Paired nociceptive blink stimuli can facilitate trigeminofacial circuit at a long inter-stimulus interval


Abstract

Backgrounds: Conditioned responses of paired nociceptive blink reflex (nBR) can reflect the excitability of trigeminofacial circuit. In the present study, we studied paired homotopic nBR with different inter-stimulus intervals (ISI). By monitoring different ISIs and consequential conditioned R2 of nBR, we aimed to investigate the impact of ISIs on the recovery cycle of nBR in normal individuals.

Methods: Twelve healthy volunteers (mean age: 29.9 ± 7.0 years; M/F: 7/5) were enrolled in this study. After individuals’ reflex threshold was determined, triple pulses were given in pairs with ISIs 125 to 10000 milliseconds randomly. We calculated the ratio of conditioned and unconditioned nBR area-under-curve (AUC) (defined as recovery index), and amplitude of each ISI.

Results: The average latency of unconditioned nR2 is 42.6 ± 5.5 ms, with amplitude of 53.4 ± 43.9 μV and the AUC of 563.5 ± 480.6 ms·μV. The conditioned nBR/unconditioned nBR response ratio was less than 100% while the ISI is shorter than 1667 ms, suggesting an inhibited conditioned response. The recovery index and the amplitude of conditioned nBR gradually increased with increasing ISI. The recovery index was greater than 100% at ISI of 10 s (p = 0.005), implying full recovery and facilitation of conditioned nBR.

Conclusion: Our study established the time-dependent dynamic recovery curve of paired nBR. The facilitated nBR at ISI longer than 10 s might be associated with temporal summation to the facial motor neurons after repeated stimuli. Our study results provided potential applications for patients with pain disorders involving trigeminofacial region.

Keywords: Electric stimulation; Nociceptive blink reflex; Recovery cycle; Temporal summation; Trigeminal nerve

1. INTRODUCTION

The blink reflex (BR) provides a non-invasive method to examine the trigeminofacial-brainstem connections. The traditional blink reflex contains three components: one oligosynaptic reflex, R1 (latency 9.1-11.8 ms), and two polysynaptic reflexes, including R2 (latency 27.1-38.6 ms) and R3 (70.7-92.0 ms). R2 is influenced by interneuronal networks and is suppressed by preceding conditioning stimulation. In general, R2 is completely abolished from 0 to 200-300 ms in response to paired stimuli, and recovers to approximately 30-50% at the 500 ms interval and 70-90% at the 1,500 ms interval. The recovery curve of a second R2 is considered reflective of the excitability of brainstem interneurons and the trigemino-facial circuit. Abnormal R2 recovery is associated with hemifacial spasm, torticollis spasmodica, and blepharospasm.

A novel method, using a concentric electrode, has been developed to selectively stimulate nociceptive fibers in order to elucidate pain-related disorders of the trigeminal nociceptive systems. The electrode consists of a small central cathode (diameter: 0.5 mm), an isolation insert (diameter: 5 mm), and an external anode ring (diameter: 6 mm), providing a stimulation area of 19.6 mm² (Walter Graphtek GmbH, Lübeck, Germany). With low stimulus intensity and high current nociceptive density, the electrode selectively activates skin nociceptive Aδ fibers. The onset latency of the BR in response to the concentric electrode is 44.7 ± 7.3 ms and is relatively prolonged in comparison with conventional electrical stimulators. The nociceptive BR (nBR) elicited by the novel concentric electrode has proven to be sensitive and specific to study the trigeminal nociception pathway. Abnormal nBR excitability has been demonstrated in patients with primary headache. However, the complete recovery curve of the nBR has not yet been determined. If nBR is activated by the concentric electrode through Aδ fibers, it is likely that the recovery of nBR to paired stimuli will be longer than the recovery time for conventional electrical stimulation (i.e. more than 1,500 ms). In the current study we applied the concentric electrode to study trigeminal nociceptive processing in the brainstem and the recovery curve of nBR.
2. METHODS

2.1. Subjects
Healthy controls without history of migraine were recruited for the study. Infrequent episodic tension-type headaches (< 1 headache day/month) were permitted.14 Subjects did not have major medical conditions nor did they take any medications on a regular basis. All subjects were nonsmokers. To avoid confounding variables, participants were asked to abstain from caffeine and alcohol for four hours prior to the study. The study was reviewed and approved by the Institutional Review Board of Taipei Veterans General Hospital. Informed consent was obtained from all subjects before participating in the study.

2.2. Nociceptive stimulation: reflex threshold (RT) intensity
Nociceptive stimulation was elicited by methods described by Kaube et al.7 Briefly, custom-made planar concentric electrodes were used to provide high current densities at low intensities. We modified the method of Bischoff et al.15 by placing the stimulator in the middle of the face, i.e. the base of the nose. This site is farther from the outlet of the ophthalmic nerve and is more sensitive to electrical stimulation than forehead skin. Stimulation at the base of the nose activates the external nasal nerve (a division of trigeminal nerve V1) and elicits a more symmetric nBR. Due to our focus on the recovery phase of the nBR, we collected data from one hemisphere for analysis. The recording electrode was placed at the right infraorbital area over the orbicularis oculi muscle and the reference electrode was placed lateral to the orbit (Fig. 1A). A grounding electrode was placed on the left arm. The signal was recorded with a sweep duration of 200 ms (Bandwidths: 1-1,000 Hz, sampling rate: 2,500 Hz).

Stimulation parameters were as follows: triple pulses with an inter-pulse interval of 5 ms and a pulse duration of 0.5 ms.16 The current intensity (of each pulse) was adjusted by 0.5 mA increments, starting from 0.5 mA, in order to detect individual pain thresholds (i.e. a corresponding verbal rating of approximately 3 on a scale of 1-10).16 An individual’s reflex threshold intensity (RT) was set at 1.5 times that of an individual’s pain threshold (0.5 - 2 mA). During the experiment, a recording electrode was placed on the right sternocleidomastoid muscle to detect contractions resulting from the startle reflex. Responses associated with a startle reflex were excluded. The measurements of nBR included latency, amplitude, duration, and area under the curve (AUC) (Fig. 1B).

2.3. Unconditioned R2 and paired nociceptive blink reflex
Paired triple pulses were administered under inter-stimulus intervals (ISIs) of 10,000, 5,000, 3,333, 2,500, 2,000, 1,667, 1,429, 1,250, 1,111, 1000, 500, 333, 250, 200, 167, 143, and 125 ms (equal to frequencies of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, and 8 Hz). Because the interval of the ISI was set at a maximum of 10 s, the built-in program of the F wave response was modified for the study. The two stimuli were presented consecutively. The ISI was derived from the stimulating frequency. For example, the ISI was 333 ms when the two stimuli were given at a frequency of 3 Hz. For each nBR, the response to the first stimulation was recorded as the unconditioned nBR (S1) and the response to the second stimulation was recorded as the conditioned nBR (S2). Each session was composed of paired nBRs of the same ISI, separated by at least 30 seconds. In total, each subject was presented with 17 sessions and 34 stimuli. After a session was complete, responses at different ISIs were tested randomly. Latencies, amplitudes and AUC of each paired nBR were recorded during all sessions (Fig. 1B). The latency, amplitude and AUC of all responses were analyzed offline. The recovery index (RI) at each ISI was defined as the conditioned nBR AUC divided by the unconditioned nBR AUC.12 The amplitude ratios of conditioned/unconditioned nBR at each ISI were also calculated.

2.4. Statistical analysis
Data were statistically analyzed by parametric testing. All values were expressed as mean ± standard deviation (SD) for continuous variables. The mean RI at each ISI was calculated. The group RI mean at each ISI was first compared to 100% with a two-tailed, one sample t-test. If the t-test showed a significant difference from 100% (p < 0.05), a one-tailed, one sample t-test followed to determine if the results were significantly less than

Fig. 1 (A) Position of recording and stimulation electrodes. ◯: stimulation, □: recording, Q: reference, Δ: sternocleidomastoid muscle recording. (B) Nociceptive blink reflex (nBR) to train stimulation. Definition of latency, amplitude and area under curve (AUC). Every column on the X-axis represent 10 ms and every column on the Y-axis represent 50 μV (Per division is 50 μV/10 ms.).
100% (indicating inhibition) or more than 100% (indicating facilitation). The current study was an explorative study to evaluate facilitation of response at longer ISIs of paired nBR, and thus, no reference was available. Nevertheless, based on previous studies on the R2 recovery curve after the traditional blink reflex, we hypothesized that at some ISI, the RI would exceed 120% with a standard deviation of 25% (effect size = 0.8). Given alpha = 0.05, and statistical power = 0.8, a sample size of 12 was necessary. Correlations of conditioned/unconditioned nBR in amplitude and AUC (RI) were calculated. A probability level of < 0.05 was chosen for significance.

To evaluate the reliability of the nBR the intra-class correlation coefficient (ICC) of amplitude and AUC was calculated using the results from the first and second stimulation, throughout the entire set of stimuli (i.e. stimuli at 17 different frequencies). A value between 0.60 and 0.74 indicates good reproducibility, while a value between 0.75 and 1.00 indicates excellent reproducibility between stimulations.

### 3. RESULTS

Twelve healthy volunteers (mean age: 29.9 ± 7.0 years; 5 females) were enrolled in the study. The average latency of unconditioned nR2 was 42.6 ± 5.5 ms, with an amplitude of 53.4 ± 43.9 μV and an AUC of 563.5 ± 480.6 ms μV.

The conditioned/unconditioned nBR responses of all subjects at each ISI are shown in Table 1. The response of the conditioned nBR gradually increased as the ISI lengthened (Fig. 2). The mean RIs at ISIs from 125 ms (8 Hz) to 1,667 ms (0.6 Hz) were significantly less than 100%, indicating an inhibited conditioned nBR response \( p < 0.05 \) (Fig. 3A). At ISIs between 1,667 ms (0.6 Hz) and 10 s (0.1 Hz), the RI increased gradually, reflecting a recovery process (Fig. 3B). The RI was significantly greater than 100% at an ISI of 10 s \( p = 0.005 \), implying full recovery and facilitation of the conditioned nBR (Fig. 3C). Although the amplitude ratios were not identical, the conditioned/unconditioned nBR amplitude was highly correlated with the RI \( r = 0.89, p < 0.05 \), Fig. 4).

The ICC of amplitude between the first and the second stimulation was 0.662, while the ICC of the AUC was 0.686. Therefore, both measures had good reproducibility.

### 4. DISCUSSION

The current study investigated nBR recovery. There were three major findings. First, the conditioned nBR amplitude recovered gradually as the ISIs lengthened. Second, after gradual recovery, the conditioned nBR was facilitated as the ISI lengthened up to 10 s. Third, the amplitude ratios of conditioned/unconditioned nBR were highly correlated with the RI, providing a new measure of nBR facilitation. These results indicate that the measurement of amplitude is easier and more time saving than the measurement of AUC in the study of conditioned nBR. Moreover, many commercialized electrodiagnostic devices are not equipped to calculate AUC. The high correlation between AUC ratio and amplitude ratio of unconditioned/conditioned nBR found in the current study indicates that the measurement of amplitude should be the recommended method for the study of paired nBR. However, in a study of unconditioned nBR, baseline-adjusted AUC showed the best correlation with subjective pain ratings.

Previous studies have shown that paired classical trigeminal stimulation can induce transient facilitation of R1, peaking at 50 ms, followed by inhibition of R2. Similarly, arising from the medullary subnuclei interpolaris and caudalis of the spinal trigeminal nucleus, the nBR is also influenced by many suprasegmental structures including the motor cortex, the post-central cortex, and the basal ganglia. Repeated painful stimuli can induce pre-synaptic control, gating of trigemino-facial reflex, and activation of segmental inhibitory interneurons. It is thought that the above-mentioned mechanisms protect the brain from experiencing sensory overload. A previous study showed that sensory gating underlying paired-stimulus suppression is specific to the affected afferent limb of a reflex. However, responsiveness of the nociceptive pathway increased when painful stimuli are given repeatedly at longer intervals.
High-frequency electrical stimulation (i.e., 5 trains of 100 Hz for 1 s) repeated after a 10 s interval has been shown to increase pain perception by eliciting long-term potentiation in the dorsal horn of the nociceptive pathway. Meanwhile, it has also been shown that blink-evoking stimuli lead to a facilitating process likely initiated in the facial motoneuron. We therefore hypothesize that the sensory gating and activated segmental inhibitory interneurons are more prominent at ISIs shorter than 2,000 ms, causing an inhibitory conditioned nBR. As the ISI lengthened, the inhibitory process diminished gradually, long-term potentiation in the nociceptive pathway was induced, and the facilitation in facial motor neurons occurred at an ISI of 10 s.

The mechanisms underlying the facilitation of the nBR response at longer ISIs are not clear. However, the activated nociceptive transmission and temporal summation at facial motor neurons may be contributing factors. The temporal summation of the lower limb flexion reflex is well studied and may help in our understanding of the mechanisms underlying nBR. The flexion reflex of the lower limb is a polysynaptic and multi-segmental pathway, consisting of an early response and a late response. The wide dynamic range (WDR) neurons, located in lamina V of the dorsal horn of the spinal cord, along with a complex network of interneurons, play a significant role in pain processing and flexion reflex mediation. The flexion response is produced after integration of descending motor commands and multi-sensory feedback in the spinal cord, which further projects the output to motor neurons. The nBR response is generated by a complex network of interneurons located from the pons to the lower medulla. Afferent impulses are conducted through the descending spinal tract of the trigeminal nerve, reaching the caudal spinal trigeminal nucleus in the lower medulla, and then ascend bilaterally via the ascending medullary pathway to reach the facial nuclei in the pons. This multi-synaptic pathway involves the reticular formation and the mechanisms responsible for the conditioned response are mainly mediated by WDR interneurons in the medullary dorsal horn.

In previous studies, wind-up of spinal cord neurons and pain sensation was not observed at frequencies below 0.2-0.3 Hz. In the present study, we observed that the nBR recovered at an ISI of 2,000-5,000 ms (stimulating frequency approximately 0.2-0.5 Hz, Fig. 2), and that the facilitated nBR recovered at an ISI of 10 s (0.1 Hz).

The current study is the first to describe the time course of paired nBR suppression at an ISI as high as 10 s in healthy subjects. We hypothesized that the facilitated trigeminofacial response is associated with temporal summation of multiple stimuli onto facial motor neurons. However, there are limitations to the study that must be considered. First, the variability in the amplitude ratio and the AUC ratio (RI) was relatively large, although they did not significantly differ from a previous study using the traditional blink reflex paradigm. Second, the peak of the facilitated nociceptive response was not determined. Future studies should examine longer ISIs. In addition, we studied the recovery curve with paired stimuli without further repeated multiple stimuli to confirm the phenomenon of temporal summation. Lastly, the sample size in the current study was relatively small and homogeneous. Further large-scale studies, incorporating parameters mentioned above (i.e. longer ISIs, multiple stimulations, subgroup studies including different gender and
Ane groups) are needed to confirm the present findings and to expand our understanding of paired nBR physiology.

In conclusion, the current study established the time-dependent dynamic recovery curve of paired nBR. In addition, the facilitated nBR at an ISI of 10 s may be associated with temporal summation at facial motor neurons after repeated stimuli. The results of the current study suggest that the evaluation of the pattern of the dynamic recovery curve of the nBR may be a useful tool in diagnosis and treatment of patients with pain-related diseases in the trigeminofacial region. The ICC between the first and the second stimulation showed good reproducibility of both amplitude and AUC. The paired nBR protocol is a reliable technique for use in longitudinal studies for the detection of differences in individuals/patients over time, and may further aid in our understanding of the underlying pathophysiology of pain-related diseases involving the trigeminal pathway.

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REFERENCES


