Journal of Veterinary Behavior 10 (2015) 556–560

Contents lists available at ScienceDirect

Journal of Veterinary Behavior

journal homepage: www.journalvetbehavior.com

Non-canine Research

Gene expression profile of cytokines in leukocytes from stereotypic horses

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ARTICLE INFO

Article history:
Received 4 May 2015
Received in revised form
16 July 2015
Accepted 26 August 2015
Available online 4 September 2015

Keywords:
horse
stereotypic behaviors
cytokine gene expression
immune system

ABSTRACT

In animals, recent studies have shown a dynamic interaction between neuroendocrine-immune systems and consequent behaviors, resulting in adaptive responses to different stimuli. Stereotypic oral and locomotor behaviors are common among intensively managed horses; however, little is known about how or whether immune responses affect such abnormal repetitive behaviors. Cytokines can be classified as proinflammatory or anti-inflammatory and they can affect brain development, neurogenesis, and synaptic plasticity. In humans and rodents, there is some evidence that cytokines can impair behavior. Our study compared leukocyte gene expression of cytokines of horses with stereotypic behavior with those exhibiting normal behavior. Blood samples were collected from 22 horses: 8 non-stereotypic controls (group C), 8 cribbers (group CR), and 6 weavers (group W). Leukocyte counts were obtained using an automated cell counter. Expression of some proinflammatory [interleukin (IL)-1, IL-2, IL-6, tumor necrosis factor (TNF)-α] and anti-inflammatory (IL-4 and IL-10) cytokines was determined by quantitative polymerase chain reaction. No differences in body temperature, heart rate, respiratory rate, total proteins in serum, or leukocyte counts were found. A significant effect of group was found for IL-4, IL-10 messenger RNA (mRNA), and TNF-α mRNA. Group CR and group W showed a similar profile of cytokines expression. The results of this study show that there are differences in cytokine mRNA expression between non-stereotypic and stereotypic horses which could be related to a different activation of the immune system.

Introduction

Abnormal repetitive behavior, or stereotypic behavior, is considered to be an indicator of poor welfare (Mason et al., 2006) and has been suggested to be a way for animals to cope with an unfavorable, stress-inducing environment (Cooper and Mason, 1998). There is some evidence that links the development of stereotypical behavior with a dysregulation of the basal ganglia, which is caused by a complex interaction of genetic predisposition and significant acute or chronic stress (McBride and Parker, 2015). In equines, the development of stereotypic behavior could be a result of a situation that induces chronic stress, such as weaning at an early age (McBride and Hemmings, 2009).

Many studies have investigated ethological and physiological characteristics of equine stereotypic behavior (Clegg et al., 2008). Stereotypic patterns of behavior are particularly associated with stabling, occurring in 10 to 40% of stabled horses (Nicol, 1999). Cribbing, also known as crib-biting, is an acquired stereotypic oral behavior in which a horse grasps a horizontal solid object with its upper incisors, contracts its ventral neck muscles, retracts the larynx, and pulls backwards (Monin, 1982). Commonly cited causes of cribbing are boredom, frustration, spasmodic colic, and pain (Fraufelder, 1981; McGreevy et al., 1995; McBride and Hemmings, 2009). Weaving is described as an obvious lateral swaying movement of the head, neck, forequarters, and sometimes the hindquarters (Mils and Davenport, 2002). The occurrence of weaving is probably associated with a horse's confinement to the stable and its frustration from failed attempts of reinstating social contact (Nicol,

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http://dx.doi.org/10.1016/j.jveb.2015.08.007
1558-7878/

1999). Pathologic repetitive behavior is mediated by a complex circuitry that involves a very large number of genes (Lewis and Kim, 2009). Mutations of one or a few of these genes could result in significant disruptions of this circuitry and a full expression of the behavioral phenotype (Welch et al., 2007). More specifically, the appearance of stereotypic behavior seems to be intimately related to the dysregulation of dopamine pathways (Lewis and Bodfish, 1998; Cooper and McGreevy, 2002; McBride and Hemmings, 2009). In horses, however, the dopamine receptor gene and some other known stereotypic genes are not major risk factors for cribbiting (Hemmann et al., 2014). Previous studies have shown that a lack of serotonin-mediated inhibition of central nervous dopaminergic pathways may be involved in the performance of stereotypic behavior patterns (Lebelt et al., 1998). The marked elevation of β-endorphin levels and the trend for lower serotonin levels in cribbing horses suggests a dysregulation between their opioid and serotonin systems (Lebelt et al., 1998). In contrast, Pell and McGreevy (1999) did not observe significant differences in plasma β-endorphin levels of stereotypic and control horses. It should, also, be noted that in human patients with chronic neuropathic pain, β-endorphin, and substance P levels were comparable to controls (Bäckryd et al., 2014). These contrasting results suggest that caution must be used when assessing the relationship between neurotransmitters and stereotypic behavior as their levels may be affected by different factors, such as when and how they are measured.

Social isolation and feeding of concentrates are important management factors related to the incidence of stereotypies (Nicol, 1999) and appear to have effects on serotonin levels in horses (Alberghina et al., 2010; 2015). Horses kept during the day in individual boxes had lower plasma serotonin levels than pasture-raised horses (Alberghina et al., 2015). Lower levels of plasma serotonin have also been reported for horses fed with high-concentrate levels when compared with those feed with low-concentrate levels (Alberghina et al., 2010). Furthermore, approximately 11% of the group of horses with low serotonin fed on high-concentrate diets cited in the previous study developed cribbing behavior after 4 weeks on the diet (Ellis et al., 2010). It has also been reported that activation of cytokines is linked to a decrease of serotonin because of an increased depletion of its precursor tryptophan (Dunn et al., 1999). Tryptophan depletion was shown to worsen repetitive motor behaviors in autistic adults (McDougle et al., 1996).

The behavioral responses to psychosensorial stimuli and immune responses to antigenic stimuli can be viewed as 2 subsystems of a larger, integrated, and complex system aimed to provide optimal conditions for the host's survival and adaptation. For instance, immune responses and inflammation are an ancestral, overlapping set of responses aimed at the neutralization of stimuli perturbing homeostasis (Franceschi et al., 2007). Neuroendocrine and immunologic mediators regulate the signaling and feedback networks of the integrated system. Data from both human and animal studies show that connections between the neuroendocrine and immune systems allow for a finely tuned regulatory system that is crucial in maintaining an individual's health (Padgett and Glaser, 2003). As such, it is possible that certain neuroendocrine and immunologic mediators play key roles in the onset of stereotypic behavior when animals are placed in captivity.

The complex interaction between the immune system and the stress or inflammation complex has mainly developed in the phylogenetic evolution of vertebrates through the development of a diversified system of cytokines and chemokines. Proinflammatory cytokines can stimulate the hypothalamic-pituitary-adrenal (HPA) axis to release the stress hormone glucocorticoids (Silverman et al., 2005). Neuroinflammation associated with the cytokine-chemokine network may also play an important role in the pathogenesis of neuropathic pain (Kiguchi et al., 2012; Bäckryd, 2015), and blood cytokines are candidates as biomarkers in pain medicine (Bäckryd, 2015). Elevated levels of certain cytokines, such as IL-1, IL-6, and TNF-α, contribute to some aspects of abnormal behaviors and atypical symptomatology in animals. For instance, elevated cytokine levels have been associated with increased levels of sleep and muscle fatigue, and reduced feeding and social and reproductive activities (Hart, 1988; Anisman et al., 2002; Dantzer et al., 2004; Dantzer, 2009). Cytokine dysregulation may also have important biological effects on activity that adversely affect behavior (Ashwood et al., 2012).

Given the role that cytokines play in mediating certain behaviors, it is quite plausible to find differences in the cytokine gene expression between stereotypic and non-stereotypic horses. The study of cytokine gene expression in leukocytes has been proposed as a useful peripheral model to study the activity of immune cells in the central nervous system (Sullivan et al., 2006). The aim of this study was to investigate leukocyte gene expression of proinflammatory cytokines (IL-1, IL-2, IL-6, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) in stereotypic and non-stereotypic horses.

Materials and methods

Subjects and management

Twenty-two adult Throughtbreds were used in this study. Animals were divided into 3 groups: group C were controls (5 mares, 3 geldings with an average age of 12 years) with no history of stereotypic behavior, group CR consisted of cribbers (6 mares, 2 geldings with an average age of 11 years), and group W consisted of weavers (4 mares, 2 geldings with an average age of 9 years). Before the start of this study, cribbers and weavers were reported by their owners to have shown stereotypical behavior for at least 6 months. The stereotypical behavior of each horse in the CR and W groups was confirmed using scan sampling. Horses were observed for 10 minutes at 60-minute intervals between 08.30 AM and 04.50 PM for a period of 2 days before and after blood sampling. The subjects were singly housed in conventional horse boxes at 2 different riding stables in East Sicily. In 1 stable, there were 4 controls, 5 cribbers, and 3 weavers, and in the second stable, there were 4 controls, 3 cribbers, and 3 weavers. Each horse received an estimated 1 kg/day of oats and 10 kg/day of hay mixture, and drinking water was available ad libitum. For each animal, body temperature was based on rectal temperature. Rectal temperature was monitored using a digital thermometer (HI92704, Hanna Instruments, Bedfordshire, UK) that was inserted 15 cm into the rectum. Heart rate was measured using a heart rate monitor (Horse Polar Trainer S610) and an oscillometric apparatus (Argus TM-7, Schiller, Barr, Switzerland). Blood samples were collected between 8 and 9 AM. All samples were collected in accordance with the standards recommended by the European Union Directive 2010/63/EU for animal experiments and informed consent from owners.

Methods

Blood was collected from each horse, via jugular venipuncture, into 2 tubes where 1 tube had no additive and the other contained ethylenediamine tetra-acetic acid (EDTA). The serum samples obtained after centrifugation were used for obtaining total protein concentration, which was measured by an automated spectrophotometer (SEAC, Slim, 50144 Florence, Italy) using the Biuret method with commercially available reagents (Biosystems S.A., 08030