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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

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

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

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

32 Several tools exist to assess tactile reactivity, the best known being the cotton swab test, the static Weber test, the
33 dynamic Weber test (Sapa et al. 2019) and also the lancet test for newborns (Gibbins et al. 2008). Nevertheless,
34 most of the research conducted with these methods was for therapeutic purposes and not for increasing knowledge
35 of the fundamentals of tactile reactivity (e.g. Gibbins et al. 2008; Bigorre et al. 2017). In addition, the body areas
36 tested with these tools are often the same, i.e. hands (humans, e.g. Dellon 1986; Bigorre et al. 2017) and the force
37 applied is not standardized. Among the different tools used, one seems to stand out thanks to its numerous
38 advantages: the von Frey filaments. Indeed, these filaments can be applied to any species, without age limit, on
39 any part of an individual's body with a constant and standardized pressure. Initially, horse hair was used to test the
40 perception of human skin, i.e. the response to tactile stimulation to this hair application. Von Frey (1894) adapted
41 this method by developing a set of calibrated filaments consisting of a rigid plastic body extended by a nylon
42 thread of variable thickness. When the end of the filament, of a given length and diameter, is applied
43 perpendicularly on the skin, the application force increases as long as the experimenter continues the pressing
44 motion, until the filament flexes. Continuing the pressing motion increases the bending but not the application
45 force. This principle therefore allows a known, measurable and reproducible force to be applied to the skin surface.
46 Von Frey filaments were initially developed for humans to define tactile perception thresholds (Bell-Krotoski et
47 al. 1995). A nylon filament delivering an average force of 0.07g was found to be a good predictor of the "normal"
48 pressure threshold of light touch on hands and on a large part of the body of adult humans (Bell-Krotoski et al.
49 1995). This tool has become widely used both for human, i.e. medicine (Jerosch-Herold 2005) and preclinical
50 neuroscience (Le Bars et al. 2001), often to assess pain with the hope of therapeutic translation (Mogil 2009) but
51 also for animal (Norrrell et al. 1999 e.g. sheep: Reddy et al. 2018; rodents: Bonin et al. 2014; dogs: Brydges et al.
52 2012; horses: Redua et al. 2002) research.

53

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

55 1. The role of tactile stimulations in animals' lives

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

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

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

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

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

107 a positive reinforcement by trainers and is used as such by some of them (e.g. Warren-Smith and McGreevy 2015).
108 On the other hand, tactile actions such as leg contact are considered negative reinforcement with the difficulty of
109 expecting horses to respond to "subtle" leg actions, but overlook potentially inappropriate tactile actions of
110 inexperienced or disabled riders (Warren-Smith and McGreevy 2015). Most current training practices involve so-
111 called "desensitization" (in fact, habituation) procedures that aim to reduce horses' responsiveness to tactile stimuli
112 (e.g. Spier et al. 2004). Moreover, the possible impact of inappropriate handling/contact actions on the emotional
113 state of horses involved in equine-assisted interventions has been questioned in recent studies (Contalbrigo et al.
114 2021; Lerch et al. in prep.). Horses therefore appear to be a very interesting animal model, providing an
115 opportunity to study the impact of intrinsic factors such as sex or breed, extrinsic factors such as season and the
116 interplay between genetic and environmental factors on their tactile reactivity (e.g. Hausberger et al. 2004).

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

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

127 **2.1. Intrinsic factors**

128 - *Sex*

129 Differences according to sex have been reported for dogs, females' tactile reactivity thresholds are higher than
130 those of males (Sanchis-Mora et al. 2017). Data concerning horses are contradictory. Hausberger et al. (2012),
131 studying play of adult horses, noted that geldings were more reactive (lower reactivity threshold) than mares to
132 von Frey filaments whereas other studies did not find any difference (Vidament et al. 2021; Veres-Nyéki et al.
133 2021; Briefer Freymond et al. 2019). Other studies tested only mares (Lansade et al. 2008; Lansade et al. 2017;
134 Lansade and Simon 2010; Valenchon et al. 2013a,b,c; Valenchon et al. 2017) while one study did not mention the
135 sex of the subjects (Lansade et al. 2016). The remaining studies did not mention whether or not they tested possible
136 sex-related differences of tactile reactivity, despite testing both mares and geldings (Fureix et al. 2012; Lansade et
137 al. 2012; Lansade et al. 2013; Lansade et al. 2014; Gonzalez-De Cara et al. 2017). It is difficult to draw conclusions
138 at this point, given the few studies that have investigated this factor. Moreover, the discrepancies between studies
139 with conflicting results might also be explained by other methodological differences as well: location (chest,
140 Hausberger et al. 2012; withers, Briefer Freymond et al. 2019 and Vidament et al. 2021; head, Veres-Nyéki et al.
141 2021) or context (home environment vs. unfamiliar stall on competition ground) of testing.

142 - *Age*

143 Several studies tested only equids of a given age (Lansade et al. 2012; Lansade et al. 2013; Lansade et al. 2014)
144 but for those using animals of different ages, results are mostly absent (not tested or not mentioned: Briefer-
145 Freymond et al. 2019, Fureix et al. 2012, Gonzalez-De Cara et al. 2017; Hausberger et al. 2012; Lansade et al.
146 2008; 2016, 2017; Lansade and Simon 2010; Valenchon et al. 2013a,b,c; 2017). However, the two studies
147 investigating effect of age converge to suggest a decrease in tactile reactivity with age. According to Saslow's
148 review (2002), older horses (over 20 years old) would have a lower tactile reactivity. Veres-Nyeki et al.'s (2021)
149 results suggest that tactile reactivity decreases with age and Vidament et al. (2021) found that 2-year-old horses
150 were more reactive than 3-year-olds.

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

157

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

173 - *Temperament*

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

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

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

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

194 Other studies have examined the relationships between chronic stress and tactile reactivity. Stereotypic behaviors
 195 are one expression of chronic stress (Mason 1991). Briefer-Freymond et al. (2019) compared the tactile reactivity
 196 of 38 horses, half of them presenting an oral stereotypic behavior (cribbing), the other half presenting no cribbing
 197 (as reported by owners). They found that the cribbing horses were more reactive to von Frey filaments and
 198 suggested that this higher tactile sensitivity may be due to altered dopamine or endogenous opioid physiology
 199 resulting from chronic stress exposure. However, there were biases that prevent definite conclusions: an owner's
 200 recognition of welfare expressions may not be entirely reliable (Lesimple and Hausberger 2014; Hausberger et al.
 201 2021) and the non-cribbing horses may have exhibited other expressions of compromised welfare (e.g. other
 202 types of stereotypic behaviors... e.g. Lesimple 2020), e.g. depressive-like states that are associated with lower
 203 sensory reactivity, in particular tactile reactivity (Fureix et al. 2012, 2015)

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

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

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

214 **2.2 Extrinsic factors**

215 - *Living conditions*

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

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

231 - *Working conditions*

232 Although no studies have focused on the potential impact of working activity on tactile reactivity, Vidament et al.
233 (2021), as mentioned above, found differences between sport and recreational ponies. While these authors favor
234 the idea of possible selection, i.e. a genetic process, it cannot be ruled out that activity *per se* can influence tactile
235 reactivity, for example, by increasing habituation or sensitization at certain body parts (Lerch et al. in prep.).
236 According to Vidament et al. (2015), horses ridden by lower level riders (beginners to moderately advanced) have
237 lower tactile reactivity than those ridden by experienced riders (first competition level and above). Of course, it is
238 again difficult to disentangle whether the horses were selected for such characteristics in the first place or whether
239 repeated inappropriate handling or tackling by beginners while the horses were told not to respond may have
240 resulted in habituation or response inhibition.

241 **3. A limiting factor: methodology**

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

248 **3.1 Context of testing**

249 - *Environmental conditions*

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

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

258 mention the location of their test. For some, the horses were tested in their familiar stall (Fureix et al. 2012,
259 Hausberger et al. 2012), in their familiar stall or in a paddock (Briefer-Freymond et al. 2019), in an unfamiliar
260 arena (without specifying whether it is indoors or outdoors) (Gonzalez-De Cara et al. 2017; Lansade et al. 2013,
261 2014), in their stall or in a mobile stall (without specifying familiarity with location) (Valenchon et al. 2013a, b,
262 c, 2017; Lansade et al. 2008), in an arena (without specifying familiarity with this location or whether it is indoors
263 or outdoors) (Lansade et al. 2012, 2017, Lansade and Simon 2010), in an outdoor arena (without specifying
264 familiarity with this location) (Lansade et al. 2016) while others do not provide any information concerning the
265 test location (Verez-Nieki et al. 2021; Vidament et al. 2020).

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

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

275 - *Season*

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

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

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

287 - *Handling conditions*

288 Although no report mentioned a possible impact of horses' handling conditions on tactile reactivity, a few studies
289 did not mention how the horse was held during the test (Briefer-Freymond et al. 2019; Fureix et al. 2012; Lansade
290 et al. 2012, 2017). Reports providing this information show that horses can be tied up to the wall (Lansade et al.
291 2008; Vidament et al. 2021), held by another person (Lansade et al. 2013; Lansade et al. 2016; Lansade and Simon
292 2010; Valenchon et al. 2013 a,b,c; 2017, Veres-Nieki et al. 2021), or free moving (Lansade et al. 2016: for the
293 "simplified personality tests"). The way horses are held (loose or tight lunge for example) might influence a horse's
294 emotional or attentional state, hence its sensory reactivity. This may add to the determinants of discrepancies
295 between studies.

296 **3.2 The tool: filaments' size**

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

300 Lansade et al. (2008) were the first to use this tool to assess tactile reactivity of equids outside a medical context.
301 They tested five different sizes of these filaments (0.008g; 0.02g; 0.6g; 8g; 300g) in order to cover a wide range
302 of pressures. They showed that the number of horses reacting to von Frey filaments increased significantly between
303 0.008g and 8 g, whereas above 8 g, fewer horses reacted. Since then, most studies (14 of 17) have used four sizes
304 of filaments (0.008g; 0.02g; 0.6g; 300g, without explaining the choice of sizes). Follow-up studies by Lansade and
305 Valenchon's groups applied the same filament sizes except for one (1g instead of 8g) and in the same way, i.e.:
306 filament 0.008g on the right side of the withers then 300g on the left side of the withers, interruption for another

307 test then application of the filament 0.02g on the right side of the withers then 1g on the left side of the withers.
308 Fureix et al. (2012) and Hausberger et al. (2012) applied these four sizes of filaments but both on the left and right
309 body sides. The authors of the remaining studies tested different, complementary and more filament sizes:
310 Vidament et al. (2021) tested four sizes of filaments (0.008g; 0.02g; 1g; 50g); Briefer Freymond et al. (2019) tested
311 five sizes (0.008g, 0.020g, 0.6g, 1g, 300g); Gonzalez-De Cara et al. (2017) tested seven sizes (0.008g, 0.02g,
312 0.16g, 0.6g, 8g, 60g, 300g). Veres-Nieki et al. (2021) did not specify which filament sizes were used, they only
313 explained that von Frey filaments were applied in an increasing order until a muscle contraction was detected in
314 response to stimulation (the average size of the filament to which the horses responded at each area is however
315 detailed). The reasons for these choices are not explicitly mentioned and most studies do not give details
316 concerning the horses' reactivity according to filament size (Lansade et al. 2012, 2013, 2014, 2016, 2017; Lansade
317 and Simon 2010; Hausberger et al. 2012; Veres-Nieki et al. 2021). Vidament et al. (2020) reported that equids had
318 responded as follows: 0.008g (18% of the population), 0.02g (29%), 1g (78%) and 50g (72%). Fureix et al. (2012)
319 found that depressed horses responded less to tactile stimuli while Briefer-Freymond et al. (2019) found that crib-
320 biter horses responded mostly to the 0.6g filament. Gonzalez De Cara et al. (2017) found that a 0.6g filament
321 induced the highest reactivity of donkeys, followed by the 0.16g filament while the 300g and 60g filaments
322 generated the lowest levels of reactivity. Discrepancies are also found in the measurements, Gonzales De Cara et
323 al. (2017) and Veres-Nieki et al. (2021) evaluated the responses in terms of number of reactions per horse while
324 the other studies measured the number of horses showing a reaction.

325 3.3 Testing procedure

326 - *Body area*

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

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

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

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

350 - *Body Side*

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

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

364 - *Filament application order*

365 In their first study of sensory sensitivity, Lansade et al. (2008) tested horses using stimuli of different intensities
366 at each test session. The five filament sizes had been selected in a preliminary study to produce satisfactory inter-
367 individual variability in responses and were used in the same order: 300g; 0.6g; 0.02g; 0.008g; 8g. This was
368 replicated by Briefer Freymond et al. (2019) who however changed the 8g filament for a 1g filament. Then all the
369 other follow-up studies by Lansade and Valenchon's team used four sizes of filaments in the following way:
370 0.008g, 300g, 0.02g and finally 1g (each size on one side of the body as mentioned above). The only justification
371 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
372 horse usually does not respond the second time (Lansade et al. 2016). Similarly, Veres-Nyeki et al. (2021) applied
373 the filaments in the same order, following the increasing order of filament size.

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

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

378 - *Repetition*

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

387 **Conclusion**

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

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

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

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

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

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

415 **MATERIALS AND METHODS**

416 **Ethics**

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

423 **Subjects**

424 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
425 old ($X \pm SE = 11 \pm 8$ years) of different breeds (Tab.1). These horses lived at or near the Paimpont Biological
426 Station (Ille-et-Vilaine, France) in the same naturalistic conditions (i.e. in stable groups of 2 to 4 individuals, in 1
427 to 5 hectare natural pastures with water ad libitum). Nine had never been ridden, five had been ridden in the past
428 but not anymore (for at least 6 years) and two were occasionally ridden on week-ends (outdoor recreational riding).
429 All of them were nevertheless used to being handled by humans (halter, walking in hand, grooming). Welfare
430 assessments (observational assessments) performed for another study (in prep.) indicated that none of these horses
431 exhibited any welfare problems (e.g. no indicators of compromised welfare: stereotypic behaviors, depressed
432 postures, aggressiveness... see e.g. Lesimple 2020 for review).

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454 *Tab. 1: Characteristics of the individuals involved in the study*
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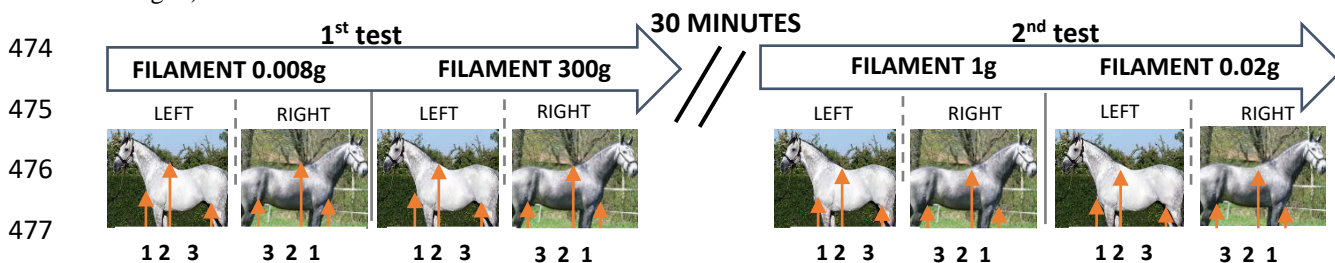
INDIVIDUAL	SEX	AGE (years)	BREED	TYPE OF EQUID
1	Stallion	3	Unregistered	Pony
2	Gelding	4	Appaloosa crossbred	Pony
3	Gelding	4	Unregistered	Horse
4	Mare	5	Unregistered	Horse
5	Mare	5	Unregistered	Horse
6	Gelding	5	Appaloosa crossbred	Pony
7	Mare	6	Unregistered	Horse
8	Mare	6	Appaloosa crossbred	Pony
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

456
 457 **Tactile reactivity test**

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

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

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



478 *Fig. 1: Example of a tactile reactivity test performed on a horse based on the following order of application of the filaments (the*
 479 *order was chosen randomly): 0.008g; 300g; 1g; 0.02g. Each filament was applied first to one side (in the example: left side) of*
 480 *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.

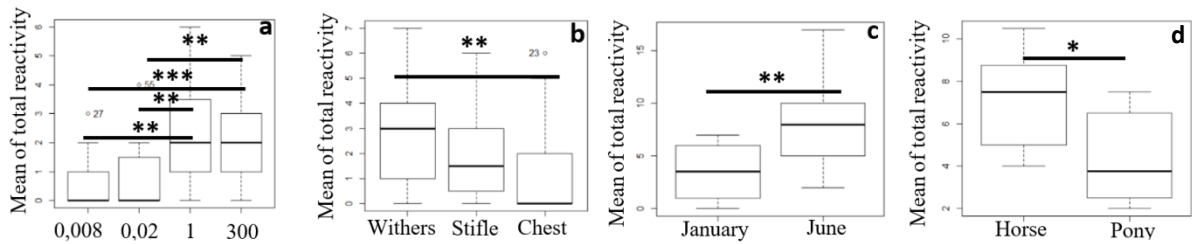
506 **RESULTS**

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).



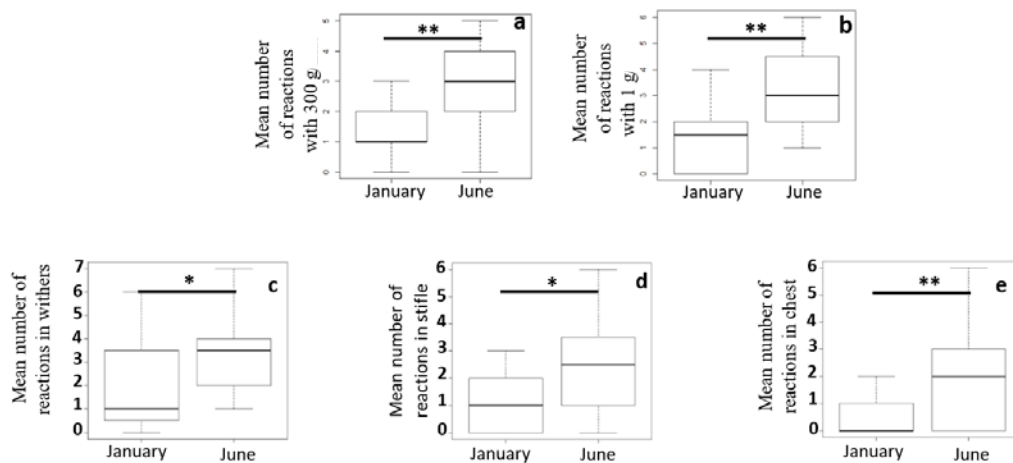
527
 528 *Fig. 2: Influence of: filament size (a), body area tested (b), period of test (c) and type of equid (d) on overall tactile responsiveness*
 529 *of equids. Friedman's, Wilcoxon's and MW test: *p<0.05, **p<0.01, ***p<0.001. The plots show median (line within box), 25th*
and 75th percentiles (box), 5th and 95th percentiles (whiskers), and outliers (°).

530 **Influence of test session**

531 We could evidence no significant differences between test sessions (1st in January, 2nd in June) concerning the
 532 number of horses that reacted at least once to a tactile stimulation (June: all 16 horses; January: 13 out of 16)
 533 (McNemar test, df=1, p>0.05), the number of horses reacting to stimulations at the different body areas (chest,
 534 withers and stifle: 10, 16, 14 horses reacted in June and 5, 12, 10 in January respectively (McNemar test, df=1,
 535 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).
 536 However, in June, more horses reacted to applications on their right side (15 *versus* 8 in January; df=1, p=0.016),
 537 and to the 1g filament (16 *versus* 10 equids; df=1, p=0.031) than in January.

538 As mentioned above, when considering all reactions to all stimuli, the subjects reacted more (number of reactions)
 539 to the tactile stimuli during the second test session (June) than during the first test session (January) (Wilcoxon
 540 Test, N=16, V=117, p=0.001). However, this result seemed to be mostly due to the larger filaments: 300g (N=16,
 541 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
 542 smaller filaments (0.008g: N=16, V=52.5, p>0.05; 0.02g: N=16, V=33, p>0.05) were considered.

543 These differences according to the testing session remained whatever the body side (right side: V=78, p=0.002;
 544 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,
 545 p=0.005) (Fig. 3c, d, e).



546
 547 *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)*
 548 *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 (°).

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

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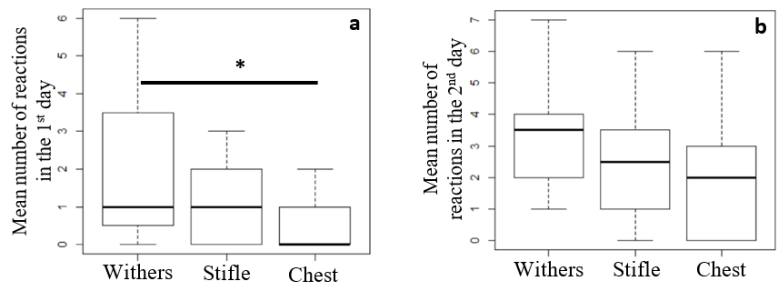


Fig. 4. Median number of reactions to applications on withers, stifle and chest during the first (January) (a) and the second (June) test sessions (b). Friedman's test: * $p < 0.05$. The plots show median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers), and outliers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

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).

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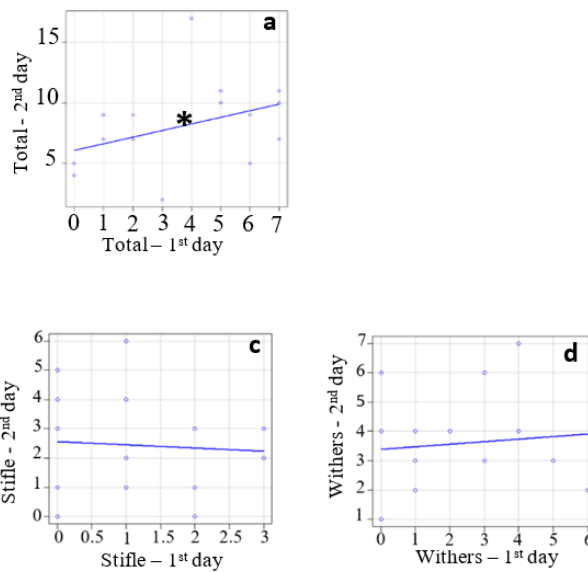


Fig 6 : Correlations between the two test sessions in horses' total tactile reactivity at: (a) all body parts pooled, chest (b), stifle (c), and withers (d). Spearman correlations: * $p < 0.05$, ** $p < 0.01$.

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

600 **Conclusion**

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

609

610

GENERAL DISCUSSION

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

616 Importance of intrinsic factors for tactile reactivity

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

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

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

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

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

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

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

655 Impact of extrinsic factors

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

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

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

672 Methodological aspects

673 Clear discrepancies emerged between studies concerning the areas and sides of the body tested, and the sizes of
674 filaments or the order they were applied. Most studies focused on tests at the base of the withers (Lansade et al.
675 2008, 2012; Valenchon et al. 2013a,b,c; Vidament et al. 2020), while other authors applied the filaments to several
676 body areas and found that responses differed and depended on the area tested (e.g. Fureix et al. 2012). Our pilot
677 study confirmed their findings, equids reacting more (both test sessions combined) to stimulations to the withers
678 than to the chest and stifle, which converges with the variations of sensory nerve receptors between body areas
679 (Mills and Nankervis 2013). However, we found this higher reactivity at the withers only for the first session
680 (January) whereas we did not find any difference related to the area tested for the second session (June). Maybe
681 horses habituate more to tactile stimulations to withers (which could explain Lansade et al.'s 2008 findings of
682 rapid habituation) or there are seasonal variations. Interestingly, allogrooming, which often occurs at withers, is
683 observed more often in winter; this could be explained by their thicker coat, which may then enclose mud or
684 ectoparasites, in particular lice, probably causing irritation (Tyler 1972). Finally, the finding that the reactivity of
685 mares and geldings differed in June only at stifle remains unexplained but worth further investigation. These
686 different results prove the importance of testing several areas in order to obtain a global score of tactile reactivity
687 and not a score for only one area that, moreover, could be biased because of the impact of the type of work, the
688 distribution of the sensory receptors on the surface of the body but also the season at the time of the tests. It is
689 worth noting that individual consistency appeared highest in our study for chest and not withers.

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

696 Finally, with a few exceptions, most studies tested the same sizes of filaments but in a different order. We found
697 that the equids reacted more to the larger filaments than to the smaller ones, but the order of application had no
698 impact on their reactivity. However, of the four filaments we tested in this pilot study the 1g filament seems
699 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 horses 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	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	
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	
FAMILIAR OR UNFAMILIAR EXPERIMENTER	N	Y Unfamiliar	Y Unfamiliar	N	N	Y Familiar	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	
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 adlibitum	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 experimenter	Y Idem lansade et simon	Y 2 experimenter	Y 2 experimenter	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	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	

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