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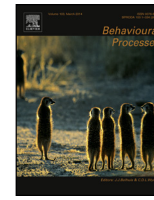

# Influence of emotional balance during a learning and recall test in horses (*Equus caballus*)

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ABSTRACT

Modern day horse–human relationships entail different types of sport and riding activities, which all require learning. In evaluating the interaction between learning and emotions, studying normal coping strategies or adaptive responses to the surroundings is critical. 34 horses were involved in a cognitive test, in the absence of physical effort, to analyze performance, as well as physiological and behavioral responses related to learning, memorization and recall, associated to the capacity to reverse a learned model. Synthetic Equine Appeasing Pheromone (EAP) was used in 17 horses in order to modulate their emotional state and evaluate differences in cognitive–emotional response during cognitive effort in comparison to the control group (placebo group). Both groups showed statistically significant changes in heart rate during the test, indicating emotional and physio-cognitive activation. The EAP group produced fewer errors and made more correct choices, showing behaviors related to increased attention, with less influence from environmental stimuli. The capacity to learn to learn, as shown in the bibliography, allows animals to establish conceptual learning, when a normal or positive emotional state (in this case modulated by semiochemicals) is used to control limbic system activation and, consequently, decrease stressful/fearful reactions, resulting in better learning capacities during the cognitive test.



## 1. Introduction

The domestication of horses by humans almost certainly occurred before 4000 B.C.E. (Ekesbo, 2011) and has represented one of the most important steps in the development of human societies. Horses were first considered as a food source, then as a working machine and a means of carriage and transport, and finally became an important military weapon (as late as the World War I; Hintz, 1995). As a key factor in improving human society through their great versatility, horses' relationship to humans slowly changed, as did their dependencies and needs in human bonding (Cooper and Albentosa, 2005).

Blake (1977) described the horse as one of the most perceptive species, regarding their remarkable ability to modulate stimuli from the environment, transforming them into a rapid fear response and related behavioral reactions (flight/fight/freeze; McLean and McGreevy, 2004). Horses have developed a phylogenetic predisposition for sensorial processing that, combined with a functional accommodation of the individual experience, leads to learning modulation (McGreevy and McLean, 2010). For example, basic learning processes include habituation, when an animal decreases or stops producing reactions to an inconsequential stimulus, after repeated exposure at similar intensity to that stimulus (Hanggi, 2005). The capacity to establish this primary filter would allow animals to better manage their environment and modulate reactions. In addition, horses would recognize subtle changes to the environment, related to visual clues (Murphy and Arkins, 2007) and easily correlate their reactions (above all fear-related ones) to the place where they were observed for the first time (McGreevy and McLean, 2010). For this reason, teaching and training horses in association with specific contexts could give trainers various types of information about their environmental perception ("place-dependent" learning; Jorgensen et al., 2011). These tools are crucial in a prey species and, in regards to domestication and current lifestyle routines with humans, constitute an uninterrupted adaptation to the environment provided by owners (Price, 1999). Learning quality and capacity are readily involved in survival and organization within the setting, and

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are strongly linked to environmental conditions (McGreevy and McLean, 2010).

Nowadays in equitation, different sporting and show activities put increasing demands on animals to be faster, more sophisticated and to demonstrate even greater cognitive and memorization capacities (Murphy and Arkins, 2007; McGreevy and McLean, 2010). Neurobiological and behavioral factors influence different aspects of learning; for example, a horse's athletic capacity and coping strategies are deeply involved in reaching goals and overcoming obstacles (Visser et al., 2003). Today, all these topics are analyzed from different points of view: learning, training, learned versus innate behavior, stereotypes and compulsive behaviors, breeding and welfare (Houpt and Rudman, 2002).

Behavioral repertoire, biological, and emotional states are three fundamental elements in analyzing the horse's capacity to demonstrate coping strategies and stress-related responses in captivity (Fraser et al., 1997). All these variables could have an impact on the subjective level of stress perceived by the animal, related to changes in its emotional state, while activating pro-active or reactive mechanisms in response to fear-inducing stimuli (Von Borell et al., 2007), and producing general negative emotional effort. Fear, for example, could be considered one of the typical emotional reactions, very common in horses, in response to any unknown or known stimulus which the animal wants to end, to be removed, or to avoid (Gray, 1987). Both active (better considered as proactive) and passive (or reactive) mechanisms in fear-related responses entail physiological changes such as alterations in heart rate and blood pressure (Malik, 1996; Physick-Sheard et al., 2000; McGreevy and McLean, 2010). Nowadays, heart rate variability (HRV) provides a much more accurate, non-invasive and detailed indicator of autonomic nervous system activity in response to psycho-physiological stress (Malik, 1996; Physick-Sheard et al., 2000; Visser et al., 2002; Rietmann et al., 2004; Von Borell et al., 2007). Hyperthermia and dehydration (Friend et al., 1998; Stull, 1999; Stull and Rodiek, 2000; Desjardins and Cadore, 2004; McGreevy, 2004; Oikawa et al., 2005), hematological parameters such as leukocytosis (McClure et al., 2005; Raidal et al., 2006), neutrophilia (Oikawa et al., 2005; Raidal et al., 2006), lymphopenia (Stull and Rodiek, 2000; Stull et al., 2004) and immunodepression (McClure et al., 2005; Raidal et al., 2006) may also be considered. Emotional-related behavioral evaluation often measures avoidance, with active (menace, aggression) or passive (freezing, displacement behaviors) responses (Christensen et al., 2002; Heitor et al., 2006; Christensen et al., 2008, 2011), scanning the surroundings with vigilance (Potter and Yeates, 1990), vocalization (Boissy, 1995), pawing (McDonnell and Poulin, 2002) and showing repeated, compulsive behaviors (Houpt, 1981; Luescher et al., 1998; Redbo et al., 1998; Cooper and McGreevy, 2002). Behaviors related to investigation and environmental exploration are also important parameters in evaluating the horse's emotional state (Araba and Crowell-Davis, 1994; Christensen et al., 2008).

Regarding emotional activation in horses, Equine Appeasing Pheromones (EAP) have been shown to modulate horses' emotional reactions (Mills and Nankervis, 2001; Falewee et al., 2006; Cozzi et al., 2011) in different situations, related to fear-eliciting stimulation, mental effort and behavioral responses (above all attempts to flee/flight). Pheromones may be considered as a subclass of semiochemicals, used in intraspecific communication (Wyat, 2003). In regards to their use in prevention (stress-related behavioral pathologies), social behavior (Van Dierendonck et al., 2004; Van Dierendonck and Goodwin, 2005) and practice (equine management in box, during transport, etc.; Falewee et al., 2006), the number of studies related to the use of semiochemicals in horses has greatly increased (Falewee et al., 2006; Van Sommeren and Van Dierendonck, 2010, Cozzi et al., 2011). The utilization of appeasing semiochemicals is generally considered a good tool for reducing stress-related responses in cats (Cozzi et al., 2010), companion dogs (Mills et al., 2003; Graham et al., 2007; Taylor and Mills, 2007; Denenberg and Landsberg, 2008; Gaultier et al., 2008, 2009), sheltered dogs (Osella et al., 2005; Tod et al., 2005; Barlow and Goodwin, 2009), pigs (McGlone and Anderson, 2002; Yonezawa et al., 2009), and chickens (Madec et al., 2006, 2008).

In this exploratory study regarding possible modifications in the emotional balance during a cognitive test and the effect of semiochemicals, we examined 34 horses, looking for cognitive–emotional interactions in response to mental effort (during a learning exercise, its recall, and reversal of the memorized model). In addition, we examined the possible effect of a specific appeasing semiochemical in modulating this emotional response, during a cognitive effort situation, in the absence of reliable physical activity.

In both instances, performance, physiological, and behavioral parameters were evaluated.

## 2. Materials and methods

### 2.1. Subjects

34 adult horses (*Equus caballus*; age 3–25 years, 14 mares and 20 geldings) were involved in the experiment. The horses were new to research studies, under regular veterinary monitoring and did not suffer from any observable hearing or vision problems. All the animals were located in the "La Catherine" equestrian center, near Isle sur la Sorgue (France). They normally participated in training for riding activities at the center with the owners, during their typical daily routine (see Fig. 1). The horses involved in the test varied in their housing arrangement (box and/or paddock) but all received the same management (same handlers, same structures,

| name | age | sex | size | activity |
|---|---|---|---|---|
| Brutus | 21 | Gelding | Horse | riding |
| Figlia | 17 | Female | Horse | riding |
| Micmac | 10 | Gelding | Horse | riding |
| Noisette | 9 | Female | Horse | riding |
| Poésie | 7 | Female | Pony | riding |
| Jiff | 13 | Gelding | Horse | riding |
| Makila | 10 | Female | Pony | riding |
| Juriste | 13 | Gelding | Pony | riding |
| Cardinal | 7 | Gelding | Horse | riding |
| Erevane | 18 | Female | Pony | riding |
| Option | 8 | Female | Horse | riding |
| Ruben | 7 | Gelding | Horse | riding |
| Made | 10 | Female | Horse | riding |
| Nectar | 9 | Gelding | Horse | riding |
| Samouraï | 5 | Gelding | Pony | riding |
| Pistache | 7 | Female | Horse | riding |
| Harmonie | 15 | Female | Horse | riding |
| Oscar | 17 | Gelding | Pony | riding |
| Dakota | 19 | Gelding | Pony | riding |
| Carolin | 20 | Gelding | Pony | riding |
| Quassia | 6 | Female | Pony | riding |
| Belle | 25 | Female | Pony | riding |
| Jaffar | 13 | Gelding | Horse | riding |
| Oliver | 8 | Gelding | Horse | riding |
| Rosi | 5 | Female | Horse | riding |
| Pepper | 7 | Gelding | Horse | riding |
| Colomba | 20 | Female | Pony | riding |
| Drink | 19 | Gelding | Pony | riding |
| Haddock | 15 | Gelding | Pony | riding |
| Gentil | 16 | Gelding | Pony | riding |
| Graffiti | 16 | Gelding | Miniature | riding |
| Niki | 20 | Female | Pony | riding |
| Ultral | 3 | Gelding | Pony | riding |
| Kalin | 12 | Gelding | Horse | riding |

**Fig. 1.** Composition of the experimental groups with age, size and activity.