Vibrissae of the horse,

a pilot study on how to assess the effect of manipulation.

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Abstract

Introduction: The horse's vibrissae are often trimmed for cosmetic purposes in the Netherlands. The function of vibrissae has been studied in certain rodents and sea mammals, but not in horses. To assess the effect of trimming vibrissae on the welfare of horses, the vibrissae's function in the horse must first be studied. However, no standardized test is available. The primary aim of this pilot study was to find parameters that could both measure the function of the horse's vibrissae as well as the effects of trimming them. The secondary aim was to create a standardized test, which would allow their function to become apparent. To this end, two tests were performed.
Test 1: **Materials and method:** Five stimuli were applied to the vibrissae of 14 horses: heat, cold, electricity, damage and traction. All stimuli and their control (i.e. "sham") stimuli were applied for 5 seconds each, in a randomized order. Behavior and heart rate were analyzed. **Results:** No significant heart rate results were seen. Results on behavior are not yet available. **Conclusion:** These results could indicate that horses do not sense the applied stimuli with their vibrissae. However, heart rate might not be the right outcome measure.
Test 2: **Materials and method:** Multiple devices were designed and evaluated for usability. The best device was a design that was adjustable to the circumference of the horse's muzzle plus 1 cm. Horses were randomly divided into three groups. Group 0: vibrissae trimmed to 0 cm (N=16), group 1: vibrissae trimmed to 1 cm (N=13), group 2: vibrissae kept at natural length (N=14). All horses were asked to move their nose through the device. Behavior, nose-bumps, heart rate, heart rate variability and latency time were investigated. **Results:** Results on behavior and latency time are not yet available. Available data showed a large within group variation. There was no significant difference between the means of the three groups. **Conclusion:** Due to a small power of our test, no conclusions can be drawn from these results regarding the effect of trimming vibrissae on the used parameters. Analyzed parameters do not appear to be useful outcome measures. Because not all results are available yet, the usability of the designed device is still under debate.

Abstract

Introductie: In Nederland worden de vibrissae van het paard op grote schaal ingekort voor cosmetische redenen. De functie van vibrissae is bij verscheidene knaagdieren en zee zoogdieren onderzocht, maar bij het paard nog niet. Om het effect van het inkorten van tastharen op het welzijn van paarden te bepalen moet eerst de functie van vibrissae bij het paard onderzocht worden. Echter is hier nog geen gestandaardiseerde test voor beschikbaar. Het primaire doel van dit onderzoek was om parameters te vinden waarmee zowel de functie van vibrissae als het effect van het inkorten van vibrissae gemeten kan worden. Het secundaire doel was om een test te ontwerpen waarmee de functie van vibrissae meetbaar gemaakt kan worden. Hiertoe werden twee testen uitgevoerd.
Test 1: **Materialen en methode:** Vijf stimuli werden toegepast op de vibrissae van 14 paarden: hitte, koude, elektriciteit, schade en tractie. Al deze stimuli en hun controle ("nep") stimuli werden elk 5 seconden lang toegediend in een gerandomiseerde volgorde. Gedrag en hartslag werden beoordeeld. **Resultaten:** Er werden geen significante hartslag resultaten gemeten. De resultaten van de gedragsbeoordeling zijn nog niet beschikbaar. **Conclusie:** De gevonden resultaten kunnen een indicatie zijn dat paarden de gebruikte stimuli met behulp van hun vibrissae niet kunnen voelen. Echter is het ook mogelijk dat hartslag niet de juiste uitkomstmaat is.
Test 2: **Materialen en methode:** Verscheidene apparaten werden ontworpen en beoordeeld op bruikbaarheid. Het beste ontwerp was aan te passen aan het formaat en de omtrek van de paardenneus plus 1 cm. De paarden werden at random verdeeld in drie groepen. Groep 0: vibrissae ingekort tot 0 cm (N=16), groep 2: vibrissae ingekort tot 1 cm (N=13), groep 2: vibrissae op natuurlijke lengte gehouden (N=14). Alle paarden werden gevraagd hun neus in het apparaat te steken. Gedrag, neus stoten, hartslag, hartslag variabiliteit en latentie tijd werden onderzocht.
Resultaten: De resultaten van gedrag en latentie tijd zijn nog niet beschikbaar. De beschikbare data liet een grote binnen-groepen variatie zien. Er was geen significant verschil tussen de gemiddelden

van de drie groepen. **Conclusie:** Vanwege een kleine power kunnen er geen conclusies getrokken worden over het effect van het inkorten van vibrissae op de gebruikte parameters. De gebruikte parameters lijken geen bruikbare uitkomstmaten te zijn. Omdat nog niet alle resultaten beschikbaar zijn kan er nog geen conclusie getrokken worden over de bruikbaarheid van het ontworpen apparaat.

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1 Introduction

In the past few decades equine welfare became more and more a subject of discussion. The first time that animal welfare was incorporated into the Dutch law was when in 1886 the criminal law stated that it was forbidden to abuse animals. In 1981 the intrinsic value of animals was for the first time acknowledged and in 1993 the 'Gezondheids en Welzijns Wet voor Dieren' (Health and Welfare Law for Animals) was established. More recently, proposals for new guidelines for legislation have been made, such as the 'Nota Dierenwelzijn' (Animal-welfare Memorandum by the government) which was presented in 2007 and 'Het Paardenbesluit' (the Horse Decree) which was presented in January 2011 by a Public Animal Welfare Society. However, these proposals were not incorporated in the Dutch law. If with the development of these new guidelines horse owners would be expected to meet new demands, these demands will have to be based on valid knowledge.

Many horse owners and riders are concerned with their horse's external appearance. With this, certain hairs of the body can be found undesirable and are therefore either shortened or fully removed by cutting, shaving or even burning them. Depending on personal flavor, these hairs may include: the hair along the base of the tail, the manes, the hair on the inner side of the ear, the long hairs on the fetlock, the entire coat when it is 'too' long, the vibrissae around the eyes and the vibrissae around the nose and mouth. This so called "grooming" by a person (unlike mutual grooming which is seen between two horses), which aims to enhance the horse's external appearance, has been described into detail in many books on horse care. Many horse owners really enjoy grooming their horse beyond the usual necessary brush of the coat and t's become an indispensable area of interest to many horse owners. Some even specialize in it. The most extreme form of grooming can be found with showhorses, like with the American Miniature Horses prepared for a show: even the eyelashes are removed to make the eyes look bigger to give it a more dramatic appearance (*figure 1 and 2)* (*Tennill*).

Certain hair of the body, namely the hairs on the inside of the ear, at the tail base, the fetlock and all vibrissae may, however, fulfill an important function by protecting the horse from insects and dirt, facilitating water drainage and in the case of tactile hair, act as a sensory organ. Shortening or even removing these hairs could therefore have a negative effect on the well being of the horse (*Koninklijke Nederlandse Hippische Sportfederatie 2008 and 2013; prof. dr. Marianne Sloet van Oldruitenborgh-Oosterbaan, personal communication*).

Figure 1 (left): American Miniature Horse groomed for a show. The hair on the inner side of the ears, the manes just behind the base of the skull, the vibrissae around the nose, mouth and eyes and the eyelashes are completely trimmed. The entire coat is trimmed to minimal length.

Figure 2 (right): Dutch Warmblood horse. The vibrissae around the eyes and the eyelashes are completely trimmed off.

1.1 Legal regulations

Currently there is no law in the Netherlands against the shortening or removal of vibrissae. In the 'Wet Dieren' (Animal Law), article 40 states that it is prohibited to “perform any physical intervention on any animal, that leads to the removal or damage of any body part(s)”. Article 41 of the 'Wet Dieren' states that it is prohibited to enroll or allow any animals that have undergone a procedure as described in article 40 of the 'Wet Dieren' in any exhibition, animal show or competition. However, it remains unclear whether trimming vibrissae is perceived as (partial) removal of a body part.

In the ‘Nota Dierenwelzijn’ (Animal Welfare Memorandum) the government recognizes manipulating vibrissae as a problem (*Ministerie van LNV, 2007*). The ‘Paarden besluit’ (Horse Decree), a proposal on horse welfare guidelines from the Dutch animal protection organization, states in article 4.7 that the removal of vibrissae should become prohibited. In answer to this proposal the 'Sectorraad Paarden' (Equine Sector Council), an independent foundation that informs the government on equine matters and communicates the interests of the equine industry, presented the “Gids voor Goede Praktijken” (Guide of Good Practices), a guide on proper horse care, to State Secretary Bleker, in 2011 (*Sectorraad Paarden, 2011*). This 'Gids voor Goede Praktijken' also states that the removal of vibrissae should be prohibited. However, neither the 'Paardenbesluit' nor the 'Gids voor Goede Praktijken' have yet resulted in a law that makes these practices an offense followed by penalty.

The 'Gids voor Goede Praktijken' lists the removal of vibrissae under “cosmetic intervention”. They explain that a horse needs these vibrissae when they search for food or explore their surroundings. The ‘Sectorraad Paarden’ and their ‘Gids voor Goede Praktijken’ is supported by several organizations, like the KNHS (Royal Dutch Equestrian Federation), that have an influence on their members, among which are horse owners, riders and grooms. (*Koninklijke Nederlandse Hippische Sportfederatie 2013*; *Sectorraad Paarden 2009*)

A ban on the removal of vibrissae still allows shortening them. Other organizations however, like the ECAHO (European Conference of Arab Horse Organisations), state that vibrissae must be left untouched (*European Conference of Arab Horse Organizations 2013*) so that shortening vibrissae it is not allowed. A second major difference that the ECAHO makes compared to the “Paardenbesluit” and the ‘Gids voor Goede Praktijken’ is that they make it a rule instead of a guideline.

In Germany, Belgium and Sweden removal of the vibrissae around the nose or the eyes of the horse is prohibited by law. In Germany, it trimming of vibrissae is legally considered as animal cruelty, unless there is a veterinary indication (*Deutsche Tierschutzrecht, 1972*). All horses that have their vibrissae removed or clipped, are excluded from competitions in Germany, regardless of their nationality (*Deutsches Olympiade-Komitee für Reiterei e.V. and Reit- und Fahrverein Warendorf e.V., 2013; Deutsche Reiterliche Verenigung FN, 2013*). The reason is that every organ of an animal has a significant function and should therefore be kept intact (*Fikuart, 1998; Ministerium für Umwelt, Landwirtschaft, Ernährung, Weinbau und Forsten, 2013*). The importance of the function of vibrissae for exploring surroundings, food and other horses is specifically mentioned.

To conclude, the Dutch government has clearly adopted a markedly different strategy by trying to convince the horse owner of the importance of tactile hair through guidelines and information. As long as a ban on trimming vibrissae is not included in the law, the choice will remain in the hands of the owner.

The government and organizations like the Sectorraad Paarden are trying to help better provide information by increasing their efforts to inform. In order to achieve this, in 2009 the ‘ministerie van LNV’ (ministry of Agriculture, Nature and Foodquality) assigned the Wageningen University and Research center to investigate the information search behavior of horse enthusiasts in the Netherlands. To this end project 'Passie voor Paarden' (Passion for Horses) was brought to life. The Sectorraad Paarden also made efforts by releasing their “Gids voor Goede” Praktijken guidelines in a glossy 'Paard & Welzijn' (Horse & Welfare) in 2012 (*Stichting Levende Have, 2012)*. In this glossy the importance of tactile hair was extensively described. They even describe that horses use their tactile hair to detect electrical currents on wires.

Since grooming aims to show the horse at its best external appearance during competitions, judges also play an important role. It is very likely to assume that if judges would refuse to judge horses with excessively shortened or removed tactile hair, the motivation to shorten tactile hair would decrease dramatically.
Moreover, we are still waiting for scientific proof of the validity of different theories assessing the function of tactile hair.

1.2 Anatomy

Vibrissae, also called 'tactile hair' or 'sinus hair', have a different anatomy than the hairs of the tail, manes or coat. Vibrissae are longer and thicker than regular coat hair, hey have a tapered shape and their root lies deeper into the skin. The vibrissae’s hair follicle is about 5-6 times larger than a regular hair follicle. (*Andres et al. 1973*) The vibrissae are usually located in groups at specific areas of the body. On the horse, these areas are limited to the head: around the lips, the nostrils and the eyes (*figure 3 and 4*).


Figure 3: Some tactile hairs, or 'vibrissae' , are located above and underneath the horse's eyes.
Figure 4: Some tactile hairs, or 'vibrissae' , are located around the horse's nostrils and lips.

Vibrissae are the first hair type that develops during the embryonic development (*figure 5*). (*Davidson and Hardy 1952*) Also, even in animals that are born naked the vibrissae are the only hairs that are present (*figure 6*) (*Halata 1993; Meyer 2008; Meyer and Roehrs 1986*). They do not shed seasonally like ordinary coat hair does during the molting period because vibrissae don't have a distinct telogen phase so that their function doesn't become disrupted (*Young et al. 1976*).

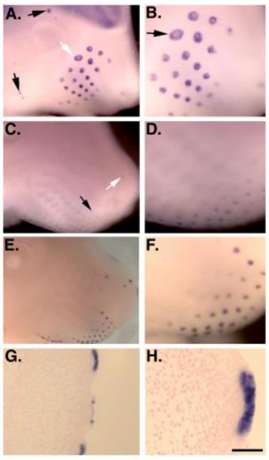


Figure 5 (left): The development of vibrissae in the embryo. In this picture, developmental proteins are stained. (Maddox et al. 2001)

Figure 6 (right): A naked baby rat. The vibrissae are the only hairtype that has developed at this age. (http://www.janbrett.com/piggybacks/whiskers.htm)

The most prominent anatomical difference between a vibrissae's follicle and other hair follicles is that a blood filled sinus surrounds the whisker's hair follicle (*Rice et al 1986)*. This blood sinus (*figure 7*) is positioned between the external shaft of the hair root follicle and the outer fibrous tissue capsule. The inner side of the blood sinus is lined with endothelium. The horse's blood sinus is completely cavernous (*von Rotz, 1995*). Inside this cavernous blood sinus multiple trabeculae cross over. These trabeculae contain many nerve fibers that run into the mesenchymal tissue that surrounds the follicle epithelium and the blood sinuses (*Halata, 1993*). Nowhere else in the skin do so many nerve fibers come together. These nerve fibres mainly originate from the infraorbital nerve (*Doerfl 1982*). They have different types of nerve endings that are located (mainly) in the 'ringwulst' area: a protrusion of the follicle epithelium (*Davidson 1952; Andres 1966*; *Rice et al 1986; Halata, 1993*) forming a collar around the follicle. The fast-adapting receptor system is represented by Pancini bodies, lancet-shaped nerve endings and unmyelinized nerve fibre endings (*Rice et al 1986)*. The slowly-adapting receptor type is represented by Merkel cells. Thanks to these different types of nerve endings every vibrissae can sense pressure, traction or pain. (*Rice et al 1986)* The design of the blood sinus functions to facilitate reinforcement of the signal that is generated. Even though it is not completely understood how this signal reinforcement works, it has become clear that afferent nerve fibers are not capable of generating signals after the blood sinus capsule is opened (*Gottschaldt et al., 1973*) It is believed that the blood pressure inside the sinus reinforces the mechanical stimulus, which means that the collaboration between blood sinus and mechanoreceptors facilitates optimal stimulus detection. Subsequently, the signal is transported to the brain, where it is processed and leads to behavioral reactions. (*Ahl 1986; Woolsey et al. 1981; Rice et al. 1986*)

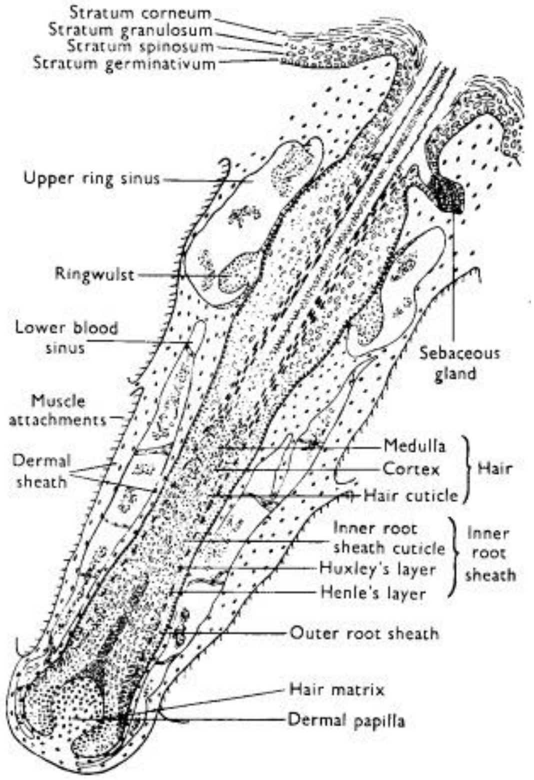


Figure 7: Schematic drawing of a vibrissal follicle in an adult mouse (Davidson and Hardy 1952)

1.3 Function

In theory, vibrissae are sensory organs located in places where fast reflexes are important like around the eye, or where sight is limited as is directly underneath and in front of the horse's nose. Horses have an elongated head and their eyes are placed laterally and quite far back. This most probably gives them an evolutionary advantage because it prevents the long grass from obstructing the hose's view when grazing (*McGreevy, 2004*). However, the disadvantage of this design is that the nose blocks their view of what's directly in front or underneath it. Depending on the breed, the anatomical placement of the eyes and how high the horse carries its head, this blind spot can extend up to almost two meters in front of the horse (*figure 8 and 9)* (*McGreevy, 2004*). It is because of this that the horse needs a different way of knowing what's directly in front of its nose. Most likely, the vibrissae around the nose and mouth fulfill this need by creating a zone (*figure 11 and 12*) where the horse, in a sense, has an alternative way of seeing. This way, for example, the horse can be aware of in incoming object (*figure 10*) (*Vernimmen 2011; Dyce et al. 2003 Knutsen 2006*).

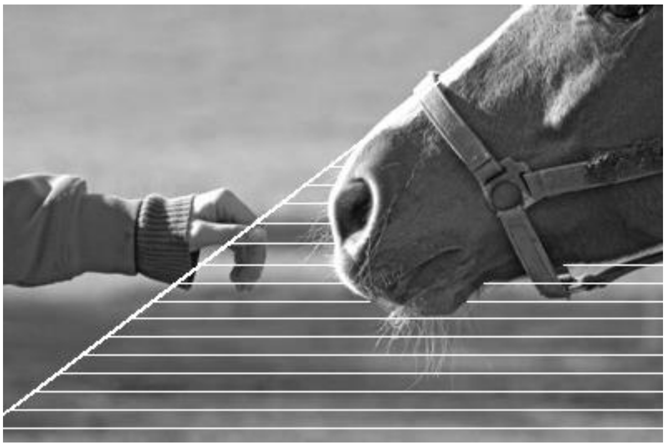


Figure 8 (left) and 9 (top right): The elongated shape of the horse's head creates a blind spot directly underneath or in front of the nose, which can extend up to almost two meters. Figure 10 (bottom right): The horse mainly uses its nose to investigate objects, as we do with our hands. However, the horse cannot see objects that are right in front or underneath it's nose.

A horse will mainly use its nose to investigate objects, food, fellow herd-members or its environment and therefore uses its nose intensively and much like we use our hands. This means that an unimpaired use of the horse's vibrissae to be able to 'see' the area around its nose would be of even greater importance.

Figure 11 (left): The long vibrissae help the horse to know what's in his blind zone.
Figure 12 (right): Some people don't like the sight of vibrissae and therefore shorten them.

Thus one is convinced that the horse actively uses its vibrissae to explore its surroundings, which contributes to finding food, assessing food, conducting social contact with fellow horses (*figure 13 and 14*) and preventing injury to the nose area. (*Sectorraad Paarden, 2011*) This theory is supported by the fact that newborn foals, which are known to have poor vision, possess more and longer vibrissae than adult horses do. It is thought that this disorganized beard of vibrissae helps them find the mare's teat (*McGreevy, 2004*).
In any case it is obvious that the horse lives in a sensory world vastly different from ours. A world difficult for people to comprehend.

Figure 13 (left) and 14 (right): The vibrissae are located around the horse's nose and mouth, where the horse can't see. Amongst other things, the horse uses them during social contact.

So far, this theory about the function of vibrissae in horses has not yet been confirmed by scientific research. One can only speculate from studies on other species, such as rodents and sea mammals. From these studies it appears that vibrissae are used to determine the depth (*Schiffman et al. 1970*), width (*Krupa et al. 2001*), shape and texture (*Harvey et al. 2001*) of an object as well as the distance to it (*Hutson et al. 1986; Jenkinson et al. 2000*).

Vibrissae vary in length and thickness (*figures 15 and 16*), rendering them more or less easy to bend. The density with which they are dispersed also varies. Two types of vibrissae can be distinguished: macro-vibrissae (longer and thicker) and micro-vibrissae (shorter and thinner). Micro-vibrissae can be found most rostral and closest to the mouth opening. They are critically involved in object recognition tasks, but are not essential for spatial tasks (*Brecht et al. 1997*). Because these vibrissae stand closer to each other, they create as it were a higher resolution of the mental image of the object that's being examined (*Grant et al. 2012*). Macro-vibrissae are critically involved in spatial tasks, but are not essential for object recognition. (*Brecht et al 1997*) Together, these features help to determine the distance to an object (*Hutson et al. 1986; Jenkinson et al. 2000*) as well as the width (*Krupa et al. 2001*), shape and texture of it (*Harvey et al. 2001*).

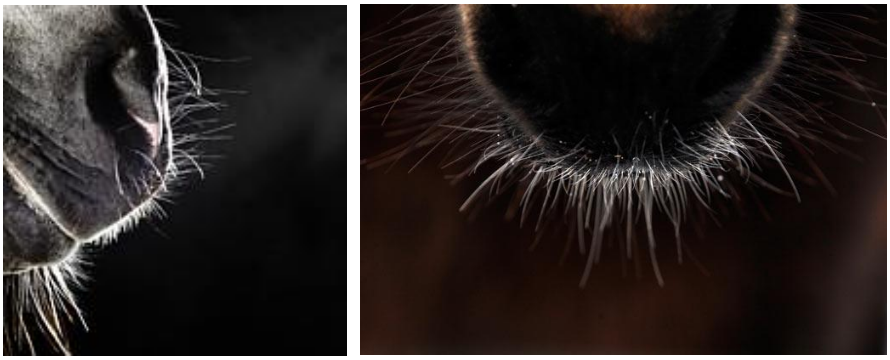


Figure 15 and 16: Vibrissae vary in length and thickness. The 'micro vibrissae' are the shortest and thinnest and can be found most rostrally around the mouth opening.

Several studies have shown that rats, walruses and sea lions can detect shape differences using only their vibrissae. Using their vibrissae, walruses could "see" the difference between round and triangular objects with a size difference of only 0.4 cm^2 (*Kastelein et al. 1990*). The Californian sea lion could even detect the difference between five shapes with the same accuracy as with eyesight (*Dehnhardt et al. 1990*) and rats could distinguish between triangular and square cookies (*Brecht et al. 1997*). Besides shape, size and distance, studies have shown that rats can discriminate different textures of objects to a high accuracy by means of their vibrissae. For example, they were able to distinguish between a smooth surface and a surface with grooves of 0.03 mm deep (*Carvell et al. 1990*) and between smooth and rough sandpaper (*Guic-Robles et al. 1989*).

The information from these studies teaches us how effective different animals can use their vibrissae to explore their environment and therefore about the important role these vibrissae play in their sensory world. This was emphasized by research on histological sections of the brains of rats. Specific areas were found on histological sections of the brains of rats, which specialize in the processing of information from the vibrissae (*Woolsey 1970; Hutson et al. 1986*). These areas are relatively large because each affected vibrissae is represented by its own separate location in the brain. The fact that such a relatively large part of the cerebral cortex is dedicated to each whisker, gives us an indication of the importance of vibrissae for the rat. In other animals with vibrissae, including horses, it cannot be excluded that they also possess specific areas like these in their sensory cerebral cortex.

From this information it becomes clear that animals use their vibrissae to gather information from their environment to a degree that can be compared to our sense of touch from our fingers and our perception of shapes, sizes and depth from our eyes. The next question is, what happens when animals are stripped from their vibrissae?

Previous research showed that blind rats cross longer elevated gaps when their vibrissae are intact than when they are clipped. (*Hutson et al. 1986*) Also when confronted with a cliff, rats will only show avoidance behavior when their vibrissae are trimmed. (*Schiffman et al. 1970*) These findings showed the importance of their vibrissae with regard to depth perception in rats. Another study showed that rats with intact vibrissae were very good swimmers. However, when their vibrissae were trimmed on either one or both sides, they showed body tilting and nose-dipping, suggesting that vibrissae help them feel when their nose is above the water surface. This means that without vibrissae rats may very well drown. (*Ahl 1982*) Other studies showed significant deficits in exploration, predation, food finding, locomotion, maze learning, fighting and other capabilities in rats when deprived of their vibrissae. (*Ahl, 1986; Gustafson et al. 1977*)

From these findings, it became clear that rodents without vibrissae were severely disabled. This leads to the question how significantly manipulation of vibrissae effects horses. It has been suggested that the inability to detect fixed objects is a contributory factor to facial trauma in horses subjected to road transport subsequent to whisker trimming *(Amy Coffman, personal communication 2002 in 'Equine Behavior, A Guide for Veterinarians and Equine Scientists').* Even though this has not yet been established with scientific research, there have been many anecdotes on facial trauma subsequent to whisker trimming. One in particular on a foal who had a severe disorientation stress reaction after it's vibrissae had been clipped (*Machteld van Dierendonck, personal communication 2013*).

1.4 Relevance

Because the act of cutting, shaving or burning of vibrissae (*figure 17, 18 and 19*) is conducted on a large scale, it is important to achieve a better understanding of the effect on equine welfare. The 'Sectorraad Paarden' continues efforts to improve the welfare of horses in the Netherlands. Information on the function of the horse's vibrissae and their role in equine welfare could give direction to the formation of new legislation and reduce vibrissae trimming practices all together.

Figure 17 (left), 18 (middle) and 19 (right): Horses groomed for different purposes. Left: competition; middle: promotional media; right: horse fairs.

In order to investigate the effect of trimming vibrissae on the welfare of horses, it is necessary to first assess their function to the horse. Momentarily there is no direct behavioral scientific proof of the function of the horse’s vibrissae. Furthermore, no standardized tests are currently available to measure these functions or how these are effected by trimming. Therefore, this pilot study is focused on finding a way to appropriately test and measure the function of the horse’s vibrissae.

1.5 Aim

The primary aim of this pilot study was to find parameters that could both measure the function of the horse’s vibrissae as well as the effects of trimming them. The secondary aim was to create a standardized test, which would allow their function to become apparent.

2 Materials and method

2.1 Parameters

In order to find a way to appropriately test and measure the function of the horse's vibrissae, the following parameters were used based on their theoretical plausibility:

2.1.1 Behaviour

As outlined in the introduction, previous studies on vibrissae have only been conducted on rodents and sea mammals. In those studies, trimming the vibrissae resulted in changed behaviour (*Ahl, 1986; Gustafson et al. 1977*). Furthermore, horses theoretically use their vibrissae to gather information and modify their behaviour. Therefore, behaviour was observed during this study, in particular sudden fright reactions, signs of stress and overall completion of given tasks. This was done by video recording the entire experiment. The video footage was then interpreted by two independent experts.

2.1.2 Nose-bumps

In theory, horses are more likely to bump their nose into an object that is in their blind zone when their vibrissae are trimmed compared to when their vibrissae are of full length. There are different components to a nose-bump that would be interesting to measure, such as the difference between the amount of nose-bumps, the maximal pressure with which the nose and the object collide and what area of the nose is effected most. During this experiment we focused on the amount of nose-bumps and the area of the nose that is affected.

2.1.3 Heart rate

Theoretically, when a horse loses its vibrissae, it would mean that the horse loses its way of knowing what's right in front of or underneath it's nose. If this were true, it would mean that trimming the horse's vibrissae makes the horse 'blind again', or if you will 'blind for the first time' in that area. This could affect stress levels and therefore heart rate. Also, if a horse would bump it's nose into objects more often because of it, this would also lead to sudden fright reactions and elevated heart rates.

2.1.4 Heart rate variability

Another parameter linked to stress is heart rate variability (HRV). HRV is measured by the change of the intervals between consecutive heartbeats. In a healthy person at rest the interval between consecutive heartbeats will vary constantly as a reaction of the body to stimuli. This process is under the influence of the autonomic nervous system. It is said that when a person is healthy, the parasympathetic and sympathetic nervous systems are both active and therefore both have an equal influence on the heart rate and HRV, so that the heart is capable of adapting to different situations. It seems that more variability means that the individual is more capable of adapting. In theory, when a person is diseased or stressed the sympathetic nervous system becomes dominant over the parasympathetic nervous system. This will result in a decrease in the variability of the beat-to-beat intervals and therefore, HRV (*Borrell et al. 2007; Rietman et al. 2004*).
Although the ECG-telemetric system is the golden standard to measure the R-R intervals needed to calculate HRV, in this study a Polar RS800CX device was used. Van Leeuwen *et al*. (2013) found a moderate significant correlation between the RMSSD (Root Mean Square of Successive Differences, HRV time domain parameter) obtained by the Polar RS800CX and the ECG at rest. The author stated that the Polar RS800CX should be compatible to be used in a field study. However, some authors still advise to use the ECG-telemetric system instead of the Polar system for HRV-data analysis (*Wallen et al. 2012*). The Polar system was better suited for this pilot study, as it

was not possible to arrange an ECG-telemetric system at location. Because the horses in this study were allowed to stand still during the tests where the Polar RS800CX was used, we did not have reasons to expect unreliable results (*van Leeuwen, 2013*). The Polar RS800CX is composed of two coated electrodes incorporated in an elastic girth that fits around the thorax of the wearer. Detection of the inter-beat-interval is carried out during recording and the resulting inter-beat-interval data are transmitted wirelessly and stored on a data logger. The collected data may then be downloaded onto a PC for later analysis of HRV.

2.1.5 Latency time

A horse that has shortened vibrissae would in theory have to get closer to an object to discover the object's presence, compared to a horse that has long vibrissae. This would theoretically imply that when two horses move their nose towards an object, the one with the longer vibrissae would decrease speed to avoid bumping into it earlier than the horse with shortened vibrissae. Therefore, during this study video recordings were made of the horse's nose to analyse time-lapse to derive speed of movement.

Another consequence of the theory that horses with shortened or, moreover, completely trimmed vibrissae would have to get closer to an object to discover its presence, is that they are often more surprised by the object's presence than a horse with long intact vibrissae. This could theoretically lead to more intense fright responses upon sudden discovery of the object. If this would be so, a horse with completely trimmed vibrissae would need more time to explore an object than a horse with long intact vibrissae, due to added recovery time. In this study, the time it took a horse to complete an exploratory task was investigated.

2.2 Tests

In order to find an apparatus which would allow the function of the horse's vibrissae to become apparent, the following tests were designed:

2.2.1 Sensitivity test

A sensitivity test was carried out to see how horses react to basic sensory stimuli that were applied to the horse's vibrissae. To this end, thermal stimuli (heat, cold), painful stimuli (damage by cutting the tactile hair, traction) and electricity were used. Heart rate and behavioral parameters were investigated.

Each horse received a sequence of stimuli to their vibrissae that each lasted 5 seconds. During this test horses were wearing an adjusted halter to block their view around their nose (for details, see paragraph 2.2.2). They were also wearing a heart rate measuring device (Polar RS800CX) to document heart rate and R-R intervals. The experiment was filmed from two angles from the side, one from which the entire horse was visible and another from which only the head was visible. All stimuli were applied to vibrissae at different locations on the muzzle (left side, right side, top, bottom). The order in which these stimuli were applied was randomly assigned using a list randomizer at www.random.org. To view the randomized list, please see Appendix I, *Table 5*.

- Heat: a hot iron (Remington 6500) commonly used to straighten human hair was used to apply heat of 190 degrees Celsius to two vibrissae at a time for five seconds. As a control, an identical hot iron was used for five seconds without being switched on while the heated hot iron was held close to make sure the horse received the same olfactory stimuli.

- Electricity: an electric three layered fly swat (no specific brand) was used to apply an electrical stimulus to two vibrissae at a time for five seconds. As a control the same fly swat was used for five seconds without being switched on.
- Traction: for a five second period two vibrissae were pulled at to a point where the skin was slightly lifted. As a control the vibrissae were only held firmly between the fingers for five seconds without being pulled.
- Cold: an ice cube was held against two vibrissae at a time for five seconds. As a control a plastic fake ice cube that can commonly be used for the same purposes (after cooling in a freezer) was used at room temperature for the same time period.
- Damage: for five seconds two vibrissae were cut using barber scissors. As a control the same scissors were used to make the same movements as if to cut a hair, but they were actually held just outside the range of the vibrissae (but where the horse still couldn't see them).

After the experiment the video footage on which the entire horse could be seen was edited so that the viewer could not see which test was done when. The video was then muted and a sound signal was added to mark the beginning and the end of each test. Two independent experts were then asked to look at the edited footage to score the horses' behavior. These scores were divided in three categories: no reaction, slight reaction and clear reaction. Behavioral signals that were looked at were: ear position and movement, eye movement, head position and movement, tail position and movement, general posture, muscle tension and movement of the horse indicating attempt to escape or avoid. The nose and mouth of the horse were not visible.

2.2.2 Templater test

The second test aimed to measure the effect of trimming the horse’s vibrissae. First, a test was designed, which would provoke the horses to use their vibrissae. Second, the test was used to measure the effect of trimming the horse’s vibrissae. Behavior, amount of nose-bumps, heart rate, heart rate variability and latency time were investigated. All tests were filmed.

2.2.2.1 Device designs

Because no devices are currently available, the first step was to create a device that would seemingly provoke the use of the horse’s vibrissae in a functional task. All devices were designed on the theories that:

- horses can sense an object's presence and shape and respond to it in order to avoid bumping into the object.
- horses use their vibrissae more effectively when investigating a novel object.
- vibrissae help prevent damage to the nose by preventing the horse from bumping in to objects.

Three horses were used to test each device on the following criteria:

- The horses were motivated to approach the device
- The horses were motivated to carry out a task which required them to place their nose through a small opening
- The horses were cautious when carrying out the task
- The device could withstand repeated execution of the experiment
- The device would allow the use of the chosen parameters
- The device would prevent the horses from using visual information to carry out the task

In case a device would not meet all criteria, a new model was designed based on gained knowledge from the previous device. The device that would meet all criteria would then be used in a group of 43 horses.

Design 1: The bucket with a treat

The first design was a convex shaped bucket. The horse was meant to move its nose into the bucket. To facilitate this, several olfactory stimuli were used in different occasions by placing them at the bottom of the bucket: a treat (apple or muesli) and horse manure. With the convex shape a similar shape as that of the horses' head was created to achieve a precise fit so that the horse could not turn its head to see the bottom of the bucket. This way, in theory, the horses would have to make use of their vibrissae to understand what is directly in front of their nose. The bucket was then placed on a slight slope to achieve the right angle in which the horses placed their nose into the bucket. To check whether the horse was able to steer it's nose in the right direction to avoid bumping into the bucket using it's vibrissae, the bucket was covered in chalk.

Design 2: The bucket with only olfactory stimulus

The second design was a similarly shaped bucket, but a second bottom layer with holes in it was added. Underneath this second bottom layer a treat was hidden. This was to stimulate the horses to place their nose into the bucket, but prevented them from actually eating the treat which hopefully would prevent the horses from becoming overly enthusiastic during the experiment. To check whether the horse was able to steer it's nose in the right direction to avoid bumping into the bucket using it's vibrissae, the bucket was covered in chalk.

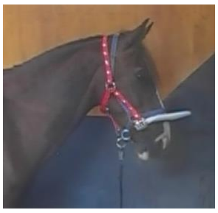
Design 3: The bucket with a surprise element, adjustable depth

The third design was a similarly shaped bucket, but with four different bottom levels that could be placed into the bucket and therefore creating a surprise element: variable depth. In theory, the horses were stimulated to have to use their vibrissae to sense how deep the bucket was and therefore how far to reach for the treat. To check whether the horse was able to sense the added bottom layer and avoid bumping into it using it's vibrissae, the bottom of the bucket was covered in chalk.

Design 4: The bucket with a surprise element, objects

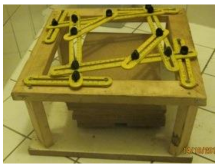
The fourth design was a similarly shaped bucket, but with different adjustable surprise elements. A brush was attached to the bottom of the bucket and several holes that could fit a stick were made in the sides of the bucket at three different positions. The brush was intended to have a negative effect when the horses moved their nose into the bucket too vigorously, so that it would make the horses more careful when exploring the bucket. The stick was another surprise element that the horses could bump their nose into. Theoretically they would be able to sense it's presence and location using their vibrissae. To check whether the horse was able to steer it's nose in the right direction to avoid bumping into the stick using it's vibrissae, the stick was covered in chalk.

The adjusted halter



To better facilitate the use of the horse's vibrissae and make sure we can rule out any use of eyesight during the next test (for example, by tilting their head), we created an adjusted halter. This halter had a fan shaped nosepiece that was attached to the noseband. To check whether the adjusted halter did indeed block the horse's sight near the nose the halter was first fitted to a test horse. This horse was first allowed to get used to the halter by letting the horse sniff and touch it. Then the adjusted halter was fitted to the horse and the horse was walked around in a round pen and presented with an object (a plastic garden chair). The horse's behavior was observed.

Design 5: The Templater device



The Templater device was designed differently than any of the buckets. It's composed of a wooden frame that holds eight rulers. These rulers are interconnected so that they form a window frame that the horse can move its nose through. The rulers are interconnected in such a way that the size and shape of the gap in the frame could be adjusted to the size and shape of the horse's nose. The depth of the Templater device was also adjustable by subtracting or adding wooden plates of 1 cm in height. The wooden frame made it possible to see the horse's nose during the experiment. This made it possible to record latency time on film by video recording the horse's nose through the wooden frame.
The horses were lured to place their nose inside the Templater device by placing treats on the bottom plate. The bottom plate that was on top had a slit in it to keep the treats in place. Because the horses could not see the Templater device and to prevent from fright or facial trauma, they were first helped by guiding the horse's nose in the right direction using a treat. After the horses succeeded at this task three times, the Templater device was turned 45 degrees. That way, the horses had to figure out how to fit their nose into the Templater device all over again by themselves, offering a certain newness to the task (*Dix et al. 1999*).

2.2.2.2 Device procedures

Three Icelandic horses, mares and geldings between the age of 6 and 23, were used to test all device designs.

Design 1: The bucket with a treat

The inside of the device was covered in chalk. The bucket was placed on a slope. The first handler was holding either two pieces of apple, a hand full of muesli or a horse dung ball. The second handler placed the test horse in the test arena. The first handler then placed the device at a set distance of two meters from the horse, the apple pieces were visibly and audibly dropped into the bucket, after which the horse was immediately set loose by the second handler to search for the treat inside the bucket. The first handler stayed in place in case the bucket needed to be secured to stay in place. After the horse had found the treat, the second handler pulled the horse back and the first handler inspected the horse's nose for chalk markings. The test was repeated with muesli and again with manure.

Design 2: The bucket with only olfactory stimulus

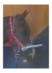
A couple of apple pieces and a hand full of muesli was placed at the bottom of the device. The second bottom layer was put in place. The inside of the device was covered in chalk. The bucket was placed on a slope. The second handler placed the test horse in the test arena. The first handler then placed the device at a set distance of two meters from the horse. The first handler audibly knocked on the bottom of the bucket to make it seem like a treat was placed inside. Then the horse was immediately set loose by the second handler to search for the treat inside the bucket. The first handler stayed in place in case the bucket needed to be secured to stay in place. After the horse had found the treat, the second handler pulled the horse back and the first handler inspected the horse's nose for chalk markings.

 *Design 3: The bucket with a surprise element, adjustable depth*

First, the test was run without a second bottom layer. Only the bottom of the bucket was covered in chalk. The bucket was placed on a slope. The first handler was holding two pieces of apple. The second handler placed the test horse in the test arena. The first handler then placed the device at a set distance of two meters from the horse, the apple pieces were visibly and audibly dropped into the bucket, after which the horse was immediately set loose by the second handler to search for the treat inside the bucket. The first handler stayed in place in case the bucket needed to be secured to stay in place. After the horse had found the treat, the second handler pulled the horse back and the first handler inspected the horse's nose for chalk markings. Then a second bottom layer was placed inside the device and the test was repeated.

 *Design 4: The bucket with a surprise element, objects*

The stick was covered in chalk and placed through one of the holes in the sides of the device. The bucket was placed on a slope. The first handler was holding two pieces of apple. The second handler placed the test horse in the test arena. The first handler then placed the device at a set distance of two meters from the horse, the apple pieces were visibly and audibly dropped into the bucket, after which the horse was immediately set loose by the second handler to search for the treat inside the bucket. The first handler stayed in place in case the bucket needed to be secured to stay in place. After the horse had found the treat, the second handler pulled the horse back and the first handler inspected the horse's nose for chalk markings.

 *The adjusted halter*

The first handler held the fan shaped adjustment up inside a round pen. The second handler led the horse up to the first handler with the fan shaped adjustment so that the horse could see, sniff and touch it. When the horse seemed relaxed, the first handler rubbed the horse's head with the fan shaped adjustment in hand. When the horse seemed relaxed, the fan shaped adjustment was unfolded so that the horse could see. The horse was allowed to sniff and touch it until it seemed relaxed. Then the unfolded fan shaped adjustment was moved around in front of the horse's nose. When the horse seemed relaxed, the unfolded fan shaped adjustment was moved around over the horse's nose and eyes and kept in place on the horse's nose a few times. When the horse seemed relaxed, the first handler fitted the adjusted halter to the horse. The horse was again allowed time to get used to the adjusted halter until it seemed relaxed. Then the second handler walked the horse around inside the round pen while the first handler placed a chair in the middle. Then the second handler led the horse up to the chair and the horse was allowed to investigate it. During the entire test, the horse's behavior was observed by the handlers.

 *Design 5: The Templater device*

Before the Templater experiment was begun, the width of the horse's nose was measured using a ruler and the dorsal-to-ventral distance was measured at the point two fingers above the corners of the mouth. The first handler then adjusted the rulers of the Templater device so that the gap was two centimeters wider than the nose (one centimeter on every side). The depth was adjusted to the

length of the mouth opening plus two fingers, by placing one or more 1cm planks on the bottom of the Templater device. The Templater device was placed on a table of 60 cm in height.
The horse was introduced to the fan shaped adjustment as mentioned under "*The adjusted halter*", paragraph 2.2.2.2, after which the adjusted halter was fitted to the horse.
The first handler placed the treats underneath the table and sat beside the Templater device. The second handler led the horse up to the Templater device so that it could easily reach it with its nose. The second handler then sat behind the Templater device at a distance of two meters, holding the end of the lead-rope.
The first handler then asked the horse to place its nose inside the Templater device by holding a treat up to its nose and letting the horse follow the treat into the Templater device. This was repeated until the horse was able to perform the task without assistance. Then the treat was audibly placed in the slit on the bottom plate and the horse was allowed to find and eat it. Then the Templater was turned 45 degrees and the task was repeated. If the horse was distracted by anything in its surroundings, we asked for its attention back by making it take one step back and one step forth and presenting it with a treat again.

2.3 Population and allocation

2.3.1 Sensitivity test

A mixed group of 14 horses of different breeds, sexes and ages was selected to participate in the Sensitivity test (for details, please see Appendix I, *Table 4*).

2.3.2 Templater test

A mixed group of 43 horses of different breeds, sexes and ages was selected and divided at random into three groups (for details, please see Appendix II, *Table 12*) using a 'list randomizor' function on an internet site (www.random.org):

1. group 0: vibrissae completely trimmed off (N=16)
2. group 1: vibrissae trimmed to 1 cm (N=13)
3. group 2: vibrissae 'sham' trimmed, so they were kept at natural length (N=14).

2.4 Protocol

1. Before the Templater experiment was begun, the width of the horse's nose was measured using a ruler. The dorsal-to-ventral distance was measured at the point two fingers above the corners of the mouth. The first handler then adjusted the rulers of the Templater device so that the gap was two centimeters wider than the nose (one centimeter on every side) so that horses from group 2 were clearly able to feel the opening with their vibrissae and horses from group 0 were only able to feel it with their skin. The depth was adjusted to the length of the mouth opening plus two fingers, by placing one or more 1cm planks on the bottom of the Templater device.

2. The Templater device was then placed on a table of 60 cm in height. All requirements for the Sensitivity experiment were placed underneath the table: the real and fake ice-cubes, the scissors, the electric fly swat and the hot irons. One of two hot irons was allowed to pre-heat.

3. The cameras were placed so that one had a wide-screen shot of the Templater device from the side and the other showed the entire experiment from the side (Templater device, handler and horse).

4. The horses were then allowed to get used to the adjusted halter. The second handler held the fan shaped adjustment up (in its folded-up state) in front of the horse so that the horse could

see, sniff and touch it. When the horse seemed relaxed, the second handler rubbed the horse's head with the fan shaped adjustment in hand. When the horse seemed relaxed, the fan shaped adjustment was unfolded so that the horse could see. The horse was allowed to sniff and touch it until it seemed relaxed. Then the unfolded fan shaped adjustment was moved around in front of the horse's nose. When the horse seemed relaxed, the unfolded fan shaped adjustment was moved around over the horse's nose and eyes and kept in its intended place on the horse's nose until the horse seemed relaxed with it. If during any of these steps the horse became anxious, the handler holding the fan shaped adjustment stopped all movements and the horse was allowed to calm down. If this did not work, the second handler would put away the fan shaped adjustment and approach and feed the horse treats by hand. When the horse calmed down, the procedure was continued from the first step again (holding the folded fan shaped adjustment up) with the use of treats to positively reinforce the horse. If a horse still showed avoidance or escape behavior, it was excluded from the study.

5. During all experiments a heart rate measuring device (Polar RS800CX) was used to document heart rate (HR) and R-R interval (RR). This heart rate measuring device was fitted to the horse using an elastic girth. On this elastic girth, two sensors were adjusted. One on the thorax behind the elbow and one behind the right shoulder blade. The hair and skin underneath these two sensors were soaked with water.

6. The adjusted halter was fitted to the horse.

7. The cameras were turned on and set to record.

8. The heart rate measuring device was turned on at the same time a signal was given to the recording cameras to make sure that the HR and RR measurements could be synchronized to the video footage. The Polar device was double checked by measuring the heart rate using a stethoscope. The horse's basic HR and RR values were measured for two minutes to record their individual reference values. After the first ten horses were tested, the protocol was adjusted. Some horses had an elevated HR after fitting the adjusted halter (step 7). In this case, the horse was allowed to return to reference values (HR 28-40 bpm (*Kuijper and van Nieuwstadt, 2008*)) before the next step in the protocol was initiated.

9. Sensitivity test was performed (see paragraph 2.2.1.) according to the randomized list (Appendix I, *Table 5*). The handler that had hold of the horse stood beside the horse’s head. The other handler stood in front of the horse, within hands reach of the equipment that was used. Every five seconds the handler holding the horse gave an audible signal to the cameras, signaling the start and the end of a stimulus applied to the horse’s vibrissae.

10. To make sure that the HR and RMSSD could be synchronized to the video footage, the time on the Polar watch was called out loud into the camera's microphones.

11. The horse's vibrissae were either trimmed or "sham" trimmed (BaByliss for men, E700YTE), depending on the group the horse was assigned to. First, the horses were allowed to sniff the hair trimmer. Then the handler stroke the horse with the hair trimmer over its shoulder, neck, back, legs, cheeks and nose until the horse didn’t seem to notice anymore. Then the hair trimmer was turned on at a distance of approximately two meters away from the horse. Then the hair trimmer was turned off again and the first steps were repeated until the horse relaxed with the hair trimmer on. Then the handler stroke the horse with the back of her hand that was holding the hair trimmer (minimizing the sense of vibrations for the horse) over the horse’s shoulder, neck, back, legs, cheeks and nose until it

seemed relaxed. Then the handler turned around her hand and stroke the horse's shoulder, neck, back, legs, cheeks and nose with the hair trimmer until it seemed relaxed. When the horse did not seem relaxed after any one of these steps, the desensitizing procedure was started again from the top. When the desensitizing procedure did not seem to work, the horse was excluded from the study. After the desensitizing protocol, the horse's vibrissae were either trimmed or sham trimmed. However, to make sure all groups were treated equally, when sham trimming the horse's vibrissae the same hair trimmer was used during the same amount of time only it was held backwards so that the stomp side of the hair trimmer was in contact with the horse instead of the side that had the blades.
After the first ten horses were tested, the protocol was adjusted. HR values were checked using a stethoscope. Some horses had an elevated HR after (sham) trimming their vibrissae. In this case, the horse was allowed to return to reference values (HR 28-40 bpm (*Kuijper and van Nieuwstadt, Het klinisch onderzoek bij paard en landbouwhuisdieren*)) before the next step in the protocol was initiated.

12. The first handler took place beside the Templater device. The second handler led the horse up to the Templater device. The second handler then took place behind the Templater device at a distance of just about two meters, holding the lead-rope.

13. The first handler then asked the horse to place its nose inside the Templater device by holding a treat up to the horse's nose and letting the horse follow the treat into the Templater device until the horse could eat it off of the bottom plate. This was repeated until the horse was able to perform the task without assistance. Then the treat was audibly placed in the slit on the bottom plate and the horse was allowed to find and eat it until it succeeded at performing the task without assistance three consecutive times. After performing the test on twelve horses, the protocol was adjusted. After the last mentioned step, the Templater device was turned 45 degrees so that the horses had to find a new way to reach for the treat.

14. Again, to make sure that the HR and RMSSD could be synchronized to the video footage, the total running time on the Polar watch was called out loud into the camera's microphones.

15. The heart rate measuring device was then switched off.

16. The cameras were switched off.

17. The adjusted halter was removed.

2.5 Statistics

First, all data was collected and mean ± SD values were calculated and graphs were drawn of every measure outcome to acquire a general overview. To determine whether the collected data was normally distributed the Shapiro-Wilk test was used as opposed to the Kolmogorov-Smirnova test because of the relatively small sample size. Q-Q plots were also viewed. When data showed to be normally distributed a One Way ANOVA test was performed to see whether there was a significant difference between the means of groups 0, 1 and 2. The One Way ANOVA test was chosen because there were three groups in the experiment and the measurements that were performed were all single, independent measurements. However, when data showed not to be normally distributed both a non-parametric test and a transformation of the data using a log10 scale was performed. Then again the data was checked for normality. Transformed data that proved to be normally distributed was then subjected to a One Way ANOVA test. As a non-parametric test the Kruskal-Wallis test was used. After all relevant data were analyzed, a power analysis (Program GPower 3.1) was used to calculate the power of our test to estimate the probability of a type 2 error occurring.

3 Results

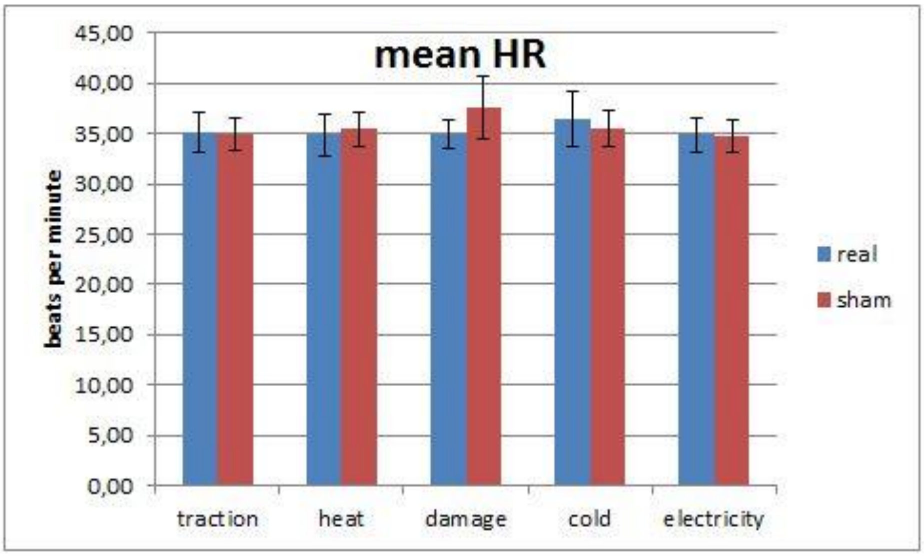
3.1 Population

Fifty horses were initially selected from different riding schools. However, seven horses had to be excluded from the test group for different reasons. For example, all horses with a secondary atrial-ventricular block had to be excluded because of unreliable HR and RR measurements. Another horse was excluded because it was practically impossible to approach the horse with a novel object such as the fly swat or the hot iron (*Sensitivity test, paragraph 2.2.1*) let alone to fit the adjusted halter for the Templater test. Yet another horse was excluded because it did not react to any of the treats that were available (apple, carrot, pellet food). The horse did not move a muscle: no ear movement, no facial movement, no sniffing and it did not follow the treat's scent. Even when an apple was placed inside its mouth the horse did not eat nor drop it. This horse did not seem to react to any other stimuli in its environment except for conditioned cues by the handler so it was not possible to do the experiment, in which the horse would have to show exploratory behavior.

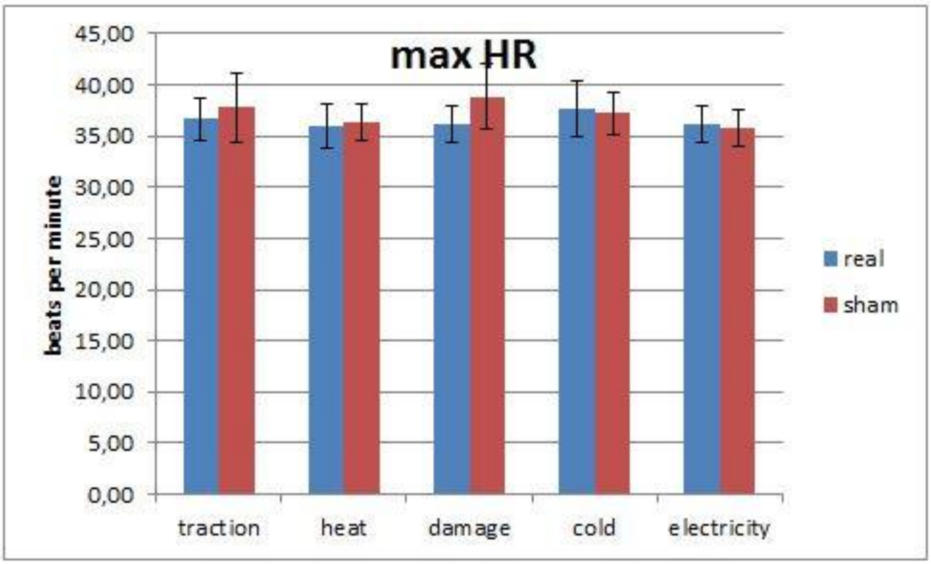
3.2 Sensitivity test

3.2.1 Heart rate

Graphs 1 and 2 show the mean and maximum heart rates of all five categories of the sensitivity test (N = 14). The graphs show that no significant difference in mean and maximum heart rate were measured during the sensitivity test is to be expected.



Graph 1: Mean heart rate ± SD (presented as lines within the bars) during the sensitivity experiment (N = 14).



Graph 2: Maximum heart rate ± SD (presented as lines within the bars) during the sensitivity test (N = 14).

Graphs 1 and 2 show that the mean heart rates for the categories ‘sham-damage' and ‘sham-heat' are higher than for 'real-damage' and 'real-heat'. Also, the maximum heart rate for ‘sham -traction', ‘sham -damage' and ‘sham -heat' seem higher than their respective 'real' categories. However, these differences are not significant.

3.2.2 Behavior

The results of the behavioral scoring by independent observers are not yet available.

3.3 Templater test

3.3.1 Designs

 *Design 1: The bucket with a treat*

The horses were able to stand still and wait at a set distance from the bucket until a treat was dropped in. After either treat was dropped into the bucket, all horses approached the bucket and found the treat. However, it was not easy to secure the bucket well enough so that it wouldn't be tipped over or broken. Also, after the horses found the treat the chalk was smeared all over the their noses. When horse manure was used, two of three horses did not approach the bucket. The other horse did, but did not place it's nose inside.

 *Design 2: The bucket with only olfactory stimulus*

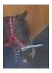
The horses were able to stand still and wait at a set distance from the bucket. During the first trials the horses were quite rough and reckless with the bucket which made it difficult to secure it well enough. However, after a couple of trials the horses were more careful with the bucket and we were able to secure it well enough to preserve it. Again, after the horses retreated from the bucket, their noses were entirely covered in chalk. After a few more trials the horses did not attempt to place their nose into the bucket at all anymore. This made it impossible to continue the experiment.

 *Design 3: The bucket with a surprise element, adjustable depth*

The horses were able to stand still and wait at a set distance from the bucket. After the treat was dropped into the bucket, all horses approached the bucket and found the treat. Again, after finding the treat their noses were completely covered in chalk. Also, it was not easy to secure the bucket well enough so that it wouldn't be tipped over or broken.

 *Design 4: The bucket with a surprise element, objects*

The horses were able to stand still and wait at a set distance from the bucket until the treat was dropped in. After the treat was dropped into the bucket, all horses approached the bucket and placed their nose inside. However, the treat fell into or next to the brush. All horses still attempted to find the treat, which made it more difficult to secure the bucket. One horse gave the bucket a shove, rolling the bucket over the floor for about two meters. The other two horses also didn't mind the stick or the broom and used the same force as before. One horse finally retreated, the other had pulled out a few pieces from the broom and had to be pulled back by the handler to save the bucket from any further damage. The three positions of the stick that was placed through the holes in the sides did not make a visible difference in strategy or chalk pattern.

 *The adjusted halter*

First, the adjusted halter was tested on a test horse. Once the horse was wearing the adjusted halter, the horse moved its head very slowly to one side and tilted it slightly after which it was able to see the ground area that was previously behind the fan-shaped adjustment. Then it started to move its head to the ground and sniffed the ground. Then the horse moved its head around a little and touched the handler's arm with its nose. When asked to walk with the handler, the horse lifted its legs up higher than usual. The horse also seemed to be reluctant to move a couple of times. When presented with an object (a chair) the horse bumped it's nose into it twice, after which the horse tilted its head making the object visible, sniffed the object and followed the object's linings with its nose.

 *Design 5: The Templater device*

During the Templater experiment all horses were able to wear the halter, stand still and have a heart rate within reference values (28-40 beats per minute (*Kuijper and van Nieuwstadt, 2008*)) at the start of the experiment. The circumference of the horses' noses could be measured repeatedly with the same outcome. It was possible to adjust the Templater device accordingly. All horses were able to stand still and wait in front of the Templater device. All horses were able and cautiously motivated to fit their nose into the Templater device without damaging the adjusted halter. The Templater device withstood all movements of the horse without getting damaged. After a few trials (*Table 1*) with assistance from the helper, all horses were able to repeat the exercise without any assistance.

Table 1: Templater test, mean amount of trials with assistance. Group 0 = vibrissae completely trimmed; group 1 = vibrissae 1cm length; group 2 = vibrissae full length.

	Group 0 N = 16	Group 1 N = 13	Group 2 N = 14
Mean ± SD	7 ± 5	6 ± 5	5 ± 2

3.3.2 Effect of trimming

During the data transportation from the Polar watch to a computer, the minRR, meanRR and maxRR data from the last three horses were lost. Thus, some of the HRV parameters could not be calculated and the HR data had N=43 and the RR data had N=40.

Graphs of all Templater measure outcomes can be found in Appendix II. From these graphs, the following data was further examined: HR, RR intervals and RMSSD (‘Root Mean Square of Successive Differences’ = HRV) of the first three successful attempts to reach for the treat without any assistance; the RMSSD of the successful attempts to reach for the treat with assistance from the helper; the amount of times that the horse bumped it's nose on the Templater device; the RMSSD from those times when the horse bumped it's nose on the Templater device; the HR, RR and RMSSD of the attempts to reach for the treat from the moment the Templater device was turned 45 degrees; the total time it took for the horses to achieve three successful attempts to reach for the treat.

3.3.2.1 Heart rate and heart rate variability

First three successful attempts without assistance
HR, RR intervals and RMSSD were examined.
The Shapiro-Wilk and Q-Q plots showed that not all data was normally distributed. The mean HR, maximum HR and RMSSD data was not normally distributed in all groups. When these data were examined using the non-parametric Kruskal-Wallis test there was no significant difference between means in the three test groups. Therefore, the null hypothesis could not be refuted (*Table 1*).
After transformation of the mean HR, maximum HR and RMSSD data, the log(mean HR) and log(RMSSD) data were normally distributed.
The results of the One Way ANOVA that was performed on all normally distributed data showed no significant difference between means in the three test groups (*Table 2*). As with the non-parametric test, the null hypothesis was retained for all data.

Table 2: Templater test. Significance values of the first three successful attempts without assistance. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed; group 1 = vibrissae 1cm length; group 2 = vibrissae full length.

	Group 0 N = 16	Group 1 N = 13	Group 2 N = 14	N Totaal	P-value Kruskal-Wallis	P-value one-way ANOVA
Mean HR ± SD	40 ± 6	40 ± 6	38 ± 5	43	0,79	0,79
Max HR ± SD	43 ± 8	43 ± 7	42 ± 6	43	0,91	0,92
Min RR ± SD	1420 ± 231	1417 ± 228	1460 ± 203	40	0,86	0,86
Mean RR ± SD	1522 ± 210	1530 ± 231	1574 ± 194	40	0,74	0,79
MaxRR ± SD	1620 ± 196	1633 ± 240	1685 ± 216	40	0,78	0,71
RMSSD ± SD	69 ± 26	77 ± 36	91 ±74	40	0,91	0,84

Successful attempts with assistance
RMSSD was examined.
The Shapiro-Wilk test and Q-Q plots showed that these data were not normally distributed. There were some outliers in each group, but these could not be excluded. The Kruskal-Wallis test showed that there was no significant difference between means in the three test groups with a P-value of 0,14, N=40. After transformation the data was normally distributed. The One Way ANOVA showed that there was no significant difference between means in the three test groups. Therefore, the null hypothesis was retained.

Nose-bumps
RMSSD was examined.
The data showed to be normally distributed within all three groups. However, the One Way ANOVA showed that there was no significant difference between means in the three test groups, with a P-value of 0,44, N=40. Therefore, the null hypothesis was retained.

Templater turned 45 degrees
HR, RR intervals and RMSSD were examined.
The Shapiro-Wilk test showed that all the data (mean HR, maximal HR, minimal RR, mean RR, maximal RR and RMSSD) from the three groups were normally distributed. However, the One Way ANOVA showed that there was no significant difference between means in the three test groups in any category (*Table 3*). Therefore, the null hypothesis was retained for all categories.

Table 3: Templater test. Significance values One-way ANOVA, Templater turned 45 degrees. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed; group 1 = vibrissae 1cm length; group 2 = vibrissae full length.

	Group 0 N = 16	Group 1 N = 13	Group 2 N = 14	N Totaal	P-value one-way ANOVA
Mean HR ± SD	40 ± 4	39 ± 4	41 ± 5	31	0,35
Max HR ± SD	43 ± 4	41 ± 4	44 ± 7	31	0,17
Min RR ± SD	1405 ± 120	1495 ± 167	1388 ± 211	28	0,15
Mean RR ± SD	1492 ± 128	1606 ± 193	1466 ± 191	28	0,32
MaxRR ± SD	1606 ± 171	1723 ± 221	1554 ± 189	28	0,71
RMSSD ± SD	75 ± 32	85 ± 32	62 ± 28	28	0,43

3.3.2.2 Nose-bumps

Total amount of nose-bumps
The data on the total amount of times the horses bumped their nose into the Templater device was only normally distributed in group 2. However, after transformation only group 0 and group 1 were normally distributed. The Kruskal-Wallis test showed no significant difference between means in the three test groups, with a P-value of 0,73, N=43. Therefore, the null hypothesis was retained.

3.3.2.3 Latency time

Time-lapse and speed of movement
The data on latency time derived from the video material is not available yet.

Total time until three successful attempts without assistance
The data from the 'sham' group showed to be normally distributed. However, the other groups were not. The Kruskal-Wallis test showed that there was no significant difference between means in the three test groups groups, with a P-value of 0,47, N=43. After transformation all three groups were normally distributed. The One Way ANOVA gave a P-value of 0,34, N=43, meaning that there was no significant difference between means in the three test groups. Therefore, the null hypothesis was preserved.

3.3.2.4 Behavior

The results of the behavioral scoring system are not yet available.

3.4 Power analysis

Power analysis for One-way ANOVA
Power analysis *Templater experiment* (alfa 0,05; power 0,8; sample size 43) gives effect size 0,5.

4 Discussion

4.1 Sensitivity test

4.1.1 Heart rate

Graph 1 and graph 2 of the sensitivity test show that a significant effect of the used stimuli on heart rate is not to be expected. This could be because horses do not sense heat, cold, electricity, damage or traction through their vibrissae. Another possibility is that heart rate is not a good parameter to measure the effect of these stimuli on the horse. An interesting stimulus that was not used during the sensitivity test is pressure. The way that horses are thought to sense objects using their vibrissae is by detecting even the slightest bend or pressure on to their vibrissae. However, keeping in mind that horses experience such pressures on to their vibrissae on a regular basis we didn't expect an effect on HR. For the same reason it would be possible that the horse can sense either one or all of the used stimuli, either through its vibrissae (thermal stimuli, electricity, damage) or its skin (traction) without experiencing an effect on HR. It would therefore be interesting to see what other parameters can be explored to measure basic stimulus detection through the horse's vibrissae.

Another possibility that would explain the lack of results is that the control stimulus (i.e. sham) tests might not have been designed well enough to discriminate from the actual stimulus tests. For example, the traction was designed to measure the horse's response to a whisker being pulled. The control test for this was to grab hold of a whisker in the same way, but without pulling it. However, during the experiment the horses would not always keep their nose still. To prevent from accidentally pulling the whisker the handler pushed it into the skin a little so that it had a little bend in it. This created pressure, which is considered a stimulus that a horse can sense. Even though a pressure stimulus is different from traction, it might not be the right control stimulus.

4.1.2 Behaviour

It is not yet possible to discuss the behavioral parameter because the results on this parameter are not yet available.

4.2 Templater test

4.2.1 Designed devices

 *Design 1: the bucket with a treat*

As with all bucket tests, it seemed as though the horses didn't see it as a novel object as we hoped they would. In fact, they were so comfortable with the bucket that they were quite rough with it. It seemed as though, in their excitement over the treat, they used a certain force intentionally. This force was enough to almost tip over or otherwise damage the bucket. Therefore it was quite difficult to secure the bucket well enough to keep it still.

 *Design 2: the bucket with only olfactory stimulus*

During this test we tried to degrade the horses' motivation enough for them to want to investigate the bucket, but not be too excited about it. However, after a few trials with this design the horses became reluctant to investigate the bucket at all, so it seemed that their motivation dropped further than we had intended.

 *Design 3: the bucket with a surprise element, adjustable depth*

During this test it seemed as though the horses did not have the intention of not bumping into the bottom with their noses. Again, it seemed as though they used a certain force intentionally.

 *Design 4: the bucket with a surprise element, objects*

During this test it became clear that the stick and the broom piece did make it more difficult for the horses to reach the treat, but it did not make the horses more careful. Apparently the motivation to reach the treat was higher than the difficulties experienced by the obstructions.

 *Design 5: the Templater device*

The Templater device combined with the adjusted halter seemed to fit all of our criteria mentioned in paragraph 2.2.2.1. The next step was to see if this design would allow the use of all parameters.

4.2.2 Templater

Even though the primary aim of a pilot study is not to look at the statistical outcome, we did perform further analysis to get a feel for the type of data drawn from this experiment.

Although no significant difference was measured in the Templater test, no definite conclusions can be drawn from this. Using a sensitivity analysis, the minimal effect size can be calculated that we would have been able to detect with our study. Using the alfa (0,05), beta (0,8) and sample size (43) values, we found that the power of our test is big enough to detect effects of 0,5 or larger (the sensitivity of our test). This means that even an effect that would be termed as 'large' (0,4) could come out as a non-significant result, which in turn means that concluding that there is no difference between the groups, based on our data, could possibly be wrong (type 2 error). In fact, by finding non-significant results, all we know is that the effect size in the target population will not be 0,5 and over. Smaller effects would probably go undetected in our sample.

However, even though no definite conclusions can be drawn from our data, there could be a few explanations for not finding any significant results.

First, it could be due to the large within groups variances in our data. When there is a large variance within groups, the difference between groups also has to be large before it comes out as significant. There are different solutions to this problem. One way to solve this problem is to use a larger sample. However, depending on the expected effect size and the power of our test, it can be difficult to gather a large enough sample. Another solution would be to use a more homogenic group of horses. For example, horses could be selected based on age catagories (foals versus adult horses) or character (reactivity). The disadvantage to this is that results coming from such a specific sample can not be generalized to the entire population. Another posibility is to use a within subjects design. This would mean that each horse would be subjected to every condition (vibrissae at natural length, at 1 cm length and completely trimmed off). The problem with this design would be that the horses would always be tested in a set order (from long to short). Since it would take too long for the vibrissae to grow back to natural length, timewise it would be unpracticle to start with the vibrissae at 1 cm length or completely trimmed off. Not being able to randomize the order of the different conditions would mean that the results could be subjected to confounding effects. The confounders that would be expected in such a study would be a learning effect, or habituation to the test because the same horse would have to repeat the test in every category. However, there are ways to overcome their confounding effect. One way would be to keep the test novel by changing some of the conditions within the test, for instance changing the position of the templater as was done in this study by turning the Templater device 45 degrees (*Dix et al. 1999*). Another way would be to let every horse perform the test several times before starting the experiment so that the learning effect, as well as habituation to the test, would be equal in each condition.

The second possible explanation to the non-significant results is that the test design or method is not adequate. What has come to our attention is that it is very difficult to design a test that provokes horses to use their vibrissae. Also, in this study multiple parameters were investigated. However, because neither the parameters nor the test design have been used in studies on vibrissae before nor have they been validated for their use on vibrissae, it is difficult to draw conclusions from this study regarding their usability. More research will be needed to find the right parameters to measure the use of vibrissae.

A third possible explanation to the non- significant test results is that there is no significant effect to be measured.

Our experiment was designed to make use of the theory that horses will use their vibrissae more abundantly when faced with a novel object. First, we aimed to achieve three successful attempts of placing their nose inside the Templater without assistance. However, the design of the Templater test has one pitfall: repeated exposure to a novel object facilitates learning, habituation and desensitization, therefore the object will automatically become less 'novel' with every exposure. In this case, the horses were asked to place their nose inside the Templater device repeatedly. Therefore, there is a possibility that the horses learned from this exposure and gained enough familiarity and confidence with the task to lose their caution and not make as much use of their vibrissae anymore. In fact, it could be argued whether the object was still novel at all at the time the horses were able to perform the task without any assistance. Dix *et al.* (1999) showed that rats that are confronted with a familiar object in a different position or a different location respond to it in the same way as they do to a novel object. Therefore, after performing the Templater experiment on 12 horses, we decided to see what would happen if we would turn the Templater device 45 degrees so that the horses had to find a new way to reach for the treat. However, the RMSSD of the Templater turned 45 degrees did not yield any significant results (P=0,43 with n=28).

4.2.3 Parameters

HR and HRV

Heart rate and heart rate variability were both examined. Again, because our sample size wasn't big enough for the power of our test, no conclusions can be drawn from our data. Moreover, it can be argued whether HRV was a good parameter in our test. Because the Templater experiment took an average time of 0:04:02 from the first attempt with assistance to the last successful attempt without assistance. This means that the recording time from the first successful attempt without assistance to the last successful attempt without assistance is even shorter. Since the standard recording time for RMSSD is 5 minutes and no less, the Templater test would officially be of too short duration to assemble reliable HRV data (*Task Force of the European Society of Cardiology, the North American Society for Pacing and Electrophysiology, 1996*). In case the Templater test will be used in the future, it would be advisable to measure RR for a 5 minute period before the test is begun. When the test is begun, testing should take place in 5 minute intervals.

Nose-bumps

The horses that had their vibrissae trimmed did seem to have a bigger fright response when suddenly bumping into the Templater by retracting faster and further than the horses that had long whiskers. Because there are several aspects to a nose-bump, for instance 1) the amount of nose-bumps 2) the force with which the horse bumps it's nose into the object 3) the spot on the nose where the horse bumped it and 4) the amplitude of the reaction to the nose-bump. It might be possible that if horses are warned in advance that they might bump their nose by stimulation of their whiskers, they might not spook as much as they would if they had no warning at all. During this pilot study we looked at the amount of nose-bumps. There were no significant results, but as has been explained before there is a high chance of making a type II error, so we cannot draw any conclusions from our data. It seems as though the parameter 'nose-bumps' can be further optimized, for instance by analysing the pressure of a nose-bump or the excursion length of head-withdrawal in response to a nose-bump. Hopefully other aspects of nose-bumps can be explored in the future.

Behaviour

Of the parameters that we used, the results on behaviour and latency time are not yet available. What might be worth mentioning however, are the behavioural observations that were made ourselves during the experiment. Although these observations were not acquired by independent experts nor were the observers blinded from the purpose of the study, they might be of value for future ideas of experimental designs.

One observation that was remarkable, was when one of the test horses first wore the adjusted halter and started to walk around with its nose just above the ground surface, blowing out short bursts of air as it moved forward. This gave us the idea that it might be possible that horses measure the distance to objects by the combination of the use of their whiskers and air bursts that are reflected back off of objects.

Another observation was that when the horse was wearing the adjusted halter and a handler touched the vibrissae on one side, the horse responded by moving its nose in that direction. This could be repeated for every side of the horse's nose that the handler would touch, indicating that horses can indeed use their vibrissae to sense objects.

Latency time

The results of the total time until three successful attempts without assistance did not show a significant difference. This can be due to the fact that, besides amount of fright responses and recovery time, several other factors contribute to this outcome measure. Focus, for example, can make a difference because some horses were focused more on the task than others. Also, environmental factors that can destract a horse will inevitably influence the time it takes to master the given task.

5 Conclusions

The sensitivity test can still be optimized by better designing the stimulus controls. It would also be interesting to include a pressure stimulus. So far, the sensitivity test did not yield significant results. However, to truly give an answer to the question 'how do horses respond to manipulation of their vibrissae?', analysis of other parameters would be needed. Therefore it would be interesting to see what results will come from the behavioral analysis of the video footage.

The Templater test also did not yield any significant results. However, no conclusions can be drawn due to a small power of our test and a high chance of a type II error. The results on latency time will still be due shortly.

It can be said that overall it proved very difficult to design a test in such a way that the horses have the right amount of motivation so that they do engage, but not so much that they neglect the use of their vibrissae. Also, it seems not all that easy to choose the right parameters to measure the use of vibrissae or the reaction to manipulation. It would be very interesting to explore more parameters in this regard.

The adjusted halter seemed to be very effective at blocking the horse’s view around it’s nose without getting in the way of other used materials. Furthermore, the horses seemed to accept it.

With this pilot study we were able to design an experiment to measure the effect of whisker trimming on HR, HRV, behavior, nose-bumps and latency time during an exploratory task. More research would be needed to further determine this effect. Because of the large variance in our results, in future it would be advisable to select a more homogenic sample and/or use within-subjects design. Furthermore, other parameters will still be analyzed, such as behaviour and latency time, or can in the future be explored, such as nose-bump pressure, nose-bump reaction amplitude. No definite conclusions on the effect of trimming of any horse's vibrissae can be drawn from our data.

6 Suggestions for future research

As has been mentioned before, it would be interesting to explore other parameters. Nose-bump parameters for instance, such as the pressure with which nose-bumps occur and the amplitude of the reaction after a nose-bump. It would also be interesting to analyse the response to pressure applied to the vibrissae, because it seemed as though horses can follow an object with their nose only by having contact with it through their vibrissae. Another interesting area would be to explore and refine more behavioural parameters.

Besides exploring other parameters, it would be interesting to learn more about the incidence of damage to the nose after trimming vibrissae and under what circumstances this happens most or least.

When vibrissae are trimmed, it leaves the horse with short rigid vibrissae that are not easily bent. It would be interesting to investigate whether this is uncomfortable to horses, as the short stumps seem to act as needles in a soft and sensitive skin.

Another way to investigate whether horses are able to use their vibrissae like rodents and sea mammals do, is to see how (partially) blind horses respond when their vibrissae are trimmed. Otherwise, an exploratory task in pitch black might give the same circumstances.

Last but not least, because it is said that new-born foals have limited eyesight and therefore need their abundantly developed vibrissae to find the teat, it would be interesting to do more behavioural research on this area.

7 References

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8 Acknowledgements

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9 Appendix I: Sensitivity test

Table 4: Sensitivity test. List of horses and their breeds, genders and ages.

Horse	Breed	Gender	Age
1	Welsch	Gelding	27
2	Dartmoor	Mare	16
3	-	Gelding	11
4	Appaloos	Mare	7
5	NRPS	Mare	7
6	NRPS	Gelding	7
7	KWPN	Gelding	8
8	Haflinger	Mare	17
9	New Fore	Mare	22
10	KWPN	Mare	27
11	KWPN	Mare	18
12	Haflinger	Gelding	10
13	KWPN	Mare	17
14	Fjord	Mare	6

Table 5: Sensitivity test. List of the (random) order in which every stimulus was applied for every individual horse. Blue: real stimulus. Red: sham stimulus.

Mando	traction	traction	heat	damage	electricity	electricity	heat	cold	cold	damage
Fleur	damage	heat	cold	traction	traction	heat	damage	cold	electricity	electricity
Joris	traction	heat	cold	electricity	traction	cold	damage	damage	heat	electricity
Missy	damage	damage	heat	heat	cold	traction	electricity	electricity	traction	cold
Poppetje	heat	traction	traction	damage	cold	damage	electricity	heat	electricity	cold
Ali	cold	traction	cold	heat	electricity	traction	damage	electricity	heat	damage
Valdo	damage	electricity	heat	electricity	damage	traction	cold	traction	heat	cold
Marma	damage	traction	cold	heat	damage	electricity	heat	electricity	traction	cold
Lusette	electricity	electricity	damage	heat	damage	traction	cold	electricity	traction	cold
Emma	damage	traction	damage	cold	heat	electricity	electricity	traction	traction	heat
Nolande	cold	heat	traction	heat	electricity	damage	heat	cold	traction	electricity
Harry	heat	heat	cold	traction	traction	electricity	damage	damage	cold	electricity
Novelle	electricity	electricity	damage	cold	traction	traction	heat	damage	cold	heat
Bibi	damage	electricity	damage	traction	electricity	traction	cold	heat	heat	cold

Table 6: Sensitivity test. Mean outcomes per category. Stimulus in blank cell = real stimulus; stimulus with red cross = sham stimulus. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability.

Mean outcomes per category							
	start HR	mean HR	max HR	min RR	mean RR	max RR	RMSSD
traction	35,6	35,1	36,6	1654,3	1724,6	1771,5	0
~~traction~~	34,9	34,9	37,8	1627,1	1737,9	1843,3	0
heat	34,6	34,9	35,9	1689,2	1673,8	1804,1	0
~~heat~~	35,9	35,4	36,3	1672,0	1716,2	1764,9	0
damage	35,2	35,0	36,1	1673,8	1727,2	1774,1	0
~~damage~~	37,8	37,6	38,8	1581,6	1636,7	1695,4	0
cold	37,0	36,4	37,6	1624,4	1683,0	1731,3	0
~~cold~~	36,9	35,5	37,2	1620,8	1702,3	1763,1	0
electricity	35,4	34,9	36,1	1673,6	1728,2	1785,6	0
~~electricity~~	34,8	34,7	35,7	1698,1	1741,2	1793,9	0

Table 7: Sensitivity test. Results for traction stimulus per individual horse. Stimulus in blank cell = real stimulus; stimulus with red cross = sham stimulus. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability; SD = standard deviation.

		start HR	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mando	traction	31	30	31	1944	2001	2078	0
	~~traction~~	28	29	30	1993	2079	2131	0
Fleur	traction	28	29	34	1788	2047	2134	0
	~~traction~~	33	29	33	1844	2070	2414	0
Joris	traction	35	35	35	1705	1721	1745	0
	~~traction~~	33	34	34	1764	1787	1812	0
Missy	traction	35	35	35	1696	1730	1749	0
	~~traction~~	35	36	36	1665	1682	1699	0
Poppetje	traction	32	32	34	1780	1864	1896	0
	~~traction~~	34	38	58	1032	1566	1778	0
Ali	traction	34	34	34	1742	1747	1756	0
	~~traction~~	38	37	38	1583	1615	1650	0
Valdo	traction	38	37	39	1542	1621	1668	0
	~~traction~~	34	36	37	1606	1665	1749	0
Marma	traction	44	41	44	1362	1455	1520	0
	~~traction~~	36	36	37	1619	1665	1755	0
Lusette	traction	38	37	39	1530	1620	1694	0
	~~traction~~	35	39	43	1380	1558	1722	0
Emma	traction	36	35	36	1646	1718	1760	0
	~~traction~~	33	33	33	1829	1841	1864	0
Nolande	traction	39	38	39	1540	1564	1578	0
	~~traction~~	41	40	41	1461	1510	1559	0
Harry	traction	34	35	36	1689	1739	1780	0
	~~traction~~	35	34	35	1719	1742	1773	0
Novelle	traction	45	43	45	1322	1389	1443	0
	~~traction~~	39	35	39	1552	1736	1989	0
Bibi	traction	30	31	32	1874	1929	2000	0
	~~traction~~	35	33	35	1732	1815	1911	0
Mean	traction	36	35	37	1654	1725	1772	0
	~~traction~~	35	35	38	1627	1738	1843	0
SD	traction	5	4	4	179	191	200	0
1/2 SD		2,4	2,0	2,1	89,5	95,6	100,0	0,0
SD	~~traction~~	3	3	7	234	173	218	0
1/2 SD		1,5	1,6	3,4	117,0	86,4	108,8	0,0

Table 8: Sensitivity test. Results for heat stimulus per individual horse. Stimulus in blank cell = real stimulus; stimulus with red cross = sham stimulus. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability; SD = standard deviation.

		start HR	mean HR	max HR	min RR	meanRR	max RR	RMSSD
Mando	heat	29	29	30	2018	2040	2067	0
	~~heat~~	29	29	30	2016	2087	2150	0
Fleur	heat	28	28	29	2078	2168	2302	0
	~~heat~~	38	36	38	1570	1665	1713	0
Joris	heat	34	35	35	1699	1726	1746	0
	~~heat~~	35	35	36	1683	1708	1720	0
Missy	heat	42	40	42	1419	1495	1562	0
	~~heat~~	37	37	38	1584	1612	1624	0
Poppetje	heat	34	32	34	1752	1873	1942	0
	~~heat~~	38	37	38	1572	1630	1771	0
Ali	heat	34	34	34	1751	1771	1784	0
	~~heat~~	36	35	36	1662	1709	1752	0
Valdo	heat	35	36	37	1640	1684	1723	0
	~~heat~~	36	35	36	1673	1737	1782	0
Marma	heat	34	36	37	1601	674	1785	0
	~~heat~~	34	35	36	1687	1732	1789	0
Lusette	heat	36	36	38	1579	1679	1810	0
	~~heat~~	32	32	33	1833	1883	1957	0
Emma	heat	36	36	37	1613	1650	1676	0
	~~heat~~	36	36	36	1669	1678	1688	0
Nolande	heat	39	39	40	1514	1539	1562	0
	~~heat~~	44	44	44	1362	1373	1380	0
Harry	heat	42	43	44	1355	1393	1437	0
	~~heat~~	41	39	41	1466	1535	1624	0
Novelle	heat	32	33	33	1798	1827	1858	0
	~~heat~~	34	34	34	1763	1784	1821	0
Bibi	heat	30	31	33	1832	1914	2004	0
	~~heat~~	32	32	32	1868	1894	1937	0
Mean	heat	35	35	36	1689	1674	1804	0
	~~heat~~	36	35	36	1672	1716	1765	0
SD	heat	4	4	4	204	355	225	0
1/2 SD		2,1	2,1	2,1	102,2	177,6	112,5	0,0
SD	~~heat~~	4	4	4	166	170	179	0
1/2 SD		1,9	1,8	1,8	83,0	85,0	89,6	0,0

Table 9: Sensitivity test. Results for damage stimulus per individual horse. Stimulus in blank cell = real stimulus; stimulus with red cross = sham stimulus. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability; SD = standard deviation.

		start HR	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mando	damage	29	30	30	1994	2021	2036	0
	~~damage~~	29	28	29	2058	2109	2139	0
Fleur	damage	30	30	33	1836	1972	2083	0
	~~damage~~	30	33	35	1714	1838	2015	0
Joris	damage	36	36	36	1656	1666	1684	0
	~~damage~~	36	36	36	1660	1676	1687	0
Missy	damage	35	35	36	1690	1701	1710	0
	~~damage~~	36	36	36	1648	1672	1683	0
Poppetje	damage	34	33	34	1771	1792	1818	0
	~~damage~~	32	33	34	1779	1804	1866	0
Ali	damage	35	36	37	1641	1681	1720	0
	~~damage~~	43	41	43	1385	1458	1544	0
Valdo	damage	36	37	37	1610	1627	1647	0
	~~damage~~	36	36	37	1628	1666	1686	0
Marma	damage	34	35	36	1664	1722	1767	0
	~~damage~~	47	46	47	1286	1307	1349	0
Lusette	damage	39	37	39	1523	1634	1745	0
	~~damage~~	35	34	35	1718	1791	1869	0
Emma	damage	34	35	36	1679	1721	1743	0
	~~damage~~	40	38	40	1505	1594	1653	0
Nolande	damage	38	38	38	1574	1589	1624	0
	~~damage~~	38	37	38	1587	1611	1625	0
Harry	damage	45	40	45	1319	1502	1611	0
	~~damage~~	46	44	46	1297	1370	1484	0
Novelle	damage	35	35	35	1696	1725	1756	0
	~~damage~~	49	52	53	1124	1160	1228	0
Bibi	damage	33	33	34	1780	1828	1894	0
	~~damage~~	32	32	34	1753	1858	1907	0
Mean	damage	35	35	36	1674	1727	1774	0
	~~damage~~	38	38	39	1582	1637	1695	0
SD	damage	4	3	3	156	140	142	0
1/2 SD		1,9	1,4	1,7	77,8	70,1	71,1	0,0
SD	~~damage~~	6	6	6	243	250	251	0
1/2 SD		3,2	3,2	3,2	121,4	124,8	125,3	0,0

Table 10: Sensitivity test. Results for cold stimulus per individual horse. Stimulus in blank cell = real stimulus; stimulus with red cross = sham stimulus. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability; SD = standard deviation.

		start HR	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mando	cold	28	29	29	2071	2100	2114	0
	~~cold~~	29	30	30	1975	2010	2035	0
Fleur	cold	32	31	35	1732	1918	2032	0
	~~cold~~	35	32	35	1692	1882	2029	0
Joris	cold	36	36	36	1658	1686	1719	0
	~~cold~~	47	43	47	1284	1392	1500	0
Missy	cold	37	37	37	1621	1627	1635	0
	~~cold~~	37	37	37	1606	1620	1638	0
Poppetje	cold	32	31	32	1868	1946	1993	0
	~~cold~~	30	31	32	1865	1933	1976	0
Ali	cold	35	35	35	1700	1713	1727	0
	~~cold~~	37	36	37	1635	1653	1683	0
Valdo	cold	42	39	42	1435	1532	1647	0
	~~cold~~	38	37	39	1542	1606	1658	0
Marma	cold	37	35	37	1629	1693	1773	0
	~~cold~~	34	34	35	1699	1750	1790	0
Lusette	cold	33	31	33	1819	1929	1999	0
	~~cold~~	37	34	37	1603	1778	1902	0
Emma	cold	35	36	37	1614	1664	1703	0
	~~cold~~	41	39	41	1467	1524	1572	0
Nolande	cold	45	44	45	1324	1372	1398	0
	~~cold~~	38	38	38	1568	1586	1613	0
Harry	cold	46	46	48	1255	1302	1334	0
	~~cold~~	38	37	38	1566	1623	1653	0
Novelle	cold	46	46	46	1291	1307	1333	0
	~~cold~~	34	34	34	1741	1775	1808	0
Bibi	cold	34	34	35	1724	1773	1831	0
	~~cold~~	41	35	41	1448	1700	1826	0
Mean	cold	37	36	38	1624	1683	1731	0
	~~cold~~	37	36	37	1621	1702	1763	0
SD	cold	6	6	6	232	245	253	0
1/2 SD		2,8	2,8	2,8	115,9	122,3	126,6	0,0
SD	~~cold~~	5	3	4	173	166	173	0
1/2 SD		2,3	1,7	2,1	86,6	83,2	86,5	0,0

Table 11: Sensitivity test. Results for electricity stimulus per individual horse. Stimulus in blank cell = real stimulus; stimulus with red cross = sham stimulus. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability; SD = standard deviation.

		start HR	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mando	electricity	30	29	30	2022	2051	2080	0
	~~electricity~~	29	29	29	2042	2081	2108	0
Fleur	electricity	30	34	37	1616	1773	2010	0
	~~electricity~~	32	32	33	1822	1885	1991	0
Joris	electricity	35	35	35	1691	1710	1733	0
	~~electricity~~	35	35	36	1683	1703	1729	0
Missy	electricity	36	36	37	1641	1649	1664	0
	~~electricity~~	38	38	39	1548	1576	1597	0
Poppetje	electricity	32	32	33	1799	1865	1889	0
	~~electricity~~	31	31	33	1841	1928	1976	0
Ali	electricity	35	34	35	1726	1747	1769	0
	~~electricity~~	35	35	35	1717	1732	1766	0
Valdo	electricity	36	36	37	1617	1657	1681	0
	~~electricity~~	36	36	37	1639	1661	1701	0
Marma	electricity	38	37	38	1559	1611	1664	0
	~~electricity~~	34	35	36	1646	1696	1767	0
Lusette	electricity	32	31	32	1847	1962	2120	0
	~~electricity~~	32	32	33	1816	1875	1994	0
Emma	electricity	34	33	34	1781	1802	1840	0
	~~electricity~~	36	35	36	1669	1704	1763	0
Nolande	electricity	38	38	38	1565	1583	1604	0
	~~electricity~~	41	41	42	1430	1472	1507	0
Harry	electricity	43	41	43	1411	1464	1491	0
	~~electricity~~	40	40	42	1424	1488	1551	0
Novelle	electricity	43	39	43	1397	1522	1568	0
	~~electricity~~	35	34	35	1711	1749	1788	0
Bibi	electricity	34	33	34	1759	1799	1886	0
	~~electricity~~	33	33	34	1786	1827	1877	0
Mean	electricity	35	35	36	1674	1728	1786	0
	~~electricity~~	35	35	36	1698	1741	1794	0
SD	electricity	4	3	4	168	164	193	0
1/2 SD		2,0	1,6	1,9	84,0	81,9	96,4	0,0
SD	~~electricity~~	3	3	4	165	169	178	0
1/2 SD		1,7	1,7	1,8	82,4	84,5	89,1	0,0

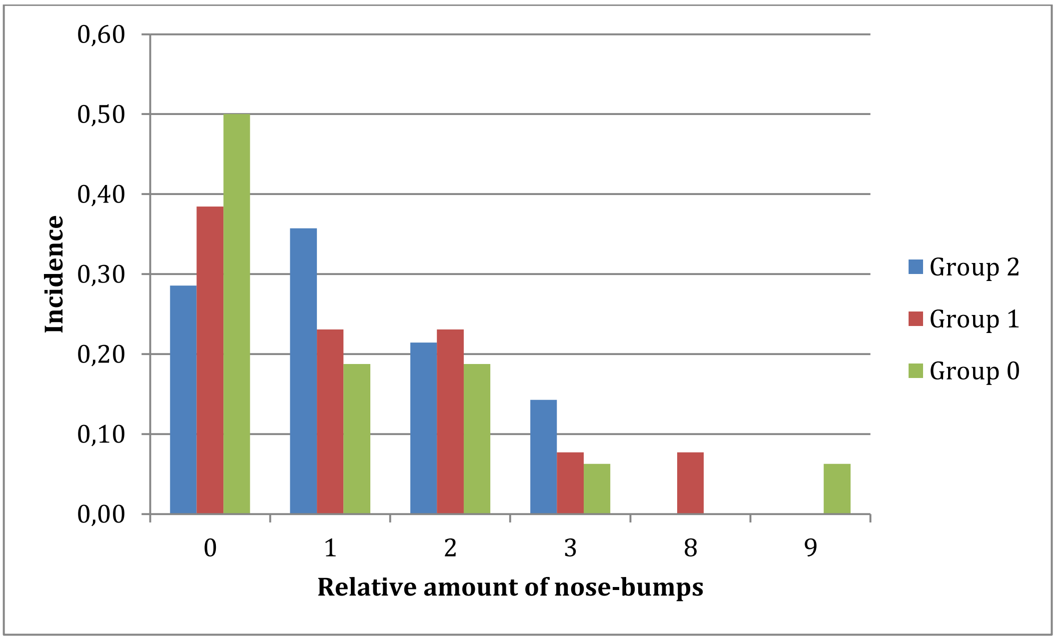
10 Appendix II: Templater test.

Table 12: Templater test. List of horses and their breeds, genders and ages. Group 0 = vibrissae completely trimmed; group 1 = vibrissae 1cm length; group 2 = vibrissae full length.

Horse	Breed	Gender	Age	Group
1	Welsch	Gelding	27	2
2	Dartmoor	Mare	16	1
3	-	Gelding	11	0
4	Appaloosa	Mare	7	2
5	NRPS	Mare	7	0
6	NRPS	Gelding	7	1
7	KWPN	Gelding	8	2
8	Haflinger	Mare	17	1
9	New Forest	Mare	22	0
10	KWPN	Mare	27	2
11	KWPN	Mare	18	1
12	Haflinger	Gelding	10	0
13	KWPN	Mare	17	2
14	Fjord	Mare	6	1
15	NRPS	Gelding	16	2
16	-	Mare	9	0
17	Appaloosa	Gelding	10	1
18	New Forest	Mare	5	1
19	NRPS	Mare	17	2
20	Welsh	Mare	13	1
21	Welsch	Mare	17	0
22	Welsch	Gelding	6	2
23	NRPS	Mare	18	0
24	NRPS	Mare	13	1
25	Conamara	Mare	5	0
26	NRPS	Mare	10	0
27	NRPS	Gelding	14	1
28	Fresian	Gelding	5	2
29	Fresian	Gelding	7	0
30	KWPN	Gelding	21	1
31	Fjord	Mare	18	2
32	-	Gelding	18	0
33	Shetland pony	Mare	18	1
34	Welsh	Mare	18	2
35	Haflinger	Mare	7	2
36	Haflinger	Mare	19	0
37	-	Gelding	23	2
38	Conamara	Mare	22	0
39	Conamara	Mare	22	0
40	KWPN	Mare	6	0
41	Conamara	Mare	21	2
42	Conamara	Mare	5	1
43	KWPN	Gelding	22	0

Table 13: Templater test. The absolute and relative amount of nose-bumps. Group 0 = vibrissae completely trimmed; group 1 = vibrissae 1cm length; group 2 = vibrissae full length.

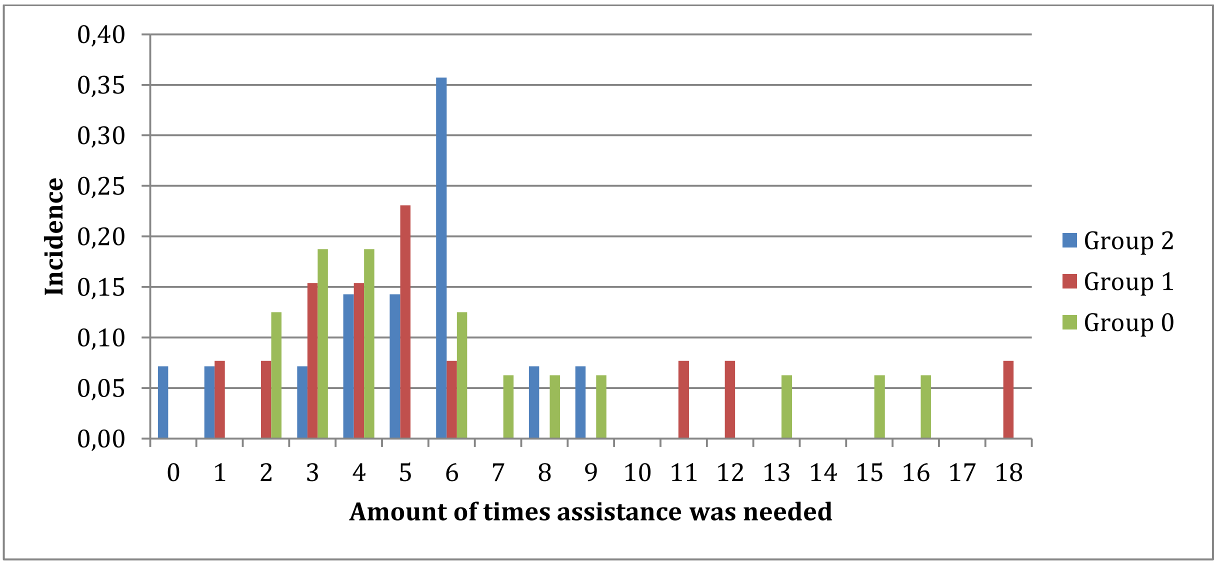
Amount of nose-bumps							
Absolute x	0	1	2	3	8	9	Total
Group 2 (N = 14)	4	5	3	2	0	0	14
Group 1 (N = 13)	5	3	3	1	1	0	13
Group 0 (N = 16)	8	3	3	1	0	1	16
Percentage	0	1	2	3	8	9	Total
Group 2 (N = 14)	29%	36%	21%	14%	0%	0%	100%
Group 1 (N = 13)	38%	23%	23%	8%	8%	0%	100%
Group 0 (N = 16)	50%	19%	19%	6%	0%	6%	100%



Graph 3: Templater test. Amount of nose-bumps. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

Table 14: Templater test. The amount of times assistance was needed until the horse was able to perform the task by itself. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

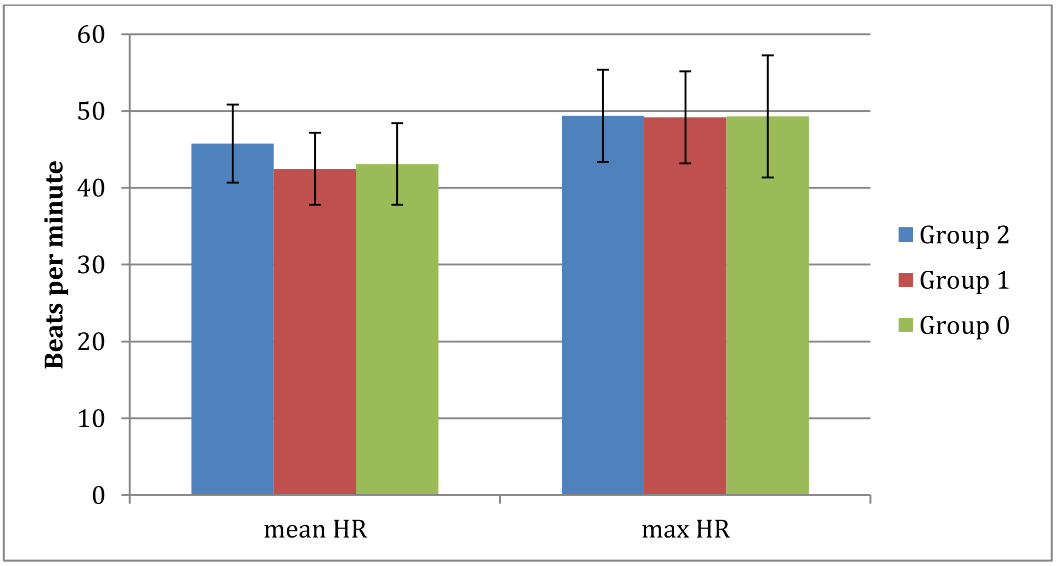
Amount of times assistance was needed			
	Group 0	Group 1	Group 2
0	0	0	1
1	0	1	1
2	2	1	0
3	3	2	1
4	3	2	2
5	0	3	2
6	2	1	5
7	1	0	0
8	1	0	1
9	1	0	1
10	0	0	0
11	0	1	0
12	0	1	0
13	1	0	0
14	0	0	0
15	1	0	0
16	1	0	0
17	0	0	0
18	0	1	0
Total	16	13	14



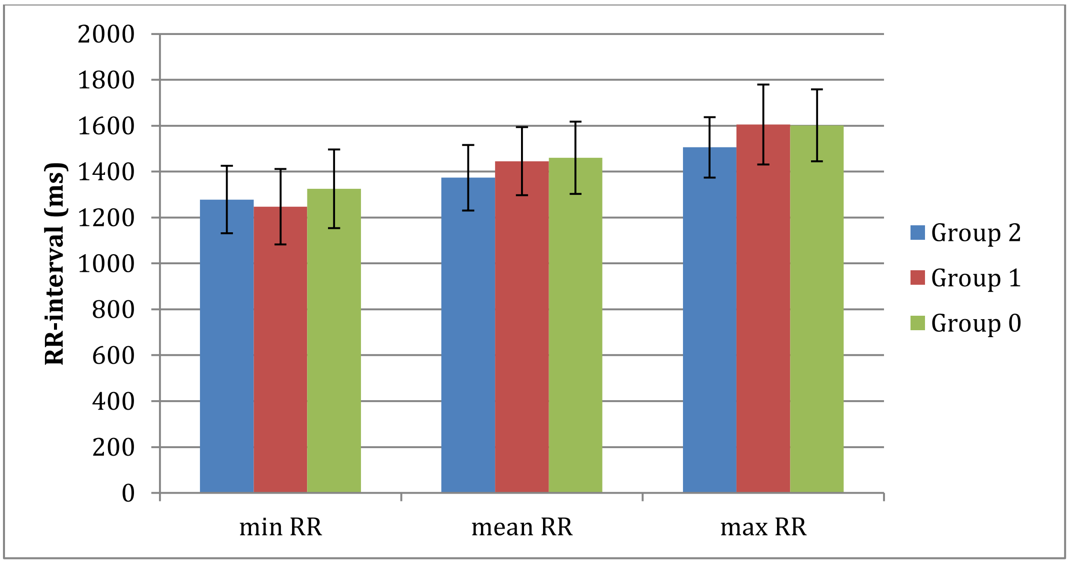
Graph 4: Templater test. Amount of times assistance was needed to perform the task. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

Table 15: Templater test, nose-bumps. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

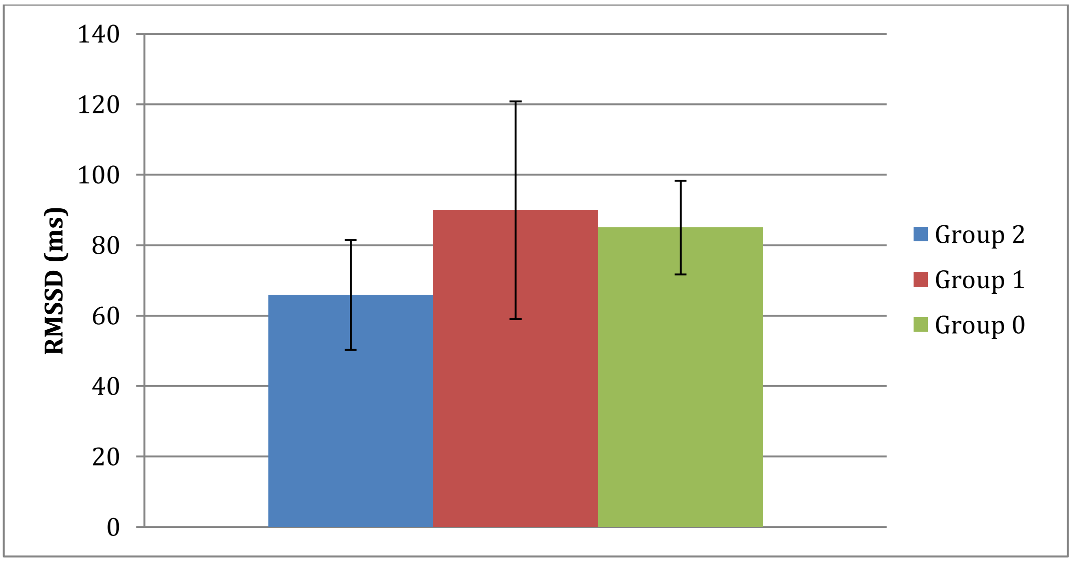
Nose-bumps		Incidence	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mean	Group 2	1,21	46	49	1278,12	1373,53	1505,95	65,89
	Group 1	1,54	42	49	1247,27	1445,13	1604,66	89,90
	Group 0	1,31	43	49	1324,83	1460,41	1601,72	85,03
	Total	1,35	44	49	1284,43	1421,38	1564,24	78,74
group 2	SD	1,05	10	12	294,59	285,93	262,78	31,17
	1/2 SD	0,53	5	6	147,30	142,96	131,39	15,58
Group 1	SD	2,18	9	12	328,19	296,68	348,25	61,76
	1/2 SD	1,09	5	6	164,09	148,34	174,13	30,88
Group 0	SD	2,27	11	16	342,37	314,66	312,74	26,58
	1/2 SD	1,14	5	8	171,18	157,33	156,37	13,29



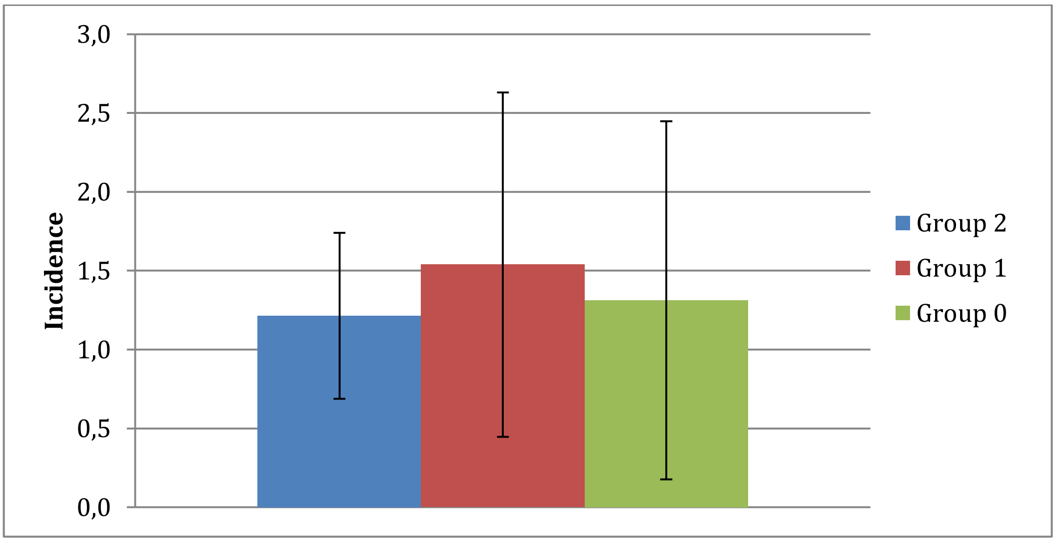
Graph 5a: Templater test, nose-bumps. Mean and maximum HR (heart rate) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



Graph 5b: Templater test, nose-bumps. Minimum, mean and maximum RR (R-R interval) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



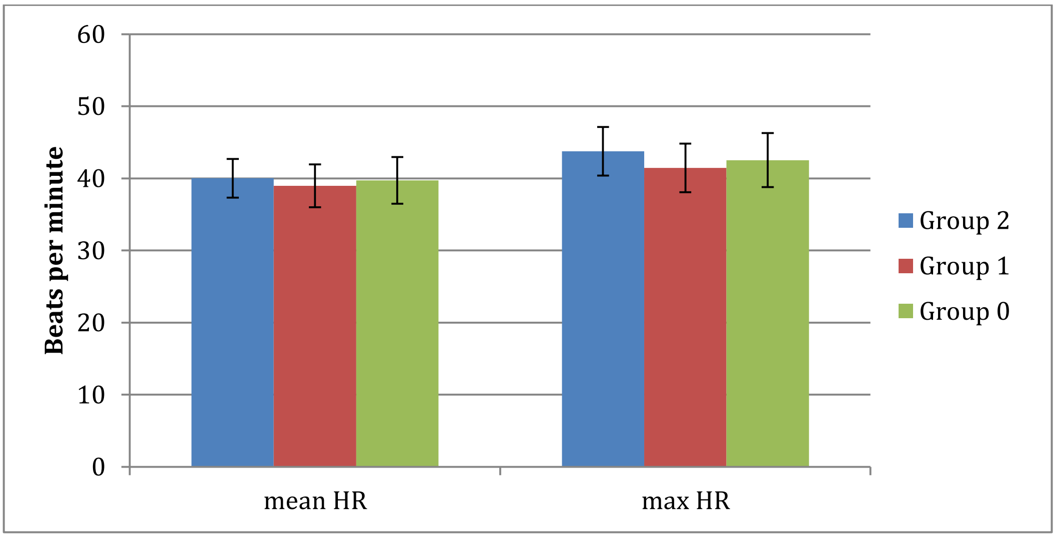
Graph 5c: Templater test, nose-bumps. RMSSD (Root Mean Square of Successive Differences, a measure for heart rate variability) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



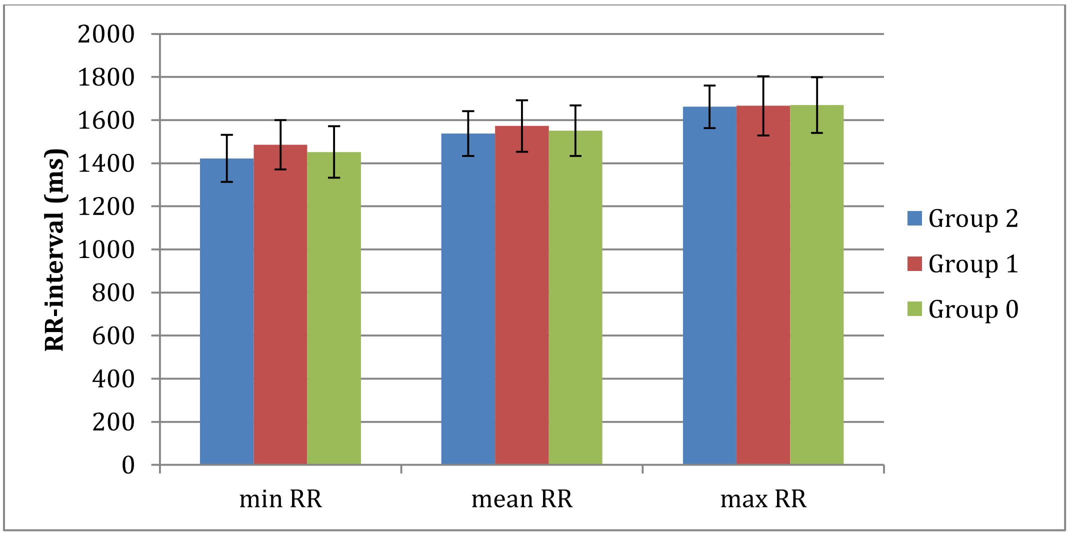
Graph 5d: Templater test, incidence of nose-bumps ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

Table 16: Templater test. Attempts to perform the task with assistance. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

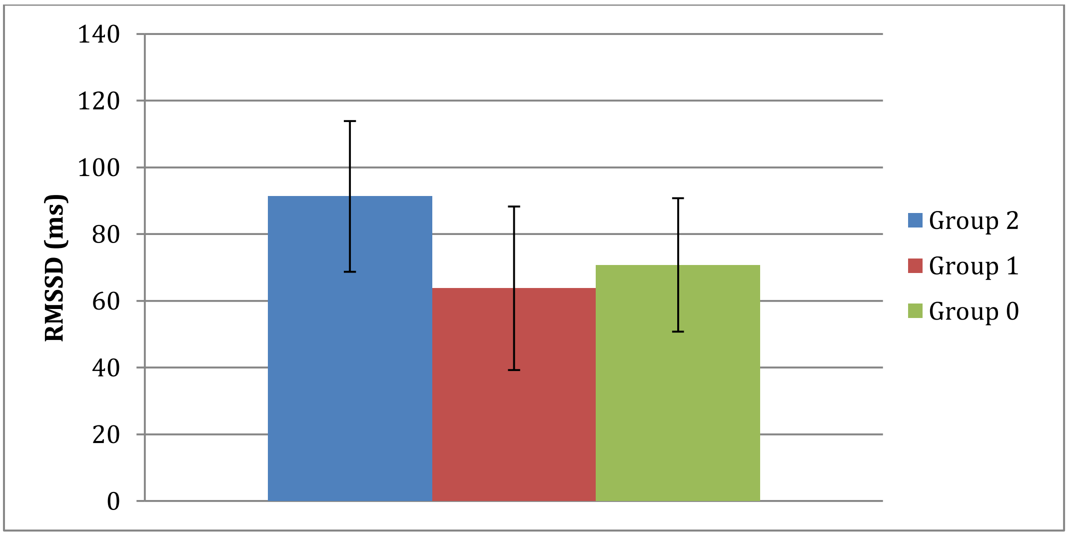
With assistance		Incidence	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mean	Group 2	4,93	40	44	1422,29	1537,79	1662,21	91,33
	Group 1	6,08	39	41	1485,93	1573,05	1666,42	63,76
	Group 0	6,56	40	43	1452,16	1550,69	1669,84	70,76
	Total	5,88	40	43	1458,81	1561,85	1677,77	77,49
group 2	SD	2,43	5	7	217,82	207,41	197,80	45,19
	1/2 SD	1,22	3	3	108,91	103,71	98,90	22,60
Group 1	SD	4,79	6	7	227,96	238,78	274,23	48,94
	1/2 SD	2,39	3	3	113,98	119,39	137,11	24,47
Group 0	SD	4,55	6	7	239,81	235,42	258,87	39,98
	1/2 SD	2,27	3	4	119,90	117,71	129,43	19,99



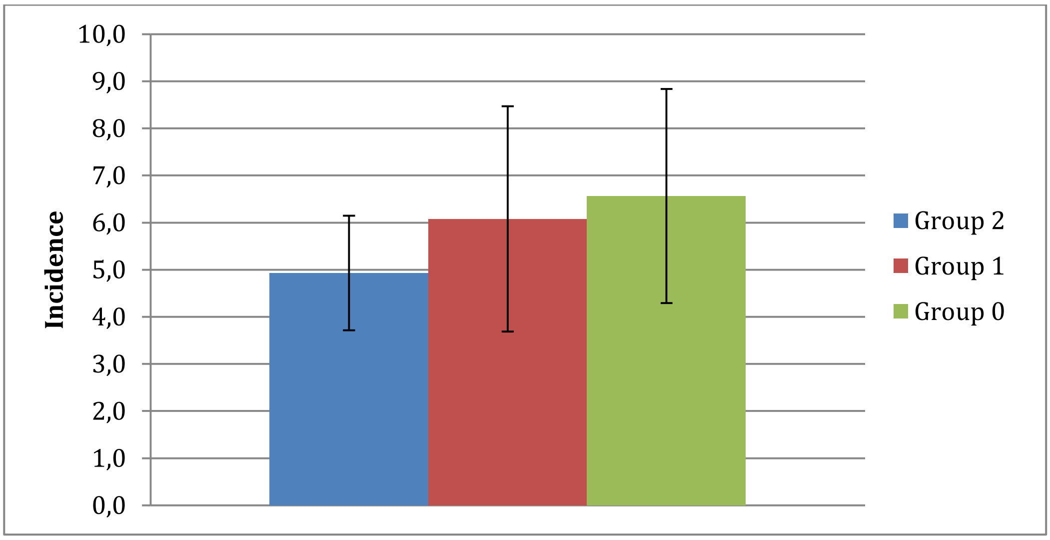
Graph 6a: Templater test, attempts to perform the task with assistance. Mean and maximum HR (heart rate) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



Graph 6b: Templater test, attempts to perform the task with assistance. Minimum, mean and maximum RR (R-R interval) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



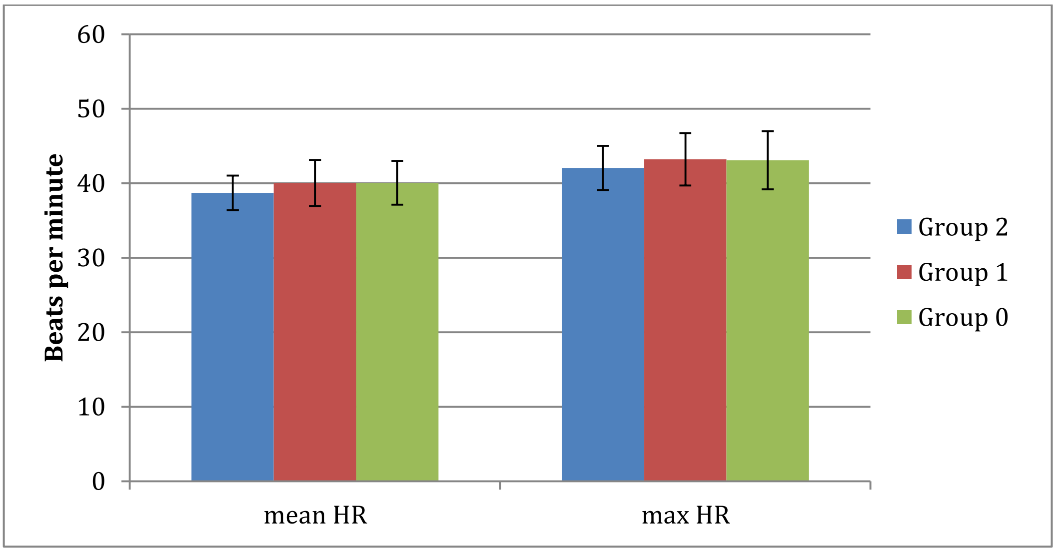
Graph 6c: Templater test, attempts to perform the task with assistance. RMSSD (Root Mean Square of Successive Differences, a measure for heart rate variability) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



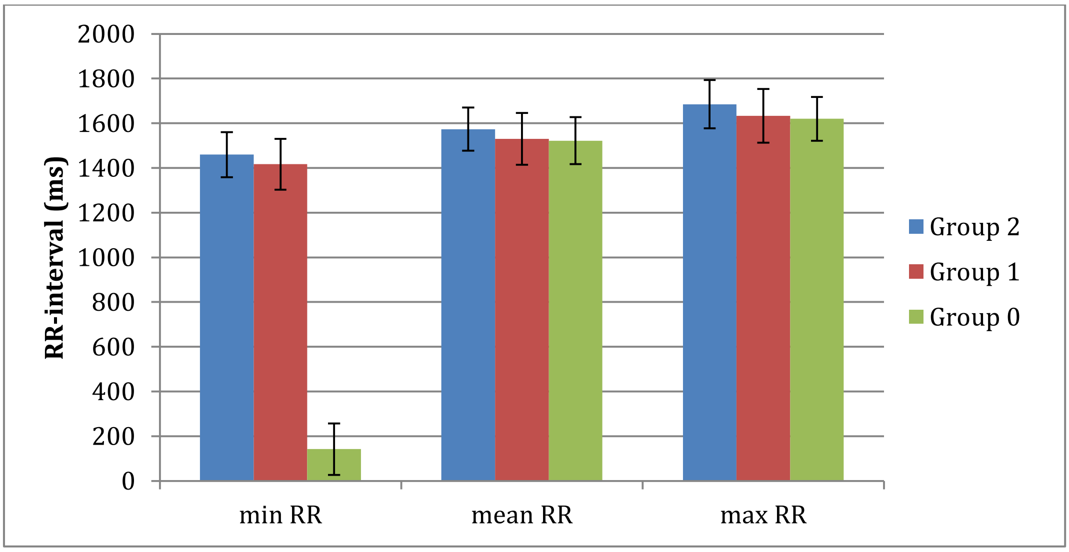
Graph 6d: Templater test, incidence of attempts to perform the task with assistance ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

Table 17: Templater test. Successful attempts to perform the task without assistance. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

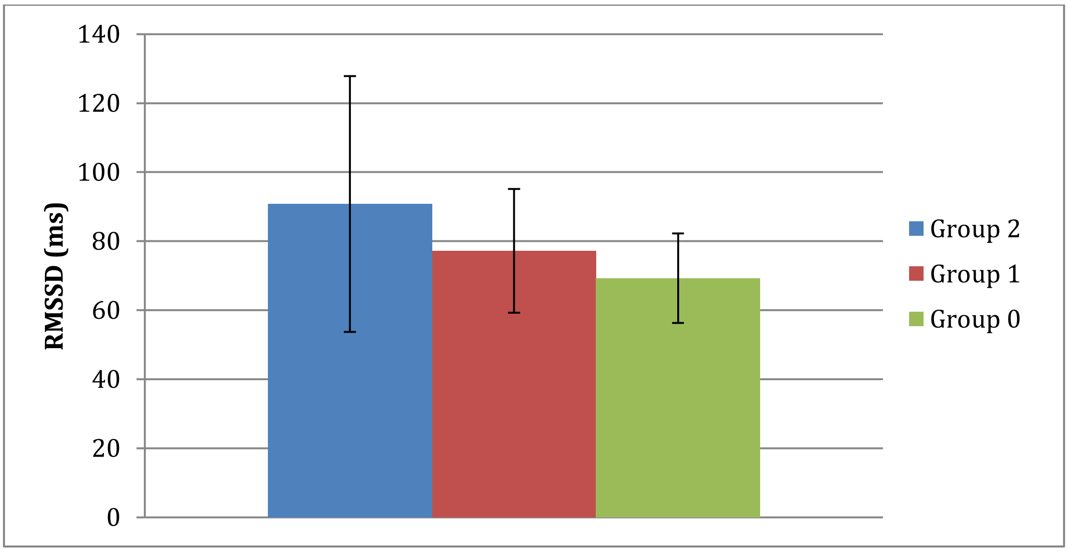
Without assistance			mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mean	Group 2		39	42	1459,50	1573,71	1685,12	90,76
	Group 1		40	43	1416,97	1530,22	1633,33	77,21
	Group 0		40	43	1420,00	1522,00	1620,02	69,30
	Total		40	43	1428,62	1539,47	1645,38	80,14
group 2	SD		5	6	202,56	193,68	215,59	74,13
	1/2 SD		2	3	101,28	96,84	107,79	37,07
Group 1	SD		6	7	227,76	230,68	240,28	35,87
	1/2 SD		3	4	113,88	115,34	120,14	17,93
Group 0	SD		6	8	230,80	210,09	196,30	25,96
	1/2 SD		3	4	115,40	105,04	98,15	12,98



Graph 7a: Templater test, three successful attempts to perform the task without assistance. Mean and maximum HR (heart rate) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



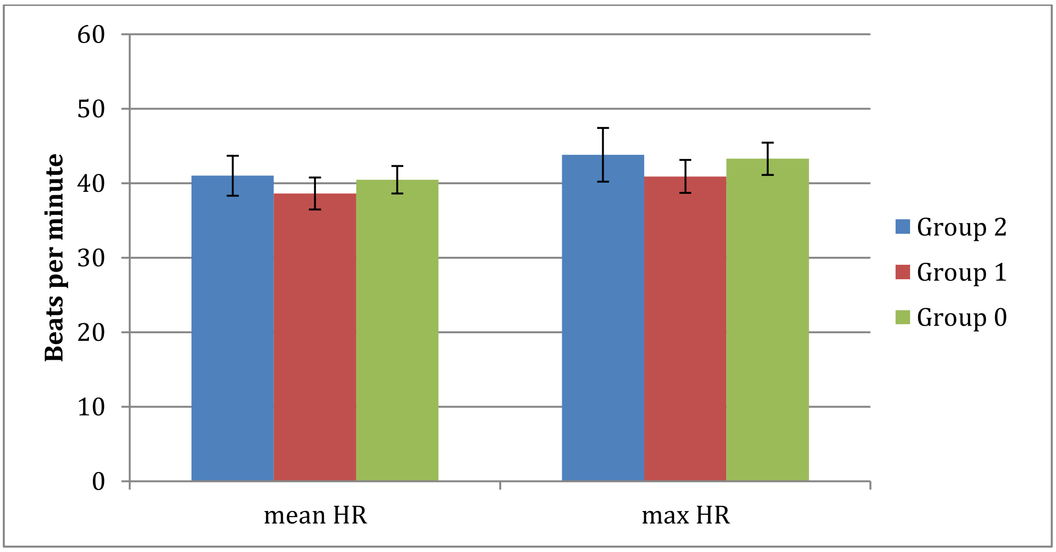
Graph 7b: Templater test, three successful attempts to perform the task without assistance. Minimum, mean and maximum RR (R-R interval) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



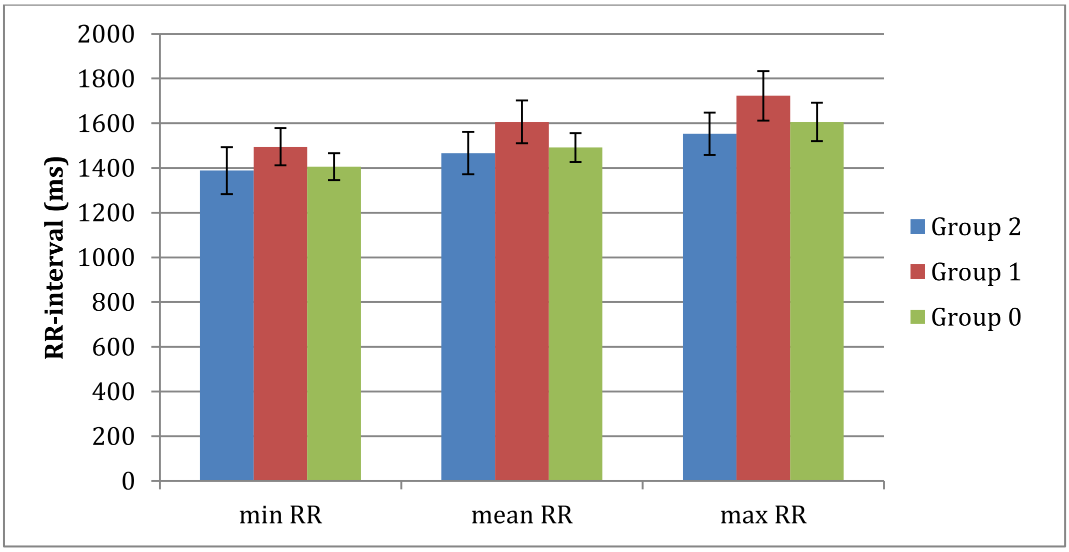
Graph 7c: Templater test, three successful attempts to perform the task without. RMSSD (Root Mean Square of Successive Differences, a measure for heart rate variability) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

Table 18: Templater test. Successful attempts to perform the task after the Templater device was turned 45 degrees. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

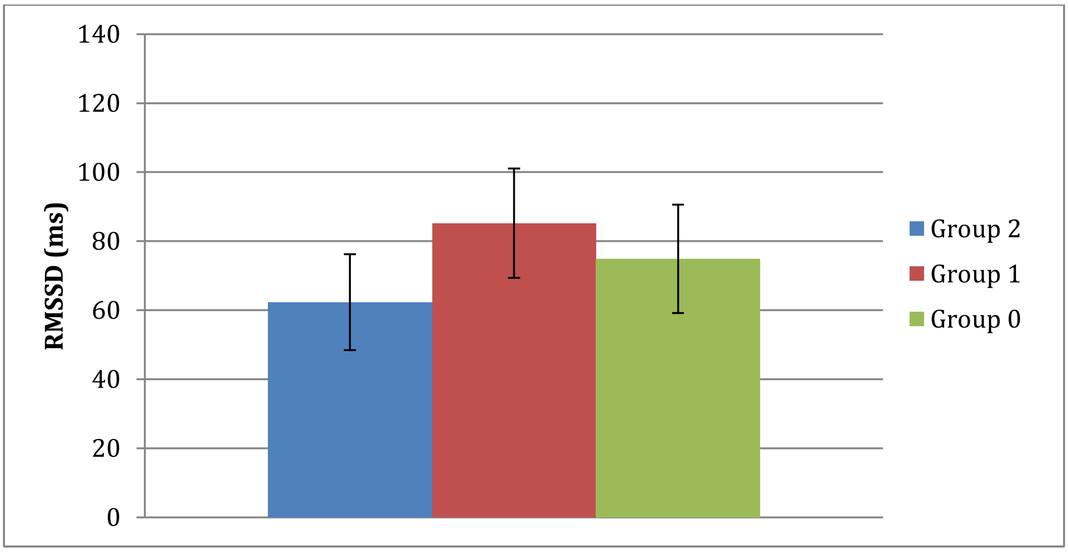
Templater turned 45°		time	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mean	Group 2	0:00:20	41	44	1388,00	1466,40	1553,50	62,31
	Group 1	0:00:11	39	41	1495,14	1606,14	1722,71	85,20
	Group 0	0:00:19	40	43	1405,33	1491,89	1606,11	74,88
	Total	0:00:17	40	43	1422,85	1512,85	1617,27	72,82
group 2	SD	0:00:30	5	7	211,00	191,02	189,28	27,76
	1/2 SD	0:00:15	3	4	105,50	95,51	94,64	13,88
Group 1	SD	0:00:12	4	4	167,33	192,86	220,97	31,63
	1/2 SD	0:00:06	2	2	83,66	96,43	110,48	15,81
Group 0	SD	0:00:24	4	4	119,85	128,34	171,42	31,49
	1/2 SD	0:00:12	2	2	59,92	64,17	85,71	15,74



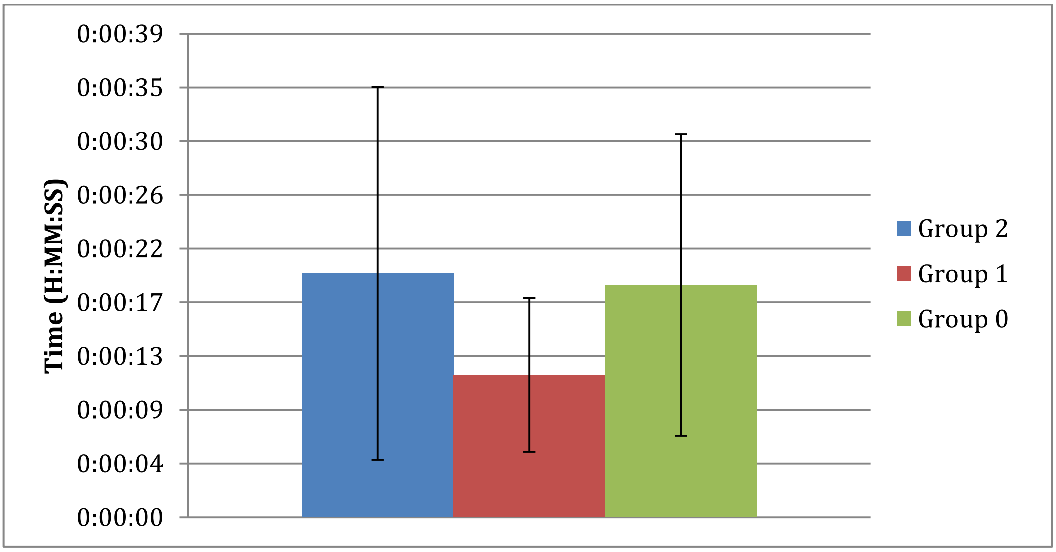
Graph 8a: Templater test, successful attempts to perform the task after the Templater device was turned 45 degrees. Mean and maximum HR (heart rate) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



Graph 8b: Templater test, successful attempts to perform the task after the Templater device was turned 45 degrees. Minimum, mean and maximum RR (R-R interval) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



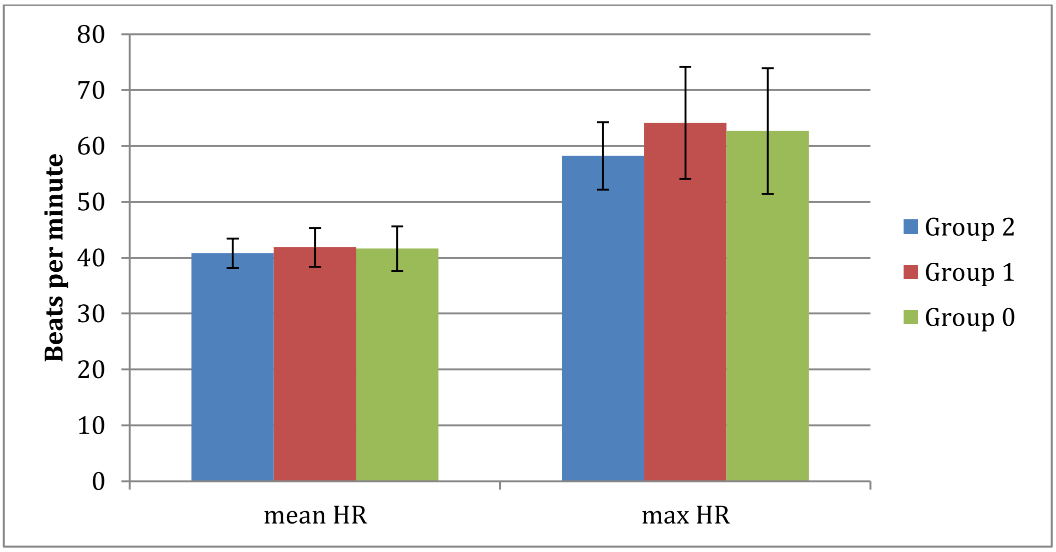
Graph 8c: Templater test, successful attempts to perform the task after the Templater device was turned 45 degrees. RMSSD (Root Mean Square of Successive Differences, a measure for heart rate variability) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



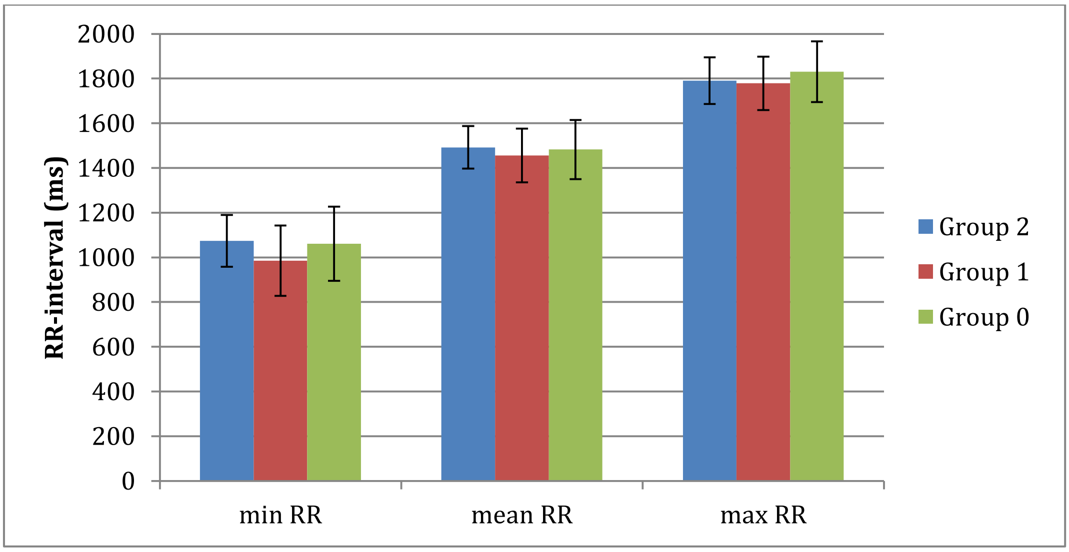
Graph 8d: Templater test, successful attempts to perform the task after the Templater device was turned 45 degrees. Time lapse ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

Table 19: Templater test. From the start of the Templater test, until the last successful attempt without assistance. HR = heart rate; RR = R-R interval; RMSSD = Root Mean Square of Successive Differences = heart rate variability. Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.

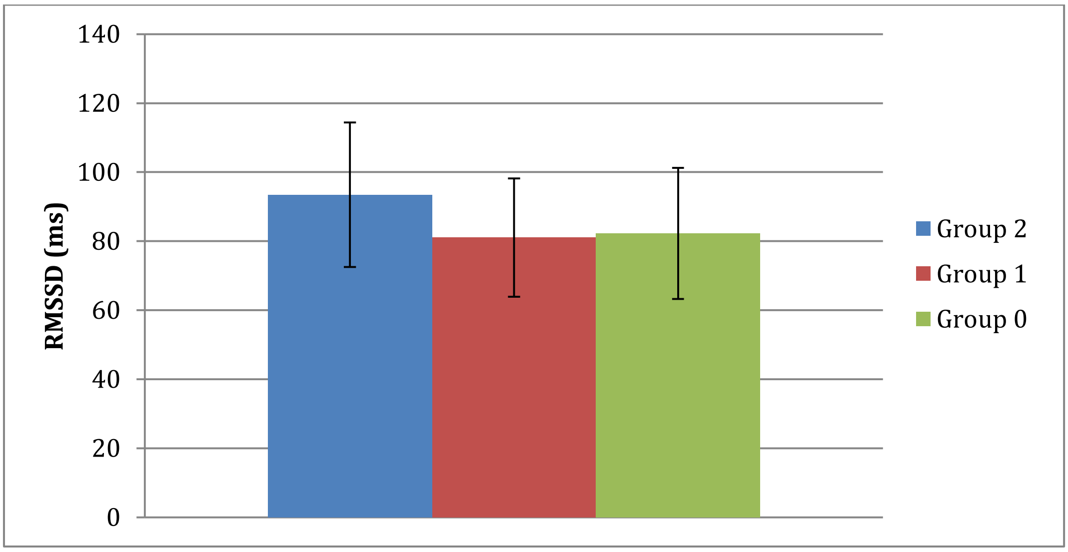
Start - end test		time	mean HR	max HR	min RR	mean RR	max RR	RMSSD
Mean	Group 2	0:02:38	41	58	1073,93	1492,07	1790,93	93,45
	Group 1	0:04:08	42	64	985,42	1455,67	1778,50	81,03
	Group 0	0:05:12	42	63	1061,21	1482,71	1830,43	82,24
	Total	0:04:02	41	62	1051,32	1483,53	1796,66	86,44
Group 2	SD	0:01:13	5	12	230,98	190,14	209,70	41,83
	1/2 SD	0:00:36	3	6	115,49	95,07	104,85	20,92
Group 1	SD	0:03:59	7	20	314,30	239,90	238,82	34,20
	1/2 SD	0:02:00	3	10	157,15	119,95	119,41	17,10
Group 0	SD	0:04:25	8	22	332,80	264,59	271,84	38,04
	1/2 SD	0:02:13	4	11	166,40	132,29	135,92	19,02



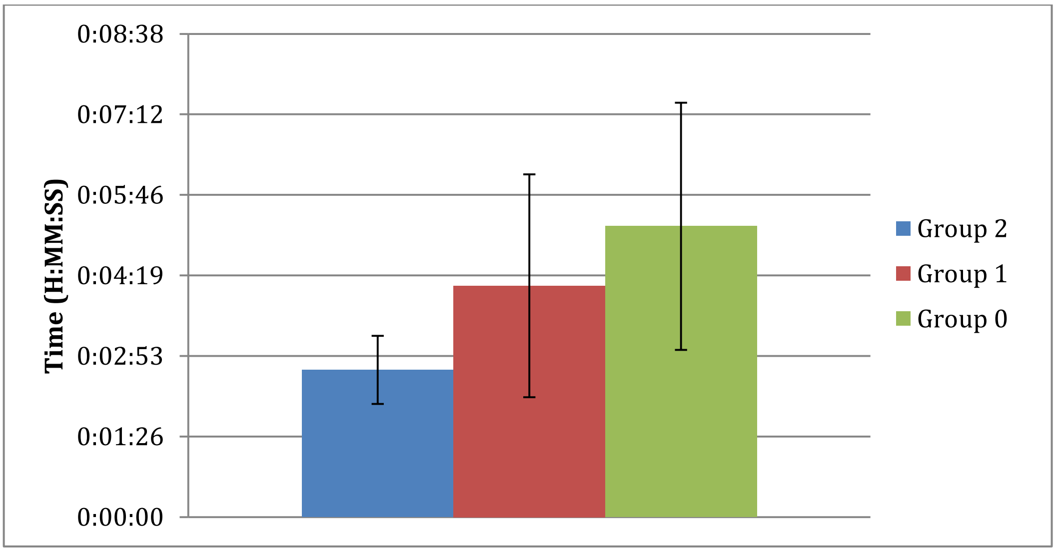
Graph 9a: Templater test, from the start of the Templater test, until the last successful attempt without assistance. Mean and maximum HR (heart rate) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



Graph 9b: Templater test, from the start of the Templater test, until the last successful attempt without assistance. Minimum, mean and maximum RR (R-R interval) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



Graph 9c: Templater test, from the start of the Templater test, until the last successful attempt without assistance. RMSSD (Root Mean Square of Successive Differences, a measure for heart rate variability) ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.



Graph 9d: Templater test, from the start of the Templater test, until the last successful attempt without assistance. Time lapse ± SD (presented as lines within the bars). Group 0 = vibrissae completely trimmed, N=16; group 1 = vibrissae 1cm length, N=13; group 2 = vibrissae full length, N=14.