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Testing individual variations of horses' tactile reactivity: when, where, how?

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Abstract

Tactile perception is involved in a variety of contexts (adaptations to climatic conditions, protection of the body against external dangers...) and is as important as the other sensory modalities for the survival of an individual.

This tactile modality has been particularly well studied in humans, revealing high individual variations modulated by a variety of intrinsic and extrinsic factors such as age, sex, pathological disorders or temperament.

Tactility is also involved in animals' social lives, although there are disparities between species. For example, social tactile contact among horses is limited, but this does not mean that they do not react to tactile stimuli but rather with their very thin skin they are able to detect minute stimuli (although they respond more to larger stimuli). Despite a fairly large effort to characterize it, there are controversies concerning equine tactile sensitivity.

In this review, we examine studies that have used the same tool (Von Frey filaments) and try to disentangle what could explain the differences observed. It appears that many aspects are poorly known or controversial and that the procedures may be so different that the results of different studies cannot be compared. We went further by testing tactile reactivity of a population of unriden horses and found that four factors influenced their tactile reactivity (type of horse, filament size, body area, time of day). These results could explain some of the discrepancies observed in the literature and suggest, in particular, that more attention should be paid to the context of the test.

Keywords: perception, equids, Von Frey filaments, methodology

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1 Introduction

The tactile system is the first sensory system developing in mammals (e.g. functional 20-24 weeks after conception in humans, (André et al. 2017)). The skin is innervated by a wide variety of sensory receptors, including nociceptors, which perceive painful stimuli; pruriceptors, which transmit itching information; thermoreceptors, for temperature information; and low-threshold mechanoreceptors, which encode non-painful mechanical stimuli, or touch (Zimmerman et al. 2014). The nerve endings of these receptors convert these stimuli into nerve impulses (Jenkins and Lumpkin 2017). The signal is then sent to the brain by different fibers, classified by diameter, speed of conduction of the impulse, and whether or not they are covered with myelin (Jenkins and Lumpkin 2017). For the sense of touch, the vast majority of information is carried to the brain by large diameter, myelin-covered fibers, conducting the impulse at high speed and carrying information about the type of the tactile stimulus experienced (Jenkins and Lumpkin 2017).

Touch allows subjects to perceive, understand and respond to the external world. As with other sensory modalities, tactile perception belongs to the "Umwelt" (perceptual and effector worlds) of species (von Uexkuell 1965). Tactile perception is ubiquitous in the daily lives of most animal species and plays a major role in determining experience and behavior (e.g. Bremner and Spence 2017). Furthermore, touch allows an individual to encode and recognize objects and their characteristics through the active contact of skin with the object. This is known as haptic touch. This function is particularly relevant in the context of transmitting the social aspects of touch, which in turn may lead to strong emotional responses, especially in early life (Bremner and Spence 2017; Field 2001).

Human tactile perception has been particularly well studied, revealing that beyond species-specific characteristics, individual variations are high and modulated by a variety of intrinsic and extrinsic factors such as age, sex, pathological disorders, temperament or conditions of development. A study has shown sex and age effects in 70 subjects aged 20 to 88 years, tested on both hands using von Frey filaments: skin thresholds increased significantly with age for older women but not for men (Bowden and McNulty 2013). Children with autism spectrum disorders (ASD) commonly present atypical sensory sensitivities (e.g. hypo- or hyper-sensitivity) with varying degrees of severity (Ben-Sasson et al. 2008). These results raise the question of whether individual variations of sensory responsiveness actually reflect differences in perception or differences in responsiveness (i.e. perceiving a stimulus but not responding to it with a particular behavior). The question of the effects of experience, and the possible habituation or sensitization effect of repeated tactile stimulations is also important. Thus, pre-term infants tested at term-age showed a greater tactile sensitivity compared to full-term neonates (André et al. 2020). One hypothesis is that these preterm infants had been exposed to repeated tactile stimulations at that time (e.g. repeated venipunctures) (Gibbins et al 2008) which may have led to sensitization.

Several tools exist to assess tactile reactivity, the best known being the cotton swab test, the static Weber test, the dynamic Weber test (Sapa et al. 2019) and also the lancet test for newborns (Gibbins et al. 2008). Nevertheless, most of the research conducted with these methods was for therapeutic purposes and not for increasing knowledge of the fundamentals of tactile reactivity (e.g. Gibbins et al. 2008; Bigorre et al. 2017). In addition, the body areas tested with these tools are often the same, i.e. hands (humans, e.g. Dellon 1986; Bigorre et al. 2017) and the force applied is not standardized. Among the different tools used, one seems to stand out thanks to its numerous advantages: the von Frey filaments. Indeed, these filaments can be applied to any species, without age limit, on any part of an individual's body with a constant and standardized pressure. Initially, horse hair was used to test the perception of human skin, i.e. the response to tactile stimulation to this hair application. Von Frey (1894) adapted this method by developing a set of calibrated filaments consisting of a rigid plastic body extended by a nylon thread of variable thickness. When the end of the filament, of a given length and diameter, is applied perpendicularly on the skin, the application force increases as long as the experimenter continues the pressing motion, until the filament flexes. Continuing the pressing motion increases the bending but not the application force. This principle therefore allows a known, measurable and reproducible force to be applied to the skin surface. Von Frey filaments were initially developed for humans to define tactile perception thresholds (Bell-Krotoski et al. 1995). A nylon filament delivering an average force of 0.07g was found to be a good predictor of the "normal" pressure threshold of light touch on hands and on a large part of the body of adult humans (Bell-Krotoski et al. 1995). This tool has become widely used both for human, i.e. medicine (Jerosch-Herold 2005) and preclinical neuroscience (Le Bars et al. 2001), often to assess pain with the hope of therapeutic translation (Mogil 2009) but also for animal (Norrrell et al. 1999 e.g. sheep: Reddy et al. 2018; rodents: Bonin et al. 2014; dogs: Brydges et al. 2012; horses: Redua et al. 2002) research.

Part I: WHAT DO WE KNOW ABOUT TACTILE PERCEPTION IN HORSES? A critical review

1. The role of tactile stimulations in animals' lives

The tactile sense is just as important for animals as sight, hearing, smell or taste, because an individual totally devoid of sensitivity would have no chance of surviving (Jenkins 1948). Tactile sensitivity allows individuals to adapt to climatic conditions (Jenkins 1948). Indeed, thermal behavior is entirely dependent on a subject's ability to detect changes in its internal (i.e., body) and external environment through the detection of changes in skin temperature and humidity (Filingeri 2016). In addition, tactile sensitivity also protects the body from external injuries and hazards (Jenkins 1948).

Tactile contact is an important part of the social life in most primate species, where it is thought to play a major role from an early age (Field 2010; Suomi et al. 1973; Harlow and Suomi 1974).

Early deprivation of physical contact (rhesus monkey: Suomi et al. 1973; rats: Davis et al. 2017) leads to impaired social and cognitive development and can be detrimental to survival as well as reproduction. Its role can vary from transferring information, developing and maintaining relationships, or alleviating an uncomfortable state (Dudzinski et al. 2019).

However, there are large disparities among both wild and domestic species, with some species presenting a large amount of tactile contact during social interactions (e.g. dogs: Siniscalchi et al. 2018; cows: Sato et al. 1993; elephants: Langbauer 2000) while others limit them to parent/juvenile interactions and very occasional allogrooming sequences (e.g. zebras: Joubert 1972; sheep and goats: Hall 1983). This is also true for horses that have less social contacts than other mammals, and spatial proximity is then the best indicator of social affinity (Waring 2003). Mutual grooming accounts for only 2-3% of a horses' time budget (Boyd 1988) and contacts are limited to specific body parts, particularly withers and neck (Feh and de Mazières 1993). Tactile contacts among conspecifics are the most frequent during early development, when mares lick actively their foal (part of the recognition process and bonding between mare and foal). Nevertheless, Diehl et al. (2002) revealed that the duration of licking by a mare after giving birth does not exceed 15 minutes during the first 4 hours after foaling and that the rate of licking decreases rapidly over time.

Horses' skin is very thin over the majority of their body and encodes information concerning pressure, temperature, and touch, allowing detection of very fine tactile stimuli (review in Hausberger and Henry 2018). For example, Lansade et al.'s (2008) reported that 73% of the horses reacted with skin tremors to the application of a 0.008 g von Frey filament to the base of their withers, whereas none of the adult human subjects tested at the pad of their index finger perceived it. In a natural environment, slight tactile stimulations can therefore be perceived by horses, allowing them to adapt to their environment and take appropriate measures, as for instance to protect themselves from biting flies that are both a health hazard and a cause of emotional distress (Saslow 2002). Thus, the presence of flies in the environment influences horses' feeding habits (Mayes and Duncan 1986), as well as their habitat choice (Duncan and Cowtan 1980) and size of their social group (Duncan and Vigne 1979). On the other hand, in domestic situations, human-horse interactions involve many tactile contacts, from grooming (i.e., cleaning), to saddling, to hand and leg contact during riding that can vary in quantity and quality depending on the audience, type of training, and work. Therefore, despite their strong ability to respond to subtle stimuli, domestic horses are subjected to a wide range of human tactile stimuli, sometimes from an early age. Although early handling is thought to decrease fear of humans in a variety of domestic species (e.g. calves: Boivin et al. 1992; piglets : Tallet et al. 2005), results from studies of early handling of foals indicate instead that this may induce fear and discomfort and have long-term detrimental effects on their social skills and emotional expression (Henry et al. 2006; Durier et al. 2012). Interestingly, neonatal manipulations induce different emotional reactions depending on the side of the body handled, with more lasting effects when the manipulation is done on the right side (de Boyer des Roches et al. 2011). Observations at later stages of development show that the horses' perception of human contact remains ambiguous. Although mimicking allogrooming patterns by scratching the withers area results in a decrease in heart rate, supposedly indicating relaxation (Feh and de Mazières 1993), this procedure does not seem to be sufficiently motivating to be a primary reinforcer in a learning task or to reduce individual variations in the perception of human touch (Sankey et al. 2010; Rochais et al. 2014). Human contact during grooming appears to induce more negative than positive emotions as Lansade et al. (2019) reported that only 5% of 69 horses showed approach or "relaxation" behavior while 21% expressed avoidance and threatening behaviors. Human perception of what horses like and dislike in terms of tactile contact leads to practices that may be more or less appropriate from a horse's point of view (Hausberger et al. 2021). On the one hand, although the majority of professionals use negative reinforcement, tactile contact, whether petting, patting the neck, or scratching the withers, is widely considered as

a positive reinforcement by trainers and is used as such by some of them (e.g. Warren-Smith and McGreevy 2015). On the other hand, tactile actions such as leg contact are considered negative reinforcement with the difficulty of expecting horses to respond to "subtle" leg actions, but overlook potentially inappropriate tactile actions of inexperienced or disabled riders (Warren-Smith and McGreevy 2015). Most current training practices involve so-called "desensitization" (in fact, habituation) procedures that aim to reduce horses' responsiveness to tactile stimuli (e.g. Spier et al. 2004). Moreover, the possible impact of inappropriate handling/contact actions on the emotional state of horses involved in equine-assisted interventions has been questioned in recent studies (Contalbrigo et al. 2021; Lerch et al. in prep.). Horses therefore appear to be a very interesting animal model, providing an opportunity to study the impact of intrinsic factors such as sex or breed, extrinsic factors such as season and the interplay between genetic and environmental factors on their tactile reactivity (e.g. Hausberger et al. 2004).

2. Horses' tactile responsiveness: the impact of intrinsic and extrinsic factors

A review of the literature was then carried out of tactile reactivity in equids and more precisely of studies using von Frey filaments because they are widely used and present many advantages as mentioned above. A search based on the following keywords: tactile reactivity and equids gave about 100 scientific studies, 17 of which used von Frey filaments. Studies for which only the abstract was found online were not considered. These 17 studies involved horses or ponies, and one focused on the tactile reactivity of donkeys (Gonzalez-De Cara et al. 2017). The primary focus studies were not necessarily tactile reactivity per se, thus, they could also fall under a broader rationale such as learning (e.g. Valenchon et al. 2017), temperament (e.g. Lansade et al. 2008), or welfare state (e.g. Fureix et al. 2012). Here we list first the available information concerning possible factors influencing equids' tactile perception and then try to identify reasons for the discrepancies observed between studies.

2.1. Intrinsic factors

- Sex

Differences according to sex have been reported for dogs, females' tactile reactivity thresholds are higher than those of males (Sanchis-Mora et al. 2017). Data concerning horses are contradictory. Hausberger et al. (2012), studying play of adult horses, noted that geldings were more reactive (lower reactivity threshold) than mares to von Frey filaments whereas other studies did not find any difference (Vidament et al. 2021; Veres-Nyéki et al. 2021; Briefer Freymond et al. 2019). Other studies tested only mares (Lansade et al. 2008; Lansade et al. 2017; Lansade and Simon 2010; Valenchon et al. 2013a,b,c; Valenchon et al. 2017) while one study did not mention the sex of the subjects (Lansade et al. 2016). The remaining studies did not mention whether or not they tested possible sex-related differences of tactile reactivity, despite testing both mares and geldings (Fureix et al. 2012; Lansade et al. 2012; Lansade et al. 2013; Lansade et al. 2014; Gonzalez-De Cara et al. 2017). It is difficult to draw conclusions at this point, given the few studies that have investigated this factor. Moreover, the discrepancies between studies with conflicting results might also be explained by other methodological differences as well: location (chest, Hausberger et al. 2012; withers, Briefer Freymond et al. 2019 and Vidament et al. 2021; head, Veres-Nyéki et al. 2021) or context (home environment vs. unfamiliar stall on competition ground) of testing.

- Age

Several studies tested only equids of a given age (Lansade et al. 2012; Lansade et al. 2013; Lansade et al. 2014) but for those using animals of different ages, results are mostly absent (not tested or not mentioned: Briefer-Freymond et al. 2019, Fureix et al. 2012, Gonzalez-De Cara et al. 2017; Hausberger et al. 2012; Lansade et al. 2008; 2016, 2017; Lansade and Simon 2010; Valenchon et al. 2013a,b,c; 2017). However, the two studies investigating effect of age converge to suggest a decrease in tactile reactivity with age. According to Saslow's review (2002), older horses (over 20 years old) would have a lower tactile reactivity. Veres-Nyeki et al.'s (2021) results suggest that tactile reactivity decreases with age and Vidament et al. (2021) found that 2-year-old horses were more reactive than 3-year-olds.

However, the Vidament et al. (2021) study does not mention a subsequent correlation with age despite Vere-Nyeki et al.'s (2021) observation that tactile reactivity decreases with each year due to a decrease in innervation, as found in dogs (Sanchis-Mora et al. 2017) whose nervous system is thought to be immature up to three years old. It is possible that beyond age itself, the increasing experience of horses with tactile stimuli along with age during riding or grooming sessions may also lead to greater habituation, and thus less reactivity. Further studies are clearly needed to disentangle the effects of maturation and experience.

Study samples included either horses of a single breed (e.g. Lansade et al. 2008, 2012, 2013, 2014, 2017, Lansade and Simon, 2010; Valenchon et al. 2013a, b, c, 2017; Veres-Nyeki et al. 2021), of a predominant breed (e.g. Fureix et al. 2012, Hausberger et al. 2012; Lansade et al. 2016) or horses of various breeds, including unregistered horses, but not tested for possible breed effects (e.g. Briefer-Freymond et al. 2019). Only two studies mention testing potential genetic effects. Vidament et al. (2021) tested the tactile reactivity of a large sample of 1345 young equids from 67 different breeds. They only found that one breed, Anglo-Arabian horses, had higher tactile reactivity than all other equids. Most differences observed were between "equine types": sport horses were more reactive than sport ponies and both were more reactive than recreational ponies. This seems to confirm earlier results from the same group, showing that Merens stallions (a leisure breed) were less responsive than sport stallions to tactile stimulation (Vidament et al. 2015). A few studies have described behavioral differences between horses and ponies, suggesting an interesting line of research (Lerch et al. 2021; Schrimpf et al. 2020; Henriksson et al. 2019). Differences related to type of activity seem to be more important than differences related to breed; this is particularly interesting and would merit follow-up studies, as different types of work may involve different quantities of tactile contact on different body parts.

173 - *Temperament*

A classical definition of temperament is that of Goldsmith et al. (1987), "a set of individual differences in behavioral patterns, called 'traits' or 'dimensions,' that are relatively stable across various types of situations and over time". Humans' tactile reactivity has been shown to be lower for introverts than for extroverts (Schaefer et al. 2012). No link has been shown between horses' or donkeys' sensory sensitivity and their emotional responses in temperament tests (horses: Lansade et al. 2008; Valenchon et al. 2013 a,b,c; Vidament et al. 2021; donkeys: Gonzalez de Cara et al. 2017). However, because of the stability over time (at 5-month intervals) of individual differences in tactile reactivity to the same stimuli, Lansade et al. (2008) proposed tactile reactivity as a temperament trait, in agreement with some authors working on humans (reactivity threshold: Plomin 1983; sensory threshold: Thomas and Chess 1977).

Until now, tactile reactivity has never been described as a temperamental trait in different species, including humans (Eysenck 1991). Thus, the question of whether sensory reactivity can be considered a temperamental trait remains open because many behaviors can remain stable over time (e.g., stereotypic behaviors) without being labeled as temperamental traits.

187 - *Internal state: tactile reactivity and acute/chronic stress*

Valenchon et al. (2013a,b,c; 2017) subjected Welsh pony mares to tests of emotionality using different stimuli (i.e. sounds: barking, bell ringing, sudden movement: opening an umbrella, introduction of a novel object in the stall) after each individual had undergone temperament tests, including the application of von Frey filaments to the base of the withers. They found no relationships between estimated stress levels and tactile reactivity. Furthermore, Lansade et al. (2012) found no differences in tactile reactivity between an experimental group of Welsh ponies socially isolated for 11 days and a control group remaining under social conditions.

Other studies have examined the relationships between chronic stress and tactile reactivity. Stereotypic behaviors are one expression of chronic stress (Mason 1991). Briefer-Freymond et al. (2019) compared the tactile reactivity of 38 horses, half of them presenting an oral stereotypic behavior (cribbing), the other half presenting no cribbing (as reported by owners). They found that the cribbing horses were more reactive to von Frey filaments and suggested that this higher tactile sensitivity may be due to altered dopamine or endogenous opioid physiology resulting from chronic stress exposure. However, there were biases that prevent definite conclusions: an owner's recognition of welfare expressions may not be entirely reliable (Lesimple and Hausberger 2014; Hausberger et al. 2021) and the non-cribbing horses may have exhibited other expressions of compromised welfare (e.g. other types of stereotypic behaviors... e.g. Lesimple 2020), e.g. depressive-like states that are associated with lower sensory reactivity, in particular tactile reactivity (Fureix et al. 2012, 2015)

This is consistent with field reports that apathetic horses show less responsiveness to human approach and contact, and overall less sensory responsiveness to environmental stimuli (Burn et al. 2010; Pritchard et al. 2005; Popescu and Diugan, 2013). However, again, horses may have expressed other forms of compromised welfare that are not mentioned. However, Hausberger et al. (2012) found that, adult horses that played when released into paddocks were characterized by both higher levels and different expressions of compromised welfare (oxidative stress, back

disorders, aggressiveness, stereotypic behaviors...) and a lower tactile reactivity (measured by von Frey filaments applied to the chest) as compared to non-playing adult horses.

Therefore, again, it is not possible to draw a direct conclusion concerning relationships between welfare state and tactile reactivity. However, these first studies are promising, particularly because they suggest potential links between expressions of compromised welfare and tactile reactivity rather than an effect of welfare state *per se*.

2.2 Extrinsic factors

- Living conditions

Lansade et al. (2014) compared tactile reactivity (von Frey filaments at base of withers) between two groups of yearlings that had spent 12 weeks either in "enriched" or "conventional" (i.e., impoverished) housing conditions. Horses in the "conventional group" lived in small individual stalls on wood shavings bedding, had access to a paddock three times a week, and had limited access to roughage while horses in the enriched group were housed in large individual stalls on straw bedding 7 hours a day and spent the rest of the time together in groups on pasture. Twice a day (morning and evening), horses were offered randomly one of two different meals: a meal composed of either 500g oat bran with 80g carrots, 70g apples and 50g alfalfa horse treats or 500g concentrated pellets flavored each time with an additive (randomly chosen between garlic, fenugreek, cumin, banana, cherry, or oregano).

They were also exposed to a variety of objects, odors and music daily. The authors found that the tactile sensitivity of the "enriched" group (i.e., with more appropriate living conditions) was lower than that of the control group (i.e., with impoverished living conditions). Many studies are therefore not entirely comparable, as the living conditions of the subjects outside tests can be very different; this factor must be considered when making comparisons. For example, Vidament et al. (2021) tested horses in the context of shows, but gave no information about their usual living conditions.

- Working conditions

Although no studies have focused on the potential impact of working activity on tactile reactivity, Vidament et al. (2021), as mentioned above, found differences between sport and recreational ponies. While these authors favor the idea of possible selection, i.e. a genetic process, it cannot be ruled out that activity *per se* can influence tactile reactivity, for example, by increasing habituation or sensitization at certain body parts (Lerch et al. in prep.). According to Vidament et al. (2015), horses ridden by lower level riders (beginners to moderately advanced) have lower tactile reactivity than those ridden by experienced riders (first competition level and above). Of course, it is again difficult to disentangle whether the horses were selected for such characteristics in the first place or whether repeated inappropriate handling or tackling by beginners while the horses were told not to respond may have resulted in habituation or response inhibition.

3. A limiting factor: methodology

In addition to a lack of information on intrinsic and extrinsic factors in the different studies on equine tactile reactivity, we also noted considerable methodological discrepancies, despite the use of a common tool (von Frey filaments) and a common indicator of reactivity (skin tremor, except Veres-Nyeky et al. 2021 who focused on muscle contraction). Nevertheless, this is true for other sensory modalities as well as is the case for knowledge dealing with the perceptual world of newborns where some factors are not mentioned or they are not considered (such as gender or arousal state) (André et al. 2017).

3.1 Context of testing

- Environmental conditions

The hygroscopic properties of monofilaments must be considered for reliable use in sensory testing. Indeed, authors have shown that 1% increase in relative humidity corresponds to a relative decrease of 1-4% in numerical bending strength depending on the monofilament diameter (Werner et al. 2011). Similarly, changes in relative humidity were found to affect filament sizing significantly, while changes in temperature had a smaller effect (Andrews 1993). These results indicate that the environmental conditions in which von Frey filaments are used must be considered.

Briefer-Freymond et al. (2019) reported that location (indoors/outdoors) influenced tactile reactivity but they do not provide data concerning the effects of location. This is interesting because most studies do not specifically

mention the location of their test. For some, the horses were tested in their familiar stall (Fureix et al. 2012, Hausberger et al. 2012), in their familiar stall or in a paddock (Briefer-Freymond et al. 2019), in an unfamiliar arena (without specifying whether it is indoors or outdoors) (Gonzalez-De Cara et al. 2017; Lansade et al. 2013, 2014), in their stall or in a mobile stall (without specifying familiarity with location) (Valenchon et al. 2013a, b, c, 2017; Lansade et al. 2008), in an arena (without specifying familiarity with this location or whether it is indoors or outdoors) (Lansade et al. 2012, 2017, Lansade and Simon 2010), in an outdoor arena (without specifying familiarity with this location) (Lansade et al. 2016) while others do not provide any information concerning the test location (Verez-Nieki et al. 2021; Vidament et al. 2020).

Distractions are used clinically to relieve humans of pain (Wismeijer and Vingerhoets 2005). Thus, the presence or absence of distractors in the environment during tactile reactivity tests could potentially alter the response of horses during filament application. The presence or absence of conspecifics may also have an influence, as it has been suggested that paired mice show empathy in response to observing pain-related distress in their cage mate (Langford et al. 2006).

Similarly, Vidament et al. (2021) studying young horses at conformation shows in 67 locations in France found that 23 of the 28 behavioral traits tested, including tactile reactivity, were significantly influenced by the show location where the tests were conducted. Therefore, there is a need to standardize test conditions for comparative studies.

- *Season*

In addition to increase in hair density, horses that gradually acclimatize to the cold have a different distribution of fat tissue that, instead of accumulating on certain parts of the body, is spread over the entire surface, allowing better insulation (Cymbaluk 1994). One could therefore expect differences in reactivity according to season.

However, Lansade et al. (2008) do not mention any differences according to season when they tested the same ponies at two periods (spring and fall) 5 months apart, but found a positive correlation between an individual's responses between the two test periods. Most of the other studies (13 out of 17) do not mention the season when the horses were tested, except two that do mention this information, but then tests were performed over a very broad period without discriminating the results according to season (Briefer-Freymond et al. 2019: September to February; Fureix et al. 2012: January to June).

Again, it is difficult to draw any conclusion concerning the impact of the test period on tactile reactivity, as these studies did not test this or specify the period when the horses were tested.

- *Handling conditions*

Although no report mentioned a possible impact of horses' handling conditions on tactile reactivity, a few studies did not mention how the horse was held during the test (Briefer-Freymond et al. 2019; Fureix et al. 2012; Lansade et al. 2012, 2017). Reports providing this information show that horses can be tied up to the wall (Lansade et al. 2008; Vidament et al. 2021), held by another person (Lansade et al. 2013; Lansade et al. 2016; Lansade and Simon 2010; Valenchon et al. 2013 a,b,c; 2017, Veres-Nieki et al. 2021), or free moving (Lansade et al. 2016: for the "simplified personality tests"). The way horses are held (loose or tight lunge for example) might influence a horse's emotional or attentional state, hence its sensory reactivity. This may add to the determinants of discrepancies between studies.

3.2 The tool: filaments' size

As mentioned in the introduction, von Frey filaments consist of a rigid plastic body extended by a nylon thread of different thicknesses, which give standardized measurements of sensitivity. Redua et al. (2002) adapted this tool to horses for the first time, in order to evaluate the preventive analgesic effect of pre-incision epidural ketamine.

Lansade et al. (2008) were the first to use this tool to assess tactile reactivity of equids outside a medical context. They tested five different sizes of these filaments (0.008g; 0.02g; 0.6g; 8g; 300g) in order to cover a wide range of pressures. They showed that the number of horses reacting to von Frey filaments increased significantly between 0.008g and 8 g, whereas above 8 g, fewer horses reacted. Since then, most studies (14 of 17) have used four sizes of filaments (0.008g; 0.02g; 0.6g; 300g, without explaining the choice of sizes). Follow-up studies by Lansade and Valenchon's groups applied the same filament sizes except for one (1g instead of 8g) and in the same way, i.e.: filament 0.008g on the right side of the withers then 300g on the left side of the withers, interruption for another

test then application of the filament 0.02g on the right side of the withers then 1g on the left side of the withers. Fureix et al. (2012) and Hausberger et al. (2012) applied these four sizes of filaments but both on the left and right body sides. The authors of the remaining studies tested different, complementary and more filament sizes: Vidament et al. (2021) tested four sizes of filaments (0.008g; 0.02g; 1g; 50g); Briefer Freymond et al. (2019) tested five sizes (0.008g, 0.020g, 0.6g, 1g, 300g); Gonzalez-De Cara et al. (2017) tested seven sizes (0.008g, 0.02g, 0.16g, 0.6g, 8g, 60g, 300g). Veres-Nieki et al. (2021) did not specify which filament sizes were used, they only explained that von Frey filaments were applied in an increasing order until a muscle contraction was detected in response to stimulation (the average size of the filament to which the horses responded at each area is however detailed). The reasons for these choices are not explicitly mentioned and most studies do not give details concerning the horses' reactivity according to filament size (Lansade et al. 2012, 2013, 2014, 2016, 2017; Lansade and Simon 2010; Hausberger et al. 2012; Veres-Nieki et al. 2021). Vidament et al. (2020) reported that equids had responded as follows: 0.008g (18% of the population), 0.02g (29%), 1g (78%) and 50g (72%). Fureix et al. (2012) found that depressed horses responded less to tactile stimuli while Briefer-Freymond et al. (2019) found that crib-biter horses responded mostly to the 0.6g filament. Gonzalez De Cara et al. (2017) found that a 0.6g filament induced the highest reactivity of donkeys, followed by the 0.16g filament while the 300g and 60g filaments generated the lowest levels of reactivity. Discrepancies are also found in the measurements, Gonzales De Cara et al. (2017) and Veres-Nieki et al. (2021) evaluated the responses in terms of number of reactions per horse while the other studies measured the number of horses showing a reaction.

3.3 Testing procedure

- *Body area*

As for humans, the body surface is the largest of horses' sensory organs (Rørvang et al. 2020) and skin sensitivity varies across a horse's body depending on the distribution of sensory nerve receptors, with the most sensitive areas being the nose, neck, withers, shoulders, lower flank, and back of the pastern (Mills and Nankervis 2013). This may explain the differences in tactile reactivity assessed with von Frey filaments found by Fureix et al. (2012) based on the body area. They found that horses responded more to stimulations to the groin and withers than to the chest: out of 45 non-depressed horses, 36 responded to the stimulation to withers: 41 at groin but only 5 at chest, revealing thus higher individual variations at the chest.

Therefore, Hausberger et al. (2012) decided to apply the filaments only at the chest in order to compare adult horse players and non-players. The most responsive areas of donkeys used in equine-assisted therapy were (in the following order): withers, back, forelimbs, ribs and stomach, while neck, rump and forehead showed the lowest sensitivity (Gonzalez-De Cara et al. 2017).

On the contrary, but without any particular explanation, most of the other studies only tested the base of the withers area (Briefer Freymond et al. 2019; Lansade et al. 2008,2012, 2013, 2014, 2016, 2017; Lansade and Simon 2020; Valenchon et al. 2013a,b,c; 2017; Vidament et al. 2020). This body area is especially stimulated in horses used for riding which could possibly create a bias in reactivity (e.g. habituation or sensitization according to experience). Only one study focused on tactile reactivity of horses' face where six areas were tested: nostril, lateral canthus of the eye, mental foramen, supraorbital foramen, infraorbital foramen, upper lip, this study showed that site of stimulation had a significant effect on tactile sensory threshold with the nostril thresholds being the most consistent (Veres-Nyéki et al. 2021).

This underlines the importance of the area(s) of the body tested on the results and suggests that more attention is paid to this factor in future studies. Moreover, testing several areas yields a global tactile reactivity score and not a score for a single area which, in addition, may be biased for instance because of the impact of work or another factor.

- *Body Side*

Tactile laterality is a potentially important aspect in mammals (Karenina et al. 2018), and there is evidence that lateralized tactile actions have different effects on foals' memory of the procedure (Durier et al. 2012). Moreover, most human actions with horses are lateralized (saddling and mounting on the left side (Larose et al. 2006)), which suggests possible influences on tactile laterality. However, some studies do not mention whether both sides were tested (Briefer Freymond et al. 2019) or test only one side (left side of the face: Veres-Nieki et al. 2021). Most studies by Lansade and Valenchon's team (appendix) tested both sides but with different filament sizes according to body side and in the same standardized order (once 0.008g on the right, 300g on the left followed by another

experimental test and then 0.02g on the right and 1g on the left). Lansade et al.'s (2016) explanation is that it is not possible to repeat stimulation on the same area twice in a row, as the horse usually does not respond the second time, which is why they switched sides between the first and second filament, and waited several minutes between the first and last two filaments. This prevents however appropriate comparison between sides. Finally, the studies that tested both sides with the same filaments do not mention whether the horses reacted more to one side than to the other. Therefore, it is difficult at this stage to conclude anything about horses' tactile laterality.

- Filament application order

In their first study of sensory sensitivity, Lansade et al. (2008) tested horses using stimuli of different intensities at each test session. The five filament sizes had been selected in a preliminary study to produce satisfactory inter-individual variability in responses and were used in the same order: 300g; 0.6g; 0.02g; 0.008g; 8g. This was replicated by Briefer Freymond et al. (2019) who however changed the 8g filament for a 1g filament. Then all the other follow-up studies by Lansade and Valençon's team used four sizes of filaments in the following way: 0.008g, 300g, 0.02g and finally 1g (each size on one side of the body as mentioned above). The only justification for this method is, as cited above, that it is not possible to repeat stimulation on the same area twice in a row, as a horse usually does not respond the second time (Lansade et al. 2016). Similarly, Veres-Nyeki et al. (2021) applied the filaments in the same order, following the increasing order of filament size.

In contrast, Fureix et al. (2012) tested the filaments, body areas and sides in a random order for each horse. The tests involved two sessions (2 hours apart) in which two von Frey filaments were tested 10 minutes apart.

The impact of the order of application of the filaments to a horse's body has not been studied, but it is important to consider these discrepancies between studies when comparing results.

- Repetition

Most authors applied the filaments only once. Lansade et al. (2008) tested the same young horses 5 months apart and found that the data were correlated over time, but did not specify whether there were differences in reactivity level between the two periods. Other authors, such as Lansade et al. (2014), repeated the tactile reactivity test three times (5, 12, and 23 weeks after the start of treatment) but they do not mention whether the results were compared within groups based on repetition. Moreover, later Lansade et al.'s (2016) team highlighted a decrease in reactivity during rapid repetition of the application of the same filament, hence the fact that the filaments were only applied once. On the other hand, Veres-Nyeki et al. (2021) applied each filament twice and only kept the tactile sensory threshold measurement when the second application of the same size filament elicited a similar response.

Conclusion

This bibliographic research revealed disparate information. In addition, important information sometimes lacks concerning certain parameters likely to influence tactile reactivity (sex not mentioned in one study, season not mentioned, contexts vary, and so on). All the information cited or missing for each of the studies using the von Frey filament tactile reactivity tests on horses is listed in Appendix.

In addition, the method itself is rarely performed in the same way for the different studies. Thus, some studies test only one area of the body whereas others test several areas, but not necessarily in the same way. Similarly, not all studies test both sides of the areas; the sizes of the filaments also vary and are not applied in the same order by the different authors.

Thus, these methodological discrepancies coupled with intrinsic and extrinsic factors that could influence the tactile reactivity of horses lead to a large variability between results and could possibly explain these differences. Thus, in order to test the relevance of some of these factors and their impact on tactile reactivity, we conducted a pilot study.

Part II: IMPACT OF SOME FACTORS ON HORSES' TACTILE REACTIVITY: A PILOT STUDY

The aim of this pilot study was to test basic factors when setting up a method such as age, sex and type of equid as well as the period when subjects were tested. Based on these factors, we tested various modalities such as body area, body side and filament size on the tactile reactivity threshold.

In order to limit extrinsic effects, which, as we have seen, can impact tactile reactivity, we kept several factors homogeneous. Indeed, the horses selected for the tactile reactivity tests in this study all lived in the same living conditions (which explains the small sample size). In addition, they had a good welfare status (Guilbaud et al. in prep.) to avoid an impact of this factor on the reaction threshold of the animals and were not at all or not routinely used for horse riding, a practice that involves many tactile stimulations that can bias the reaction threshold obtained with von Frey filaments.

MATERIALS AND METHODS

Ethics

The experiments were carried out in 2020 in accordance with the European Parliament and the European Union Council recommendations related to protection of animals used for scientific purposes (directive 2010/63/UE) and complied with the current French laws related to animal experimentation (decree n° 2013-118 1 February 2013) and its five implementation rules (JO 7 February 2013), integrated in the Code rural and the Code of the maritime fishing (n° R. 214-87 to R.214-137). According to these European and French laws, our experiment did not require an authorization request. Indeed, our manipulations did not cause any physical or mental pain.

Subjects

This study included 16 equids (8 horses: > 1.48 m at withers and 8 ponies: ≤ 1.48 m at withers), aged 3 to 25 years old ($X \pm SE = 11 \pm 8$ years) of different breeds (Tab.1). These horses lived at or near the Paimpont Biological Station (Ille-et-Vilaine, France) in the same naturalistic conditions (i.e. in stable groups of 2 to 4 individuals, in 1 to 5 hectare natural pastures with water ad libitum). Nine had never been ridden, five had been ridden in the past but not anymore (for at least 6 years) and two were occasionally ridden on week-ends (outdoor recreational riding). All of them were nevertheless used to being handled by humans (halter, walking in hand, grooming). Welfare assessments (observational assessments) performed for another study (in prep.) indicated that none of these horses exhibited any welfare problems (e.g. no indicators of compromised welfare: stereotypic behaviors, depressed postures, aggressiveness... see e.g. Lesimple 2020 for review).

Tab. 1: Characteristics of the individuals involved in the study

INDIVIDUAL	SEX	AGE (years)	BREED	TYPE OF EQUID
1	Stallion	3	Unregistered	Pony
2	Gelding	4	Appaloosa crossbred	Pony
3	Gelding	4	Unregistered	Horse
4	Mare	5	Unregistered	Horse
5	Mare	5	Unregistered	Horse
6	Gelding	5	Appaloosa crossbred	Pony
7	Mare	6	Unregistered	Horse
8	Mare	6	Appaloosa crossbred	Pony
9	Mare	7	French Saddlebred x Friesian	Horse
10	Stallion	9	Unregistered	Pony
11	Gelding	16	Unregistered	Pony
12	Mare	16	Appaloosa crossbred	Pony
13	Mare	19	Thoroughbred x Friesian	Horse
14	Mare	22	Unregistered	Horse
15	Mare	23	Unregistered	Horse
16	Gelding	25	Haflinger x Connemara	Pony

Tactile reactivity test

Tactile reactivity was estimated using von Frey filaments (Stoelting, IL, USA), a procedure first used by Redua et al. (2002). These filaments consist of a hard-plastic body extended by nylon threads of variable thickness and are calibrated to apply a specific force on the skin (here 0.008g, 0.02g, 1g and 300 g), making it possible to obtain standardized measures. Indeed, when the tip of the filament, of given length and diameter, is applied perpendicularly against the skin, the application force increases as long as the experimenter continues the pressure, until the filament flexes and the filament is then removed.

Tactile reactivity was tested by the same experimenter (LG, unknown to the horses at the time of testing) while the subject was held lightly with a halter and loose lead rope by another experimenter, in its usual environment (home pasture). In order to avoid reactions due to separation, the individual was tested with other group members near-by but testing occurred only while the conspecifics were busy foraging and not interfering.

In order to obtain a general score of tactile reactivity and not a score for a particular body area we applied the von Frey filaments to the base of the withers, to the stifle and chest (Fig. 1). The tactile reactivity test was performed in two sessions at least 30 minutes apart. For each session, the order of the body sides, the body areas and the size of the filaments were predefined in a random order for each individual. Thus, for each session, two filaments were applied, first on one side for the three areas and then on the other side for the same three areas (example described in Fig. 1).

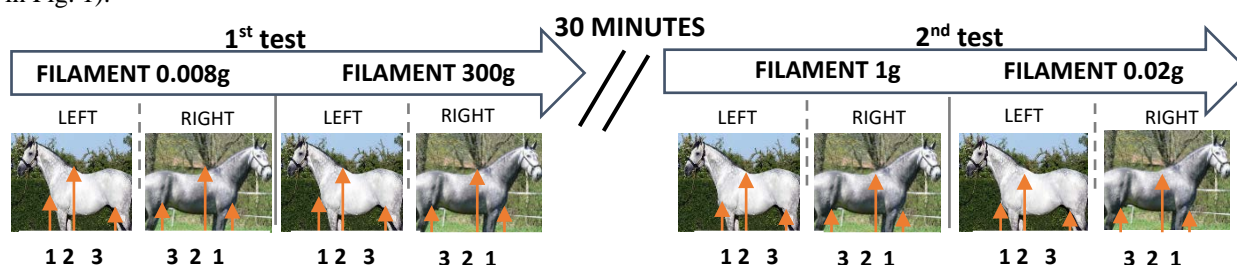


Fig. 1: Example of a tactile reactivity test performed on a horse based on the following order of application of the filaments (the order was chosen randomly): 0.008g; 300g; 1g; 0.02g. Each filament was applied first to one side (in the example: left side) of the chest, withers and stifle and then to the other side (in the example: right side) of the chest, withers and stifle.

481 These tests were conducted over two days, between 10:00 a.m. and 3:30 p.m., on 20 January (winter) 2020 and 9
482 June (spring) 2020, 5 months apart.

483 **Statistical data and analyses**

484 All statistical analyses were performed using R 3.6.2 software (alpha threshold set at $p < 0.05$) (R Core Team, 2019).

485 An analysis by generalized linear mixed models (GLMM) (using package {car}) (Fox and Weisberg 2019) was
486 performed to evaluate the influence of the day of the test, filament size, body side and area, as well as sex and age
487 of the individuals on the tactile reactivity of equids. The factor “individual” was included as a random effect to
488 avoid pseudo-replication.

489 The horses' responses to each von Frey stimulation were coded in binary form (tremor = 1/no tremor = 0). Several
490 measures were analyzed:

- 491 1) Total reactivity: sum of all responses, up to a maximum of 24 (4 filaments tested on both sides on 3 areas
492 of the body).
 - 493 2) The reactivity by filament range: the sum of the responses for each range of filaments tested. For example:
494 sum of the responses recorded with the filament 1g all sides and areas combined.
 - 495 3) Reactivity per side of the body: the sum of the responses on the left and right sides of the body (areas and
496 filaments confused).
 - 497 4) Reactivity by body area: the sum of the responses recorded for each body area tested (sides and filaments
498 confused).
- 499

500 Further post-hoc statistical analyses based on non-parametric statistical tests were performed as the data were not
501 normally distributed (Siegel 1957). McNemar tests were performed to compare the number of equids reacting to
502 filaments between the two test days. Friedman tests, followed by post hoc Wilcoxon tests, were carried out to
503 evaluate possible differences in the levels of tactile reactivity between the two days. In addition, Spearman
504 correlations were performed between the responses on the two days to estimate whether individual differences
505 remained stable.

506 **RESULTS**

507 **General tactile reactivity of our horse population**

508 Over the two test sessions (January and June), all subjects reacted at least once to a tactile stimulus (all filaments,
509 body areas and sides combined: mean \pm standard deviation: 5.75 ± 3.9 reactions for a maximum of 24 possible
510 reactions). All 16 equids reacted at least once to stimulation to the withers ($X \pm SD = 2.7 \pm 1.9$ reactions), 15 to
511 stimulation on the stifle ($X \pm SD = 1.7 \pm 1.5$ reactions) and 10 to stimulation at the chest ($X \pm SD = 1.2 \pm 1.6$ reactions).
512 These 16 equids reacted at least once when tested on the left side ($X \pm SD = 3.0 \pm 1.9$ reactions) and 15 of them when
513 tested on the right side ($X \pm SD = 2.7 \pm 2.4$ reactions). All our equids reacted at least once to the two largest filaments
514 (1g: 2.3 ± 1.7 reactions; 300g: $X \pm SD = 2.1 \pm 1.4$ reactions), 11 reacted to the 0.02g filament ($X \pm SD = 0.8 \pm 1$
515 reactions) and 12 to the smallest filament (0.008g) ($X \pm SD = 0.6 \pm 0.8$ reactions).

516 As revealed by the GLMM analysis, four of the seven factors tested influenced the overall tactile reactivity of
517 equids (number of responses): filament size (GLMM: $X^2 = 64.1$, $df = 3$, $p = 7.8e-14$); body area ($X^2 = 30.1$, $df = 2$, $p =$
518 $4.3e-08$); day of test ($X^2 = 40.4$, $df = 1$, $p = 2.06e-10$); and type of equid (horse/pony) ($X^2 = 7.8$, $df = 1$, $p = 0.005$)
519 whereas there was no effect of age (GLMM: $X^2 = 0.34$, $df = 1$, $p = 0.56$), sex (GLMM: $X^2 = 5.5$, $df = 2$, $p = 0.06$) nor of
520 body side (GLMM: $X^2 = 0.37$, $df = 1$, $p = 0.54$) considered separately.

521 These results were further confirmed by post hoc statistical analyses and comparisons. Thus, the horses responded
522 more to the largest than the smallest filaments (Friedman Test, $N = 16$, $df = 3$, $p = 0.0000004$) (Fig. 2a); more to
523 applications to the withers than to the chest (Friedman's Test, $N = 16$, $df = 2$, $p = 0.005$) (Fig 2b), and more during the
524 second (June) than during the first (January) test session (Wilcoxon Test, $N = 16$, $V = 3$, $p = 0.001$) (Fig 2c). In
525 addition, horses were more responsive than ponies (Mann Whitney U-test $N_1 = 8$, $N_2 = 8$, $W = 12.5$, $p = 0.045$) (Fig.
526 2d).

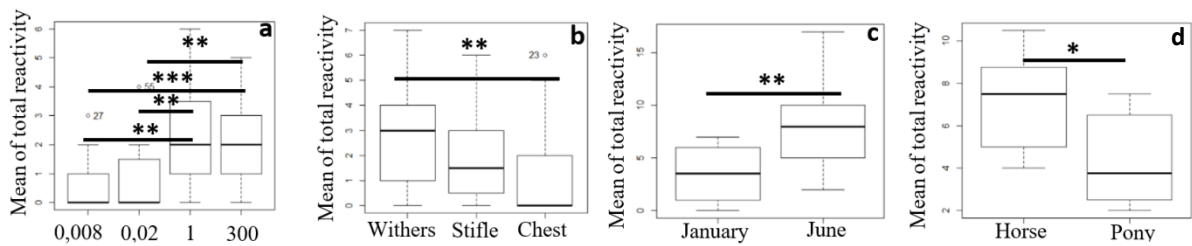


Fig. 2: Influence of: filament size (a), body area tested (b), period of test (c) and type of equid (d) on overall tactile responsiveness of equids. Friedman's, Wilcoxon's and MW test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The plots show median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers), and outliers (°).

Influence of test session

We could evidence no significant differences between test sessions (1st in January, 2nd in June) concerning the number of horses that reacted at least once to a tactile stimulation (June: all 16 horses; January: 13 out of 16) (McNemar test, $df=1$, $p > 0.05$), the number of horses reacting to stimulations at the different body areas (chest, withers and stifle: 10, 16, 14 horses reacted in June and 5, 12, 10 in January respectively (McNemar test, $df=1$, $p > 0.05$) or to applications on the left side of their body (16 in June and 13 in January; McNemar test, $df=1$, $p > 0.05$). However, in June, more horses reacted to applications on their right side (15 *versus* 8 in January; $df=1$, $p=0.016$), and to the 1g filament (16 *versus* 10 equids; $df=1$, $p=0.031$) than in January.

As mentioned above, when considering all reactions to all stimuli, the subjects reacted more (number of reactions) to the tactile stimuli during the second test session (June) than during the first test session (January) (Wilcoxon Test, $N=16$, $V=117$, $p=0.001$). However, this result seemed to be mostly due to the larger filaments: 300g ($N=16$, $V=94$, $p=0.009$) and 1g ($N=16$, $V=117$, $p=0.001$) (Fig 3a,b), as no such differences were observed when only the smaller filaments (0.008g: $N=16$, $V=52.5$, $p > 0.05$; 0.02g: $N=16$, $V=33$, $p > 0.05$) were considered.

These differences according to the testing session remained whatever the body side (right side: $V=78$, $p=0.002$; left side: $V=117$, $p=0.001$) or body area (withers: $V=87$, $p=0.03$; stifle: $V=75$, $p=0.04$; and especially chest $V=55$, $p=0.005$) (Fig. 3c, d, e).

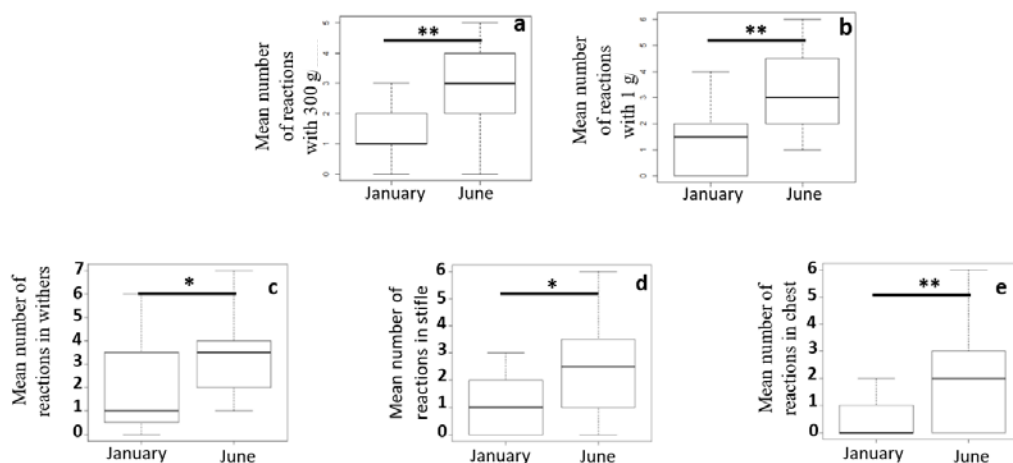


Fig. 3. Reaction data: to applications of the 300g filament (a), and of the 1g filament (b), on withers (c), stifle (d) and chest (e) during the first and the second test sessions. Wilcoxon's test: ** $p < 0.01$. The plots show median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers), and outliers (°).

Moreover the subjects' reactions differed significantly according to the body area tested during the first test session (January) (Friedman test, $df=2$, $p=0.006$), but not during the second test session (June) ($df=2$, $p > 0.05$) (Fig 4).

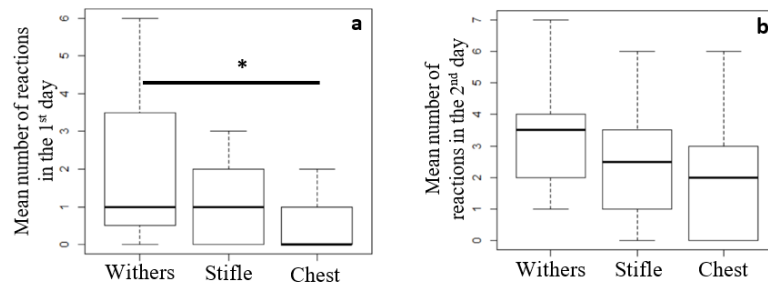


Fig. 4. Median number of reactions to applications on withers, stifle and chest during the first (January) (a) and the second (June) test sessions (b). Friedman's test: * $p < 0.05$. The plots show median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers), and outliers (°).

Sex differences varied also according to test session: geldings showed more reactions than mares to applications to stifle (filament size and side combined) during the second test session in June (Mann Whitney U-test $N_1=5$, $N_2=9$, $W=38.5$, $p=0.034$) whereas no such difference was found in the first session of January ($W=24.5$, $p>0.05$) (Fig. 5).



Fig. 5: Reactions of geldings and mares to applications on stifle during the first (January) (a) and the second (June) test sessions (b). Mann Whitney's test: * $p < 0.05$. The plots show median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers), and outliers (°).

Individual consistency over time

The horses' overall tactile reactivity was moderately correlated between both test sessions (January/June) (total number of reactions: Spearman test of correlation: $\rho=0.51$, $p=0.042$) (Fig. 6a).

Actually, the correlation between sessions was high for data for the right side ($\rho=0.78$, $p=0.0004$) but there was no correlation of the data for the left side ($\rho=0.19$, $p=0.49$). Moreover, this was observed only for the 1g filament ($\rho=0.59$, $p=0.017$) and not for the others (0.008g, 0.02g and 300g: $\rho=0.006$ to 0.28 , $p=0.287$ to 0.981). Finally, the number of responses to stimulation was consistent between test sessions only for the chest area ($\rho=0.71$, $p=0.002$) but not for the other areas (stifle: $\rho=-0.04$, $p=0.877$; withers: $\rho=0.062$, $p=0.82$) (fig. 6b, c, d).

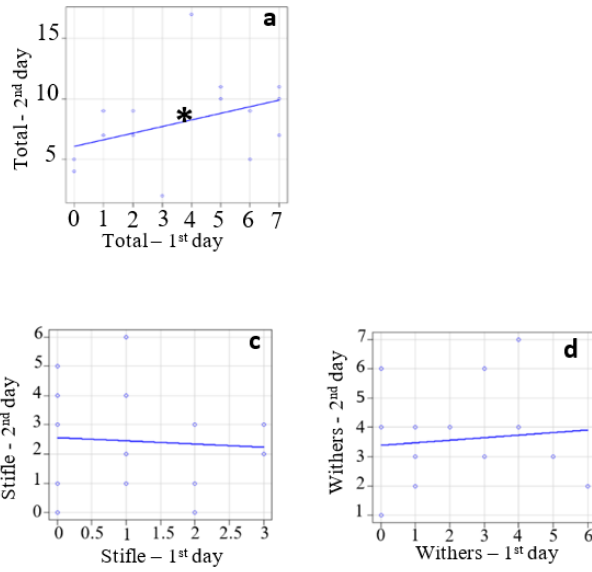


Fig 6 : Correlations between the two test sessions in horses' total tactile reactivity at: (a) all body parts pooled, chest (b), stifle (c), and withers (d). Spearman correlations: * $p < 0.05$, ** $p < 0.01$.

In summary, testing modalities may enhance or diminish the evidence of individual consistency (Tab. 2). Individual consistency appeared to be enhanced when using larger filaments applied to the right side whereas the tests to stifle or on the left side with small filaments seemed poorly predictive. Actually, the most reliable approach for individual consistency appeared to be the application of the 1g filament to the chest and on the right body side.

Tab 2: Correlations between the number of reactions observed according to body zone and filament size between testing days. Only the significant ones are noted.

	0.008g	0.02g	1g	300g	Left	Right	Stifle	Chest	Wither	Total
0.008g										
0.02g										
1g			p=0.02 r=0.59			p=0.01 r=0.61				p=0.04 r=0.51
300g			p=0.01 r=0.62		p=0.04 r=0.51		p=0.047 r=0.50			p=0.03 r=0.53
Left										
Right			p=0.02 r=0.57			p=0.0004 r=0.78		p=0.03 r=0.53		p=0.01 r=0.62
Stifle										
Chest			p=0.02 r=0.057	p=0.009 r=0.63		p=0.0003 r=0.79		p=0.002 r=0.71		p=0.007 r=0.64
Wither			p=0.01 r=0.62				p=0.049 r=0.50			
Total			p=0.02 r=0.58			p=0.03 r=0.54				p=0.04 r=0.51

Conclusion

This pilot study, where a homogeneous population of horses and ponies (same housing conditions, same welfare state), enabled us to confirm that horses reacted more to the larger von Frey filaments than to the smaller ones, more to stimulations to the withers than to the chest, but it also revealed that our subjects' number and location of reactions differed between test sessions separated by 6 months. Intrinsic factors seemed also to be influential: ponies responded overall less than horses while geldings responded more than mares, but only to stimulations to the stifle and during the second test session in June. Finally, comparisons of individual variations between sessions confirmed the existence of some individual consistency in overall responsiveness over time, but that this was enhanced when stimulating the chest area, the right side, and with the 1g filament.

GENERAL DISCUSSION

This article highlights contradictory results between studies testing equine reactivity with von Frey filaments. Moreover, investigations concerning the possible influence of many factors are clearly lacking. When examining thoroughly the publications, it appeared clearly that a variety of factors, from the context of tests, the sex or age of the subjects are often not taken into consideration and that major methodological discrepancies exist thus prevent drawing any conclusions.

Importance of intrinsic factors for tactile reactivity

First of all, intrinsic factors, such as sex or age, are not always mentioned by the authors and attempts to make a link between these factors and tactile reactivity are rarely made. However, our pilot study highlights the importance of taking these factors into account and the impact they can have on tactile reactivity.

Thus, only one study (Hausberger et al. 2012) noted that geldings were more responsive than mares to von Frey filaments while the other authors found no differences (Vidament et al. 2020; Veres-Nyéki et al. 2021, Briefer-Freymond et al. 2019) or did not seem to test these factors (e.g. Fureix et al. 2012; Lansade et al. 2012; Lansade et al. 2013). However, our pilot study revealed a non-significant trend, in line with Hausberger et al.'s (2012) report, with more reactions by geldings than by mares, and most of all a significant sex difference that depended upon season.

Whether this was related to order of tests or to season cannot be determined at this stage, but variations in sensory perception according to season are known for other modalities (e.g. hearing in songbirds, and generally related to hormonal changes (e.g. androgen and estrogen hormones, (Caras et al. 2010 ; Cousillas et al. 2013).

The only three studies that tested the possible impact of age suggested a decrease in tactile reactivity with age but our pilot study testing animals of different ages could not evidence any link with tactile reactivity. However, our equine subjects had never been ridden, had not been ridden for several years or were not routinely ridden. Possibly, increasing experience with age of tactile stimuli perceived during work or grooming sessions could also lead to greater habituation, and thus lower responsiveness (van Iwaarden et al. 2012). Further studies are clearly needed to disentangle the effects of maturation and experience.

Subjects, in most studies (e.g. Lansade et al. 2008, 2012; Valenchon et al. 2013a,b,c; Veres-Nyeki et al. 2021; Fureix et al. 2012), were of a single breed or a predominant breed, thus the authors were not able to test the impact of breed on tactile reactivity. Studies including equids of various breeds and having tested for links with tactile reactivity yield several results indicating on the one hand that the tactile reactivity of Anglo-Arabs was higher than that of all the other equids (different breeds of horses and ponies) tested, and on the other hand that sport horses were more reactive than sport ponies and both were more reactive than leisure ponies (Vidament et al. 2021). These results suggest that the type of work (i.e. an extrinsic factor) may be more influential than breed. Nevertheless, our pilot study indicated an impact of the type of equid, as horses' tactile reactivity was higher than that of ponies, thus suggesting that there may be an interplay between genetic parameters and environmental factors.

Finally, because they found a stability of individual variations of tactile reactivity to the same stimuli over time (5 months apart) Lansade et al. (2008) proposed tactile reactivity as a temperament trait, in agreement with some authors studying humans (reactivity threshold, Plomin 1983; sensory threshold, Thomas and Chess 1977). As a reminder, the classic definition of temperament is that of Goldsmith et al. (1987): "a set of individual differences

in behavioral patterns, called 'traits' or 'dimensions', that are relatively stable across various types of situations and over time." Our pilot study confirmed individual consistency when testing on the right side with rather gross filaments. There is therefore a clear need for more investigation on this question. Moreover, since tactile reactivity is affected by environmental conditions and welfare state (e.g. Fureix et al. 2012), the question of whether it can be considered a temperament trait remains open. Moreover no clear influence of measures of temperament traits on those of tactile reactivity is reported in the literature (e.g. Eysenck 1991).

Impact of extrinsic factors

Extrinsic factors include living, training and working conditions as well as environmental factors at the time of testing, such as season. Actually, only one study looked at the impact of general equine living conditions (Lansade et al. 2014), but none have looked at possible effects of working conditions; this means that there is a real need for further testing and that, in any case, studies cannot be compared when the horses' living conditions are not similar.

This raises a methodological question and the importance of standardizing tests for comparative studies to avoid the impact of the place where the test is performed as much as possible. Indeed, in addition to the usual living conditions of the subjects, the place and time of a test seem important and should be taken into consideration. The fact that our pilot study showed that equids reacted more during the second session in June than during the first session in January proves that our subjects did not habituate to the test after the first session. Several hypotheses can explain this result: the properties of the horses' coat vary seasonally, becoming thicker in the winter and this may explain why horses respond less to tactile stimuli during this season (Osthaus et al. 2018). However, as the various studies carried out the tests at different times of the year this could have impacted the results and therefore make it impossible to compare studies and even sometimes within studies.

The location of a test may have an influence (Vidament et al. 2021), and even whether it is indoor or outdoor (Briefer-Freymond et al. 2019). Nevertheless, no details are provided on the final outcome, nor which condition had which effect.

Methodological aspects

Clear discrepancies emerged between studies concerning the areas and sides of the body tested, and the sizes of filaments or the order they were applied. Most studies focused on tests at the base of the withers (Lansade et al. 2008, 2012; Valenchon et al. 2013a,b,c; Vidament et al. 2020), while other authors applied the filaments to several body areas and found that responses differed and depended on the area tested (e.g. Fureix et al. 2012). Our pilot study confirmed their findings, equids reacting more (both test sessions combined) to stimulations to the withers than to the chest and stifle, which converges with the variations of sensory nerve receptors between body areas (Mills and Nankervis 2013). However, we found this higher reactivity at the withers only for the first session (January) whereas we did not find any difference related to the area tested for the second session (June). Maybe horses habituate more to tactile stimulations to withers (which could explain Lansade et al.'s 2008 findings of rapid habituation) or there are seasonal variations. Interestingly, allogrooming, which often occurs at withers, is observed more often in winter; this could be explained by their thicker coat, which may then enclose mud or ectoparasites, in particular lice, probably causing irritation (Tyler 1972). Finally, the finding that the reactivity of mares and geldings differed in June only at stifle remains unexplained but worth further investigation. These different results prove the importance of testing several areas in order to obtain a global score of tactile reactivity and not a score for only one area that, moreover, could be biased because of the impact of the type of work, the distribution of the sensory receptors on the surface of the body but also the season at the time of the tests. It is worth noting that individual consistency appeared highest in our study for chest and not withers.

None of the studies, including the present one, which studied a possible lateralization of tactile reactivity in adult equids, found one, although procedures differed largely (e.g. some filaments on one side and the others on the other side, e.g. Lansade et al. 2008, Vidament et al. 2021). This is surprising since newborn foals are differently affected by handling according to the body side touched (de Boyer des Roches et al. 2011). Interestingly, although we did not find any side bias, individual consistency appeared higher when the test was performed on the right side.

Finally, with a few exceptions, most studies tested the same sizes of filaments but in a different order. We found that the equids reacted more to the larger filaments than to the smaller ones, but the order of application had no impact on their reactivity. However, of the four filaments we tested in this pilot study the 1g filament seems interesting to use since it was the one that seems to reveal best individual coherence.

700 This review of tactile reactivity in equids, one of the rare mammal species for which an extensive effort has been
701 made to understand its perceptual (and especially tactile) world, reveals how far we are still from having a complete
702 view of the factors involved and to know the best methodological approach to develop. These information and
703 pilot experimental results suggest that more attention must be paid to the context of testing, the type of animal
704 considered and even its working/living conditions. This more extensive view of tactile reactivity could help
705 understand how it fits with social and non-social behavioral patterns observed in natural conditions. It also
706 constitutes a basis for future studies on other non-laboratory animal.

PARAMETERS / STUDIES	Briefer Freymond et al. 2019	Fureix et al. 2012	Hausberg et al. 2012	Lansade and Simon 2010	Lansade et al. 2008	Lansade et al. 2012	Lansade et al. 2013	Lansade et al. 2014	Lansade et al. 2016	Lansade et al. 2017	Valencho n et al. 2013a	Valencho n et al. 2013b	Valencho n et al. 2013c	Valencho n et al. 2017	Veres-Nyéki et al 2021	Vidame nt et al. 2020	Gonzalez De Cara et al. 2017
	N=37	N=59	N=29	N=36	N=26	N=25	N=19	N=19	N=24	N=29	N=30	N=26	N=49	N=60	N=34	N=1345	N=36
AGE Y=years old	Y 4-24 y	Y 5-20 y	Y 7-17 y	Y 5-7 y	Y 3-4 y	Y 10 months	Y 12 months	Y Idem Lansade et al., 2012	Y 16 horses of 4 years old and 8 of 5 years old	Y 10.1±1.7 y	Y 7.2±0.6 y	Y 6±1 y	Y 7±1 y	Y 5- 2 y	Y 1-23 y	Y 2-3 y	Y 5.79±2.71 y
SEX (G: Gelding, M: mare, S: Stallion)	Y G+M+S	Y G+M	Y M+G	Y M	Y M	Y M+S	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	N	Y M	Y M	Y M	Y M	Y M	Y G+M+S	Y G+M+S	Y M
BREED	Y Varied	Y Majority French Saddlebred	Y Majority French Saddlebred	Y Idem Lansade et al., 2012	Y Welsh	Y Welsh	Y Anglo arabe	Y Idem Lansade et al., 2012	Y Majority French Saddlebred	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Warmblood	Y Sport horsesn anglo arabians sport ponies, leisures ponies	Y Donkeys Andalusian and crossbred
FILAMENT RANGE	Y 0.008, 0.02, 0.6, 300	Y 0.008, 0.02, 1, 300	Y 0.008, 0.02, 1, 300	Y Idem Lansade et al., 2012	Y 300, 0.6, 0.02, 0.008, 8	Y 0.008, 300, 0.02, 1	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	N	Y 0.008, 0.02, 1, 50	Y 0.008, 0.02, 0.16, 0.6, 8, 60, 300
SIDE (L: left, R: right)	N Withers height	Y L+R	Y L+R	Y Idem Lansade et al., 2012	N (At the base)	Y L+R	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y L	Y L+R	Y L+R
BODY AREA	Y Withers	Y Withers, chest, groin	Y Chest	Y Idem Lansade et al., 2012	Y Withers	Y Withers	Y Withers	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Face	Y Withers	Y front, neck, withers, back, forelimbs, ribs, stomach, rumps, buttocks and hind limbs
TEST PERIOD/SEASON	Y Between September & February	Y Between January & June	N	N	Y May and October	N	N	N	Y February and March	N	N	N	N	N	N	N	N
TIME OF DAY	N	N	N	N	Y 14 horses in the morning and 12 in the afternoon	N	N	N	N	N	N	N	N	N	Y After daily exercise, between two meals	N	Y From 9 am to 2pm

ORDER OF APPLICATION OF THE FILAMENTS	Y Same order 300, 0.6, 0.02, 0.008, 1	Y Random	Y Random	Y Idem Lansade et al., 2012	Y Same order : 300, 0.6, 0.02, 0.008, 8	Y Same order 0.008g was applied to the right side of the horse, 300g to the left side, 0.02g to the right side, 1g to the left side	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	N	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	N	Y Same order: 0.008g on the left, 0.02g on the right, 1g on the left and 50g on the right	N
TIME INTERVAL BETWEEN THE APPLICATION OF 2 SUCCESSIVE FILAMENTS	Y 30s	Y 10min	N	N	N	Y 60s	N	N	N	N	N	N	N	N	N	N	N
REPETITION	N	N	N	N	Y 2 times (5 months apart)	N	N	Y 3 times (few weeks apart)	N	N	N	N	N	N	N	N	N
FAMILIAR OR UNFAMILIAR EXPERIMENTER	N	Y Unfamiliar	Y Unfamiliar	N	N	Y Familiar	N	N	N	N	N	N	N	N	Y Familiar	N	N
ATTACHED/FREE /HELD DURING TEST	N	N	Y Held	Y Idem Lansade et al., 2013	Y Attached	N	Y Held	Y Idem Lansade et al., 2013	Y Free or held depending on the type of test (complete or simplified)	N	Y Idem Lansade et al., 2012	Y Idem Lansade and Simon	Y Idem Lansade et al., 2013	Y Idem Lansade et al., 2013	Y Idem Lansade et al., 2013	Y Idem Lansade et al., 2008	Y Held
HORSES THAT WORK OR NOT	Y Ridden	Y Ridden	Y Ridden	Y Idem Lansade et al., 2008	Y Handling	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Ridden	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade and Simon	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2012	N	N	N
TEST LOCATION: FAMILIAR OR NOT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y Unfamiliar
TEST LOCATION: INDOOR OR OUTDOOR	N	N	N	Y Idem Lansade et al., 2017	N	N	N	N	Y Outside	Y Inside	Y Idem Lansade et al., 2017	Y Idem Lansade et al., 2017	Y Idem Lansade et al., 2017	Y Idem Lansade et al., 2017	N	N	N
SHAVED / NOT SHAVED	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y Shaved or not	N	N

HORSES NEARBY	Y Alone	N	N	Y In the presence of a horse	Y A horse in front and a side but not seen	N	N	N	Y In the presence of a horse	Y An unfamiliar horse present	Y In the presence of a horse	Y Idem Lansade and Simon	Y In the presence of a familiar horse	Y In the presence of a familiar horse	N	N	N
LIVING CONDITIONS OF THE HORSES: ACCOMMODATION	Y Stall	Y Stall	Y Stall	Y They were housed in seven adjacent boxes in random groups of three or four and spent 4 hours a day together in a large outdoor paddock	Y In pasture	Y Out for half an hour a day, in groups or individually, in the same paddock	Y Kept together on pasture. 5 days a week they were individually housed in indoor boxes with adjacent outdoor areas for 4 hours	Y Individual stall, individual paddock or group paddock depending on the group	Y Individual box and went out to the paddock 2 3 times a week	Y Information on accommodation before the experience (group paddock in summer, individual boxing and group regular outing in the paddock in winter) but no information on accommodation during the experience	Y Accommodation for half-days in groups of three in boxes	Y Idem Lansade and Simon	Y Randomly housed in groups of 3 in a small stable or 10 in a large stable. All horses spent 2 hours a day together in a large outdoor paddock	Y Housed in adjacent stalls in groups of three random mixes each day and spent 4 hrs per day in a large outdoor enclosure	N	N	N
BEDDING	N	Y Straw	N	Y Idem Lansade et al., 2008	Y Straw	Y Idem Lansade et al., 2008	Y Wood shavings	Y Wood shavings or straw	N	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	N	N	N	N
LIVING CONDITION OF THE HORSES : FEEDING	N	Y Pellets	Y Pellets	Y Pellets and hay	Y Pellets	Y Pellets and hay	Y Hay ad libitum	Y Pellets and hay	Y Pellets and hay	Y Hay and mineral block ad libitum	N	Y Idem Lansade and Simon	Y Pellets	Y Pellets and hay	N	N	N
WATER	N	Y Ad libitum	Y Ad libitum	Y Idem Lansade et al., 2008	Y Ad libitum	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	N	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	Y Idem Lansade et al., 2008	N	N	N
ACCOMMODATION - SOCIAL	Y Alone or in a group	Y Individual box	N	Y In group	Y 2 horses per box	Y Alone or in a group	Y In group	Y Together in the paddock at certain times of the day	Y Individual box, not specified if went out in a group to the paddock	Y Information on before experience but not during	Y In group	Y In group	Y In group	Y In group	N	N	N

1 OR MORE EXPERIMENTERS	Y 2 experimenters	Y 1 experimenter	Y 2 experimenters	Y 2 experimenters	Y 1 experimenter	Y 2 experimenters	Y 2 experimenters	Y Idem Lansade et al., 2012	Y 1 ou 2 en fonction des test (complet, simplifié)	N	Y 2 experimenters	Y Idem lansade et simon	Y 2 experimenters	Y 2 experimenters	N	N	Y 2 experimenters
HEALTH PROBLEMS OF HORSES	Y Discussed with owners	N	N	N	N	N	N	N	N	N	N	N	N	N	Y Clinically healthy	N	Y Clinically healthy
WEANING / EARLY HANDLING	N	N	N	N	N	Y From birth to weaning, they lived in the same group with their mother in grass paddocks. They were weaned at 6 months ± 1 month then kept together in a large stall and walked together in a paddock for 6 hours a day.	Y Idem Lansade et al., 2012.	Y They were weaned at 7 months of age and raised together until the start of the experiment.	N	N	N	N	N	N	N	N	N
STEREOTYPIC BEHAVIORS	Y 19 stereotypics and 18 control	N	Y Presence of stereotypical behavior	N	N	N	N	N	N	Y No stereotypical behavior	N	N	N	N	N	N	N
HORSES WERE FROM THE SAME PLACE OR NOT	Y 19 farms	Y 3 riding school	Y Same riding school	Y Idem Lansade et al., 2012	N	Y Same place	Y Same place	Y Same place	N	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade and Simon	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	N	Y 67 breeding shows in France	N
TIME IN THE STRUCTURE	Y > 1 yr	Y > 1 yr	N	Y Idem Lansade et al., 2012	N	Y Since their birth	Y Since their birth	Y Idem Lansade et al., 2012	N	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	Y Idem Lansade Simon	Y Idem Lansade et al., 2012	Y Idem Lansade et al., 2012	N	N	N
AGE OF CASTRATION	N	N	N	N	N	N	Y 10 mois	N	N	N	N	N	N	N	N	N	N
AMBIENT TEMPERATURE AT THE TIME OF TESTING	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y Between 3-13 degrees	N	N

- André, V., Durier, V., Beuchée, A., Roué, J.-M., Lemasson, A., Hausberger, M., Sizun, J., & Henry, S. (2020). Higher tactile sensitivity in preterm infants at term-equivalent age: A pilot study. *PLoS ONE*, *15*(3), e0229270. <https://doi.org/10.1371/journal.pone.0229270>
- André, V., Henry, S., Lemasson, A., Hausberger, M., & Durier, V. (2017). The human newborn's umwelt: Unexplored pathways and perspectives. *Psychonomic Bulletin & Review*. <https://doi.org/10.3758/s13423-017-1293-9>
- Andrews, K. (1993). The effect of changes in temperature and humidity on the accuracy of von Frey hairs. *Journal of Neuroscience Methods*, *50*(1), 91–93.
- Bell-Krotoski, J. A., Fess, E. E., Figarola, J. H., & Hiltz, D. (1995). Threshold Detection and Semmes-Weinstein Monofilaments. *Journal of Hand Therapy*, *8*(2), 155–162. [https://doi.org/10.1016/S0894-1130\(12\)80314-0](https://doi.org/10.1016/S0894-1130(12)80314-0)
- Ben-Sasson, A., Cermak, S. A., Orsmond, G. I., Tager-Flusberg, H., Kadlec, M. B., & Carter, A. S. (2008). Sensory clusters of toddlers with autism spectrum disorders: Differences in affective symptoms. *Journal of Child Psychology and Psychiatry*, *49*(8), 817–825. <https://doi.org/10.1111/j.1469-7610.2008.01899.x>
- Bigorre, N., Petit, A., Saint-Cast, Y., Jeudy, J., Cesari, B., Rabarin, F., Fouque, P. A., & Raimbeau, G. (2017). Interest of the “compress test” in diagnosis of nerve injury in hand wounds. *Orthopaedics & Traumatology: Surgery & Research*, *103*(4), 505–507. <https://doi.org/10.1016/j.otsr.2017.02.010>
- Boivin, X., Neindre, P. L., & Chupin, J. M. (1992). Establishment of cattle-human relationships. *Applied Animal Behaviour Science*, *32*(4), 325–335. [https://doi.org/10.1016/S0168-1591\(05\)80025-5](https://doi.org/10.1016/S0168-1591(05)80025-5)
- Bonin, R. P., Bories, C., & De Koninck, Y. (2014). A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. *Molecular Pain*, *10*, 26. <https://doi.org/10.1186/1744-8069-10-26>
- Bowden, J. L., & McNulty, P. A. (2013). Age-related changes in cutaneous sensation in the healthy human hand. *Age*, *35*(4), 1077–1089.
- Boyd, L. E. (1988). Time budgets of adult Przewalski horses: Effects of sex, reproductive status and enclosure. *Applied Animal Behaviour Science*, *21*(1–2), 19–39. [https://doi.org/10.1016/0168-1591\(88\)90099-8](https://doi.org/10.1016/0168-1591(88)90099-8)
- Bremner, A. J., & Spence, C. (2017). The development of tactile perception. *Advances in Child Development and Behavior*, *52*, 227–268.
- Briefer Freymond, S., Bardou, D., Beuret, S., Zuberbühler, K., Bachmann, I., & Briefer, E. F. (2019). Elevated sensitivity to tactile stimuli in stereotypic horses. *Frontiers in Veterinary Science*, *6*(162), 1–14. <https://doi.org/10.3389/fvets.2019.00162>
- Brydges, N. M., Argyle, D. J., Mosley, J. R., Duncan, J. C., Fleetwood-Walker, S., & Clements, D. N. (2012). Clinical assessments of increased sensory sensitivity in dogs with cranial cruciate ligament rupture. *The Veterinary Journal*, *193*(2), 545–550. <https://doi.org/10.1016/j.tvjl.2012.01.019>
- Burn, C. C., Dennison, T. L., & Whay, H. R. (2010). Relationships between behaviour and health in working horses, donkeys, and mules in developing countries. *Applied Animal Behaviour Science*, *126*(3–4), 109–118.
- Caras, M. L., Brenowitz, E., & Rubel, E. W. (2010). Peripheral auditory processing changes seasonally in Gambel's white-crowned sparrow. *Journal of Comparative Physiology A*, *196*(8), 581–599.
- Contalbrigo, L., Borgi, M., De Santis, M., Collacchi, B., Tuozi, A., Toson, M., Redaelli, V., Odore, R., Vercelli, C., & Stefani, A. (2021). Equine-Assisted Interventions (EAIs) for Children with Autism Spectrum Disorders (ASD): Behavioural and Physiological Indices of Stress in Domestic Horses (*Equus caballus*) during Riding Sessions. *Animals*, *11*(6), 1562.
- Cousillas, H., George, I., Alcaix, S., Henry, L., Richard, J.-P., & Hausberger, M. (2013). Seasonal female brain plasticity in processing social vs. Sexual vocal signals. *European Journal of Neuroscience*, *37*(5), 728–734. <https://doi.org/10.1111/ejn.12089>
- Cymbaluk, N. F. (1994). Thermoregulation of horses in cold, winter weather: A review. *Livestock Production Science*, *40*(1), 65–71. [https://doi.org/10.1016/0301-6226\(94\)90266-6](https://doi.org/10.1016/0301-6226(94)90266-6)
- Davis, E. P., Stout, S. A., Molet, J., Vegetabile, B., Glynn, L. M., Sandman, C. A., Heins, K., Stern, H., & Baram, T. Z. (2017). Exposure to unpredictable maternal sensory signals influences cognitive development across species. *Proceedings of the National Academy of Sciences*, *114*(39), 10390–10395.
- de Boyer des Roches, A., Durier, V., Richard-Yris, M.-A., Blois-Heulin, C., Ezzaouia, M., Hausberger, M., & Henry, S. (2011). Differential outcomes of unilateral interferences at birth. *Biology Letters*, *7*(2), 177–180. <https://doi.org/10.1098/rsbl.2010.0979>
- Dellon, A. L. (1986). Sensory recovery in replanted digits and transplanted toes: A review. *Journal of Reconstructive Microsurgery*, *2*(02), 123–129.
- Diehl, K. D., Egan, B., & Tozer, P. (2002). Intensive, Early Handling of Neonatal Foals: Mare-Foal Interactions. Proceedings of the Havermyer Foundation Horse Behavior & Welfare Workshop. Hólar, Iceland, 23–26 June.

- Dudzinski, K. M., Heather, H., & Botero, M. (2019). Methodological Considerations for Comparison of Cross-species Use of Tactile Contact. *International Journal of Comparative Psychology*, 32.
- Duncan, P., & Cowtan, P. (1980). An unusual choice of habitat helps Camargue horses to avoid blood-sucking horse-flies. *Biological Behaviour*, 5, 55–60.
- Duncan, P., & Vigne, N. (1979). The effect of group size in horses on the rate of attacks by blood-sucking flies. *Animal Behaviour*, 27, 623–625. [https://doi.org/10.1016/0003-3472\(79\)90201-X](https://doi.org/10.1016/0003-3472(79)90201-X)
- Durier, V., Henry, S., Sankey, C., Sizun, J., & Hausberger, M. (2012). Locomotor Inhibition in Adult Horses Faced to Stressors: A Single Postpartum Experience May be Enough! *Frontiers in Psychology*, 3. <https://doi.org/10.3389/fpsyg.2012.00442>
- Eysenck, H. J. (1991). Dimensions of personality: 16, 5 or 3?—Criteria for a taxonomic paradigm. *Science Direct*, 12, 773–790. [https://doi.org/10.1016/0191-8869\(91\)90144-Z](https://doi.org/10.1016/0191-8869(91)90144-Z)
- Feh, C., & de Mazières, J. (1993). Grooming at a preferred site reduces heart rate in horses. *Animal Behaviour*, 46(6), 1191–1194. <https://doi.org/10.1006/anbe.1993.1309>
- Field, T. (2001). Touch. Cambridge, MA: MIT Press.
- Field, T. (2010). Touch for socioemotional and physical well-being: A review. *Developmental Review*, 30(4), 367–383. <https://doi.org/10.1016/j.dr.2011.01.001>
- Filingeri, D. (2016). Neurophysiology of Skin Thermal Sensations. *Comprehensive Physiology*, 6(3), 1429. <https://doi.org/10.1002/cphy.c150040>
- Fox, J., & Weisberg, S. (2019). An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Fureix, C., Beaulieu, C., Argaud, S., Rochais, C., Quinton, M., Henry, S., Hausberger, M., & Mason, G. (2015). Investigating anhedonia in a non-conventional species: Do some riding horses Equus caballus display symptoms of depression? *Applied Animal Behaviour Science*, 162, 26–36. <https://doi.org/10.1016/j.applanim.2014.11.007>
- Fureix, C., Jegou, P., Henry, S., Lansade, L., & Hausberger, M. (2012). Towards an Ethological Animal Model of Depression? A Study on Horses. *PLoS ONE*, 7(6), e39280. <https://doi.org/10.1371/journal.pone.0039280>
- Gibbins, S., Stevens, B., Beyene, J., Chan, P. C., Bagg, M., & Asztalos, E. (2008). Pain behaviours in extremely low gestational age infants. *Early Human Development*, 84(7), 451–458.
- Goldsmith, H. H., Buss, A., Plomin, R., Rothbart, M. K., Alexander, T., & Chess, S. (1987). *Roundtable: What Is Temperament? Four Approaches*. 58(2), 505–529.
- Gonzalez-De Cara, C. A., Perez-Ecija, A., Aguilera-Aguilera, R., Rodero-Serrano, E., & Mendoza, F. J. (2017). Temperament test for donkeys to be used in assisted therapy. *Applied Animal Behaviour Science*, 186, 64–71. <https://doi.org/10.1016/j.applanim.2016.11.006>
- Hall, M. J. (1983). Social organization in an enclosed group of red deer (*Cervus elaphus* L.) on Rhum. I. The dominance hierarchy of females and their offspring. *Zeitschrift Für Tierpsychologie*, 61(3), 250–262.
- Harlow, H. F., & Suomi, S. J. (1974). Induced depression in monkeys. *Behavioral Biology*, 12(3), 273–296. [https://doi.org/10.1016/S0091-6773\(74\)91475-8](https://doi.org/10.1016/S0091-6773(74)91475-8)
- Hausberger, M., & Henry, S. (2018). Equine Sensory Systems. *Encyclopedia of Animal Cognition and Behavior*, 1–11. https://doi.org/10.1007/978-3-319-47829-6_934-1
- Hausberger, M., Bruderer, C., Le Scolan, N., & Pierre, J.-S. (2004). Interplay Between Environmental and Genetic Factors in Temperament/Personality Traits in Horses (*Equus caballus*). *Journal of Comparative Psychology*, 118(4), 434–446. <https://doi.org/10.1037/0735-7036.118.4.434>
- Hausberger, M., Fureix, C., Bourjade, M., Wessel-Robert, S., & Richard-Yris, M.-A. (2012). On the significance of adult play: What does social play tell us about adult horse welfare? *Naturwissenschaften*, 99(4), 291–302. <https://doi.org/10.1007/s00114-012-0902-8>
- Hausberger, M., Lesimple, C., & Henry, S. (2021). Detecting Welfare in a Non-Verbal Species: Social/Cultural Biases and Difficulties in Horse Welfare Assessment. *Animals*, 11(8), 2249. <https://doi.org/10.3390/ani11082249>
- Henriksson, J., Sauveroché, M., & Roth, L. S. (2019). Effects of size and personality on social learning and human-directed behaviour in horses (*Equus caballus*). *Animal Cognition*, 22(6), 1001–1011.
- Henry, S., Richard-Yris, M.-A., & Hausberger, M. (2006). Influence of various early human–foal interferences on subsequent human–foal relationship. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, 48(8), 712–718.
- Jenkins, B. A., & Lumpkin, E. A. (2017). Developing a sense of touch. *Development (Cambridge, England)*, 144(22), 4078–4090. <https://doi.org/10.1242/dev.120402>
- Jenkins, W. L. (1948). *Studying the skin senses*.
- Jerosch-Herold, C. (2005). Assessment of sensibility after nerve injury and repair: A systematic review of evidence for validity, reliability and responsiveness of tests. *Journal of Hand Surgery*, 30(3), 252–264.
- Joubert, E. (1972). The social organization and associated behaviour in the Hartmann zebra *Equus zebra hartmannae* part 1. *Madoqua*, 1972(6), 17–56.

- Karenina, K., Giljov, A., & Malashichev, Y. (2018). Lateralization of mother-infant interactions in wild horses. *Behavioural Processes*, 148, 49–55. <https://doi.org/10.1016/j.beproc.2018.01.010>
- Langbauer Jr, W. r. (2000). Elephant communication. *Zoo Biology*, 19(5), 425–445. [https://doi.org/10.1002/1098-2361\(2000\)19:5<425::AID-ZOO11>3.0.CO;2-A](https://doi.org/10.1002/1098-2361(2000)19:5<425::AID-ZOO11>3.0.CO;2-A)
- Langford, D. J., Crager, S. E., Shehzad, Z., Smith, S. B., Sotocinal, S. G., Levenstadt, J. S., Chanda, M. L., Levitin, D. J., & Mogil, J. S. (2006). Social modulation of pain as evidence for empathy in mice. *Science*, 312(5782), 1967–1970.
- Lansade, L., & Simon, F. (2010). Horses' learning performances are under the influence of several temperamental dimensions. *Applied Animal Behaviour Science*, 125, 30–37.
- Lansade, L., Bonneau, C., Parias, C., & Biau, S. (2019). Horse's emotional state and rider safety during grooming practices, a field study. *Applied Animal Behaviour Science*, 217, 43–47. <https://doi.org/10.1016/j.applanim.2019.04.017>
- Lansade, L., Coutureau, E., Marchand, A., Baranger, G., Valenchon, M., & Calandreau, L. (2013). Dimensions of temperament modulate cue-controlled behavior: A study on pavlovian to instrumental transfer in horses (*Equus caballus*). *PLoS One*, 8(6), e64853.
- Lansade, L., Marchand, A. R., Coutureau, E., Ballé, C., Polli, F., & Calandreau, L. (2017). Personality and predisposition to form habit behaviours during instrumental conditioning in horses (*Equus caballus*). *PLoS One*, 12(2), e0171010. <https://doi.org/10.1371/journal.pone.0171010>
- Lansade, L., Neveux, C., & Levy, F. (2012). A few days of social separation affects yearling horses' response to emotional reactivity tests and enhances learning performance. *Behavioural Processes*, 91(1), 94–102.
- Lansade, L., Philippon, P., Hervé, L., & Vidament, M. (2016). Development of personality tests to use in the field, stable over time and across situations, and linked to horses' show jumping performance. *Applied Animal Behaviour Science*, 176, 43–51. <https://doi.org/10.1016/j.applanim.2016.01.005>
- Lansade, L., Pichard, G., & Leconte, M. (2008). Sensory sensitivities: Components of a horse's temperament dimension. *Applied Animal Behaviour Science*, 114(3–4), 534–553. <https://doi.org/10.1016/j.applanim.2008.02.012>
- Lansade, L., Valenchon, M., Foury, A., Neveux, C., Cole, S. W., Layé, S., Cardinaud, B., Lévy, F., & Moisan, M.-P. (2014). Behavioral and Transcriptomic Fingerprints of an Enriched Environment in Horses (*Equus caballus*). *PLoS One*, 9(12), e114384. <https://doi.org/10.1371/journal.pone.0114384>
- Larose, C., Richard-Yris, M.-A., Hausberger, M., & Rogers, L. (2006). Laterality of horses associated with emotionality in novel situations. *Laterality: Asymmetries of Body, Brain, and Cognition*, 11(4), 355–367. <https://doi.org/10.1080/13576500600624221>
- Le Bars, D., Gozariu, M., & Cadden, S. W. (2001). Animal models of nociception. *Pharmacological Reviews*, 53(4), 597–652.
- Lerch, N., Cirulli, F., Rochais, C., Lesimple, C., Guilbaud, E., Contalbrigo, L., Borgi, M., Grandgeorge, M., & Hausberger, M. (2021). Interest in Humans: Comparisons between Riding School Lesson Equids and Assisted-Intervention Equids. *Animals*, 11(9), 2533. <https://doi.org/10.3390/ani11092533>
- Lesimple, C. (2020). Indicators of Horse Welfare: State-of-the-Art. *Animals*, 10(2), 294. <https://doi.org/10.3390/ani10020294>
- Lesimple, C., & Hausberger, M. (2014). How accurate are we at assessing others' well-being? The example of welfare assessment in horses. *Front Psychol*, 5, 21. <https://doi.org/10.3389/fpsyg.2014.00021>
- Mason, G. J. (1991). Stereotypies: A critical review. *Animal Behaviour*, 41(6), 1015–1037. [https://doi.org/10.1016/S0003-3472\(05\)80640-2](https://doi.org/10.1016/S0003-3472(05)80640-2)
- Mayes, E., & Duncan, P. (1986). Temporal Patterns of Feeding Behaviour in Free-Ranging Horses. *Behaviour*, 96(1–2), 105–129. <https://doi.org/10.1163/156853986X00243>
- Mills, D. S., & Nankervis, K. J. (2013). *Equine behaviour: Principles and practice*. John Wiley & Sons.
- Mogil, J. S. (2009). Animal models of pain: Progress and challenges. *Nature Reviews Neuroscience*, 10(4), 283–294.
- Norrsell, U., Finger, S., & Lajonchere, C. (1999). Cutaneous sensory spots and the “law of specific nerve energies”: History and development of ideas. *Brain Research Bulletin*, 48(5), 457–465.
- Osthaus, B., Proops, L., Long, S., Bell, N., Hayday, K., & Burden, F. (2018). Hair coat properties of donkeys, mules and horses in a temperate climate. *Equine Veterinary Journal*, 50(3), 339–342. <https://doi.org/10.1111/evj.12775>
- Plomin, R., 1983. Developmental behavioral genetics. *Child Dev.* 54, 253–259.
- Popescu, S., & Diugan, E.-A. (2013). The relationship between behavioral and other welfare indicators of working horses. *Journal of Equine Veterinary Science*, 33(1), 1–12.
- Pritchard, J. C., Lindberg, A. C., Main, D. C. J., & Whay, H. R. (2005). Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. *Preventive Veterinary Medicine*, 69(3), 265–283. <https://doi.org/10.1016/j.prevetmed.2005.02.002>

- Reddy, C. G., Miller, J. W., Abode-Iyamah, K. O., Safayi, S., Wilson, S., Dalm, B. D., Fredericks, D. C., Gillies, G. T., Howard, M. A., & Brennan, T. J. (2018). Ovine model of neuropathic pain for assessing mechanisms of spinal cord stimulation therapy via dorsal horn recordings, von Frey filaments, and gait analysis. *Journal of Pain Research*, 11, 1147–1162. <https://doi.org/10.2147/JPR.S139843>
- Redua, M., Valadao, C., Duque, J., & Balestrero, L. (2002). The pre-emptive effect of epidural ketamine on wound sensitivity in horses tested by using von Frey filaments—Rédua—2002—Veterinary Anaesthesia and Analgesia—Wiley Online Library. *Vet Anaesth Analg*, 29, 200–206.
- Rochais, C., Henry, S., Sankey, C., Nassur, F., Gorecka-Bruzda, A., & Hausberger, M. (2014). Visual attention, an indicator of human-animal relationships? A study of domestic horses (*Equus caballus*). *Frontiers in Psychology*, 5. <https://doi.org/10.3389/fpsyg.2014.00108>
- Rørvang, M. V., Nielsen, B. L., & McLean, A. N. (2020). Sensory abilities of horses and their importance for equitation science. *Frontiers in Veterinary Science*, 7, 633.
- Sanchis-Mora, S., Chang, Y.-M., Abeyesinghe, S., Fisher, A., Volk, H. A., & Pelligand, L. (2017). Development and initial validation of a sensory threshold examination protocol (STEP) for phenotyping canine pain syndromes. *Veterinary Anaesthesia and Analgesia*, 44(3), 600–614.
- Sankey, C., Richard-Yris, M.-A., Leroy, H., Henry, S., & Hausberger, M. (2010). Positive interactions lead to lasting positive memories in horses, *Equus caballus*. *Animal Behaviour*, 79(4), 869–875. <https://doi.org/10.1016/j.anbehav.2009.12.037>
- Sapa, M.-C., Sagot, J.-C., Touvenot, G., Xavier, F., Hidalgo Diaz, J.-J., Facca, S., Zaré, M., & Liverneaux, P. (2019). Comparison of tactile sensitivity measured with a new digital esthesiometer (Beam Test) relative to Semmes–Weinstein monofilament analog esthesiometer. *Hand Surgery and Rehabilitation*, 38(4), 242–245. <https://doi.org/10.1016/j.hansur.2019.06.003>
- Saslow, C. A. (2002). Understanding the perceptual world of horses. *Applied Animal Behaviour Science*, 78(2–4), 209–224. [https://doi.org/10.1016/S0168-1591\(02\)00092-8](https://doi.org/10.1016/S0168-1591(02)00092-8)
- Sato, S., Tarumizu, K., & Hatae, K. (1993). The influence of social factors on allogrooming in cows. *Applied Animal Behaviour Science*, 38(3), 235–244. [https://doi.org/10.1016/0168-1591\(93\)90022-H](https://doi.org/10.1016/0168-1591(93)90022-H)
- Schaefer, M., Heinze, H.-J., & Rotte, M. (2012). Touch and personality: Extraversion predicts somatosensory brain response. *NeuroImage*, 62(1), 432–438. <https://doi.org/10.1016/j.neuroimage.2012.05.004>
- Schrimpf, A., Single, M.-S., & Nawroth, C. (2020). Social Referencing in the Domestic Horse. *Animals*, 10(1), 164. <https://doi.org/10.3390/ani10010164>
- Siegel, S. (1957). Nonparametric statistics. *The American Statistician*, 11(3), 13–19.
- Siniscalchi, M., d’Ingeo, S., Minunno, M., & Quaranta, A. (2018). Communication in Dogs. *Animals : An Open Access Journal from MDPI*, 8(8), 131. <https://doi.org/10.3390/ani8080131>
- Spier, S. J., Berger Pusterla, J., Villarroel, A., & Pusterla, N. (2004). Outcome of tactile conditioning of neonates, or “imprint training” on selected handling measures in foals. *The Veterinary Journal*, 168(3), 252–258. <https://doi.org/10.1016/j.tvjl.2003.12.008>
- Suomi, S. J., Collins, M. L., & Harlow, H. F. (1973). Effects of permanent separation from mother on infant monkeys. *Developmental Psychology*, 9(3), 376.
- Tallet, C., Veissier, I., & Boivin, X. (2005). Human contact and feeding as rewards for the lamb’s affinity to their stockperson. *Applied Animal Behaviour Science*, 94(1), 59–73. <https://doi.org/10.1016/j.applanim.2005.02.007>
- Thomas, A., Chess, S., 1977. Temperament and Development. Brunner Mazel, New York.
- Tyler, S. J. (1972). The Behaviour and Social Organization of the New Forest Ponies. *Animal Behaviour Monographs*, 5, 87–196. [https://doi.org/10.1016/0003-3472\(72\)90003-6](https://doi.org/10.1016/0003-3472(72)90003-6)
- Valenchon, M., Lévy, F., Fortin, M., Leterrier, C., & Lansade, L. (2013a). Stress and temperament affect working memory performance for disappearing food in horses, *Equus caballus*. *Animal Behaviour*, 86(6), 1233–1240.
- Valenchon, M., Lévy, F., Górecka-Bruzda, A., Calandreau, L., & Lansade, L. (2013b). Characterization of long-term memory, resistance to extinction, and influence of temperament during two instrumental tasks in horses. *Animal Cognition*, 16(6), 1001–1006.
- Valenchon, M., Lévy, F., Prunier, A., Moussu, C., Calandreau, L., & Lansade, L. (2013c). Stress Modulates Instrumental Learning Performances in Horses (*Equus caballus*) in Interaction with Temperament. *PLOS ONE*, 8(4), e62324. <https://doi.org/10.1371/journal.pone.0062324>
- Valenchon, M., Lévy, F., Moussu, C., & Lansade, L. (2017). Stress affects instrumental learning based on positive or negative reinforcement in interaction with personality in domestic horses. *PloS One*, 12(5), e0170783.
- van Iwaarden, A., Stubbs, N.C., Clayton, H.M., 2012. Topographical Anatomy of the Equine M. Cutaneus Trunci in Relation to the Position of the Saddle and Girth. *J. Equine Vet. Sci.* 32, 519–524. <https://doi.org/10.1016/j.jevs.2011.12.005>
- Veres-Nyéki, K. O., Nyéki, J., Bodó, G., & Spadavecchia, C. (2021). Quantitative sensory testing of the equine face. *Equine Veterinary Journal*, 53(1), 177–185. <https://doi.org/10.1111/evj.13270>

- Vidament, M., Lansade, L., Danvy, S., Priest, B. D. S., Sabbagh, M., & Ricard, A. (2021). Personality in young horses and ponies evaluated during breeding shows: Phenotypic link with jumping competition results. *Journal of Veterinary Behavior*. <https://doi.org/10.1016/j.jveb.2020.09.003>
- Vidament, M., Yvon, J. M., Bon, M. le, Saint Priest, B. D., Danvy, S., & Lansade, L. (2015). The temperament of horses measured by standardized tests: Relationship with age, race and rider level. *41ème Journée de La Recherche Équine, Jeudi 12 Mars 2015, Paris, France*, 15–24.
- von Frey, M. (1894). Beitrage zur Physiologie der Schmerzsinnes. *Ben Math Phys KI Konigl Sachs Ges Wissensch Leipzig*, 46, 185–196.
- von Uexkuell, J. (1965). *Mondes animaux et monde humain, suivi de théorie de la signification* (Pocket).
- Waring, G. H. (2003). *Horse Behavior* (2nd ed). Noyes Publishing.
- Warren-Smith, & McGreevy, P. (2015). *Equestrian Coaches' Understanding and Application of Learning Theory in Horse Training: Anthrozoös: Vol 21, No 2*. <https://www.tandfonline.com/doi/abs/10.2752/175303708X305800>
- Werner, M. U., Rotbøll-Nielsen, P., & ELLEHUUS-HILMERSSON, C. (2011). Humidity affects the performance of von Frey monofilaments. *Acta Anaesthesiologica Scandinavica*, 55(5), 577–582.
- Wismeijer, A. A., & Vingerhoets, A. J. (2005). The use of virtual reality and audiovisual eyeglass systems as adjunct analgesic techniques: A review of the literature. *Annals of Behavioral Medicine*, 30(3), 268–278.
- Zimmerman, A., Bai, L., & Ginty, D. D. (2014). The gentle touch receptors of mammalian skin. *Science*, 346(6212), 950–954.