

## The responses of horses to predator stimuli

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### Key Words:

Horse behavior; fear test; Caspian pony; physiological responses.

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Received 22 April 2009,  
Accepted 9 September 2009

### Abstract

It is not known whether the instincts of wild horses have remained strong during their centuries of domestication. Knowledge of this matter would give riders the opportunity to know more about the behavior of horses and consequently about safety for both horses and riders. In this current research, we studied the behavior of fifteen Caspian miniature horses of different ages and sexes using stimuli from predators under standardized conditions. We explored whether olfactory (lion feces) or auditory (lion roars) stimuli affected horses to a greater extent. The test arena was an appropriately equipped grass paddock, in which horses spent between 5 and 8 min. The experiments were designed to investigate behavioral responses in locomotive activity (alertness, standing, walking, trotting, exploration and other), eliminatory behavior (defecation, urination) and physiological responses (heart rates before and after the predator stimuli) of horses to novel auditory and olfactory stimuli. In the olfactory experiment, we found that the horses showed significantly more behavioral reactions compared to the control experiment (where horses were not exposed to any stimuli); the only behavioral reaction the horses did not show was flight reaction. Additionally, heart rate was significantly increased after olfactory stimuli compared to auditory stimuli. In the auditory experiment, we found that horses showed more behavioral reactions in response to the roar of the lion compared to the olfactory stimulus, including flight reactions. We concluded that the auditory stimuli caused significantly higher heart rate responses when compared to the olfactory stimuli.

### Introduction

The appropriate response of a horse towards a potentially dangerous stimulus has been important in ensuring its survival through millions of years in the wild. Domestic horses respond to perceived threats and novelty in much the same way as their wild ancestors. For instance, horses tend to react with a rapid flight response when alarmed and avoid potentially fear-eliciting situations in general. They do not approach threatening stimuli and they tend to respond nervously to novelty within a known environment. Responses towards novelty have often been used in tests of fearfulness or emotionality in animals (Gray, 1987; Boissy, 1995). In order to survive, an animal must respond to a wide variety of stimuli. They must recognize external signals, such as danger or the overtures of a potential mate, and seasonal changes that provide information about when to migrate or hibernate. Responses to all stimuli require an internal communication system. There are two main kinds of

internal signals: nerve impulses (vision) and chemical messages (smelling), (Bailey, 1995). It is important to assess both the behavioral and physiological responses, as the immediate physiological reaction to perceived danger is characterized by activation of the fight or flight response within the sympathetic neurons of the autonomic nervous system (Guyton and Hall, 1997; Korte, 2001).

Many species have developed specific behaviors to facilitate the recognition of predators, avoidance of danger, and defenses against predation. Such anti-predator behavioral systems are fundamental to survival, and natural selection has favored mechanisms that enable prey to detect predators prior to their attack, which increases the probability of escape or avoiding an encounter outright (Kats and Dill, 1998; Apfelbach *et al.*, 2005; Monclus *et al.*, 2005). Anti-predator defenses can involve responses to specific chemical cues that predators produce, and avoidance of predator odors, such as fur, urine, feces, or anal gland secretions; these have been observed in several mammalian

species, particularly in rodents (Apfelbach *et al.*, 2005). Nevertheless, there is limited research on the anti-predator behavioral systems of horses.

In the present study, we have recorded the behavioral reactions in combination with the recordings of the changes in the heart rate of horses when confronted with olfactory and auditory threat stimuli. The sudden paradox of novelty is that it will cause an intense behavioral and physiological reaction when suddenly introduced to an animal with a flighty, excitable temperament, but the same animal may be the most attracted to a novel object when allowed to approach it voluntarily. Numerous studies in many species have shown that animals raised in a variable environment are less likely to be stressed when confronted with novelty (Darmon, 2002). Equids typically live in open grasslands with a good view of the surrounding environment and they use vision as a major sensory avenue for the detection of predators. Equids are also sensitive to auditory signals of danger, such as sounds of predators, as they have a good sense of hearing (Heffner and Heffner, 1983; MacDonald, 1995).

The present experiment was designed to explore which stimuli (olfactory and/or auditory) caused an increase in heart rate, and which type of stimuli (olfactory and/or auditory) from the same species of predator affects horses to a greater extent.

## Materials and Methods

### Animals

Fifteen Caspian ponies of different ages and both sexes were used in this study. The mean age was 11 years old. This group consisted of nine female horses (three fillies and six mares) and six stallions. We used 15 horses for the control test, the same 15 horses in the olfactory test, and 13 horses in the auditory test.

The horses were selected from a herd which was kept in a pasture (the horse breeding centre of Mrs. Firooz) before transferring them to the test area at the Agricultural Research Station, Khojir. In the test area, the horses were housed in sawdust-bedded group boxes during the winter and kept in a paddock during summer. Some ponies had been in the previous mentioned location for 16 years. These horses were kept in pastures at young ages, after being transferred to Khojir, they were only handled for other research experiments; therefore, they were unaccustomed to separation from their group. Experiments were carried out in the summer of 2008 at the Agricultural research station, Khojir.

### Sample collection

The feces were collected from an eight-year-old lion, which was kept at the Darabad Wildlife Museum, to the north of Tehran. Feces were collected from a donor lion from the area where it had deposited feces

on the ground. The samples were collected one week before the experiment and were kept in special laboratory bottles to prevent odor transfer. Feces were frozen at  $-18^{\circ}\text{C}$  and removed from the freezer to defrost 18 hours prior to testing. The roar of the lion was a 2 min sound recording from an unknown lion.

### Test environment

We used the modified method described by Christensen *et al.* (2007). All experiments were carried out in an outdoor rectangular arena (30 m length and 15 m width), which was full of weeds. Each horse was fastened by a 3 m length rope to a particular part of the fence. The arena was equipped with an upside-down bucket to seat the observer and a plate and three loudspeakers according to the treatment type (see below).

### Experiment 1: Control test

For the control test, the calmest horses ( $n=15$ ) were selected from 45 non-trained horses. The aim of this test was not only to habituate the horses to the observer and heart rate monitor equipments, but also to estimate the heart rate of horses in normal conditions (without the presence of olfactory and auditory stimuli). The control test lasted two days. Nine mares were tested on the first day and six stallions were tested on the second.

### Experiment 2: Olfactory test: Lion feces

In the second experiment, the horses ( $n=15$ ) were exposed to lion feces. Prior to the test, the horses were habituated to the seated observer and to being fastened by a rope in the test arena for the duration of the test. The experiment was performed on a single day and each test lasted for 5 min. Before taking the horses to the test arena, feces were placed on a metal plate under the fence where the horses were to be fastened. A new fecal sample was put on the plate after five horses had been tested to maintain the strength of the olfactory stimulus.

### Experiment 3: Auditory test: Lion roar

In the third experiment, horses were exposed to 2 min of continuous auditory stimulus of a lion roaring. The entire test duration for each horse was 5 minutes. Three loudspeakers and a laptop were placed outside the paddock behind a handmade hay wall to prevent them being seen by the horse.

Experiment 3 was carried out one day after the olfactory test (Test 2). The horses ( $n=13$ ) used in this experiment were the same horses used in Experiment 1 and 2, except for the three stallions excluded from the experiment due to a behavioral problem (depression).

After Experiment 2, we were very careful to remove odorous ground material (the feces plate and any possible feces that may have fallen on the ground) to prevent the odor from remaining during Experiment 3.

### Data quantification and analysis

All experiments lasted for 5 min. We recorded the behavioral reactions and heart rate during each test as follows:

Behavioral reactions (Table 1): The observer sat quietly opposite the horse, 6 m away, while recording the behavioral reactions of the horse on a check sheet. All horses had been habituated to the observer during the control test.

Heart rate (HR): The HR was recorded with Equine Polar s810i ([http://www.horsebeat.co.uk/horse\\_heart\\_rate\\_monitors\\_polar\\_equine\\_s810i.htm](http://www.horsebeat.co.uk/horse_heart_rate_monitors_polar_equine_s810i.htm)), which consisted of two electrodes, a transmitter and a wristwatch receiver. Water and a lubricant gel were used to achieve a better contact between the electrodes and the skin of the horse. Each horse was taken to the entrance of the stable where they were housed prior to testing wash the relevant part of its body, adding lubricant gel and fitting the electrodes and transmitter. The receiver stored data from the transmitter (each second). Subsequently, data were downloaded via a Polar Interface to a personal computer. Then data were analyzed (average HR during the 5 min test) using Equine Polar SW and SAS software.

After fitting the HR monitoring equipment to the horse, the horses' groom took the horse to the test paddock and fastened it by 3 meters rope to the fence. The test was started as soon as the groom left the paddock.

**Table 1:** Recorded behaviors in response to predator stimuli.

| Behavior                       | Description  |
|--------------------------------|--|
| Alertness to stimulus          | Horse is alert with elevated neck, head and ears oriented towards the stimulus, with or without chewing.   |
| Alertness to their stables     | Horse is alert with elevated neck, head and ears are oriented towards the stable, with or without chewing. |
| Focus remains on other stimuli | Horse remains focused on other stimuli, such as other external sounds or the observer.                     |
| Eating with low head and neck  | Represents that horse is relaxed.  |
| Walking                        | Walking relaxed or energetically   |
| Trotting or cantering          | Cantered around the paddock  |
| Flight reaction                | Typically followed by trotting/ galloping and alertness.   |
| Neighing                       | In response to auditory and olfactory stimuli  |
| Flehmen                        | Head and neck elevated, upper lip curled (olfactory investigation).  |
| Paw bout                       | Striking the ground with a forelimb  |
| Defecation                     | Elimination of feces   |
| Urination                      | Elimination of urine   |
| Back                           | Stepping backwards   |

Behavioral data and the mean average HR responses were analyzed for effect of stimuli on horses. Behavioral data was analyzed by descriptive analysis. The behavior of each horse was recorded in a table. As the total test time for each horse was 5 minutes, this table was divided into five sections, in which each section represented 1 min of the test. HR was analyzed by a one-way analysis of variance (ANOVA). The level of significance was set at  $p < 0.001$  throughout data analysis.

## Results

### Control test

The following behavioral reactions of horses were recorded:

- Alertness to being out of paddock
- Alertness to the stables
- Focus other stimuli
- Eating with low head and neck
- Eating with elevated neck
- Walking
- Neighing
- Paw bout

In the Control test the horses did not show the flight reaction, trotting or cantering, flehmen, defecation or urination.

### Olfactory test

The behavioral reactions of horses were recorded as follows:

- Alertness to out of paddock
- Alertness to the stables
- Focus other stimuli
- Eating with low head and neck
- Eating with elevated neck
- Walking
- Neighing
- Paw bout
- Defecation
- Urination
- Trotting/cantering
- Back

In the olfactory test horses didn't show a flight reaction.

### Auditory test

The behavioral reactions of horses were recorded as follows:

- Alertness to out of paddock
- Alertness to the stables
- Focus other stimuli
- Eating with low head and neck
- Eating with elevated neck
- Walking
- Neighing

- Paw bout
- Defecation
- Urination
- Trotting/cantering
- Back
- Flight reaction

Table 2: shows that the model was significant ( $F_{4,40}=7.52, p=0.0001$ ).

Analysis of the parameters that was entered into the model (Table 3) showed the significant effect of olfactory stimuli on HR data (beats per minute) ( $F_{2,40}=12.77, p=0.0001$ ).

To investigate the influence of stimuli on heart rate, the next analysis is performed (SNK test). The results (Table 4) show that the sound had a significant influence on HR changes (Group A).

Table 2: Analysis of variance of the model

| Source          | DF | Sum of squares | Mean square | F-value | Pr > F |
|-----------------|----|----------------|-------------|---------|--------|
| Model           | 4  | 3295.74        | 823.93      | 7.52    | 0.0001 |
| Error           | 40 | 4380.57        | 109.51      |         |        |
| Corrected total | 44 | 7676.31        |             |         |        |

Table 3: Analysis of variance of influence of parameters on heart rate (beats per minute).

| Source | DF | Type III SS | Mean square | F-value | Pr > F  |
|--------|----|-------------|-------------|---------|---------|
| Treat  | 2  | 2797.64     | 1398.82     | 12.77   | <0.0001 |
| Sex    | 1  | 252.47      | 252.47      | 2.31    | 0.1368  |
| Age    | 1  | 418.061119  | 418.06      | 0.82    | 0.0577  |

Table 4: Student Newman Koil "SNK" mean comparison test between treatments

| SNK Grouping | Mean   | N  | Stimulus  |
|--------------|--------|----|-----------|
| A            | 88.067 | 15 | Sound     |
| B            | 76.967 | 15 | Olfactory |
| B            | 70.000 | 15 | Blank     |

## Discussion

It was concluded that there was significant difference between the responses of horses in the control group and the two stimuli (olfactory and auditory) that were used in the experiments. Additionally, SNK mean comparison displayed that sound stimulus had a significantly larger effect than the control treatment and olfactory stimulus. Moreover, the horses did not express any novel behavioral responses to the olfactory stimulus; however, they showed both distinct behavioral and physiological reactions to the auditory stimulus.

Our results demonstrate that horses do not show certain behavioral reactions to a set of controlled condition with no predatory stimuli in a familiar environment (flight reaction – trotting/cantering – flehmen – defecation - urination). However, the introduction of lion feces caused the horses to show more behavioral reactions, which had not been seen in the control test (defecation – urination –

trotting/cantering - back). They also showed stronger levels of certain preceding reactions (eating with elevated neck – walking – neighing - paw bout) and a shorter duration of eating. In the same manner, several studies on deer (Sullivan *et al.*, 1985; Swihart *et al.*, 1991) and many studies on rodents (including Sullivan *et al.*, 1988; Nolte *et al.*, 1994; Rosell, 2001) have reported decreased feeding rates after the presentation of predator urine.

However, HR analyses showed no significant increase when encountered compared to olfactory stimuli. The results of the lion feces experiment corresponded with those of Christensen *et al.* (2007 and 2005). Christensen (2007) concluded that, in their first two experiments of urine from wolves and lions or blood from slaughtered conspecifics and fur-derived wolf odors, horses showed significant changes in their behavior but no increase in HR.

The introduction of an auditory stimuli, a lion's roar, caused remarkably increased HR responses and the horses showed more behavioral reactions than in the olfactory experiment (alertness to being out of paddock - alertness to the stables - focus other stimuli - eating with elevated neck – walking – neighing - paw bout – defecation – urination – trotting/cantering – back-flight reaction). Horses showed a flight reaction only in confrontation to auditory stimuli in our study.

Although the selection pressure for predator recognition has inevitably relaxed through domestication, the latter result support the hypothesis that innate responses towards predators may not be lost altogether (Christensen *et al.*, 2007). Moreover, studies on sheep showed that anti-predator strategies that evolved in wild sheep persist in domesticated animals, even in the absence of natural predators (Byers, 1997). Similarly, several studies suggest that domestic horses express the same movement and social behaviors as wild horses if provided with an appropriate physical and social environment in which to show their full behavioral repertoire (Christensen *et al.*, 2002; Waring, 2003; Boyd and Keiper, 2005; Feh, 2005). Dwyer (2004) argued that although the threshold for expression of some behaviors (e.g. fear responses) may be elevated in domestic animals, there is no evidence that these behaviors are not expressed once a threshold has been reached. It is currently unknown which predator odor causes more behavioral reactions in horses and this question will require further studies.

In conclusion, horses show considerable changes in their behavioral reactions when confronted with olfactory stimulus but no increase in HR. However, the behavioral changes of horses are more intense when presented with auditory stimuli.

## Acknowledgements

Many thanks go to numerous organizations that helped with this study: the Agricultural Research

Station, Khojir allowed us to use their horses; the manager and employees of the Darabad Wildlife Museum helped us to collect predator odors; the University of Applied Science and Technology gave us access to their laboratory instruments. Thanks go to Dr. Christensen, University of Aarhus, Prof. Cregier, University of Prince Edward Island, Prof. Friend, Texas A&M University, Prof. Beaver, Texas A&M University, Dr. McGreevy, University of Sydney, Dr. Waran, Unitec, New Zealand, Dr. Heleski, Michigan State University and Dr. Creighton, University of Chester, and to my dear family for their precious help and comments during the writing of this manuscript.

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