Authors:

Rajath Sasidharan Pillai^{1,6}, Cung May Thai², Laura Zweers², Michail Koutris², Frank Lobbezoo², Yuri Martins Costa³, Maria Pigg^{4,6}, Thomas List^{5,6}, Peter Svensson^{1,6}, Lene Baad-Hansen^{1,6}

Title:

Feasibility and reliability of intraorally evoked “nociceptive-specific” blink reflexes

Author affiliations:

¹Section of Orofacial Pain and Jaw Function,

Department of Dentistry and Oral Health,

Aarhus University,

Denmark

²Department of Orofacial Pain and Dysfunction,

Academic Center for Dentistry Amsterdam (ACTA),

University of Amsterdam and Vrije Universiteit Amsterdam,

Amsterdam, The Netherlands

³Department of Physiological Sciences,

Piracicaba Dental School,

University of Campinas,

Piracicaba, Brazil

⁴Department of Endodontics,

Faculty of Odontology,

Malmö University,

Malmö, Sweden

⁵Department of Orofacial Pain and Jaw Function,
Faculty of Odontology,
Malmö University,
Malmö, Sweden

⁶Scandinavian Center for Orofacial Neurosciences (SCON),
Denmark/Sweden

Corresponding author address:

Rajath Sasidharan Pillai,
Section of Orofacial Pain and Jaw Function,
Department of Dentistry and Oral Health,
Aarhus University,
Vennelyst Boulevard 9, DK-8000,
Aarhus C, Denmark
Tel.: +4550188248
Fax: +4589424297
Email: raj.pillai@dent.au.dk
Institutional URL: <http://au.dk/raj.pillai@dent>

Abstract

Objectives:

The “nociceptive-specific” blink reflex (nBR) evoked by extraoral stimulation has been used to assess trigeminal nociceptive processing in patients with trigeminal nerve damage regardless of the site of damage. This study aimed to: test the feasibility of nBR elicited by intraoral stimulation, compare intraoral and extraoral nBR, and assess the intrarater and interrater reliability of the intraoral nBR for the maxillary (V2) and mandibular (V3) branches of the trigeminal nerve.

Materials and methods:

In 17 healthy participants, nBR was elicited by stimulation of two extraoral and two intraoral sites by two operators and repeated intraorally by one operator. Main outcome variables were intraoral stimulus-evoked pain scores and nBR R2 responses at different stimulus intensities. Intraclass correlation coefficients (ICC) were used to assess reliability.

Results:

Dependent on the stimulus intensity, intraoral stimulation evoked R2 responses in up to 12/17 (70.6%) participants for V2 and up to 8/17 (47.1%) participants for V3. Pain scores ($p<0.003$) and R2 responses ($p<0.004$) increased with increasing intensities for V2, but not V3. The R2 responses were significantly smaller with intraoral stimulation compared to extraoral stimulation ($p<0.014$). Overall, ICCs were fair to excellent for V2 but poor for V3.

Conclusion:

Intraorally-evoked nBR was feasible in a subset of healthy participants and was less responsive than nBR with extraoral stimulation. The V2 nBR showed better reliability than V3.

Clinical relevance:

The nBR can be used to assess nerve damage to the maxillary intraoral regions, though other measures may need to be considered for the mandibular intraoral regions.

Keywords:

Nociceptive blink reflex, Intraoral, Reliability, Trigeminal nerve

Introduction

The trigeminal nerve branches that innervate the extraoral and intraoral tissues of the face may be damaged due to traumatic, infectious, inflammatory, iatrogenic, and neoplastic processes [1]. The diagnosis of trigeminal nerve damage generally involves the patients' clinical history, questionnaires, clinical examination, and psychophysical testing such as qualitative sensory testing (QualST) and quantitative sensory testing (QST), all of which require subjective responses from the patients [2]. Therefore, more objective investigative options may be valuable adjunct tools in the diagnostic repertoire to assess and confirm for definite neuropathic pain [3–5]. One such objective diagnostic test that can be used to attain the level of certainty to designate a diagnosis of definite neuropathic pain is the “nociceptive-specific” blink reflex (nBR) test [4]. The blink reflex is a protective trigemino-facial brainstem reflex which, when evoked by primarily stimulating the nociceptors, is referred to as the nBR [6]. The electrophysiological nBR test has been used as a non-invasive method to test the trigeminal nociceptive transmission in primary headaches and chronic orofacial pain [7–12]. Electrical stimulation in the cutaneous innervation territory of the trigeminal nerve branches, with the use of a concentric electrode that limits the electrical pulse to the superficial layers of the skin, leads to selective activation of superficial nerve fibers, i.e. primarily nociceptors [13]. The electromyographic (EMG) recordings from the orbicularis oculi muscles after stimulation with the concentric electrode show two separate bilateral responses of these muscles, i.e. the R2 and R3 responses [14]. This way, the nociceptive pathways of the trigeminal system may be assessed through evaluation of the R2 component evoked by electrical stimulation with different intensities [15].

In patients with damage to the distal endings of trigeminal nerve branches supplying the intraoral region, the QST and QualST investigations may be performed *in situ* [16–18] and are able to reveal local sensory abnormalities [16, 17]. However, the stimulation site of nBR investigations in these conditions has so far been located to the skin overlying the more proximal branches of the damaged nerve in patients and healthy controls [7, 12, 19, 20]. Due to the difference between the site of nerve damage (intraoral) and the point of stimulation (extraoral), it may be speculated that the resulting nBR response may likely reflect the response to stimulation of both intact and damaged afferent nerve fibers. From a diagnostic viewpoint, nBR responses elicited by stimulation at the actual site of nerve damage may provide a more valid representation of the patient's condition. While a recent study concluded that the extraorally-evoked nBR is a sufficient and reliable electrophysiological test to assess the nociceptive function of the trigeminal system [19], as yet no study has assessed the reliability of nBR evoked by intraoral stimulation.

Therefore, the aims of this study were: a) to test the feasibility of nBR elicitation by intraoral electrical stimulation of the maxillary (V2) and mandibular (V3) branches of the trigeminal nerve, b) to compare the nBR responses from intraoral and extraoral stimulation, and c) to evaluate the intrarater and interrater reliability of the intraorally-evoked nBR.

Methods

The present study was performed at the Section for Orofacial Pain and Jaw Function, Department of Dentistry and Oral Health, Aarhus University, Denmark. The participants were recruited for participation in the study by posting advertisement on web pages and flyers at and around the university. The participants were financially compensated with 100 Danish kroner (~15 USD) per hour for their time and effort invested to the study.

Participants

A total of 17 healthy adult participants (mean age $\pm$ SD: 24.6 ± 4.4 years; 10 women, 7 men) were included in the study. All the participants completed the feasibility session of the study (please see section 2.2 below). One participant dropped out due to reasons unrelated to the study and did not complete the reliability session. Therefore, reliability assessment of intraoral nBR included data of 16 participants (mean age $\pm$ SD: 24.7 ± 4.6 years; 10 women, 6 men). Exclusion criteria for the participants were: usage of snus, snuff, or dipping tobacco, history of surgery or trauma in the test area, signs or symptoms of inflammation or any other pathosis related to the oral mucosa, having a pacemaker, systemic diseases, neurological illness, orofacial pain conditions, headache disorders, and/or use of medications such as antidepressants, anticonvulsants.

The participants were provided with written information about the study and their rights as a research participant at least 24 hrs before commencement of the study and verbal information was given prior to obtaining signed informed consent. The study was conducted in accordance with the Declaration of Helsinki II and was approved by the regional ethical committee (approval no. 1-10-72-105-16).

Study protocol

The participants underwent two sessions separated by at least 24 hrs. In the first session, the nBR tests were performed by a single operator. In the second session, the same operator repeated the nBR tests at the intraoral sites to test the intrarater reliability and a second operator performed the test intraorally to assess the interrater reliability. Half the participants were tested first by the first operator and the other half were tested first by the

second operator. Both operators were trained together and were aware that their results would be used in the assessment of interrater reliability. However, the operators were unaware of each other's results.

Test sites

The tests were performed on two extraoral and two intraoral sites. Extraorally, the test sites were the skin over the exit zone of right infraorbital (V2_{EO}) branch and the skin supplied by the mental (V3_{EO}) branch of the trigeminal nerve [19]. Intraorally, the test sites were the gingival mucosa mesial to the maxillary canine in order to stimulate the superior alveolar branches of the infraorbital nerve (V2_{IO}) (Fig. 1A) and the gingival mucosa overlying the mental foramen (V3_{IO}) to stimulate the mental nerve (Fig. 1B). The intraoral stimulation sites were selected based on initial testing, which revealed the selected sites as most suitable based on ease of access, moisture control, and participant comfort.

Test procedure

The nBR was performed with the participant sitting in a comfortable position in a quiet, temperature-controlled (~20° C) room. After wiping the skin using alcohol wipes, two self-adhesive EMG electrodes (Neuroline 720, Ambu A/S, Denmark) were placed over the orbicularis oculi muscle region on both sides to record the muscle activity [7, 8, 13, 19, 21]. A common reference electrode was attached to the wrist of the left arm [7, 8, 19, 21]. The recorded signals were amplified and bandpass filtered between 20 and 1,000 Hz, with a sampling rate of 2,000 Hz (Nicolet Viking, Natus Medical Incorporated, CA., USA) [7, 8, 19]. A custom-built planar concentric electrode, consisting of a metal cathode of 0.5 mm diameter, surrounded by an isolation plate and external anode ring of 5 mm diameter was used to elicit the nBR [13]. The electrode provided a short electrical stimulus containing a train of three pulses of 0.3 ms duration and at a rate of 333 Hz to the test sites [7, 8]. For the intraoral sites, moisture control was achieved initially by using a cotton wipe and pressurized air and subsequently maintained using constant suction and Hygoformic® (Orsing, Sweden) saliva ejectors. A double-sided cheek retractor was used to keep the lips and cheeks away from the area where the stimulus electrode was placed. The stimulus electrode was secured intraorally using Urihesive® (ConvaTec, UK) strips with surrounding cotton pads for additional moisture control (Fig. 2).

The sensory (I₀) and pinprick (I_P) thresholds were determined at the intraoral and extraoral sites by application of a train of electrical stimuli in an ascending-descending manner, starting from 0.1 mA with 0.2 mA increments [13, 19]. To determine the I₀, participants gave a positive response by raising their arm to signal when they first

felt a slight sensation. For I_p determination, participants gave a positive response when they felt a sharp, needle-like, pinprick sensation [7, 8, 19]. The thresholds were recorded when participants provided three consecutive positive responses.

Based on their I_p , electrical stimuli at five different intensities (100%, 150%, 200%, 300%, and 400% of I_p) were applied to the test sites in a randomized fashion [7, 19]. The nBR responses to stimulus intensities relative to the intraoral I_p were used to assess the feasibility of nBR evoked by intraoral stimulation, whereas the stimulus intensities relative to the extraoral I_p were used to be able to perform direct comparisons with nBR responses from extraoral stimulation. This meant that the intraoral sites were stimulated electrically with intensities relative to both their intraoral and extraoral I_p 's. For each stimulus intensity, the participants were stimulated six times with approximately 15 s inter-stimulus interval to minimize habituation [7, 9, 11, 13, 22, 23]. The root mean square (RMS) value of the averaged EMG signals between the time window of 27 to 87 ms quantified the R2 component of the nBR [8, 13, 19, 24]. Prior to recording the R2 values at each stimulus intensity, the participants were familiarized with the intensity to avoid overlapping and consequent contamination of the R2 response with the startle reaction and the related R3 response [19]. Additionally, after the sixth stimulus of each stimulus intensity, the participants were asked to score the evoked pain intensity on a numerical rating scale (NRS) from 0 to 100, where 0 indicated “no pain at all”, and 100 indicated the “worst pain imaginable” [25].

Statistics

A sample size estimate suggested that with the 17 participants, a 25% difference in mean R2 values could be detected with an $\alpha=0.05$ and $\beta=0.20$ with an intra-individual variability of 25%. The outcome variables assessed were: (a) R2 occurrence (yes/no), (b) I_0 (mA), (c) I_p (mA), (d) ipsilateral and contralateral R2 values (R2i, R2c) (μV), (e) NRS pain scores at each stimulus intensity, and (f) the slope of the stimulus-response curves of outcome variables (d) and (e). All the outcome variables were analyzed for influence of stimulation *Site* (extraoral and intraoral), and the outcome variables (c) and (d) were analyzed for influence of *Stimulus intensity* (100%, 150%, 200%, 300%, and 400% of I_p).

All data are presented as mean $\pm$ standard deviations. Data distribution was assessed using quantile-quantile plots. The presence or absence of R2 at any stimulus intensity was coded as ‘yes’ or ‘no’, respectively, and compared between extraoral and intraoral stimulation using McNemar’s test. The NRS pain scores were not normally distributed and hence were log transformed prior to analyses. To avoid loss of values rated as 0, a small constant of 0.1 was added to all NRS pain scores prior to log transformation [26]. Comparisons between extraoral and

intraoral I_0 and I_P was performed using one-way repeated measures analyses of variance (ANOVA). Two-way repeated measures ANOVA with Tukey's Honestly Significant Difference (HSD) post hoc tests were used to analyse the R2 values and NRS pain scores with *Site* and *Stimulus intensity* as the two factors. Statistical significance was set at $p < 0.05$.

The intrarater and interrater reliability of the nBR data was estimated using intraclass correlation coefficient (ICC, two-way random-effects model) along with standard error of measurement (SEM) and smallest detectable change (SDC; the smallest change in variable outcomes that can be considered a real change above the measurement error) [27]. The ICC values were graded as follows: < 0.40 was considered poor reliability, $0.40 - 0.59$: fair, $0.60 - 0.75$: good, and > 0.75 : excellent reliability [28]. To assess the effect on reliability from placement of EMG electrodes on same day versus different day, interrater reliability data obtained by the second operator in the second session was compared to data obtained from the first operator in the same session and the first operator in the first session. An arbitrarily selected difference of more than 30% in ICC value was considered as 'markedly different' interrater reliability. The changes in ICC grades were not used as indicators of marked difference in order to avoid misconstruing the differences by small changes at the upper and lower limits of the grades with larger changes. For example, a change of ICC value from 0.38 to 0.41 and a change from 0.1 to 0.59 would both be equated as a change from poor reliability to fair reliability.

Results

Feasibility of intraorally-evoked nBR

The averaged EMG traces showing the R2i on 300% of I_P for a single participant at different stimulation sites are presented in Fig. 2. The distribution of participants with R2 occurrences based on site and stimulus intensity is shown in Fig. 3. An R2 response could be evoked with stimulation with at least one intensity in 12/17 (70.6%) participants at the $V2_{IO}$ site, and in 8/17 (47.1%) participants at the $V3_{IO}$ site. In comparison, an R2 response could be evoked in all participants at the $V2_{EO}$ site and in 16/17 (94.1%) participants at the $V3_{EO}$ site. The proportion of participants with an R2 occurrence at any stimulus intensity was not significantly different between extraoral and intraoral stimulation for the V2 branch ($V2$: $p > 0.063$). However, a significantly higher number of participants had an R2 occurrence on extraoral stimulation than with intraoral stimulation ($V3$: $p = 0.008$) (Fig. 3).

The I_0 and I_P for both V2_{IO} and V3_{IO} branches were similar (V2_{IO}: $I_0 = 0.4 \pm 0.1$ mA, $I_P = 1.0 \pm 0.5$ mA; V3_{IO}: $I_0 = 0.4 \pm 0.2$ mA, $I_P = 1.1 \pm 0.6$ mA, $p > 0.261$). The NRS pain scores and R2i values at different stimulus intensities relative to the intraoral I_P thresholds for V2_{IO} and V3_{IO} branches are presented in Fig. 4. A statistically significant increase in NRS pain scores was seen at 300% and 400% of I_P at both the branches when compared to 100% of the respective I_P ($p < 0.003$). Similarly, significantly higher R2i and R2c values were seen for stimulation of the V2_{IO} site at 300% and 400% compared to 100% of I_P ($p < 0.004$). For stimulation of the V3_{IO} site, significantly higher responses were seen for R2i values but not for R2c values at 400% compared to 100% of I_P ($p = 0.026$).

Extraorally- vs. intraorally-evoked nBR

V2_{EO} vs. V2_{IO}

The I_0 from intraoral stimulation was significantly lower than from extraoral stimulation (I_0 extraoral: 0.6 ± 0.2 mA, I_0 intraoral: 0.4 ± 0.1 mA; $p < 0.001$). However, no statistically significant difference was seen in the I_P between extraoral and intraoral stimulation (I_P extraoral: 1.0 ± 0.3 mA, I_P intraoral: 1.0 ± 0.5 mA; $p = 0.844$).

For the electrically-evoked NRS pain scores (Fig. 5), statistically significant main effects were seen of *Site* ($F(1,16) = 6.52$, $p = 0.021$) and *Stimulus intensity* ($F(4,64) = 43.16$, $p < 0.001$). No significant interaction was found between both factors ($F(4,64) = 1.06$, $p = 0.386$). Post hoc analysis of the main effect of *Site* showed significantly, albeit slightly lower NRS pain scores with intraoral stimulation compared with extraoral stimulation ($p = 0.021$). The post hoc analysis of the main effect of *Stimulus intensity* showed that the NRS pain scores were significantly increased at all suprathreshold intensities (150%, 200%, 300%, and 400% of I_P) when compared with 100% of I_P ($p < 0.001$).

For the R2i values, there were statistically significant main effects of *Site* ($F(1,16) = 92.71$, $p < 0.001$) and *Stimulus intensity* ($F(4,64) = 23.33$, $p < 0.001$). Also, a significant interaction was seen between both factors ($F(4,64) = 8.90$, $p < 0.001$). Post hoc analysis of the main effect of *Site* showed significantly lower R2i values for intraoral than for extraoral stimulation ($p < 0.001$). Post hoc analysis of the main effect of *Stimulus intensity* showed a significant increase at 200%, 300%, and 400% of I_P when compared to 100% of I_P ($p < 0.001$). The post hoc analysis of the interaction between *Site* and *Stimulus* indicated that R2i values were significantly increased at all suprathreshold values when compared to 100% of I_P ($p < 0.012$) with the use of extraoral stimulation but not using intraoral stimulation ($p > 0.316$). In addition, the R2i values from extraoral stimulation were significantly higher when compared to intraoral stimulation at all stimulus intensities ($p < 0.008$) (Fig. 5).

A similar pattern was found for the R2c values, where statistically significant main effects of *Site* ($F(1,16) = 11.65, p < 0.004$) and *Stimulus intensity* ($F(4,64) = 13.43, p < 0.001$) were demonstrated. A significant interaction was found between *Site* and *Stimulus intensity* ($F(4,64) = 6.10, p < 0.001$). The post hoc analysis of the main effect of *Site* showed significantly lower R2c values for intraoral stimulation compared with extraoral stimulation ($p = 0.004$). The post hoc analysis of the main effect of *Stimulus intensity* showed a significant increase in the R2c values at 200%, 300%, and 400% of I_P when compared to 100% of I_P ($p < 0.001$). The post hoc analysis of the interaction between *Site* and *Stimulus intensity* showed a significant increase in the R2c values using extraoral stimulation for 200%, 300%, and 400% of I_P when compared to 100% of I_P ($p < 0.001$), whereas, no statistically significant change was seen in R2c values using intraoral stimulation for any suprathreshold intensity when compared to 100% of I_P ($p > 0.514$). Significantly higher R2c values were seen using extraoral stimulation at 150%, 200%, 300%, 400% of I_P compared to intraoral stimulation ($p < 0.001$) (Fig. 5).

V3_{EO} vs. V3_{IO}

Similar to the V2, the intraoral I_0 was significantly lower than the extraoral I_0 (extraoral: 0.7 ± 0.2 mA, intraoral: 0.4 ± 0.2 mA; $p < 0.001$) for the V3. However, no statistically significant difference was seen between intraoral and extraoral I_P (extraoral: 1.2 ± 0.3 mA, intraoral: 1.1 ± 0.6 mA; $p = 0.400$).

For the NRS pain scores (Fig. 5), a statistically significant main effect was seen for *Stimulus intensity* ($F(4,64) = 44.93, p < 0.001$), but not for *Site* ($F(1,16) = 3.08, p = 0.098$). No significant interaction was detected between *Site* and *Stimulus intensity* ($F(4,64) = 0.72, p = 0.579$). The post hoc analysis of the main effect of *Stimulus intensity* showed a significant increase in the NRS pain scores at all suprathreshold intensities when compared to 100% of I_P ($p < 0.001$).

For the R2i values, significant main effects of *Site* ($F(1,16) = 15.56, p = 0.001$) and *Stimulus intensity* ($F(4,64) = 12.05, p < 0.001$) were found. Also, a significant interaction between factors was seen ($F(4,64) = 7.63, p < 0.001$). The post hoc analysis of the main effect of *Site* showed significantly lower R2i values from intraoral stimulation compared to extraoral stimulation ($p = 0.001$). The post hoc analysis of the main effect of *Stimulus intensity* showed a significant increase in R2i values at 300% and 400% when compared to 100% of I_P ($p < 0.001$). The post hoc analysis of the interaction between *Site* and *Stimulus intensity* showed a significant increase in R2i values at 300% and 400% of I_P when compared to 100% of I_P on extraoral stimulation, whereas no increase was seen for R2i values using intraoral stimulation when compared to 100% of I_P ($p = 1.000$). The R2i values from extraoral

stimulation were significantly higher at 200%, 300%, and 400% of I_P compared to intraoral stimulation ($p < 0.014$) (Fig. 5).

For the R2c values, statistically significant main effects of *Site* ($F(1,16) = 14.38, p = 0.002$) and *Stimulus intensity* ($F(4,64) = 6.37, p < 0.001$) were detected. A significant interaction was also seen between both factors ($F(4,64) = 4.43, p = 0.003$). Post hoc analysis of the main effect of *Site* showed significantly lower R2c values using intraoral stimulation compared with extraoral stimulation ($p = 0.002$). Post hoc analysis of the main effect of *Stimulus intensity* showed significantly increased R2c values at 400% compared to 100% of I_P ($p < 0.001$). The post hoc analysis of the interaction between *Site* and *Stimulus intensity* showed significantly increased R2c values at extraoral sites for 300% and 400% of I_P when compared to 100% of I_P ($p < 0.025$). The R2c values were significantly higher using extraoral stimulation at 300% and 400% of I_P when compared to intraoral stimulation ($p < 0.010$) (Fig. 5).

Reliability of nBR using intraoral stimulation

The intrarater and interrater reliability, SEM, and SDC of the psychophysical parameters (I_0 , I_P , NRS pain scores) and the EMG responses (R2i and R2c) of the nBR evoked by stimulation of the V2_{IO} and V3_{IO} branches are presented in the Tables 1 and 2.

Psychophysical parameters

For both the V2_{IO} and V3_{IO} stimulus sites, the low ICC scores seen for the I_0 denoted a poor intrarater and interrater reliability. However, the I_P showed higher ICC values, denoting fair to good interrater and intrarater reliability (Table 1). Poor interrater and intrarater reliability were seen for the NRS pain scores at 150% and 200% of I_P for both intraoral test sites. The intrarater and interrater reliability ICC values for both intraoral test sites were higher (ICC: 0.57 to 0.95) at higher stimulation intensities of 300% and 400% of I_P . Additionally, the slopes of the stimulus-response curve of the NRS pain scores for both the V2_{IO} and V3_{IO} showed a good to excellent interrater and intrarater reliability (ICC: 0.73 to 0.92).

EMG responses

The R2i and R2c showed a fair to excellent intrarater reliability on stimulation of the V2_{IO} site (ICC: 0.44 to 0.82) (Table 2). The lowest interrater reliability for the V2_{IO} site stimulation was seen at 150% and 300% of I_P (-0.02 to 0.59). With stimulation of the V2_{IO} branch, the slopes of the R2i and R2c stimulus-response functions showed good to excellent reliability (ICC: 0.62 to 0.76). Poor reliability for the R2i and R2c was seen in 12.5% of all measurements at the V2_{IO} site. In comparison, the V3_{IO} site stimulation showed poor intrarater and interrater reliability of R2i and R2c for 62.5% of all measurements. The slope of the R2i and R2c stimulus-response functions showed poor intrarater and interrater reliability (ICC: 0.12 to 0.33). Stimulation of the V3_{IO} site at 200% of I_P showed fair to good R2i and R2c intrarater and interrater reliability (ICC: 0.45 to 0.63) and fair R2i and R2c interrater reliability at 100% of I_P (ICC: R2i = 0.55, R2c = 0.54).

Comparison of the interrater reliability of the psychophysical parameters and the EMG responses obtained between sessions and within the same session was performed to control for the effect of time on the reliability (Fig. 6 and 7). For the V2_{IO}, the I₀ showed a markedly lower reliability between different sessions compared to within the same session, whereas reliability for I_P stayed in the fair reliability range irrespective of EMG electrode placement for both V2_{IO} and V3_{IO}. Overall, the reliability for most of the nBR responses was not markedly affected by time, with 28 of the 34 variables showing no marked difference (Fig. 6). Additionally, there were no marked reliability differences regarding the slopes for NRS pain scores, R2i, and R2c for both trigeminal nerve branches (Fig. 7).

Discussion

The present study is the first to assess the feasibility and reliability of nBR elicited by intraoral stimulation. The main findings were: a) the intraorally-evoked R2 component of the nBR was seen in up to 70% of the participants on stimulation of the V2 branch, and up to 47% of the participants on stimulation of the V3 branch, depending on the stimulus intensity; b) the I_P and NRS pain scores of nBR were not significantly different between extraoral and intraoral stimulation, but electrophysiological responses (R2i, R2c) were significantly smaller using intraoral stimulation; c) both psychophysical and electrophysiological responses showed fair to excellent reliability using intraoral stimulation of the V2 branch, and poor reliability with stimulation of the V3 branch.

Feasibility of intraorally-evoked nBR

In the present study, intraoral test sites were decided based on ease of maintaining moisture control, access to the test site for electrode placement, and participants' comfort. Occurrence of R2 response was seen in the V2_{IO} site for the majority of participants, demonstrating the feasibility of nBR on intraoral stimulation at this site. However, a higher stimulus intensity was required to evoke an R2 response for the V3_{IO} site. Additionally, a significantly lower proportion of R2 occurrence was seen with intraoral stimulation compared to extraoral stimulation of the V3 branch. Therefore, intraorally-evoked nBR test cannot be considered feasible for the V3 branch. For both the V2_{IO} and V3_{IO} test sites, the NRS pain scores were significantly higher at 300% and 400% when compared to 100% of I_P. A similar stimulus-response pattern was seen for the R2 responses with stimulation of the V2_{IO} site but not the V3_{IO} site. This means that even though the participants perceived the increasing intensities of the intraoral stimuli as increasingly painful at the V3_{IO} site, it did not lead to a corresponding increase in the R2 response. Lower R2 responses by extraoral stimulation of the V3 branch has been reported previously [7, 19, 21]. Further studies may be needed to address the effect of longer inter-stimulus intervals on evoking R2 responses by intraoral stimulation of the V3 branch [19]. Moreover, it could also be speculated that the reduced R2 responses on stimulation of V3_{EO} and V3_{IO} may be due to fewer afferents from V3 to the central blink reflex circuits. Nevertheless, the primary purpose of the reflex would be to shield the eyes from exteroceptive threats [29, 30]. It has been shown that stronger and more effective defensive responses occur relative to the location of the threatening stimuli in the external defensive peripersonal space, and relative to the distance from the eye [31–34]. Thus, the reduced or absent R2 responses from intraoral stimulation may possibly be explained by the fact that no harm to the eyes may be expected to occur from within the oral cavity.

Extraoral vs. intraoral evocation of nBR

The extraoral I₀ and I_P in the present study were similar to previous studies [7, 19]. The electrically-evoked I₀ were significantly reduced in both branches with intraoral stimulation compared to extraoral stimulation. This is in agreement with a previous study which showed reduced electrically-evoked sensory thresholds on the gingival mucosa compared to the labial skin [35]. It should be noted that the cutaneous and mucosal structures differ in terms of innervation characteristics and their biophysical properties [18, 36–38]. Also, the morphology and density of the sensory afferents have been shown to vary considerably within the oral cavity, with a higher sensitivity seen in the anterior part of the mouth [39, 40]. For these reasons, future studies may benefit from further assessing the effect of stimulation site on evoking nBR responses.

For direct comparisons of extraorally- and intraorally-evoked nBR responses, the present study assessed the intraorally-evoked nBR stimulus-responses to the stimulus intensities relative to extraoral I_p . Assessment of the main effect of the site of stimulation (i.e. extraoral and intraoral sites) on the NRS pain scores showed an overall slightly but statistically significantly higher pain scores with extraoral stimulation compared to intraoral stimulation for the V2 branch but not the V3 branch. However, no significant interactions were seen in the NRS pain scores between site of stimulation and the stimulus intensities. Similarities between extraorally- and intraorally-evoked pain measures have also been seen with other stimulus modalities such as mechanical, thermal, and laser stimulation [18, 42, 43]. However, the R2 values were significantly lower using intraoral stimulation compared with extraoral stimulation at all stimulus intensities for both the branches. The difference in responses may likely be due to the reduced amount of appropriate synaptic connections of the intraoral afferent fibers with the interneurons in the trigeminal subnucleus caudalis responsible for the R2 component [44, 45].

Reliability

As the present study is the first to assess nBR elicited by intraoral stimulation, comparisons of reliability can only be made with the few previous studies that have assessed the interrater and intrarater reliability of extraorally-evoked nBR [19, 22]. Where Katsarava et al. assessed the intrarater reliability of the R2 onset latency exclusively in the V1 region, Costa et al. assessed the intrarater and interrater reliability of the psychophysical and electrophysiological measures similar to the present study on all three branches of the trigeminal nerve [19, 22]. The present study is in agreement with Costa et al., who found lower ICC values (poorer reliability) for I_0 than for I_p . The intraoral I_0 of the study participants had relatively lower variance than I_p . This lower variance between the participants made distinguishing the true reliability from measurement error difficult, thereby leading to lower ICC values [27]. Also, the negative ICC values as seen in Table 1 and 2, can be attributed to a lower within-group variance compared to the between-group variance [46]. Overall, higher ICC values were seen for the psychophysical and electrophysiological measures at higher stimulus intensities and for the slopes of the stimulus response curves, in agreement with Costa et al [13].

The present study reports the SDC based upon the interrater reliability. The SDC contributes to interpretation of the changes in the R2 values that can be considered to be true changes and not due to random error. Additionally, SDC can be used for sample size calculation in future studies assessing nBR from intraoral stimulation [47].

An additional analysis of the effect of same-day versus different-day testing was performed for the interrater reliability with the second operator as the anchor. Overall, the present study revealed no marked effect of same and different day testing on interrater reliability of the intraorally-evoked nBR responses. Other studies assessing surface EMG measures on facial muscles have also reported adequate reproducibility [48–50].

Limitations and recommendations for future research

The present study has certain methodological issues driven by technical limitations. Primarily, to avoid moisture contamination of the stimulus electrode, double-sided cheek retractors were used for the intraoral sessions. After the placement of the cheek retractors, testing began only after the participants reported that they were comfortable. One of the test sites were the attached gingiva in the V2_{IO} site, which would not be overtly affected by the cheek retractors and so the effect of the retractors in the particular site may have been minimal. However, the test site for V3_{IO} was the more pliable alveolar mucosa overlying the mental foramen which may have become stretched with the use of the retractor and may have influenced the gingival somatosensation. Furthermore, the stimulus electrode used in this study was developed for the skin and may not be directly transferable to the oral mucosa. Further research, beginning with the development of a self-adhesive “nociceptive-specific” stimulus electrode specifically for intraoral use, may be of clinical and diagnostic value. In the present study, the test sites were based on the ease of placement of stimulus electrode and the participant comfort. Consequently, the results presented are specific to the sites assessed. Regional differences in sensitivity within the oral cavity dictates that future research on intraorally-evoked nBR take multiple intraoral test sites into consideration [40, 51, 52]. Additionally, some studies have suggested gender based differences in certain blink reflex responses on extraoral stimulation [53, 54]. However, the sample size of the present study was insufficient for investigation of differences between the genders, which should be considered while interpreting the results. Furthermore, the present study was performed on healthy participants and therefore only assessed the feasibility and reliability of the intraorally-evoked nBR. Future studies with *in situ* testing of nBR in patients with nerve damage to the intraoral region may prove beneficial in establishing the validity of intraorally-evoked nBR. Since evoking nBR with stimulation of the V3_{IO} site is shown to be not feasible, development of other suitable electrophysiological measures may be needed.

Conclusion

In conclusion, the main finding of the present study was that the “nociceptive-specific” blink reflex could be evoked in up to 70% of healthy participants by intraoral stimulation of distal branches of the V2 branch, and in up to 47% of participants by intraoral stimulation of distal V3 branches. The R2 components were lower in amplitude when using intraoral stimulation compared with extraoral stimulation, and they showed fair to excellent reliability for the V2 branch, but low reliability for the V3 branch. Therefore, intraoral stimulation of the V2 branch, but not of the V3 branch, can be used reliably to elicit the nBR and may be useful for the assessment of damage to the distal nerves supplying the maxillary intraoral region.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This study was funded by the Danish Dental Association.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. Peñarrocha M, Cervelló M, Martí E, Bagán J (2007) Trigeminal neuropathy. *Oral Dis* 13:141–150. <https://doi.org/10.1111/j.1601-0825.2006.01356.x>
2. Baron R, Binder A, Wasner G (2010) Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol* 9:807–819. [https://doi.org/10.1016/S1474-4422\(10\)70143-5](https://doi.org/10.1016/S1474-4422(10)70143-5)
3. Cruccu G, Iannetti GD, Marx JJ, et al (2005) Brainstem reflex circuits revisited. *Brain J Neurol* 128:386–394. <https://doi.org/10.1093/brain/awh366>
4. Finnerup NB, Haroutounian S, Kamerman P, et al (2016) Neuropathic pain: an updated grading system for research and clinical practice. *Pain* 157:1599–1606. <https://doi.org/10.1097/j.pain.0000000000000492>
5. Galeotti F, Truini A, Cruccu G (2006) Neurophysiological assessment of craniofacial pain. *J Headache Pain* 7:61–69. <https://doi.org/10.1007/s10194-006-0287-1>
6. Ellrich J, Bromm B, Hopf HC (1997) Pain-evoked blink reflex. *Muscle Nerve* 20:265–270. [https://doi.org/10.1002/\(SICI\)1097-4598\(199703\)20:3<265::AID-MUS1>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1097-4598(199703)20:3<265::AID-MUS1>3.0.CO;2-9)
7. Baad-Hansen L, List T, Kaube H, et al (2006) Blink reflexes in patients with atypical odontalgia and matched healthy controls. *Exp Brain Res* 172:498–506. <https://doi.org/10.1007/s00221-006-0358-1>
8. Baad-Hansen L, List T, Jensen TS, et al (2005) Blink reflexes in patients with atypical odontalgia. *J Orofac Pain* 19:239–247
9. Katsarava Z, Giffin N, Diener H-C, Kaube H (2003) Abnormal habituation of “nociceptive” blink reflex in migraine--evidence for increased excitability of trigeminal nociception. *Cephalalgia* 23:814–819. <https://doi.org/10.1046/j.1468-2982.2003.00591.x>
10. Marin J, Gantenbein A, Paemeleire K, et al (2015) Nociception-specific blink reflex: pharmacology in healthy volunteers. *J Headache Pain* 16:87
11. Peddireddy A, Wang K, Svensson P, Arendt-Nielsen L (2009) Blink reflexes in chronic tension-type headache patients and healthy controls. *Clin Neurophysiol* 120:1711–1716. <https://doi.org/10.1016/j.clinph.2009.06.024>
12. Taiminen T, Kuusalo L, Lehtinen L, et al (2011) Psychiatric (axis I) and personality (axis II) disorders in patients with burning mouth syndrome or atypical facial pain. *Scand J Pain* 2:155–160. <https://doi.org/10.1016/j.sjpain.2011.06.004>
13. Kaube H, Katsarava Z, Käufer T, et al (2000) A new method to increase nociception specificity of the human blink reflex. *Clin Neurophysiol* 111:413–416. [https://doi.org/10.1016/S1388-2457\(99\)00295-3](https://doi.org/10.1016/S1388-2457(99)00295-3)
14. Kimura J (2005) The blink reflex. In: *Electrodiagnosis in Clinical Neurology*, 5th ed. pp 371–387
15. Obermann M, Yoon M-S, Eise D, et al (2007) Impaired trigeminal nociceptive processing in patients with trigeminal neuralgia. *Neurology* 69:835–841. <https://doi.org/10.1212/01.wnl.0000269670.30045.6b>

16. Baad-Hansen L, Pigg M, Ivanovic SE, et al (2013) Intraoral somatosensory abnormalities in patients with atypical odontalgia—a controlled multicenter quantitative sensory testing study. *Pain* 154:1287–1294. <https://doi.org/10.1016/j.pain.2013.04.005>
17. Baad-Hansen L, Pigg M, Ivanovic SE, et al (2013) Chairside intraoral qualitative somatosensory testing: reliability and comparison between patients with atypical odontalgia and healthy controls. *J Orofac Pain* 27:165–170
18. Pigg M, Baad-Hansen L, Svensson P, et al (2010) Reliability of intraoral quantitative sensory testing (QST). *Pain* 148:220–226. <https://doi.org/10.1016/j.pain.2009.10.024>
19. Costa YM, Baad-Hansen L, Bonjardim LR, et al (2017) Reliability of the nociceptive blink reflex evoked by electrical stimulation of the trigeminal nerve in humans. *Clin Oral Investig* 21:2453–2463. <https://doi.org/10.1007/s00784-016-2042-6>
20. Baad-Hansen L, List T, Jensen TS, Svensson P (2006) Increased pain sensitivity to intraoral capsaicin in patients with atypical odontalgia. *J Orofac Pain* 20:107–114
21. Jääskeläinen SK (1995) Electrophysiological study of blink reflex in humans: differences in mental and supraorbital nerves. *Acta Physiol Scand* 154:143–150. <https://doi.org/10.1111/j.1748-1716.1995.tb09896.x>
22. Katsarava Z, Ellrich J, Diener HC, Kaube H (2002) Optimized stimulation and recording parameters of human “nociception specific” blink reflex recordings. *Clin Neurophysiol* 113:1932–1936. [https://doi.org/10.1016/S1388-2457\(02\)00307-3](https://doi.org/10.1016/S1388-2457(02)00307-3)
23. Rossi B, Risaliti R, Rossi A (1989) The R3 component of the blink reflex in man: a reflex response induced by activation of high threshold cutaneous afferents. *Electroencephalogr Clin Neurophysiol* 73:334–340. [https://doi.org/10.1016/0013-4694\(89\)90111-9](https://doi.org/10.1016/0013-4694(89)90111-9)
24. Ellrich J, Treede R-D (1998) Characterization of blink reflex interneurons by activation of diffuse noxious inhibitory controls in man. *Brain Res* 803:161–168. [https://doi.org/10.1016/S0006-8993\(98\)00646-5](https://doi.org/10.1016/S0006-8993(98)00646-5)
25. de C Williams AC, Davies HT, Chadury Y (2000) Simple pain rating scales hide complex idiosyncratic meanings. *Pain* 85:457–463. [https://doi.org/10.1016/S0304-3959\(99\)00299-7](https://doi.org/10.1016/S0304-3959(99)00299-7)
26. Bartlett MS (1947) The use of transformations. *Biometrics* 3:39–52
27. de Vet HCW, Terwee CB, Knol DL, Bouter LM (2006) When to use agreement versus reliability measures. *J Clin Epidemiol* 59:1033–1039. <https://doi.org/10.1016/j.jclinepi.2005.10.015>
28. Rosner B (2011) *Fundamentals of biostatistics*, 7th ed. Brooks/Cole, Cengage Learning, Boston
29. Esteban A (1999) A neurophysiological approach to brainstem reflexes. Blink reflex. *Clin Neurophysiol* 29:7–38. [https://doi.org/10.1016/S0987-7053\(99\)80039-2](https://doi.org/10.1016/S0987-7053(99)80039-2)
30. Lang PJ, Bradley MM, Cuthbert BN (1990) Emotion, attention, and the startle reflex. *Psychol Rev* 97:377–395. <https://doi.org/10.1037/0033-295X.97.3.377>
31. Bufacchi RJ, Iannetti GD (2016) Gravitational cues modulate the shape of defensive peripersonal space. *Curr Biol* 26:R1133–R1134. <https://doi.org/10.1016/j.cub.2016.09.025>

32. Bufacchi RJ, Liang M, Griffin LD, Iannetti GD (2015) A geometric model of defensive peripersonal space. *J Neurophysiol* 115:218–225. <https://doi.org/10.1152/jn.00691.2015>
33. Sambo CF, Forster B, Williams SC, Iannetti GD (2012) To Blink or Not to Blink: Fine Cognitive Tuning of the Defensive Peripersonal Space. *J Neurosci* 32:12921–12927. <https://doi.org/10.1523/JNEUROSCI.0607-12.2012>
34. Sambo CF, Liang M, Cruccu G, Iannetti GD (2011) Defensive peripersonal space: the blink reflex evoked by hand stimulation is increased when the hand is near the face. *J Neurophysiol* 107:880–889. <https://doi.org/10.1152/jn.00731.2011>
35. Cadden SW, Newton JP (1988) A comparison of reflex depressions of activity in jaw-closing muscles evoked by intra- and peri-oral stimuli in man. *Arch Oral Biol* 33:863–869. [https://doi.org/10.1016/0003-9969\(88\)90014-3](https://doi.org/10.1016/0003-9969(88)90014-3)
36. Capra NF (1995) Mechanisms of oral sensation. *Dysphagia* 10:235–247. <https://doi.org/10.1007/BF00431416>
37. Naganawa T, Baad-Hansen L, Ando T, Svensson P (2015) Influence of topical application of capsaicin, menthol and local anesthetics on intraoral somatosensory sensitivity in healthy subjects: temporal and spatial aspects. *Exp Brain Res* 233:1189–1199. <https://doi.org/10.1007/s00221-015-4200-5>
38. Posnick JC, Zimble AG, Grossman JAI (1990) Normal Cutaneous Sensibility of the Face. *Plast Reconstr Surg* 86:429
39. Desjardins RP, Winkelmann RK, Gonzalez JB (1971) Comparison of nerve endings in normal gingiva with those in mucosa covering edentulous alveolar ridges. *J Dent Res* 50:867–879. <https://doi.org/10.1177/00220345710500041401>
40. Dixon AD (1962) The position, incidence and origin of sensory nerve terminations in oral mucous membrane. *Arch Oral Biol* 7:39–IN9. [https://doi.org/10.1016/0003-9969\(62\)90046-8](https://doi.org/10.1016/0003-9969(62)90046-8)
41. Bufacchi RJ, Iannetti GD (2018) An Action Field Theory of Peripersonal Space. *Trends Cogn Sci* 22:1076–1090. <https://doi.org/10.1016/j.tics.2018.09.004>
42. Pigg M, Svensson P, List T (2011) Orofacial thermal thresholds: time-dependent variability and influence of spatial summation and test site. *J Orofac Pain* 25:39–48
43. Svensson P, Bjerring P, Arendt-Nielsen L, Kaaber S (1993) Sensory and pain thresholds to orofacial argon laser stimulation in patients with chronic burning mouth syndrome. *Clin J Pain* 9:207–215
44. Aramideh M, Ongerboer De Visser BW (2002) Brainstem reflexes: Electrodiagnostic techniques, physiology, normative data, and clinical applications. *Muscle Nerve* 26:14–30. <https://doi.org/10.1002/mus.10120>
45. Sessle BJ, Greenwood LF (1976) Inputs to trigeminal brain stem neurones from facial, oral, tooth pulp and pharyngolaryngeal tissues: I. responses to innocuous and noxious stimuli. *Brain Res* 117:211–226. [https://doi.org/10.1016/0006-8993\(76\)90731-9](https://doi.org/10.1016/0006-8993(76)90731-9)

46. Bliese PD (2000) Within-group agreement, non-independence, and reliability: Implications for data aggregation and analysis. In: Multilevel theory, research, and methods in organizations: Foundations, extensions, and new directions. Jossey-Bass, San Francisco, CA, US, pp 349–381
47. Kovacs FM, Abraira V, Royuela A, et al (2008) Minimum detectable and minimal clinically important changes for pain in patients with nonspecific neck pain. *BMC Musculoskelet Disord* 9:43. <https://doi.org/10.1186/1471-2474-9-43>
48. Castroflorio T, Icardi K, Torsello F, et al (2005) Reproducibility of Surface EMG in the Human Masseter and Anterior Temporalis Muscle Areas. *Cranio* 23:130–137. <https://doi.org/10.1179/crn.2005.018>
49. Ferrario VF, Sforza C, D’addona A, Miani A (1991) Reproducibility of electromyographic measures: a statistical analysis. *J Oral Rehabil* 18:513–521. <https://doi.org/10.1111/j.1365-2842.1991.tb00074.x>
50. Suvinen TI, Malmberg J, Forster C, Kemppainen P (2009) Postural and dynamic masseter and anterior temporalis muscle EMG repeatability in serial assessments. *J Oral Rehabil* 36:814–820. <https://doi.org/10.1111/j.1365-2842.2009.01999.x>
51. Grossman RC, Hattis BF, Ringel RL (1965) Oral tactile experience. *Arch Oral Biol* 10:691–IN36. [https://doi.org/10.1016/0003-9969\(65\)90014-2](https://doi.org/10.1016/0003-9969(65)90014-2)
52. Svensson P, Baad-Hansen L, Pigg M, et al (2011) Guidelines and recommendations for assessment of somatosensory function in oro-facial pain conditions--a taskforce report. *J Oral Rehabil* 38:366–394. <https://doi.org/10.1111/j.1365-2842.2010.02196.x>
53. Kofler M, Kumru H, Schaller J, Saltuari L (2013) Blink reflex prepulse inhibition and excitability recovery: Influence of age and sex. *Clin Neurophysiol* 124:126–135. <https://doi.org/10.1016/j.clinph.2012.07.001>
54. Peddireddy A, Wang K, Svensson P, Arendt-Nielsen L (2006) Influence of age and gender on the jaw-stretch and blink reflexes. *Exp Brain Res* 171:530–540. <https://doi.org/10.1007/s00221-005-0300-y>

Legends

Fig. 1 Intraoral placement of the stimulus electrode (red wire) using a Urihesive® (ConvaTec, UK) strip at (a) the mesial to the maxillary canine eminence to stimulate the anterior superior alveolar nerve (V2) and (b) near the mental foramen to stimulate the mental nerve (V3). Black arrow points to the location of the electrode under the Urihesive strip

Fig. 2 Averaged traces of the ipsilateral R2 component at 300% of pinprick threshold (I_p) for extraoral and intraoral V2 and V3 branches, as obtained from a single participant. The site and R2 amplitude in the time window of 27 to 87 ms of the “nociceptive-specific” blink reflex is presented on the left

Fig. 3 The distribution of participants with the R2 occurrences based on site and stimulation intensity. The extraoral and intraoral test sites of V2 and V3 branches of the trigeminal nerve ($V2_{EO}$, $V3_{EO}$, $V2_{IO}$, and $V3_{IO}$) were electrically stimulated at different intensities (100%, 150%, 200%, 300%, and 400% of the respective extraoral and intraoral pinprick thresholds). *indicates significant difference in proportion of R2 occurrence between extraoral and intraoral sites at any stimulus intensity, $p < 0.05$

Fig. 4 The numerical rating scale (NRS) pain scores (a) and the ipsilateral and contralateral R2 values presented as the root mean square (RMS) and assessed at 100%, 150%, 200%, 300%, and 400% of the *intraoral* pinprick threshold (I_P) at intraoral sites of stimulation for V2 (b) and V3 (c) branches of the trigeminal nerve. White markers denote significant difference from 100% of I_P .

Fig. 5 Electrically-evoked numerical rating scale (NRS) pain scores (a) and the ipsilateral (b) and contralateral (c) R2 values presented as the root mean square (RMS) and assessed at 100%, 150%, 200%, 300%, and 400% of the *extraoral* pinprick threshold at the extraoral and intraoral sites of stimulation for V2 and V3 branches of the trigeminal nerve. #indicates significant differences from stimulation with 100% of I_P , $p < 0.05$; *indicates significant differences between the extraoral and intraoral sites, $p < 0.05$; white markers indicate a significant difference from stimulation with 100% of I_P for each site, $p < 0.05$. **Only interactions are presented here. Please refer the results section for the main effects.**

Fig. 6 Comparisons of the interrater reliability for ‘nociceptive-specific’ blink reflex responses between operators in the same session (O_B and O_{A2}) and operators in different sessions (O_B and O_{A1}). Presented as intraclass correlation coefficient (ICC) for the V2 and V3 branches of trigeminal nerve. (a) Electrically-evoked sensory and pinprick thresholds. (b) Pain scores on a 0-100 numerical rating scale at different stimulus intensities. (c) Ipsilateral and (d) contralateral R2 values at different stimulus intensities. *A difference of more than 30% between the ICCs was considered as a marked difference

Fig. 7 Comparisons of the interrater reliability for ‘nociceptive-specific’ blink reflex responses between operators in the same session (O_B and O_{A2}) and operators in different sessions (O_B and O_{A1}), presented as intraclass correlation coefficient (ICC) for the V2 and V3 branches of trigeminal nerve. (a) Slope of numerical rating scale

(NRS) pain scores calculated from increasing stimulus intensities. (b) Slope of ipsilateral and (c) contralateral R2 values calculated from increasing stimulus intensities

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Table 1 Intrarater and interrater reliability of the psychophysical parameters of the ‘nociceptive-specific’ blink reflex (nBR) on intraoral stimulation of V2 and V3 branches.

	ICC	ICC-95% CI	SEM	ICC	ICC-95% CI	SEM	SDC
	Intrarater			Interrater			
Stimulation threshold (mA)							
V2							
I ₀	0.01	(-2.10 - 0.67)	0.1	-0.12	(-2.72 - 0.56)	0.1	0.4
I _P	0.50	(0.03 - 0.88)	0.3	0.58	(0.23 - 0.91)	0.3	0.7
V3							
I ₀	0.31	(-0.60 - 0.82)	0.1	-0.15	(-2.81 - 0.52)	0.3	0.7
I _P	0.67	(0.44 - 0.93)	0.3	0.62	(0.28 - 0.92)	0.4	1.2
Pain intensity (NRS: 0-100)							
V2							
100%	0.81	(0.70 - 0.96)	0.7	0.66	(0.41 - 0.93)	1.2	3.4
150%	-0.13	(-2.85 - 0.55)	3.6	-0.27	(-4.51 - 0.41)	4.1	11.2
200%	0.02	(-1.70 - 0.66)	9.8	0.43	(-0.14 - 0.86)	8.1	22.5
300%	0.70	(0.51 - 0.94)	11.8	0.57	(0.18 - 0.90)	12.6	34.9
400%	0.92	(0.89 – 0.99)	8.2	0.95	(0.93 – 0.99)	6.6	18.3
Slope	0.89	(0.84 - 0.98)	3.5	0.92	(0.88 - 0.98)	2.9	8.1
V3							
100%	0.04	(-1.54 - 0.67)	7.4	0.90	(0.85 - 0.98)	0.8	2.3
150%	0.18	(-0.96 - 0.76)	12.2	0.33	(-0.45 - 0.82)	4.7	13.2
200%	0.17	(-0.98 - 0.76)	12.1	0.14	(-1.38 - 0.74)	7.1	19.8

300%	0.69	(0.47 - 0.94)	11.9	0.78	(0.65 - 0.96)	9.8	27.1
400%	0.81	(0.71 – 0.96)	12.6	0.86	(0.79 – 0.97)	11.2	30.9
Slope	0.73	(0.56 - 0.95)	4.5	0.87	(0.81- 0.98)	3.8	10.5

ICC intraclass correlation coefficient, *CI* confidence interval, *SEM* standard error of measurement, *SDC* smallest detectable change, *I₀* sensory threshold, *I_p* pinprick threshold, *NRS* numeric rating scale, *V2* intraoral anterior superior alveolar nerve, *V3* intraoral mental nerve. Please refer to the text (section 2.5) for grading of the ICC values.

Table 2 Intrarater and interrater reliability of the R2 ipsilateral and contralateral values (μV) of the ‘nociceptive-specific’ blink reflex using intraoral stimulation.

% of I _P	Intrarater			Interrater			
	ICC	ICC 95% CI	SEM	ICC	ICC 95% CI	SEM	SDC
V2 (ipsilateral / contralateral)							
100%	0.52/0.73	(0.15 - 0.89)/ (0.56 - 0.95)	0.68/0.91	0.67/0.49	(0.42 - 0.93)/ (0.02 - 0.88)	0.64/1.45	1.77/4.02
150%	0.61/0.44	(0.31 - 0.92)/ (-0.18 - 0.87)	1.66/1.77	-0.02/0.05	(-2.19 - 0.64)/ (-1.88 - 0.70)	2.03/1.95	5.63/5.41
200%	0.50/0.65	(0.02 - 0.89)/ (0.40 - 0.93)	3.06/1.43	0.79/0.73	(0.68 - 0.96)/ (0.56 - 0.95)	1.83/1.73	5.08/4.79
300%	0.64/0.58	(0.36 - 0.93)/ (0.16 - 0.91)	4.10/2.50	0.31/0.59	(-0.31 - 0.81)/ (0.30 - 0.91)	4.48/2.82	12.42/7.82
400%	0.78/0.82	(0.65 - 0.96)/ (0.73 - 0.97)	3.12/1.67	0.72/0.43	(0.52 - 0.94)/ (-0.08 - 0.86)	3.76/4.23	10.43/11.74
Slope	0.76/0.76	(0.62 - 0.95)/ (0.61 - 0.95)	1.10/0.75	0.72/0.62	(0.54 - 0.94)/ (0.32 - 0.92)	1.14/1.21	3.17/3.35
V3 (ipsilateral / contralateral)							
100%	0.16/0.48	(-1.27 - 0.75)/ (-0.05 - 0.88)	0.81/1.06	0.55/0.54	(0.17 - 0.90)/ (0.20 - 0.89)	0.50/1.09	1.39/3.01
150%	0.24/0.60	(-0.86 - 0.79)/ (0.28 - 0.91)	0.80/0.91	0.01/0.50	(-2.17 - 0.67)/ (0.10 - 0.88)	1.45/0.95	4.02/2.62
200%	0.46/0.45	(-.02 - 0.87)/ (0.00 - 0.86)	0.91/1.01	0.63/0.48	(0.37 - 0.92)/ (-0.06 - 0.88)	0.82/1.18	2.27/3.28

300%	0.12/0.20	(-1.47 - 0.73)/ (-1.07 - 0.77)	1.48/0.98	0.01/0.09	(-2.05 - 0.67)/ (-1.07 - 0.69)	1.87/1.56	5.18/4.31
400%	0.33/-0.01	(-0.52 - 0.83)/ (-2.35 - 0.66)	1.41/1.53	0.25/0.29	(-0.76- 0.79)/ (-0.54 - 0.80)	3.49/2.85	9.68/7.91
Slope	0.33/0.12	(-1.51 - 0.73)/ (-0.28 - 0.66)	0.49/0.64	0.21/0.14	(-0.90 - 0.77)/ (-1.25 - 0.74)	1.02/0.98	2.83/2.72

ICC intraclass correlation coefficient, *CI* confidence interval, *SEM* standard error of measurement, *SDC* smallest detectable change, *I₀* sensory threshold, *I_P* pinprick threshold, *NRS* numeric rating scale, *RMS* root mean square, *V2* intraoral anterior superior alveolar nerve, *V3* intraoral mental nerve. Please refer to the text (section 2.5) for grading of the ICC values.