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Nociceptive trigeminal reflexes in non-sedated horses

K.O. Veres-Nyéki [a,*], M. Leandri [b], C. Spadavecchia [a]

[a] Department of Clinical Veterinary Medicine, Anaesthesiology Division, Vetsuisse-Faculty, University of Berne, Länggassstrasse, Berne CH-3012, Switzerland
[b] Interuniversity Centre for Pain Neurophysiology, University of Genova, Via Dodecaneso 35, 16132 Genova, Italy

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ABSTRACT

Electrically induced reflexes can be used to investigate the physiology and pathophysiology of the trigeminal system in humans. Similarly, the assessment of the trigemino-cervical (TCR) and blink reflexes (BR) may provide a new diagnostic tool in horses. The aim of this study was to evoke nociceptive trigeminal reflexes and describe the electrophysiological characteristics in non-sedated horses.

The infraorbital (ION) and supraorbital nerves (SON) were stimulated transcutaneously in 10 adult Warmblood horses in separate sessions using train-of-five electrical pulses. The current was increased gradually until the TCR threshold was found. The stimulus–response curve of the TCR was evaluated. At the same time as TCR, the BR response was also assessed. Surface electromyographic (EMG) responses were recorded from the orbicularis oculi, splenius and cleidomastoideus muscles. Latency, duration, amplitude of the reflexes and behavioural responses were analysed.

Noxious electrical stimulation of the ION or SON evoked reflex EMG responses, with similar features regardless of the nerve that had been stimulated. Stimulations of increasing intensity elicited reflexes of increasing amplitude and decreasing latency, accompanied by stronger behavioural reactions, therefore confirming the nociceptive nature of the TCR. These findings provide a reference for the assessment of dysfunction of the equine trigeminal system.

Introduction

Recently, there has been a growing interest in idiopathic headshaking in horses. The symptoms of the disease, including nasal rubbing, snorting and sudden flipping of the head, may deteriorate during exercise and make the horse unrideable. Many affected animals are euthanased on humanitarian grounds. Although the pathophysiology behind the clinical signs is not known (Newton, 2005) neuropathic pain originating from the trigeminal nerve could be responsible for the symptoms observed (Newton et al., 2000; Roberts et al., 2009). Further, success in the treatment of headshaking in some horses has been obtained using a therapeutic regimen that is commonly used to treat trigeminal neuralgia in humans (Newton et al., 2000).

To investigate the physiology and pathophysiology of the trigeminal system in humans, electrically induced reflexes can be used. Percutaneous electrostimulation of the trigeminal afferents, the supraorbital (SON), infraorbital (ION) and mental nerve (MN) evokes activity of the muscles innervated by the efferent neurons in the reflex pathway. Evaluating the electrophysiological characteristics of the reflexes may help to localise a lesion within the reflex pathway (Cruccu and Deuschl, 2000; Aramideh and Ongerboer de Visser, 2002; Valls-Sole, 2005).

For diagnostic and research purposes, the blink reflex (BR) after stimulation of the SON is probably the most commonly evoked trigeminal reflex. In both humans and horses, three components, generated by different pathways, have been described, with the late components being commonly interpreted as nociceptive in origin. In addition to the BR, the trigemino-cervical reflex (TCR) has been investigated and described in humans (Sartucci et al., 1986; Ertekin et al., 1996, 2001; Leandri et al., 2001; Milanov et al., 2001; Serrao et al., 2005). By stimulating the SON, late EMG activity in the neck muscles is induced when the stimulus becomes painful, which was suggestive of a nociceptive nature of the TCR. Furthermore, increasing stimulation intensities corresponded to enlarged reflex size and stronger pain sensation (Serrao et al., 2003). Consequently, both the late component of the BR and the TCR appear to have a primary nociceptive physiological function that might enable them to be used for the investigation of neuropathic pain syndromes (Ertekin et al., 1996).

To the best of our knowledge, TCR has not yet been described in horses and BR has only been described in sedated horses, although assessment of the related electrophysiological parameters may provide a diagnostic tool for diseases affecting the trigeminal system in non-sedated horses. The aims of this study were as follows: (1) to assess if noxious transcutaneous electrical stimulation of the ION or

* Corresponding author. Tel.: +41 31 631 2111.
E-mail address: kata.veres@vetsuisse.unibe.ch (K.O. Veres-Nyéki).

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SON would be suitable to evoke TCR in horses as in humans; (2) to describe its electrophysiological characteristics and stimulus–response function in non-sedated horses, and (3) to evaluate the BR while eliciting the TCR.

Material and methods

Animals

Ten adult Warmblood horses (6 geldings, 4 mares; 7 Swiss Warmblood, 2 Freiberger and 1 Hanoverian) were included in the study. The horses, aged 14–23 years and weighing 540–640 kg, were judged to be clinically healthy with no known neurological disorders. Experiments were performed with the permission of the Bernese Committee for Animal Experimentation, Switzerland (Tierversuche/Bewilligung Nr. 92/08).

Definitions of experimental objectives

The BR is the response of orbicularis oculi muscles to the stimulation of the cutaneous area innervated by the trigeminal nerve. Its afferent pathway is formed by the sensory branches of the trigeminal nerve and the efferent arch is provided by the facial nerve motor fibres. According to Anor et al. (1996) the BR in horses consists of three components, called R1, R2 and R3.

The TCR is the response of the neck muscles to the stimulation of the cutaneous area innervated by the trigeminal nerve. Its afferent pathway is formed by the sensory branches of the trigeminal nerve and the efferent arch is provided by cervical nerve motor fibres. In human studies, the reflex induced by superficial noxious electrical stimulation occurs quite late after stimulation onset, in the mean range of 40–50 ms (C3) (Ertekin et al., 1996, 2001; Serrao et al., 2003), whereas the earlier components (C1, C2) are usually elicited only by mechanical, percutaneous or non-noxious electrical stimulation (Di Lazzaro et al., 1996; Ertekin et al., 2001; Leandri et al., 2001).

Instrumentation

Experiments were performed between two feeding times and after daily exercise to reduce the stress for the animals and in order to standardise the procedure. A venous catheter was placed into the left jugular vein to allow prompt sedation if necessary since the horses were restrained in stocks for stimulation and recording. The same investigators (CS and KV) performed the electro-physiological recordings and scored the behavioural reactions.

Scoring the behavioural reactions

One observer (KV) judged the behavioural reaction to stimulation using a numerical rating scale (NRS) (Table 1) and a visual analogue scale (VAS). The VAS was consisting of a 100 mm line, where no reaction corresponded to the zero on the left end and worst possible reaction on the right end of the line. Measuring the distance in millimetres of the assigned score on the scale from its left end gave a measure of the intensity of the reaction observed.

Stimulating technique

In order to evoke the TCR and the BR, either the SON or the ION was transcutaneously stimulated using two pairs of self-adhesive electrodes (Ambu 700 05-J). The electrode sites above the left supraorbital and infraorbital foramens were shaved and cleaned with alcohol (Softasept N, B. Braun Medical). The stimulating electrodes were applied with the cathode over the respective foramen and the anode 20 mm dorsally.

Recording technique

In order to simplify the experiments as far as possible, and to provide the minimum discomfort for the horses, both BR and TCR were only recorded ipsilateral to the stimulus. To record the BR from the orbicularis oculi muscle, one pair of self adhesive electrodes was placed on the shaved and degreased skin of the lateral canthus of the left eye over the orbicularis oculi muscle (OO).

Table 1
Numerical rating scale (NRS) to evaluate behavioural reaction to stimulation.

Score	Observed behaviour
0	No reaction
1	Blinking, but no other reaction
2	Blinking and mild retraction of the head
3	Blinking and powerful retraction of the head
4	Sudden violent reaction of the whole body to the stimulus
5	Unmanageable reaction of the whole body

To record the TCR, two 7.5 cm long purpose-made stainless steel un-insulated needle electrodes (0.35 mm diameter) were placed approximately 5 cm apart subcutaneously above the middle of the left splenius muscle (SPL) while another pair was placed above the middle of the left cleidomastoideus muscle (CM) as perpendicular to the muscle fibres as possible. The ground electrode was fixed on the left side behind the withers on previously moistened hair. Flexible leads were connected to the electrodes and secured to the skin and the mane to prevent displacement of the electrodes and disturbances of the horses. The distances between electrodes and the base of the ear, considered as the projection of the trigeminal nerve origin on the skull surface, were measured for estimation of conduction time. The resistance of the stimulating electrodes had to be lower than 2 kΩ during the whole experiment.

Electrical stimulation and reflex threshold

The stimulator was activated manually when the behaviour of the horse and the position of the head were deemed adequate, i.e. when there were no movements, a straight neck and minimal background activity of the SPL. Electromyographic activity was recorded from 100 ms before (background activity) to 400 ms after stimulation started. Inter-stimulus intervals were random within the range of 30–60 ms to prevent habituation. A standard stimulus consisted of a 1 ms train-of-five constant current square wave pulses delivered at the frequency of 200 Hz. Stimulation was always started at the lowest intensity of 1 mA. Then, in order to define the TCR threshold, the current was gradually increased in steps of 0.5 mA until a clear aversive backward movement of the head was elicited, which corresponded to behavioural score of 3. Adjustments were made by increasing the stimulation intensity in steps of 0.1 mA from the last sub-threshold stimulation to the point at which threshold intensity (I_t) for the TCR was confirmed.

To be considered a threshold reflex, the amplitude of the EMG activity burst recorded from the neck muscles had to be at least three times the background activity. A second stimulation at I_t was performed to verify reproducibility of the response. Once the threshold was defined, intensities of 0.9, 1, 1.1, 1.2, 1.3, 1.4 and $1.5 \times I_t$ were applied, with a minimum interval of 30 s between stimulations, to evaluate the stimulus–response curve. Stimulations were stopped if NRS score of 4 was reached.

Definition of variables and signal analysis

The latency of the reflex or of its individual components was defined as the time measured from the start of electrical stimulation to the onset of the muscle response. The duration of the reflex or of its individual components was measured as the interval from the beginning of the first deflection to its final return to the baseline. The peak-to-peak amplitude (PTP) was calculated as a difference between the highest and lowest peak of the evoked EMG burst. Moreover, the root mean square amplitude (RMS) of the EMG activity was calculated for the 100 ms epoch before stimulation, and over the individual reflex bursts.

Data analysis

In order to describe the reflex characteristics when stimulating at I_t, medians and interquartile ranges (IQR) were calculated for reflex latency, duration, PTP, RMS, stimulation intensity, NRS and VAS scores. Three EMG records per horse were analysed both in case of ION and SON stimulation. Wilcoxon Signed Rank Test was used to compare the records obtained while stimulating the two nerves (ION and SON) from each muscle and from the two muscles (SPL and CM) for each stimulated nerve. To evaluate the stimulus–response curve, the Friedman Repeated Measures Analysis of Variance on Ranks was applied. The Spearman Rank Order Correlation was used to evaluate correlation between parameters.

Results

Reflexes at I_t

Surface electrical stimulation of the trigeminal afferents ION or SON evoked clear EMG reflex responses in the OO, SPL and CM (Fig. 1). The horses tolerated the stimulation series without any sign of discomfort or need for additional sedation until I_t was reached. Technical problems were responsible for the loss of 1/3 threshold EMG recordings of one horse for ION and SON stimulation, so only 29 sets of data at I_t for each nerve could be analysed.

The occurrence of the different components of BR (Anor et al., 1996) was inconsistent when stimulating at I_t. The R1 component was recorded in 14 cases (48%) after SON stimulation and 20 cases (69%) after ION stimulation. The R2 and R3 components were present in 19 (66%) and 25 (86%) cases after SON stimulation and in 19