Heart rate response towards fear-eliciting stimuli in horses

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Abstract

Finding the right horse for each rider is a difficult task as it is just as important that the temperament of the horse fits the rider as it is that the horse is of the right size. Even though it is a commonly experienced problem, no objective method of easily measuring the horse's temperament has yet been developed. If it is possible to test horses and get an objective measure of how reactive (emotional) they are, it could be a big help in finding the right horse for each rider. It would be desirable to have a way of testing the horse's reaction in an unfamiliar (and potentially frightening) situation. In practice this test should be just as easy as it is getting a judgement of its conformation and gaits.

The aim of the present study was to measure individual variation in HR response to different novel objects in horses of the same age, breed and reared in the same environment. We wanted to see whether certain horses (*i.e.* more emotional horses) react more to novel stimuli, in general, than other horses (*i.e.* less emotional), irrespective of the type of stimulus. We also wanted to see if different novel stimuli elicited different responses within individuals. The hypothesis was that individuals will react in a similar way to various stimuli.

Twenty four Danish warmblood horses were included in this study. All horses were 2 year-old stallions, reared under similar environmental conditions. They had received a minimum of handling prior to the experimental period. Three different stimuli were used. They were chosen because they were novel to the horses and would elicit measurable fear-reactions in all horses, but not so much that the horses did not approach the feed within the duration of the test. The visual stimulus consisted of a 1meter high orange traffic cone with reflex stripes, placed 1 m in front of the tub, the olfactory stimulus was eucalyptus oil and the auditory stimulus was a radio tuned to white noise. The control was an empty arena.

The result was that only the HR response to the auditory and visual stimuli differed significantly from the control days. The olfactory stimulus did not seem to alarm the horses the way the other stimuli did. We found a tendency towards a correlation in reaction between the olfactory and auditory stimuli and between the auditory and visual stimuli within individuals. These results indicate that horses do not generalize completely in their reaction between different stimuli.

Sammanfattning

Att hitta rätt häst till rätt ryttare är en svår uppgift och det är lika viktigt att hästens temperament passar ryttaren som att den är lagom stor. Trots att det är ett vanligt problem, så finns det ännu inget bra objektivt sätt att bedöma hästens temperament. Det vore önskvärt att ha ett sätt att testa hästens reaktion i en okänd (och potentiellt skrämmande) situation och på det sättet få ett mått på hästens temperament. Det borde vara lika lätt som att få en bedömning av hästens exteriör och gångarter. Om det vore möjligt att testa hästar och få en objektiv bedömning av hur reaktiva de är skulle det kunna vara en stor hjälp i jakten på rätt häst.

Syftet med den här studien var att mäta individuell variation i hjärtfrekvens som svar på nya stimuli hos hästar av samma ras, ålder och uppfödda i samma miljö. Vi ville se om vissa hästar (de mer emotionella) reagerade mer på nya stimuli generellt än andra (de mindre emotionella), oavsett typen av stimulus. Vi ville också se om olika nya stimuli utlöste olika svar hos samma individ. Hypotesen var att individen reagerar likadant på olika stimuli. I studien användes tjugofyra Danska varmblod. Alla hästarna var 2-åriga hingstar som växt upp i samma miljö. De hade hanterats minimalt innan experimentperioden.

Vi använde tre olika stimuli. De valdes ut så att de skulle vara helt nya för alla hästarna och med avsikten att de skulle utlösa mätbara rädsle-reaktioner hos alla hästarna, men inte så mycket att hästarna inte skulle gå fram till fodret under den två minuter långa testperioden. Som visuellt stimulus valdes en 1 meter hög, orange trafik kon med reflexränder, till luktstimulus valdes eukalyptusolja och det auditoriska stimulus var ”white noise”. Som kontroll användes den tomma arenan.

Resultatet var att bara vid ljudet och det visuella stimulus skiljde sig hjärtfrekvensen signifikant från kontrollen. Lukten tycktes inte skrämma hästarna som de andra stimuli gjorde. Vi fann också en tendens till korrelation i reaktion mellan ljud och lukt och mellan ljud och visuellt stimulus, på individnivå, men inte mellan lukt och visuellt. Resultatet indikerar att hästarna inte generaliserar mellan olika stimuli så som vi först hade trott.

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1. Introduction

Horses have evolved over millions of years to escape predators. They have developed a body that is made to run away from danger, with long legs, strong muscles and a deep chest with room for large lungs for good oxygen uptake. Their senses are optimised to early detection of danger, with good hearing and vision developed especially to detect movement in the periphery. Ever since we domesticated the first horse some 5500 years ago, we have made numerous alterations to the exterior of the horse and we have continuously found new fields of application for the horse. They have gone from being just a prey animal to carrying and pulling our heavy loads in farming and transportation and accompanying us in war and hunting. Today most horses are held as companion animals and used in sports, asked to jump high fences, run fast, be calm and patient with children and sometimes we simply want them to be beautiful for shows. However, just as for many of our domesticated species, we have had a very small impact on the behaviour of the horse. However inconvenient it might be in modern society, horses are still grazing prey animals that will respond to perceived threats and novelty in much the same way as their wild ancestors (Winther Christensen, 2002).

The aim of this study was to measure individual variation in heart rate response to different novel stimuli. We wanted to see whether certain horses react more to novel stimuli in general than other horses and to see whether they would react in a similar way to various stimuli. This study was part of a larger study on fear reactions in horses, recording both physiological and behavioural data.

1.1 The body's reaction to fear

When confronted with any fear eliciting stimulus horses, as well as other animals, react either by running away or staying to take up a fight. The brain gets information from one or more sense organs, processes it and decides whether the individual needs to react and, if so, how. The body has two principally different pathways of reaction to fear; the immediate reaction of the sympathetic nervous system and the slower secretion of cortisol governed by adrenocorticotropic hormone (ACTH) (Guyton & Hall, 1997, Keeling & Jensen, 2002).

The fight or flight reaction of the sympathetic nervous system is started by stimulation of the hypothalamus which sends out signals that are transmitted via the reticular formation in the brain stem and into the spinal cord to cause sympathetic discharge. This immediately results in a number of physiological changes, all of which make the horse ready to stay and fight or to run. Sympathetic stimulation increases both the rate and force of contraction of the heart, as well as the arterial blood pressure. Blood flow is redirected, less goes to the viscera and more to the muscles and the brain. The glycolysis in both liver and muscle increases and blood glucose levels rise (Guyton & Hall, 1997). All these changes result in a greater physical and mental ability so that the horse can perform more strenuous physical activity than would otherwise be possible.

The sympathetic system is counteracted and modulated by the parasympathetic system, whose actions are principally opposite to those of the sympathetic system (Guyton & Hall, 1997).

It is well known that cortisol levels in the blood rise markedly in stressful situations, but it is not clear how this is important to the animal. One explanation is that cortisol causes a rapid mobilization of amino acids and fats from the cellular stores. This would give the body access to extra energy needed to respond to the stressful situation (Guyton & Hall, 1997). The release of cortisol is controlled by the hypothalamus. The hypothalamus produces corticotrophin-releasing factor (CRF) in response to external or internal stimuli. CRF stimulates the pituitary gland to produce adrenocorticotropic hormone (ACTH), which travels with the blood to the adrenal cortex where cortisol is secreted. Cortisol then enters the bloodstream and affects many different organs. The reaction of the hypothalamic-pituitary-adrenal-cortical system takes a few minutes and is somewhat slower than the sympathetic response, which happens in a matter of seconds (Guyton & Hall, 1997).

1.2 The senses

1.2.1 Hearing

Horses hear a wide range of frequencies (65Hz to 33.5 kHz), this includes ultrasound that we cannot hear, but excludes some of the low-frequency sounds that we can detect. With their movable pinnae (earflaps) they have a good ability to localise sounds. The ears are not only important to receive auditory signals, but also an important part of the body language of the horse (Mills & Nankervis, 1999).

1.2.2 Vision

With the eyes placed laterally, horses have a rather small binocular field; hence their perception of depth is less than humans. But their total visual field is wide with only a very small blind zone (about 5 degrees) straight behind them. When the horse is grazing it can see 360 degrees needing only to move the head slightly.
Horses do not have acuteness of vision to the same extent as humans. Instead of a small centrally placed fovea on the retina where the light is focused as in humans, their fovea is a wide line along a horizontal axis. The fovea is the area where the density of photoreceptors is at its highest, thus where the visual acuity is at its best. Horses are long-sighted compared to humans and when the eye is "at rest" it is focused on more distant objects than the human eye (Mills & Nankervis, 1999).

The round shape of the eyeball and extensive lining of the retina allow very good peripheral vision. Also the ganglion nerve cells have higher sensitivity towards the periphery of the retina. These characteristics together with the large visual field combine to an extreme ability to detect moving objects at a distance, especially in the periphery of the visual field (Mills & Nankervis, 1999). This is of course a protective trait developed to alert horses to approaching predators.

In order to have full colour vision an animal needs to have three types of visual pigment in the cones of the retina. Each pigment responds to different wavelengths of light, with considerable overlap. The difference in response from the different cones make up the colour perception. Horses seem to lack one of the pigments, *i.e.* the one corresponding to green light. This means that horses can discriminate between red and blue, but can not tell green from grey. Colour vision is difficult to study, and it is not clear how complete the colour vision of horses is. Horses can see in lower light densities than humans, this is aided by the tapetum lucidum that reflects light back onto the retina. This is useful since in the wild the horse is most active around dawn and dusk. The downside of this function is that horses are easily blinded by bright light (Mills & Nankervis, 1999).

1.2.3 Smell

As in most prey animals, smell is important to horses to detect novel chemicals, which may indicate danger. It seems that it is the detection of new chemicals in the environment which is most important and not continuous monitoring of air quality, this is shown by the fact that the olfactory receptors are rather rapidly adapting to new smells.

The sensory area covers the back of the nasal cavity and the total sensory area is enlarged by folding of the mucosa. This gives the horse a much more extensive sense of smell than we have. Sniffing increases the intake of air, snorting is thought to help clear the sensory areas so that the next inhalation presents a true impression of the current aerial environment. These behaviours are also used as part of the body language, snorting for example is an alarm signal used to alert other horses of danger (Mills & Nankervis, 1999).

1.3 How can research be of practical use to horses and horse owners?

Even though horses have been domesticated for about 5500 years, domestication has had very little effect on their behaviour. They are still prey animals that react to danger much like their wild ancestors. Today horses are held mainly as sport and companion animals (in Sweden). In sport, light, athletic horses with a lot of energy, lively temperament and high rideability are in demand. The breeding of sport horses has been focused on performance in competition for several decades. The horses have become lighter and more energetic, mainly by mixing English thoroughbred horses in the breeding of Warmblood sport horses. With this you also get a more emotional horse, and the high responsiveness that we have bred for is not always positive, as explained below.

The term emotional will be used further in this text and needs to be defined. In research as well as among horse owners there are many terms used to describe horses that are more reactive to their environment. Commonly used terms are emotional, fearful and nervous. Archer (1973) listed a number of definitions of the term emotionality, one of them was behavioural and peripheral changes presumed to accompany high sympathetic nervous activity. It was explained as an inherited predisposition of the autonomic nervous system allowing reacting in a particularly strong and lasting way to some stimuli classes. This definition was also used by Le Scolan et al (1997); they included fearfulness, gregariousness, nervousness, learning and memory abilities in the definition of emotionality.

In human psychology persons are sometimes referred to as “Type A” or “Type B” personalities, where Type A persons are more prone to react to a stressor with sympathetic discharge, while Type B persons will show less sympathetic activity to the same stressor. This has been paralleled by observations in many different domestic species, and it seems to be comparable in most species (Keeling & Jensen, 2002). In their research McCann et al (1988) divided young horses in to two groups as being of a normal or more highly reactive emotionality level. This might be compared to types A and B as mentioned above.

An emotional horse does not usually present a problem for experienced riders since they can handle more nervous horses and can use the energy of the horse in a positive way for maximum performance. The problem starts when a horse with a more emotional temperament is paired with a less experienced recreational rider or a child. For example if it lacks some of the other qualities needed for top performance, and therefore is not wanted by more experienced riders. The less experienced rider might find it difficult to handle a horse that is easily spooked and the horse might require more challenging training than the rider can offer. This is when accidents start to happen. The horse shies at something along the track and because the rider is not prepared and does not have good enough balance, falls off. Or the horse is very forward going which the rider finds slightly frightening and tries to slow the horse down by pulling the reins. The horse might protest by rearing, bucking, running even faster or refusing to move at all which results in the rider becoming even more frightened and a downward spiral is started. Both horse and rider suffer from this.

The other side of the problem is when a competition rider is paired with a calm, what might be considered, lazy horse. If the horse does not have the right “fighting spirit” and “will to work”, it will be unable to perform at the top level, no matter how excellent it’s gaits or jumping technique are, and no matter how experienced the rider is. On the other hand this horse would make an excellent companion for a recreational rider or might become a nice school horse.

Also emotionality might interfere with the learning abilities of the horse and make it more difficult to train (McCann, 1988). This is readily acknowledged by horsemen, however very little research has been done so far in this area. In a moderate amount, a heightened level of emotionality improves attentiveness and learning, but too high levels will impair learning and could even reduce the welfare of the horse by constantly raised stress levels.

Finding the right horse for each rider is a difficult task and even though it is a commonly experienced problem, no objective method of easily measuring a horse’s temperament has yet been developed. It would be desirable to have a way of testing the horse’s temperament just as easily as it is getting a judgement of its conformation and gaits. If it is possible to test horses and get an objective measure of how reactive (emotional) they are, it could be a big help in finding the right horse for each rider. This in turn would help making horse riding a less accident prone sport and it would probably save numerous horses from unnecessary suffering and wrong handling.

1.4 Research on emotionality and fear reactions in horses.

Le Scolan et al (1997) compared experimental tests with ratings by riding teachers of adult horses and showed that the experimental tests were good predictors of individual characteristics as scored by the riding teachers. Visser *et al* (2002) tested young horses and found an increase in HR and a decrease in heart rate variability (HRV) in horses when challenged with a novel object.

The aim of the present study was to measure individual variation in response to different novel objects in horses of the same age, breed and reared in the same environment and so investigate whether certain horses (*i.e.* more emotional horses) react more to novel stimuli in general than other horses (*i.e.* less emotional), irrespective of the type of stimulus. Of interest also was whether different novel stimuli elicited different responses within individuals and the hypothesis was that individuals react in a similar way to various stimuli.

Habituation to the test arena before and between test days was used to see whether there was a carry-over effect from the previous test day. This would show in higher mean HR than during the habituation before the testing started and would imply that the horses remember the negative event (the test) and that they expected something "negative" to happen again the next time they enter the arena.

This study focuses on heart rate responses, behavioural observations were also carried out but because this project had a rather tight time limit, the behavioural observations and the comparison will be presented in another study and will not be commented on further in this report.

2. Materials and methods

2.1 Animals

Twenty four Danish warmblood horses were included in this study. All horses were 2 year-old stallions owned by Viegaard Stutteri in Skals, Denmark. The horses were kept on pasture from the beginning of May until the end of September. During the winter they are stabled in straw bedded, group stables. All horses were weaned and kept under similar environmental conditions. They received a minimum of handling prior to the experimental period.

All horses were identified with different coloured plastic rings, fastened in the mane to ease recognition. Detailed descriptions of all horses, including colour, white marks, scars and the locations of whorls were also made.

It was not possible to test all 24 horses on one day, therefore the group was divided in to two halves. To avoid long time laps between test and habituation the horses were tested in two different sessions. Twelve horses were tested in the first session and all twelve horses were tested each day; four with each of the three different stimuli. The test was planned so that the horses would receive the stimuli in different orders, see Table 1. The different stimuli are described later. The remaining twelve horses were tested by another researcher in a later session, using the methods developed with the first half of the horses. Kindly I was allowed to use the HR data from this session as well in my study.

Table 1. *Order of the stimuli for each horse, four horses received the same order of the stimuli*

Horse	Day 1	Day 2	Day 3
1, 7, 13, 19	Visual	Auditory	Olfactory
2, 8, 14, 20	Visual	Olfactory	Auditory
3, 8, 15, 21	Auditory	Olfactory	Visual
4, 9, 16, 22	Auditory	Visual	Olfactory
5, 10, 17, 23	Olfactory	Visual	Auditory
6, 11, 18, 24	Olfactory	Auditory	Visual

2.2. Experimental set-up

The arena and collecting paddock was built within the enclosure. The arena was round, 10 m diameter with 5 m high walls built out of straw bales with a solid door at the entrance. See appendix 1. The collecting paddock was approximately 10x12m and it was connected to the arena by a passageway (2,5x6m) built with wooden railings. The observer sat on top of the straw bales next to the entrance. Next to the arena and collecting paddock was a larger enclosure where all horses that were not used for tests on the particular day were kept during the test days.

The arena was empty other than for the fact that at the end of the arena opposite the entrance, a tub with feed was placed and behind it the cordless radio, covered by a black plastic bag. The radio was used in the auditory test; it was left in the arena during the entire test period so that the radio itself would not be a novel visual stimulus during the auditory test.

2.3 Testing procedures

Before the testing started the horses were trained to be caught, led and enter the arena alone and stay calmly alone in the arena for two minutes. The horse was regarded as habituated when it ate within 30 seconds and ate for at least 1 minute during the 2-minute habituation period.

On the test day all horses were collected in the larger enclosure next to the arena and fed hay and concentrate. The horses that were to be tested on that day were caught and led to the collecting paddock, where they were fed some hay.

The HR equipment was placed on the first horse. This equipment will be described later. The horse was led from the collecting paddock to the passageway when the HR had been less than 60 beats per minute for at least 60 seconds. The handler held the horse while the observer took her place on the straw bales. The horse was released and at the same time the door to the arena was opened by the observer and the test started. During the test, the handler waited silently outside the arena and the observer recorded the behavioural observations. When the two minute test period had passed, the handler entered the arena and caught the horse. It was led back to the passageway where the HR equipment was taken off. Then the horse was released in the collecting paddock and the next horse was caught.

Before the novel stimuli were introduced we did observations on one day of habituation (with an empty arena). Between the test days there was always one day of habituation and after the last test day there was also one day of habituation. Thus, the final order was training, habituation 1, test day 1, habituation 2, test day 2, habituation 3, test day 3, habituation 4. The observations in the empty arena (habituation) were used as control to the tests.

2.4 Stimuli

We wanted to test three different stimuli that were novel to the horses and would elicit measurable fear-reactions in all horses, but not so much that the horses did not approach the feed within the duration of the test. Before the tests started, all stimuli were tested on two horses that would not be included in the test to make sure the reactions were on the expected level.

The visual stimulus consisted of a 1m high orange traffic cone with reflex stripes, placed 1 m in front of the tub. We chose the traffic cone because it is an object that a lot of riders know horses react to and the horses in our test group would not have come in contact with any object similar to it before this test.

The olfactory stimulus was eucalyptus oil. The oil was spread over the inside of the tub and a sponge soaked in oil was left in the tub next to the feed. Another, identical tub was used in the other tests and during habituation since it was not possible to remove the smell of the oil completely.

The auditory stimulus was a radio tuned to white noise sound that the horses probably could not hear until they entered the arena and came within a few feet of the feed tub.

2.5 Data acquisition and processing

The heart-rate was recorded with Polar Vantage NVTM (Polar Electro OY, Kemple, Finland), which consisted of an electrode belt (made for use on horses) with built-in transmitter and a wristwatch receiver. The receiver stored data, which were later downloaded via a Polar Interface to a PC, using the software Polar Precision PerformanceTM SW 4. Due to technical problems during the first session, the transmitter was replaced (with a T61, made for use on humans) during the second session. The transmitter was fastened with an elastic girth, water and exploratory gel was used to ensure contact between the skin and electrode. Data was recorded as an average heart rate every 5 seconds.

The behaviour was recorded by the observer with a hand-held computer (Workabout, PSION PLC, UK) using a preformed ethogram. The behavioural observations were recorded for later use in another study and are not reported further in this report.

2.6 Statistical analysis of HR data

The data were not normally distributed and therefore non-parametric statistics were used. Kruskal-Wallis tests were used for comparisons between several groups and Mann Whitney U tests for comparisons between two groups. For pair wise comparison between tests Dunn's Method was used.

3. Results

Due to technical problems, mainly during the first session, HR data is missing from individual tests. Therefore not all horses are included in all the comparisons. We could only use horses where all data needed for the current analysis was present. Also one of the horses was badly frightened during the first habituation day, after that we could no longer put the elastic girth on him; hence this horse is not included in the analysis. Thus the total number of horses reported here is twenty-three. A typical example of the HR curve is shown in figure 2.

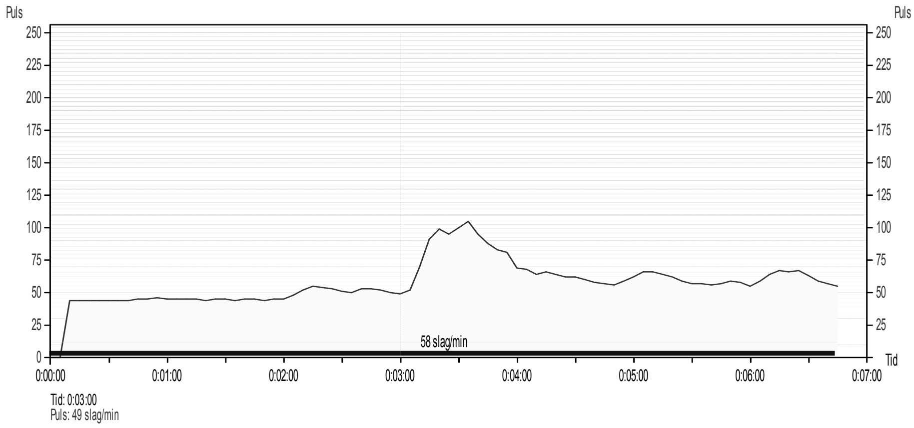

Figure 2 Example of a HR curve during a test. The test starts after 3 minutes, shown by the vertical line in the picture.

3.1 Difference in HR between habituation-days

The values of all horses were combined to a mean for each habituation day.
As figure 3 shows there was no significant difference (P=0.275) in the HR between habituation days (day 1: 52.6 ± 6.2; day 2: 52.7 ± 3.6; day 3: 52.6 ± 4.3; day 4: 50.2 ± 3.7). This means there was neither habituation nor sensitisation during the test procedure. For this reason the results from all habituation days were combined to a mean for use in the following analyses.

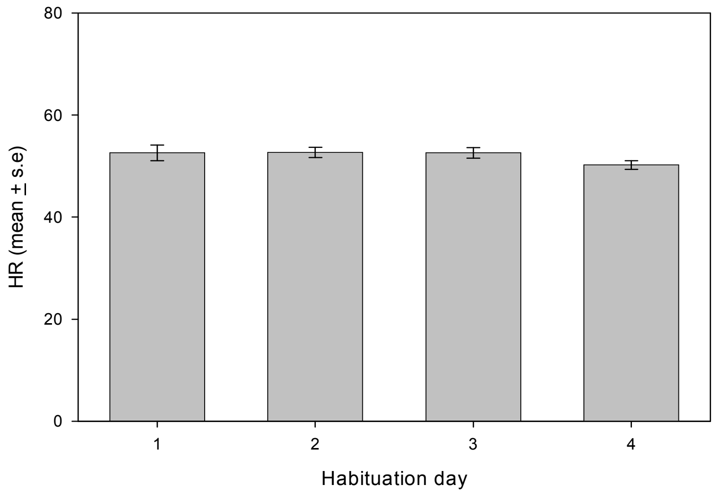

Figure 3. Mean HR (± s.e,) during the different habituation days

3.2 Effects of different novel stimuli on heart-rate.

The individual HR responses varied considerably between horses. For some horses the HR rose very high but went back to the base level quickly, e.g. after only 20-30 seconds, whereas others rose more moderately but stayed elevated for a longer period of time, the curve in figure 2 is of this type. Because of this pattern difference we chose to use the elevation from minimum to maximum HR during the test instead of mean HR, when comparing the different tests, to give a truer picture of the reaction to the stimulus.

The elevation in HR on habituation days (15.7±1.3) was significantly lower than with auditory (28.6±4.8) (H=4.32, P=0.015) and visual (27.7±4.2) (H=3.9, P=0.031) stimuli, but not than the olfactory (19.5±6.7) (H=1.1, P=0.85) stimulus. The visual (bar 3 in figure 4) and auditory (bar 4 in figure 4) stimuli did not differ significantly (H=2.3, P=039) from each other.

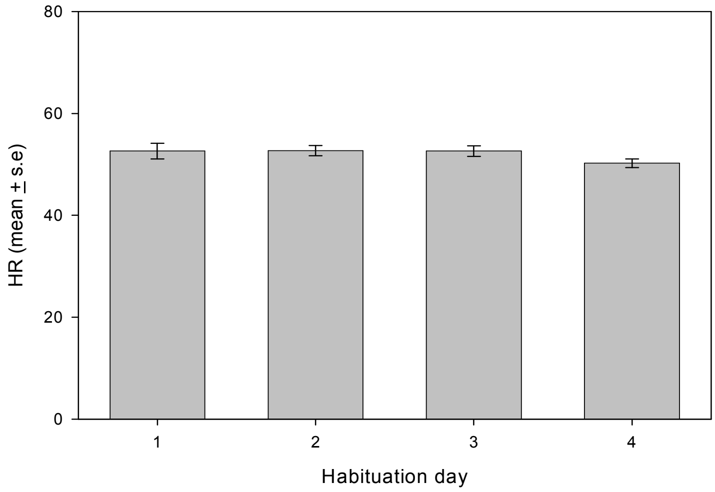

Figure 4. Elevation in HR (±s.e.) during the different test compared to habituation. Different letters indicate a significant difference at P<0.05.

3.3 Effect of the test day on HR response.

The average HR of all horses on each individual test day (day 1, 2 and 3) was compared to each other. This was irrespective of the test order each horse received and in which session they were, i.e. the results from day 1 equals the results from the first day of testing in both sessions. During day 1 a total of eight horses received each of the different stimuli (visual, olfactory and auditory).

The figure indicates a higher reaction on the first test day (mean 67.1±6.9), but the difference only tends towards significance (H=2.5, P=0.091), see figure 5. Between the second (mean 54.2±1.4) and third test-day (56.9±2.2) there was no measurable difference. On day 1 there was a greater variation in HR response than during day 2 and 3, see the bars indicating the standard error in figure 5.

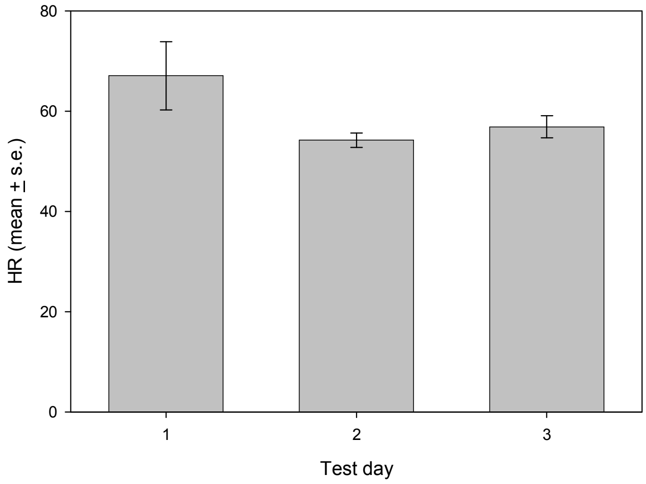

Figure 5. Mean HR (±s.e.) during each test day.

3.4 Correlation between tests

Each test was correlated with each of the other two tests in order to investigate similarities in response. See figures 6-8. We used the Spearman Rank Order Correlation (correlates pairs of data, which are not normally distributed):

Test	Correlation coefficient	P-value
Visual-Olfactory	-0.140	0.651
Visual-Auditory	0.481	0.0566
Olfactory-Auditory	0.477	0.0809

We did find a tendency towards correlation between the olfactory and auditory tests (P=0.0809) and also between the auditory and the visual tests (P=0.0566). The graphs show two distinct outliers. We chose not to exclude them in this analysis, this is further commented in the discussion. However it is worth mentioning that if we had excluded the two outliers, the correlations would have been significant.

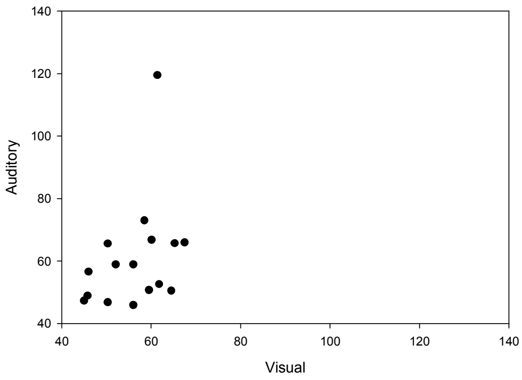

Figure 6. Correlation between HR in the auditory and visual test. Each horse is represented by one dot.

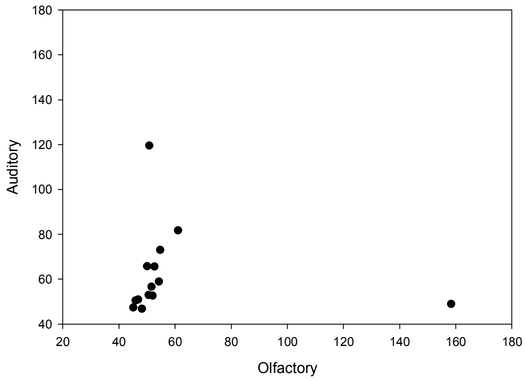

Figure 7. Correlation between HR in the auditory and olfactory test. Each horse is represented by one dot.

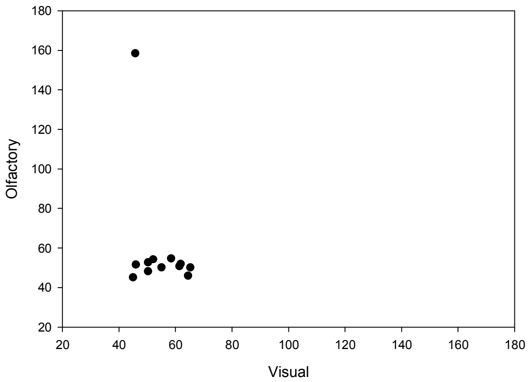

Figure 8. Correlation between HR in the olfactory and visual test. Each horse is represented by one dot.

4. Discussion

This study investigated novelty responses in horses. To do this, we compared changes in heart rate to three different stimuli; one visual, one auditory and one olfactory. We selected stimuli that were novel to the horses and would elicit measurable fear-reactions in all horses, but not so much that the horses did not approach the feed within the duration of the test. Overall, this was successful. Only on two occasions did horses react stronger than desired to the stimuli, however we had expected a stronger reaction to the olfactory stimulus than the observed. We found that horses did not generalize completely in their reaction to these three stimuli and there was a relatively large variation in response between individuals, even though we had chosen a quite homogenous group of horses. This study can be viewed as a first step in studying emotionality in horses and further research is needed before the techniques can be taken into practice as a method to help in the selection of horses.

4.1 Experimental set-up

For this study we tried to find stimuli that we were sure would be novel to all our test horses, and that could not be connected to anything they had ever come across before. The reason for choosing three different stimuli was that we wanted to test different senses of the horse. However, to be able to tell if the sense stimulated has any impact on the level or type of reaction to the novel stimulus, it would be necessary to repeat the test with many different stimuli influencing the same sense. This was not the aim with the present study. We wanted to measure HR because HR is an objective measure of the reaction, it is relatively easy to measure and the tests can easily be repeated. Behavioural observations alone have the disadvantage that the result may be affected by the observer, but it is also important to recognize that HR is influenced by physical activity, therefore it is necessary to combine behavioural observations with the HR to some extent. The idea of comparing HR with behavioural responses in novel object tests is not new (Visser et al, 2002), but this field needs more research before the techniques and results can be brought into practical use when buying or selecting horses.

It is an important part of science that experiments are repeatable with comparable results and also that tests are repeated with slight changes in the test set up, e.g. different stimuli or horse population. In the present study all horses had similar previous experiences, but if this is to be taken in to practical use it must be taken in to consideration that horses in the general population will have had very different experiences. It seems likely that the behavioural responses would vary more than the HR, meaning that HR might be a better measure in a diverse population, however this remains to be proven.

Only one stimuli of each type was used in this study. In future, it would be worthwhile to repeat the test with other stimuli. After having tested only these three stimuli it is not possible to compare the size of the reaction between different stimuli and between horses. For example, the olfactory stimulus did not give the response we anticipated. As discussed in paragraph 4.3 there are a number of possible explanations for this. Further testing will be able to tell if it was this particular stimulus that was not apprehended as frightening or, if it is in fact so that horses react less to olfactory stimuli in general than they do to visual and auditory stimuli.

4.2 Difference in HR between habituation-days

We found no difference in HR between habituation days. This implies that the horses did not learn to expect a negative experience in the arena after the tests, just as we had expected. There is of course a risk that this results from not choosing strong enough stimuli, however it was important that none of the horses found the test so frightening that it would leave a permanent negative memory. Also we did in fact see an alteration in HR in response to the novel stimuli and the fact that the HR did not get gradually lower between habituation days shows that the horses did not habituate further during the tests.

Habituation between test days was used to see whether there was a carry-over effect from the previous test day. This would show in higher mean HR than during the habituation before the testing started. This would imply that the horses remember the negative event (the test) and that they expect something "negative" to happen again the next time they enter the arena.

4.3 Effects of different novel stimuli on heart-rate.

In this study the horses reacted to the novel sound and the novel visual stimulus by an increase in HR (see figure 4). However the degree of elevation in HR varied according to the stimulus. Why they did not react to the smell in the same way is not clear. It might be that this specific smell was not particularly interesting or was not considered frightening. For example, blood and pig smell are smells that many horses react adversely to and before the test started we tried different smells on other horses, including pig smell. But, the horses we used at that time did not seem to react to these although eucalyptus oil did seem to elicit a reaction in the trial horses. In addition, we chose eucalyptus oil because we wanted a scent that we were sure was novel to all test horses and that could not be connected with anything they had come across before.

Another explanation for the difference in reaction to the different stimuli might be that the horse needed to come close to the tub before he noticed the sound or the smell, whereas he could see the visual stimulus as soon as the door to the arena was opened. This difference in surprise effect might be the reason for the difference in reactions to the different stimuli. It will be interesting to see the results of the behavioural responses and whether there are other similarities in response to the stimuli that could support this theory.

4.4 Effect of the test day on HR response.

There was a tendency to higher HR elevation on the first test day, and a greater variation between individuals (see figure 5). This might be explained by the fact that the horses had been trained to enter the arena and expect to find food in there without any changes in the environment. The first test day was the first time anything had changed in the arena and hence there was in a way an extra stimulus on this test day than on the following test days. There was the actual stimulus that we intended to test as well as the fact that something (anything) had changed. It is possible that for example moving the feed tub within the arena could have caused a similar reaction. After this first time, the horses would be prepared that there might be some kind of novelty in the arena, and the extra surprise effect disappeared.

We had not expected to find any difference between the test days, the fact that there was a tendency to higher reaction on the first day emphasises the importance of a random test order, as pictured in Table 1. If we had given all horses the same test order then all horses would have had the same stimuli on the first day and we would not have been able to tell if the higher reaction on the first test day was caused by the particular stimulus or because it was the first day.

4.5 Correlation between tests

We only saw a tendency towards correlation in reaction between the olfactory and auditory stimuli and between the auditory and visual stimuli (figures 6 and 7). Hence the hypothesis that a more emotional horse would show a greater reaction to all the stimuli was not proven in this study. The results indicate that horses do not generalize completely in their reaction between different stimuli. However it is likely that the correlations had been significant, if we had chosen to eliminate the two outliers. Why we chose not to eliminate them is explained in paragraph 4.6. This will need to be investigated further, as mentioned in paragraph 4.3. The analysis of the behavioural data in combination with the HR data might also give a better view.

4.6 Outliers

Despite these general effects, some individual horses showed extreme reactions on individual days. The first horse reacted very strongly to the olfactory stimulus, which was his first test. He showed a number of behavioural responses, galloping, snorting, neighing etc, and most importantly he started rearing against the walls to try to get out. The next day he was as calm as ever again and did not show any extreme reactions to the following tests. During the training of the horses before the tests started, this horse had reacted in a similar way, with rearing against the walls, the first time he was left alone in the arena. However during the rest of the training and habituation, he did not show any signs of anxiety or discomfort when left alone in the arena.

The second horse reacted extremely to the auditory stimulus. This was his last test, and he had not shown any extreme reactions before, not during the test or habituation days, nor during the training period. This was in fact one of the horses we considered to be very calm. In the two previous tests he had hardly exhibited any noticeable behaviour changes at all in response to the stimuli. During the auditory test however, he would not approach the tub to eat during the entire 2 minute test period. At the habituation the next day he seemed to be back to normal.

These horses can be seen as the outliers in figures 6-8. If we had noticed any random event (i.e. gunshot, bark, birds flying above the arena etc) simultaneously with these particular tests, we could have excluded these results from the analysis. However we could not exclude that this was a true reaction to the stimulus by the individual horse and therefore we chose to present the results with the outliers included. Repeated testing of these individuals, with the same stimulus as well as with other stimuli of the same kind, might reveal if it was an accidental event or a true reaction.

4.7 Further analysis of data

One purpose of this experiment was to compare HR and behavioural response to novel stimuli. However, analyzing this data is much too great a task to be completed in my exam project, therefore I did the analysis of the HR alone and I left the analysis of the behaviour, and the comparison of the two, for others to complete.

The comparison will tell how much of the HR elevation is due to physical activity and how much is attributed to the fear response. However already casual observation during the testing showed that there was not so much physical activity, that the physical activity alone can explain the rise in HR.

It will also show if the HR response is correlated to the extent of behavioural changes, i.e. if the horses that had the highest HR elevations were the ones that exhibited the most extensive changes in behaviour.

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