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Research

Validation of a fear test in sport horses using infrared thermography

Francesca Dai*, Nathalie Hélène Cogi, Eugenio Ugo Luigi Heinzl, Emanuela Dalla Costa, Elisabetta Canali, Michela Minero

Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano, Milano, Italy

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ABSTRACT

The aims of the present study were to assess feasibility and validity of a fear test in adult sport horses and to investigate whether the exposure to a fearful stimulus induces a change in eye temperature. Fifty horses, aged 14 ± 6 years, of different breed and gender, entered the study. For each horse, a caretaker was asked to fill in a validated temperament questionnaire. A novel object fear test (NOT), has been selected from literature to examine fearfulness. Temperature of the lacrimal caruncle was measured before the test and after the test on 22 horses, representative of the whole sample. To assess discriminant validity of the NOT, 3 human-animal relationship tests were performed on the same horses. Data were analyzed with descriptive, nonparametric, and multivariate statistic methods. No significant differences were found between females and geldings for any of the measured variables. Horses that were described by caretakers as more prone to panic, vigilant, excitable, skittish, and nervous ($P < 0.001$) needed significantly longer time to reapproach the novel object ($P < 0.01$). Eye temperature was significantly higher after the NOT compared to basal ($P < 0.01$), with subjects who did not reapproach the novel object tending to present larger increases ($P < 0.10$). Horses showing more fear-related responses to the NOT did not show more negative reactions to humans during the human-animal tests. These results suggest that, to some extent, the NOT predicts horses' behavior in real on-farm situations. Our findings reject the hypothesis that reactivity to humans and general fearfulness belong to the same basic feature of temperament. Importantly, infrared thermography proved to be useful in assessing physiological reactions of fear in horses.

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Introduction

Fear in domestic animals has been defined by Boissy (1998) as a reaction of the perception of actual danger. Fear responses are characterized by behavioral and physiological modifications (Forkman et al., 2007): active defense (attack, menace), active flight (hiding, escape), and passive avoidance (freezing) are some of the behaviors that are frequently related to an underlying emotion of fear in animals (Erhard and Mendl, 1999). When experiencing fear, cardiovascular changes occur in different parts of the body with the ultimate effect of increasing perfusion pressure and redirecting blood flow to the central nervous system and skeletal musculature. Sport horses may be subject to different fearful events, for example, being transported and competing in different environments with novel stimuli and sounds (McGreevy and McLean, 2010), being approached by unfamiliar people, or undergoing many handling and management procedures. Horses, as prey animals, have a tendency to escape from frightening stimuli and may show flight reactions which can be dangerous for both the horse and the man (Christensen et al., 2008, 2005; McGreevy and McLean, 2010). Keeling (1999) demonstrated that in equitation sports many serious human injuries occur as a result of unexpected horse fear reactions. Because owners often misunderstand the reason for the development of such behaviors in their horses, attempts at correcting them often involve suppression- or punishment-based approaches (Hothersall and Casey, 2012). Although repeated subjugation of undesirable fear responses may ultimately appear to solve the overt behavioral reaction, this method can cause short- or long-term stress (McGreevy and McLean, 2010) and can worsen the problem or lead to the development of alternative avoidance strategies such as abnormal behaviors (Hothersall and Casey, 2012). Besides possible problems caused by inappropriate human reactions to fear

* Address for reprint requests and correspondence: Francesca Dai, Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano, Via Celoria 10, Milano 20133, Italy, Tel: +39 0250318033; fax: +39 0250318030.
E-mail address: francesca.dai@unimi.it (F. Dai).

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displays, a long-term negative emotional state related to fear can per se cause chronic stress and reduced welfare (Dantzer and Mormede, 1983; Désiré et al., 2006; Minch et al., 2008; Willner et al., 1992).

Because of the aforementioned reasons, it is blatantly obvious that fear in horses plays an important role in their welfare, and thus, it is important that it is recognized and assessed accordingly. Various fear tests have been used to determine temperament characteristics in horses, such as novel object (e.g., Anderson et al., 1999; Christensen et al., 2008, 2005; Seaman et al., 2002; Visser et al., 2003b, 2002; Wolff et al., 1997), novel arena (e.g., Le Scolan et al., 1997; Seaman et al., 2002; Wolff et al., 1997), and restraint and human fear tests (e.g., Le Scolan et al., 1997; Visser et al., 2003b, 2001; Wolff et al., 1997). The novel object test (NOT) is an experimental situation in which the animal is exposed to an unknown stimulus to provoke a fear reaction. Although it is not possible to attribute a given measure to any single emotion, time to approach the new stimulus appears to be one of the most appropriate indicators of fearfulness (Górecka-Bruzda et al., 2011; Wolff et al., 1997). Feasibility under field conditions and ease and duration of fear tests are important characteristics for them to be applied as well as reliability and validity (Górecka-Bruzda et al., 2011). Validity means the degree to which a test measures what it purports to measure (Martin and Bateson, 1993; Weiblinger et al., 2006). Predictive validity measures the ability of an indicator to predict some later criterion (Cronbach and Meehl, 1955). To assess predictive validity of fear tests, different studies investigated their correlation with surveys via questionnaires which aimed to detect those characteristics of temperament in horses that influence their habitual behavior (e.g., Anderson et al., 1999; Le Scolan et al., 1997; Momozawa et al., 2007, 2003; Morris et al., 2002a, 2002b). Respondents were generally caretakers or riding teachers who were familiar with horses; thus, their responses were based on long-term observation and were not influenced by a temporary change in equine behavior, which may occur in behavioral tests (Momozawa et al., 2005).

Discriminant validity analyzes the divergence between measures of conceptually unrelated concepts, for instance, fear and human-animal relationship, and has seldom been evaluated for fear tests (Górecka-Bruzda et al., 2011; Visser et al., 2003b). Convergent validity regards the relationships between independent measures of the same conceptually related construct (Weiblinger et al., 2006). Assessment of convergent validity of fear tests usually considers whether their outcome is related to physiological changes due to fear. Some of the most frequently used physiological indicators are heart rate (e.g., Christensen et al., 2008; Momozawa et al., 2003), heart rate variability (e.g., Rietmann et al., 2004; Stewart et al., 2008c; Visser et al., 2002; von Borell et al., 2007), cortisol concentration (e.g., Anderson et al., 1999; Cook et al., 2001; Flauger et al., 2010; Stewart et al., 2008a), and infrared thermography (IRT). Infrared thermography can be used to detect changes in peripheral blood flow (which causes changes in body heat) as a response to fear-induced stress. Studies in different animal species have revealed that after a stressing event the small areas around the posterior border of the eyelid and the caruncula lacrimalis change temperature. This area has rich capillary beds innervated by the sympathetic system (e.g., McGreevy et al., 2012; Stewart et al., 2009, 2007) and thus represents an ideal place for measuring local changes in blood flow resulting from tuning of the autonomic nervous system. Stewart et al. (2007) measured an increase in eye temperature in cows after intramuscular injection of adrenocorticotropic hormone, corticotropin-releasing hormone, and epinephrine. Research carried out on different species correlated increased eye temperature with cortisol concentrations in response to pain (Stewart et al., 2008b, 2008c), stress (Ludwig et al., 2007; Stewart et al., 2007; Valera et al., 2012), and fear (Stewart et al., 2008a). In a study on horses undergoing stressful situations, Valera et al. (2012) found that the eye temperature increased as a consequence of stress. Similar results were found by Hall et al. (2010) who found a higher eye temperature in horses lunged with the Pessoa training aid (held responsible for increasing the stress during training) than horses without. Bartolomé et al. (2013) were able to demonstrate a correlation between an increase in heart rate and eye temperature after jumping competitions. Cook et al. (2001) investigated the underlying causes of increase in eye temperature in horses and found that it was correlated to activation of the hypothalamic–pituitary–adrenal axis.

To our knowledge, changes in superficial temperature during fear exposure have never been studied in horses. This study aims to assess the feasibility and predictive, convergent, and discriminant validity of a fear test in adult sport horses and investigates whether the exposure to a fearful stimulus induces a thermographic change in eye temperature.

Methods

This study was conducted in agreement with ISAE ethical guidelines (ISAE Ethics Committee, 2002) on adult nonpregnant horses, and no animals underwent more than the minimal distress. In addition, if horses displayed any hyper-reactive behavior that could compromise the horse or the assessor's safety, the test was immediately ended and the observer left the box (this was recorded as a result).

Animals

Experiments took place from January to May 2013 at 6 different riding centers in Northern Italy. A total of 50 adult riding horses (mean age, 14 $\pm$ 6 years) of different sex (30 geldings, 16 mares, 4 stallions) were used in the study. Horse breeds were variously distributed and comprised warmblood horses, draft horses, and thoroughbreds. All horses were stabled in single boxes with daily access to group paddocks for 1-10 hours. Straw bedding was used in 2 centers, whereas horses were kept on wood shavings in the remaining 3 centers. Horses were fed 3 times a day with hay and concentrated industrial feed depending on the type of activity they carried out. Water was provided ad libitum.

Questionnaire survey

Six caretakers (1 per riding center) completed the questionnaires for the 50 tested horses; the number of questionnaires filled in per caretaker varied from 6 to 10. The questionnaire was developed and validated by Momozawa et al. (2005) and contained 20 questions regarding horse temperament (Table 1). The responses were ranked on a scale from 1 to 9, with 1 being the lowest rank for each item. Two animal welfare experts translated the questionnaire into Italian; the mother tongue of both translators was Italian and their level of English was advanced. In a second round, the authors discussed and refined some of the items, which they felt might be difficult to interpret.

Behavioral tests

Four behavior tests were chosen and are described in sections from "Fear test (NOT)" to "Forced human approach test." All tests were conducted on the same day and in the same housing conditions. Horses were tested at least 1 hour before work and between meals to avoid possible distractions and confounding food motivation. A map of the facility was drawn before testing the horses to facilitate the randomization of the testing order. To avoid

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