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Assessment of the wind-up phenomenon in the equine nociceptive trigeminal system ☆

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ABSTRACT

Repeated sub-threshold nociceptive electrical stimulation resulting in temporal summation of the limb nociceptive withdrawal reflex is a well-established non-invasive model to investigate the wind-up phenomenon in horses. Due to structural similarities of the trigeminal sensory nucleus to the dorsal horn of the spinal cord, temporal summation should be evoked by repeated transcutaneous electrical stimulation of trigeminal afferents. To evaluate this hypothesis repeated transcutaneous electrical stimulation was applied to the supraorbital and infraorbital nerves of 10 horses. Stimulation intensities varied between 0.5 and 1.3 times the trigemino-cervical reflex threshold defined for single stimulation. Evoked electromyographic activity of the orbicularis oculi, splenius and cleidomastoideus muscles was recorded and the signals analysed in the previously established epochs typical to the early and late component of the blink reflex and to the trigemino-cervical reflex. Behavioural reactions were evaluated with the aid of numerical rating scale.

The nociceptive late component and the trigemino-cervical reflex were not elicited by sub-threshold intensity repeated transcutaneous electrical stimulation. Furthermore, the median reflex amplitude for the 10 horses showed a tendency to decline over the stimulation train so temporal summation of afferent trigeminal inputs could not be observed. Therefore, the modulation of trigeminal nociceptive processing attributable to repeated Aδ fibre stimulations seems to differ from spinal processing of similar inputs as it seems to have an inhibitory rather than facilitatory effect. Further evaluation is necessary to highlight the underlying mechanism.

Introduction

The nociceptive withdrawal reflex (NWR) has become a relevant tool in animal pain research. Several recent studies have focused on the determination of the physiological characteristics of the NWR to investigate spinal nociceptive processing linked to Aδ fibres activity in different species (Spadavecchia et al., 2002; Bergadano et al., 2006) and to assess the antinociceptive effectiveness of pharmacological interventions (Spadavecchia et al., 2005, 2007; Bergadano et al., 2009a,b; Rohrbach et al., 2009; Levionnois et al., 2010; Lervik et al., 2012). Using a standard electrical stimulus, the NWR threshold can be defined as the minimal current intensity needed to evoke a NWR in a given subject.

As part of the NWR model, the repeated stimulation paradigm can be applied to evaluate the modulation of temporal summation, an important phenomenon most likely resulting in wind-up and central sensitisation (Arendt-Nielsen et al., 1994). Subthreshold repeated stimulation elicit temporal summation of synaptic potentials at the level of the secondary wide dynamic range (WDR) neurons of the afferent pathway thus facilitating the NWR (Arendt-Nielsen et al., 2000). Originally, wind-up was believed to result from repetitive C-fibre stimulation only (Li et al., 1999). Later on, it was proved in humans that the wind-up phenomenon involved the Aδ fibres as well, when these were stimulated at a minimum frequency of 0.3 Hz (Andersen et al., 1994). Stimulation of C-fibres would necessitate a high stimulus intensity and long duration due to the characteristics of C-fibres, which would be neither acceptable nor tolerable for subjects in research settings (Arendt-Nielsen et al., 2000), especially if the subjects are non-medicated animals.

The facilitation of limb NWR by repeated electrical stimulation has been successfully evaluated in horses (Spadavecchia et al., 2004) and dogs (Bergadano et al., 2007), thus providing indirect insight into the role of the wide dynamic range (WDR) neurons in the dorsal horn of the spinal cord. In the trigeminal system, the subnucleus caudalis, which is considered to be the nociceptive specific part of the trigeminal brainstem sensory nuclear complex,

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resembles the dorsal horn in its structure and basic function ('medullary dorsal horn') and also contains WDR neurons (Sessle, 2000). So far there have been no investigations to see whether repeated electrical stimulation of trigeminal afferents is able to evoke temporal summation in the trigeminal system. A better understanding of the trigeminal nociceptive physiology could facilitate the diagnostics and treatment of diseases associated with trigeminal nerve dysfunction.

The nociceptive trigemino-cervical reflex evoked by a single train-of-five transcutaneous electrical stimulus activating Aδ fibres has recently been described in horses (Veres-Nyéki et al., 2012) and may be used as a reference for further neurophysiological investigations in this species. We hypothesised that due to structural similarities of the trigeminal sensory nucleus with the dorsal horn of the spinal cord, temporal summation should be evoked by repeated transcutaneous electrical stimulation of trigeminal afferents and could be quantified by the measurement of the electromyographic activity recorded from muscles involved in the reflex arch.

This study was designed to define the trigeminal temporal summation threshold and to evaluate the electrophysiological properties of trigeminal nociceptive reflexes evoked by repeated electrical stimulation in non-medicated horses.

Material and methods

Animals

Ten healthy adult horses (6 geldings and 4 mares; 7 Swiss Warmblood, 2 Freiberger and 1 Hanoverian), aged 14–23 years and bodyweight (BW) 540–640 kg, were included in this study and were the same subjects previously used to determine trigemino-cervical reflex threshold to single stimulation (T_{SS}) (Veres-Nyéki et al., 2012). The experiments were performed with the approval of the Bernese Committee for Animal Experimentation, Switzerland (Tierversuche/Bewilligung 92/08).

Instrumentation

The conditions of the experiment, the recording technique, the type and placement of the electrodes have been described elsewhere (Veres-Nyéki et al., 2012). Briefly, stimulation electrodes were positioned over the infraorbital (ION) and supraorbital nerve (SON) and recording electrodes over the orbicularis oculi (OO), splenius (SPL) and cleidomastoideus (CM) muscles to evaluate the blink reflex (BR) and trigemino-cervical reflex (TCR), respectively. The resistance between the pairs of electrodes had to be lower than 5 kΩ during the experimental session.

Behavioural reactions

A numerical rating scale (NRS) (Table 1) was used by the same observer (KV) to evaluate behavioural reactions to stimulation. In case of violent reaction, corresponding to a value of four on the NRS, the stimulation was stopped.

Electrical stimulation

In order to perform the repeated stimulation, the standard single stimulus (SS) consisting of 1 ms train-of-five (200 Hz) constant current square wave pulses previously used in the evaluation of nociceptive reflexes (Veres-Nyéki et al., 2012) was delivered 10 times with a frequency of 5 Hz over 2 s (Fig. 1). Each repeated stimulation (RS) was delivered at progressively increasing intensities, in particular at 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2 and 1.3 times the previously reported T_{SS} intensity (Veres-Nyéki et al., 2012). To rule out relevant changes of the reflex threshold, each RS was evaluated shortly after the assessment of T_{SS} in the same experimental session (Veres-Nyéki et al., 2012). To avoid habituation, at least 30 s were allowed between each RS.

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

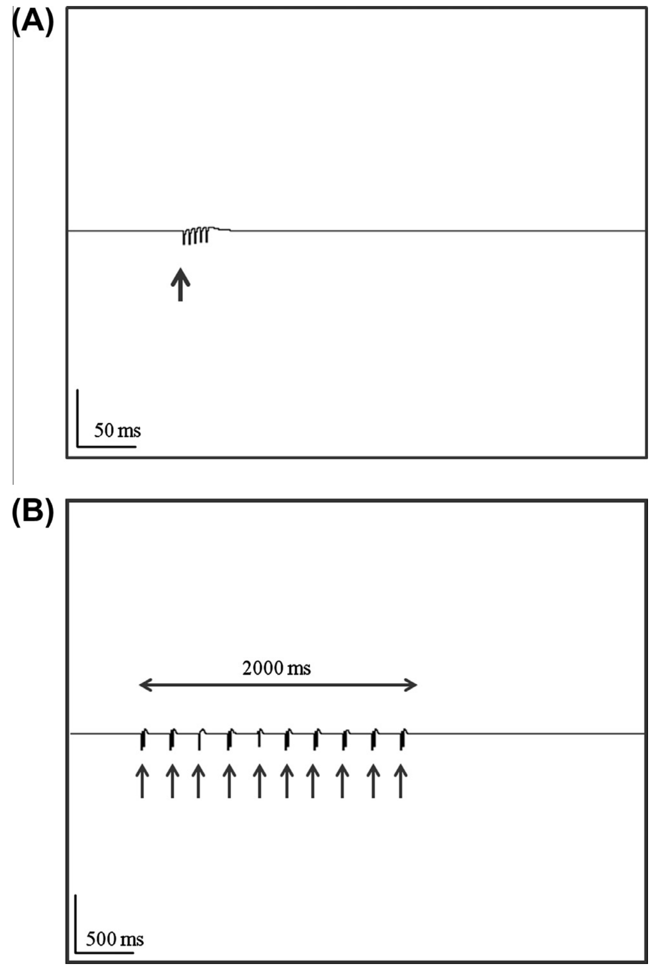

Fig. 1. (A) The standard single stimulus SS used to define the trigemino-cervical reflex threshold (T_{SS}) in the previous study: 1 ms train-of-five constant current square wave pulses (shown here as stimulation artefact on an EMG recording). The arrow indicates the stimulus onset. (B) The repeated stimulation (RS) paradigm used to evaluate temporal summation: 10 standard single stimuli (SS) delivered in a frequency of 5 Hz over 2000 ms (shown here as stimulation artefact on an EMG recording). The arrows indicate the stimuli onset.

Signal analysis

The total electromyographic (EMG) recording time was 4000 ms, including 500 ms before stimulation onset. The 500–2500 ms interval was analysed during RS and was divided into 10 × 200 ms sections to investigate the response to each stimulus separately. The epochs of the reflex components were determined according to the reference values acquired by SS: (1) 10–40 ms and 40–140 ms after the onset of each stimulus to analyse the early (BR early) and late components (BR late) of the blink reflex, and (2) 50–200 ms interval after the onset of each stimulus to analyse the trigemino-cervical reflex (TCR SPL and TCR CM). The root-mean-square (RMS) amplitudes of these epochs were calculated to quantify the magnitude of the electromyographic responses. To reduce the inter-individual variability, the reflex size was normalised to the baseline activity of muscle. The post-stimulation activity was recorded for the interval 2500–4000 ms (Fig. 2).

The reflex threshold was defined as the intensity of each SS necessary to elicit electromyographic reflex activity with amplitude of at least three times the baseline activity and a behavioural score of BR = 1 and TCR = 3. Reflex threshold of TCR SPL, TCR CM, BR early and BR late (T_{RS}) were defined for each nerve and the reported values were normalised to the single stimulation threshold (T_{SS}) of the TCR. In addition, the stimulus number able to evoke the first reflex and the maximal reflex response were recorded.

Analysis of the data

Non-parametric tests were used for statistical evaluation of the data. Descriptive statistics were used to define individual reflex threshold to repeated stimulation. One sided exact Wilcoxon Signed Rank Test was applied to investigate the effect of repeated stimulation on the reflex threshold intensity. The Brunner–Langer