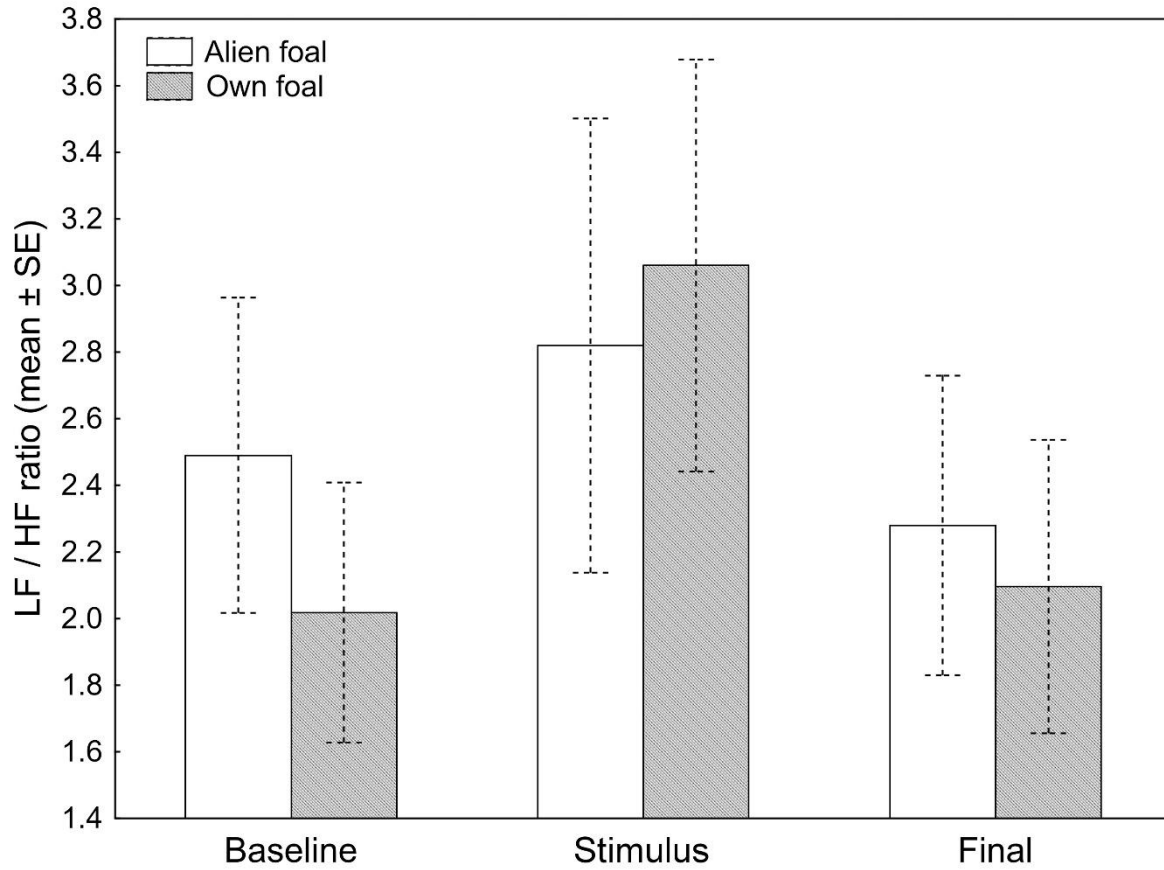


Figure 14. LF/HF ratios mean values (\pm SE) during the playback of alien foal and own foal's separation calls.

DISCUSSION

In this study, we measured behavioral and physiological (HRV) response of mares to playbacks of separation calls of their own and alien foals to investigate whether they can differentiate between them based solely on auditory cues. We found that, in several behavioral variables, mares reacted differently, indicating that they can distinguish between the two types of separation calls. We also found only one HRV measure (RMSSD) was elevated during the playback of the stimuli, but not differently to own and alien calls, which suggest a general stress response to the separation calls. Mares that participated in this study were allowed to establish social bonds with other mares and their offspring, constantly hearing vocalizations from other individuals as often as their own foal's. Thus, all alien calls were assumed to be familiar to the focal mares too.

A whinny is a call that is important for maintaining or regaining contact with affiliates or offspring, while a nicker is more commonly used by mares when she affirms the maternal-infant bond as her foal returns to her side (McGreevy, 2004). Taking this into consideration, we assumed mares would emit whinnies and/or nickers more often when they hear the own foal's separation calls. As expected, during the Baseline and Final parts, mares almost never vocalized, however they did during the playback of the stimuli. Additionally, they vocalized significantly more during the presentation of the own foal stimulus compared to the alien one. Since horses are large herbivores, but still prey species, they often communicate with low vocal signals while using other sensory modalities in order to avoid attracting predators (McGreevy, 2004). Loud calls like whinnies are used in stressful situations, such as the mother-foal separation which helps them to locate each other under emergency situations. Therefore, our results suggest the differential rate of response to the different type of vocalizations (own vs. alien) is a good and biologically relevant indicator of mother offspring recognition in the horse.

Wolski and collaborators in 1980 found similar results, mares tend to whinny more frequently to playbacks of whinnies of their own foals rather than alien's, although the difference was not statistically significant. However, they only used one vocalization from

each foal in the playback test, which gives the mare a poor chance to distinguish among a wider range of vocalizations of each foal. In our study, we tested the mares with several vocalizations obtained from the own foal and the alien foal. This allows the mare to have the opportunity to ensure the foal's identity based in many vocalizations and not just one.

Mares showed signs of alertness during the whole test (Baseline, Stimulus and Final parts), but it was significantly higher during the presentation of the vocalizations (Stimulus part), especially when the playback was from the own foal. Generally, as an initial response to a new environmental stimulus, a horse becomes alert and attempts to orient its sensory organs of the head towards the source of the stimulus. Recurring or minor sounds for example may cause only an ear to rotate while the horse continues its activities. Yet, often, stimuli are of sufficient intensity that the horse raises its head and investigates more extensively, while other activities, such as walking or chewing may cease (Waring, 2003). Our results show that vocalizations of the own foal induced a more intense/stronger state of alertness than similar vocalizations from an alien one.

The time mares turned their head towards their foal was longer during the Stimulus part compared to the Baseline and Final parts, however, there was no significant difference depend the own or the alien foal vocalization. Thereby, mares always checked their foals, independently of the origin of the calls. One possible explanation of the lack of difference is that a mare will turn its head toward her own foal if she hears its separation calls, but also will turn her head towards her own foal if the separation calls are from an alien foal to ensure her foal is not away as the alien foal that she is hearing. Another explanation might be that mares have to appeal to multi-modal individual recognition by both auditive and visual channels to guarantee it is not her foal who is vocalizing, (Proops et al., 2009; Yorzinski, 2017).

The mare's behavior of maintaining direct contact with the foal was also analyzed in this study given that it is considered to be related to the need of closeness during distress situations. However, no differences were found between the time mares spent in contact with the foal during the Stimulus, the Baseline and the Final parts nor comparing the presentation of the own and the alien foal stimuli. The fact that mares had been in body

contact with their foals at the Baseline, the Stimulus and the Final part equally can be explained by the age of the foal, in which it's still necessary for the young to remain close to the mother for protection (Waring, 2003).

In the HRV measures, we only found one variable, the RMSSD, that was elevated during the playback of the stimuli. It may reflect the possible stress response of the mares during the playback, but no differentially to own and alien calls.

The other HRV variables measured in this study did not show significant differences between the Baseline, the Stimulus, and the Final parts; nor for the own and the alien foal. This can be due to the practical difficulties that are often encountered when trying to document reliable measures of HRV in field like conditions on horses. Heart rate monitors have the benefit of not requiring invasive surgery, however, they also have inherent limitations and can often register false values. In this study, we had to exclude three mares out of eleven because at least in one of their tests the HRV recordings were heavily affected by artefacts. The spontaneous activities and movements of the animals, which we were not able to control for, could lead to a significant amount of false heart beat registrations. We also have noticed that the foals started to nibble the sensor on their mother's body several times. In some recording systems, the presence of artefacts caused by movements of electrodes on the skin or by muscle contractions can be detected and corrected automatically by software algorithms, nevertheless using such automatic correction tools must be done with special care and attention. Therefore, it is difficult to perform an appropriate identification and correction of errors that can considerably affect the interpretation of HRV. Furthermore, basal values of HRV in horses seem to have large interindividual variations and the exact origin of this differences is unknown but is likely due to a multitude of factors including genotype, behavior, temperament, and nutritional status. Clinical conditions and training regimes are also factors affecting HRV in horses (von Borell et al., 2007). Another possibility is that, as the process of domestication may affect animal behavior by reducing the responsiveness to environmental changes as an adaptation to living in a biologically "safe" environment (Price, 1999), the physiological response to stressing situations might have been affected as well.

Although the results obtained in this study suggest that mares are able to recognize the calls of their own foals and add to the compelling body of evidence that –in most social species of birds and mammals– mothers can recognize their offspring by voice, more studies on vocal recognition of the offspring by the mother needs to be done in horses. In order to assess the nature of this phenomenon and the ecological validity of our results, those studies might be performed under different conditions (e.g. during the time mares and foals spent together in the open area), as well as in wild living populations of horses, given that, in their natural habitat, animals have the opportunity to perform a much wider variety of responses (Seyfarth & Cheney, 2003). Additional experimental studies might also be useful to determine vocal features that are important for individual recognition in horses. Searby and Juventin (2003) studied the effectiveness of mutual acoustic recognition between mother and offspring on a follower species (sheep, *Ovis aries*). By spectrographic analysis of their vocal signatures they found that ewes and their lambs can recognize each other based on only some features of the calls. Moreover, longitudinal studies can be also useful to know if the response of the mothers to the calls of their foal reduces as it grows up to discern the most suitable time of weaning. On the other hand, as foals were present in the test stall during the playback of the stimulus, we cannot reject the possibility that mares' behavioral response had been a consequence of the behavioral changes of the foals during the test situation. Further studies in order to assess foal's behavior may be useful to confirm our hypothesis.

Horses develop on a complex social system that requires them to memorize social experiences, distinguish between familiar and unfamiliar individuals and even identify familiar horses and recognize their social status relative to their own group (Krueger & Heinze, 2008). They exhibit excellent long term memory of members of their bands (Waring, 2003), and also are able to retain learned categories and concepts for up to 10 years (Hanggi & Ingersoll, 2009). They also exhibit ability to recall things and have an adequate spatial short-term memory (Hanggi, 2010). Horses have proved successful on at least some quantity discrimination tasks as well (Uller & Lewis, 2009) and have been shown to remember people (particularly trainers who use positive reinforcement) and conspecifics for up to eight

months, even after contact with the individual had ceased (Hanggi & Ingersoll, 2009; Sankey, Richard-Yris, Leroy, Henry, & Hausberger, 2010).

The behavioral results obtained in this study show evidence for individual vocal recognition of the foal by its mother. This mechanism of individual recognition is considered cognitively demanding, especially to the receiver (the mares in our study) (Sheehan & Bergman, 2016), suggesting that there is a selection pressure on the mares to recognize their offspring even only by its voice because it is necessary to locate it when it is distant, out of view or during the night. By allowing a rapid and selective response by the mother to the alarm calls of her offspring may prevent the foal from being predated, injured by other mares or stallions or even from getting lost.

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