UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

YURI MARTINS COSTA

Systematic analysis of the nociceptive blink reflex: reliability and association with psychological factors

Análise sistemática do reflexo de piscar nociceptivo: confiabilidade e associação com fatores psicológicos

BAURU
2015

YURI MARTINS COSTA

Systematic analysis of the nociceptive blink reflex: reliability and association with psychological factors

Análise sistemática do reflexo de piscar nociceptivo: confiabilidade e associação com fatores psicológicos

Tese constituída por artigos apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no programa de Ciências Odontológicas Aplicadas, área de concentração Estomatologia e Biologia Oral.

Orientador: Prof. Dr. Paulo César Rodrigues Conti

BAURU
2015

Costa Martins, Yuri
C823s Systematic analysis of the nociceptive blink reflex: reliability and association with psychological factors / Yuri Martins Costa. – Bauru, 2015.
80 p.: il. ; 31cm

Tese (Doutorado) – Faculdade de Odontologia de Bauru. Universidade de São Paulo

Orientador: Prof. Dr. Paulo César Rodrigues Conti

Comitê de Ética Jutlândia
nº 1-10-72-257-13
Data: 02 de Outubro de 2013

ACKNOWLEDGMENTS

I believe that eventually it comes the point in our lifetime where we realize how insignificant we are. Our inner substance was given to us and we do not have any consistency because "for dust you are and to dust you shall return – Genesis 3:18". So, in the end, there is absolutely nothing to be proud of. Everything that we have, know, realize, and so on, was obtained from external sources. One of the synonyms for pride is vainglory and it is exactly what pride is: a glory in vain, i.e., an empty glory. Therefore, I would like to express my deepest gratitude to all the ones who have so far contributed to absolute everything I am and every accomplishment I have made.

First and foremost it would be unfair not thinking that God has been merciful on me and has been seeking after me all the time. So, I would like to praise and thank God for that.

I am especially grateful to my supervisor Professor Paulo César Rodrigues Conti for all opportunities, guidance and valuable help offered to me during the last 5 years and I am also thankful for the example he has provided as a successful professor.

My period in Denmark was a lifetime experience: full of new discoveries, challenges and, certainly, a milestone in my scientific journey. Hence, I would like to express all my appreciation and gratitude to my Danish supervisor Professor Peter Svensson for having accepted me as one of his students. It was a great honor to be part of the Section of Orofacial Pain and Jaw Function, Department of Dentistry, Aarhus University, a.k.a. "OSK family" under his major leadership. His work is definitely an inspiration to me. Also, I am deeply thankful to my co-supervisor Professor Lene Baad-Hansen. Together with Peter, she has taught me how good orofacial neuroscience research is done. I appreciate all her contributions and efforts, which surely made my researches more productive and stimulating.

Professor Eduardo Castrillon and Karina Bendixen, which are also part of the leadership of the OKS family, had an outstanding participation in my professional development during my one-year period in Aarhus. I warmly thank them for their friendly help and contributions. I also wish to thank my "5th floor's" colleagues Abhishek Kumar, Akiko Shimada, Bahram Ranjkesh, Jimoh Agbaje, Keita Ishizuka, Kayo Suzuki, Lilja Dagsdóttir, Line Larsen, Marina Roscoe, Mika Honda, Mohit and Simple Kothari, Shun-ich Suzuki, Xinwen Zhou and Xuemei Liu for their kindly support and fellowship. Also, I would like to thank Hanne Langgaard and Bente Haugsted for their tenderness and kindness towards me, which certainly made my work happier and nicer. I also would like to express my

gratitude to the senior researcher Páll Karsson. It was an honor to work with him.

A very special thanks goes to my dearest friend Rubens Spin-Neto and his wife Carolina Silverio. I'll never forget their loving-kindness. Surely, they made my "Danish life" easier and much better. He is also a living example of a brilliant scientist.

Finally, I would like to thank my dear friend and brother in faith Anders Madsen for all his benevolence and encouragement. His values and example motivated me to become a better person. Also, my deepest gratitude to all seventh-day Adventists in Aarhus, in special to my good friends Stephen Baffour, Pernille Rasmussen, Bjarne Rasmussen, Tina Maria, John Vidrine and Per Christiansen. I'm very flattered with their sympathy and friendship.

Back to Brazil, I will now thank the people who supported me during all my period abroad. Undoubtedly, I wouldn't have made it without them. First and foremost, I owe the greatest thank you to my mom and dad. They are the main drivers behind all my accomplishments and the promoters of all my dreams. Certainly, nothing would be possible without their support, love and selflessness. Similarly, I would like to thank my beloved sister for her colossal consideration towards me during the difficult times.

Likewise, I wish to express all my thankfulness to my friend and professor Leonardo Bonjardim for bearing with me all those days when I was sad, anxious and afraid. His support was refreshment to my mind and his friendship is of utmost value for me.

My warmest gratitude goes to my lifelong friend Rodrigo Safatle. I am very flattered for all his support, advices and friendship throughout the last 13 years.

I would also like to thank my beloved friends and partners André Porporatti and Juliana Stuginski-Barbosa for their close and inspiring working relationship. It has been a great pleasure to work with them. Similarly, I want to express my gratefulness to my all colleagues from the Department of Prosthodontics, Bauru School of Dentistry, in particular to Adriana Braga, Aline Baía, Carolina Ortigosa, Denise Gusmão, Fernanda Araújo, Lívia Maria, Naila Machado and Vinícius Fardin. Their companionship is highly appreciated.

I further would like to thank the professors and staff at Bauru School of Dentistry, in particular, the Department of Prosthodontics and the Post-Graduation Section for their assistance in all steps of my studies and activities. Their support is of greatest importance. Also, I would like to thank the Coordination for the Improvement of

Higher Education Personnel (CAPES) for the financial support during my one year abroad and the Graduate School at Health, Aarhus University for the 5-month grant. Finally, my deepest appreciation goes to all participants of my experiments. Their contributions were absolutely essential.

"Two things fill me with constantly increasing admiration and awe, the longer and more earnestly I reflect on them: the starry heavens without and the moral law within".

Immanuel Kant

ABSTRACT

Systematic analysis of the nociceptive blink reflex: reliability and association with psychological factors

The present study aimed to estimate the reliability of the nociceptive blink reflex (nBR) and to evaluate the possible association between the nBR and various pain-related psychological measures: the Anxiety Sensitivity Index-3 (ASI-3), the Fear of Pain Questionnaire III (FPQ-III), the Pain Vigilance and Awareness Questionnaire (PVAQ), the Somatosensory Amplification Scale (SSAS), the Pain Catastrophizing Scale (PCS) and the Situational Pain Catastrophizing Scale (S-PCS). Twenty-one healthy participants were evaluated in two sessions. The nBR was elicited by a so-called "nociceptive-specific" electrode placed over the entry zone of the right supraorbital (V1R), infraorbital (V2R) and the mental (V3R) nerve and left infraorbital (V2L) nerve. The outcomes were: (a) individual electrical sensory (I_0) and pain thresholds (I_P); b) root mean square (RMS), area-under-the-curve (AUC) and onset latencies of R2 responses; and c) stimulus-evoked pain on a 0-10 numerical rating scale. The questionnaires ASI-3, FPQ-III, PVAQ, SSAS, PCS and S-PCS were also applied. Intraclass Correlation Coefficients (ICCs) and Kappa statistics were computed as a measure of the reliability (α=5%). Besides, Pearson correlation coefficient was used to associate the average of nBR measurements among all sites and the questionnaires. The significance level was set up after a Bonferroni correction (adjusted α=0.8%). ICCs were fair to excellent in 82% of the psychophysical measures and in 86% of V1R, V2R and V2L nBR parameters, whereas the V3R showed poor reliability in 52%. ICCs for intrarater reliability were fair to excellent in 70% of measurements (V3R showed the lowest values) and in 75% of interrater measurements. All kappa values showed at least fair agreement and the majority of the nBR measures (93%) were considered to have moderate to excellent reliability. There was no correlation for any pair of variables considering the adjusted significance level ($p>0.008$) and only a single significant correlation considering the standard significance level ($p < 0.05$), where the pain intensity (NRS) at 50% of I_P presented a positive and small to moderate correlation with the PCS ($r = 0.43$, $p = 0.047$). The nBR and its associated psychophysical measures can be considered a sufficiently reliable test to assess the trigeminal nociceptive function. On the other hand, it seems not associated with psychological factors in healthy participants.

Key words: Nociceptive Blink Reflex. Reliability. Biopsychosocial Pain Model.

RESUMO

Análise sistemática do reflexo de piscar nociceptivo: confiabilidade e associação com fatores psicológicos

O presente estudo teve como objetivo estimar a confiabilidade do reflexo de piscar nociceptivo (nBR, sigla em inglês) e avaliar a possível associação entre o nBR e várias medidas psicológicas relacionadas à dor: o Anxiety Sensitivity Index-3 (ASI-3), o Fear of Pain Questionnaire III (FPQ-III), o Pain Vigilance and Awareness Questionnaire (PVAQ), o Somatosensory Amplification Scale (SSAS), o Pain Catastrophizing Scale (PCS) e o Situational Pain Catastrophizing Scale (S-PCS) (siglas e nomes em inglês). Vinte e um participantes saudáveis foram avaliados em 2 sessões. O nBR foi estimulado por meio de um eletrodo "nociceptivo específico" posicionado na zona de entrada do nervo supraorbital direito (V1D, sigla em inglês), infraorbital direito (V2D) e esquerdo (V2E) e mentual direito (V3R). As variáveis analisadas foram: a) limar elétrico sensorial (I_0) e doloroso (I_P); b) raíz quadrática média (RMS, sigla em inglês), área sobre a curva (AUC, sigla em inglês) e as latências da respostas R2; e c) dor provocada pelo estímulo em uma escala numérica de O a 10. Os questionários ASI-3, FPQ-III, PVAQ, SSAS, PCS e S-PCS também foram aplicados. Coeficiente de Correlação Intraclasse (ICC, sigla em inglês) e estatística Kappa foram calculados como medidas da confiabilidade (α=5%). Além disso, coeficiente de correlação de Pearson foi usado para associar a média do nBR entre todos os sítios de avaliação e os questionários. O nível de significância foi ajustado após correção de Bonferroni (α ajustado=0.8%). ICCs foram razoáveis à excelentes em 82% das medidas psicofísicas e em 86% dos parâmetros do nBR em V1D, V2D e V2E, enquanto que 52% das medidas em V3D apresentaram pobre confiabilidade. ICCs para confiabilidade intra-examinador foram razoáveis à excelente em 70% das medições (V3D apresentou os menores valores) e em 75% das medidas inter-examinadores. Todos os coeficientes Kappa apresentaram pelo menos razoável concordância e a maioria das medidas do nBR (93%) foram consideradas moderadas à excelente em termos de confiabilidade. Não houve correlação para nenhum par de variáveis considerando os valores ajustados de significância ($p>0,008$) e somente foi constatada uma correlação significante considerando o nível de significância padrão ($p<0,005$), em que a intensidade de dor em 50% do I_P apresentou uma correlação positiva entre pequena e moderada com o PCS. O nBR e suas medidas psicofísicas associadas pode ser considerado um teste com suficiente confiabilidade para avaliar a função nociceptiva trigeminal. Por outro lado, parece que o nBR não está associado com fatores psicológicos em participantes saudáveis.

Palavras-chave: Reflexo de Piscar Nociceptivo. Confiabilidade. Modelo Biopsicossocial da Dor.

TABLE OF CONTENTS

1 Introduction

1 INTRODUCTION

The British physician Walker Overend in a letter published in Lancet on March 7th, 1896, made the first description of eyelid twitches elicited by a gentle tap with a stethoscope (FINE; SENTZ; SORIA, 1992). Later on, in the beginning of 1950s, Erik Kugelberg first established the electrophysiological mechanisms and pathways of the so-called blink reflex (BR) using an oscilloscope and electrical stimulations (KUGELBERG, 1952). Nowadays, the BR is one of the most accessible neurophysiological methods for evaluating functions either integrated in or mediated by the brainstem (VALLS-SOLE, 2005). In particular, the BR has been used to assess the trigeminal function (ELLRICH, 2000).

The physiology and anatomical distribution of the BR can be described as follows: a) the peripheral pathways consist of trigeminal cutaneous fibers, responsible for the afferent limb and a group of motor fibers of the facial nerve, which constitute the efferent limb; b) the central pathways consist of interneurons located at the main and spinal trigeminal and the facial nucleus (ESTEBAN, 1999). Furthermore, there are some particularities related to recordable electromyography (EMG) responses that justify the diagnostic value of the BR. These EMG parameters can be divided into three categories: an early ipsilateral component (R1) with onset latency of 11 ms and two bilateral components (R2 and R3) with onset latencies of, respectively, 33 and 84 ms (ELLRICH; HOPF, 1996). The R1 response is mainly mediated by low-threshold mechanoreceptors (A□ fibers) and the R2 is mediated by both mechanical and nociceptive afferents (A□ fibers) (ELLRICH, 2000). Besides these differences in the peripheral pathways, the central components of R1 and R2 are also different: oligosynaptic connections between the main trigeminal and facial nucleus are related to the R1 responses whereas polysynaptic connections between the spinal trigeminal and the facial nucleus drive the R2 responses. Finally, the R3 responses, which is also part of the startle reaction, share similar pathways of R2 (ESTEBAN, 1999). Therefore, the analysis of these different response patterns and their anatomical arrangement may help in the topographical classification of lesions that could affect the brainstem (VALLS-SOLE, 2005).

Given the highly non-selective nature of the BR, the nociceptive blink reflex (nBR) is an alternative method where the electrical stimulation is applied with a specific type of electrode designed to almost selectively activate neuronal receptors associated with small diameter fibers, i.e., A-delta and C fibers. The electrode design and the proof of concept for

its nociceptive-specific nature were first published fifteen years ago (KAUBE et al., 2000). Since then, this approach has been proven useful to evaluate chronic orofacial and headache pain conditions (KAUBE et al., 2002; BAAD-HANSEN et al., 2005; BAAD-HANSEN et al., 2006; PEDDIREDDY et al., 2009). Yet, despite its clinical utility and application in the pain field, there is a lack of either a detailed report regarding the reliability of the nBR or the assessment of psychological factors that could affect the nBR responses.

The quality of measurement instruments is crucial for the development of the Health Sciences (BANNIGAN; WATSON, 2009). Well-designed and appropriate instruments form the basis of diagnosis, prognosis and evaluation of the results of medical interventions. In such scenario, the reliability can be considered an important measurement property and should be properly established for any medical test. The reliability of a measurement can be defined as the proportion of the total variance in the measurements which is because of "true" differences among patients, i.e., is the average score that would be obtained if the scale were given an infinite number of times (MOKKINK et al., 2010). So, the reliability expresses how well subjects can be distinguished from each other despite the presence of measurement error.

The medical field has evolved, since the beginning of the modern science, with the focus in fractional-analytic way on biological process and assuming that the diseases should be investigated in terms of causal chains of materially and conceptually comprehensible parts (ENGEL, 1977). Taking together with the abstract aspect of the human mind and the lack of appropriate methods to probe it, it is conceivable that this mechanistic view, i.e., the biomedical model in which the biological phenomena can be fully explained by physical variables, has been the mainstream of medicine for so long. However, the human pain experience, in particular the chronic pain conditions, is a vulnerable point for the biomedical model, considering that it is not fully possible to explain the disability and the burden of pain only in terms of bodily impairments and biochemical reactions (DUNCAN, 2000). Hence, the need for a new medical model, e.g., the biopsychosocial model, have risen in the late 70s as an attempt to elucidate how somatic processes affect the mental states and vice-versa (ENGEL, 1977). Within this paradigm, painful experiments or experiences could be better understood together with their cultural, social, psychological and behavioral dimensions.

Based on that, the purposes of the two studies that constitute this thesis were: 1) to estimate the reliability of the nBR and; 2) to evaluate the association of psychological factors with the nBR responses. Finally, our overall hypotheses were: the nBR is a reliable test and the psychological factors are correlated with the nBR responses.

2 Articles

2 ARTICLES

The article 1 presented in this thesis is currently under review in the journal *Clinical Oral Investigations* (Annex 1) and the article 2 was recently accepeted for publication in the journal *Journal of Oral & Facial Pain and Headache* (Annex 2). Both articles had the approval from the Regional Ethics Committee as well as the Danish Data Protection Agency (Annex 3).

2.1. ARTICLE 1

Reliability of the nociceptive blink reflex evoked by electrical stimulation of the trigeminal nerve.

Authors: Yuri Martins Costa[* a,b,c], Lene Baad-Hansen [a,b], Leonardo Rigoldi Bonjardim [d], Paulo César Rodrigues Conti [c], Peter Svensson [a,b,e].

[a] Section of Orofacial Pain and Jaw Function, Department of Dentistry, Aarhus University, Aarhus, Denmark.

[b] Scandinavian Center for Orofacial Neurosciences (SCON).

[c] Department of Prosthodontics, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

[d] Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

[e] Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden.

Abstract

Objective: To determine the reliability of the nociceptive blink reflex (nBR) of the trigeminal nerve.

Materials and Methods: Twenty-one healthy participants were evaluated in two sessions. The nBR was elicited by a so-called "nociceptive-specific" electrode placed over the entry zone of the right supraorbital (V1R), infraorbital (V2R) and mental (V3R) and left infraorbital (V2L) nerve. The outcomes were: individual electrical sensory (I_0) and pain thresholds (I_P); root mean square (RMS), area-under-the-curve (AUC) and onset latencies of R2 responses; stimulus-evoked pain on a 0-10 numerical rating scale. Intraclass Correlation Coefficients (ICCs) and Kappa statistics were computed (α=5%).

Results: ICCs were fair to excellent in 82% of the psychophysical measures and in 86% of V1R, V2R V2L nBR parameters, whereas the V3R showed poor reliability in 52%. ICCs for intrarater reliability were fair to excellent in 70% of measurements (V3R showed the lowest values) and in 75% of interrater measurements. All kappa values showed at least fair agreement and the majority of the nBR measures (93%) presented moderate to excellent reliability.

Conclusion: The nBR and its associated psychophysical measures can be considered a sufficiently reliable test.

Clinical Significance: The nBR can be prescribed to assess trigeminal nociceptive function in patients with orofacial pain complaints.

Keywords: Nociceptive Blink Reflex, Trigeminal Nerve, Reliability, Intraclass Correlation Coefficient.

1. Introduction

In general, clinical pain assessment rely on methods dependent on patient participation [1, 2] but there are some options for an objective investigation of the trigeminal nociception [3]. The blink reflex (BR) is an electrophysiological test routinely used to evaluate the cranial nerves and brainstem functions [4]. Originally evoked by mechanical stimulation (gentle tap on the forehead), this brainstem reflex is now typically elicited by electrical stimulation of the trigeminal nerve, usually the ophthalmic division [5]. Briefly, standard electrical stimulation and the electromyography (EMG) records of the BR consist of three responses: an early ipsilateral component (R1) with onset latency of 11 ms and two bilateral components (R2 and R3) with onset latencies of, respectively, 33 and 84 ms [6]. Given the largely non-selective nature of the BR (the R1 response is mediated by low-threshold mechanoreceptors (Aβ fibers) and the R2 can be elicited by both mechanical and nociceptive afferents (Aδ fibers) [7], a valid selective assessment of the trigeminal nociceptive fibers is not possible through standard electrical stimulation. However, concentric surface electrodes which produce high current density at low intensities have been used as a non-invasive method to more selectively activate the nociceptive fibers and thereby increasing the “nociceptive” specificity of the BR [8].

The “nociceptive” specificity of the BR elicited by different electrode configurations is a topic under debate [9]. Neurophysiological evidence has pointed out a “contamination” with Aβ fibers activity using concentric surface electrodes [10]. However, since the subjective pinprick sensation evoked by concentric electrode stimulation is consistent with activation of Aδ fibers and clearly different from sensations obtained with standard electrical stimulation. this technique can be regarded as useful to investigate the nociceptive trigeminal reflex [11]. Thus, the so-called “nociceptive” blink reflex (nBR) could be used to evaluate several aspects of the pain experience, e.g., modulation of the pain perception and alterations in pain processing [12,

13]. Consequently, some pathophysiological aspects of primary headaches and chronic orofacial pain have been addressed and elucidated by the nBR evoked by stimulation of all three branches of the trigeminal [14-17]. Nevertheless, despite its clinical relevance and regular use, previous studies have not in detail reported the reliability of the nBR.

Estimating the reliability of any medical measurement is an essential step towards the elaboration of an appropriate clinical instrument [18]. A consensus initiative defines reliability as "the extent to which scores for patients who have not changed are the same for repeated measurement under several conditions: for example, using different sets of items from the same health-related patient-reported outcomes (internal consistency), over time (test–retest), by different persons on the same occasion (interrater) or by the same persons (i.e., raters or responders) on different occasions (intrarater)" [19]. In other words, the reliability quantifies how much a measurement is the same each time it is measured and by whoever measures it. In this context, only two studies have estimated the reliability of the nBR. One study evaluated the reliability over time of the onset latency (intrarater) and the response area (test-retest) of the nBR elicited by stimulation of the supraorbital nerve and the authors reported high reliability for both onset latencies (Crohnbach's alpha = 0.85) and response areas (Crohnbach's alpha = 0.94) [20]. Another study evaluated, which EMG parameters were more reliable to define the nBR threshold and found that Blink Interval Peak z score showed the best performance [21]. However, to the best of our knowledge there is no available data regarding the reliability of the nBR considering the stimulation of all three branches of the trigeminal nerve and its associated psychophysical measures.

Based on that, the aim of this study was to estimate the reliability of the nBR evoked by electrical stimulation of the three branches of the trigeminal nerve under the following conditions: over time (test-retest and intrarater reliability) and by two examiners on the same day of observation (interrater reliability). According to our objective the overall hypothesis

was: the nBR evoked by electrical stimulation of the three branches of the trigeminal nerve is a reliable test.

2. Methods

2.1 Participants

Twenty-one healthy participants of both genders were recruited from the staff members at Aarhus University and the local community (convenience sampling method). Inclusion criteria were: age > 18 years and good health with no orofacial pain complaints or headache disorders. Exclusion criteria for healthy participants were: serious dental or medical illness, regular intake of medication, such as, antidepressants, anticonvulsants or non-steroidal anti-inflammatories and psychiatric or personality disorders assessed by means of medical interview/anamnesis.

This study was performed in accordance with the Helsinki Declaration II and had the approval from the Regional Ethics Committee as well as the Danish Data Protection Agency. All participants gave their voluntary consent after a full explanation of all procedures.

2.2 Study Design

All participants were evaluated in two sessions (baseline and 24 hours after) by the same examiner (test-retest and intrarater reliability). A second examiner, blinded for the participant information, performed an off-line analysis of both sessions (interrater reliability). The choice of the examiners was made by convenience and both were not blinded regarding the comparison of their judgments, i.e., they had previous knowledge that their results would be compared. However, the ratings were conducted independently. Furthermore, the training level and experience of the examiners were different, and a calibration session between them was conducted prior to data collection of the first session and a re-calibration was conducted

prior to data collection of the second session. The calibration sessions consisted of discussion and consensus achievement regarding the upper and lower time limits for making the decision about the onset latencies, the amplitude display to read the reflex and how to distinguish noise contamination.

2.3 Measurement process

The nBR was performed in a quiet room with the temperature around 20^{o}C and recorded by placing two surface self-adhesive EMG electrodes (Neuroline 720, Ambu ®, Denmark) on both orbicularis oculi muscles (infraorbital region and the corner of the eye) and the ground electrode was attached to the wrist. The recorded signals were amplified and band-pass filtered between 20-1000 Hz and the sampling rate was 2000 Hz (Nicolet Viking™, Natus Medical Inc., USA). A custom built planar concentric electrode [8] with a central metal cathode (diameter (D) = 0.5 mm) and external anode ring (D = 5 mm) was used to elicit the nBR by stimulation of all three branches of the trigeminal nerve. Each stimulus sweep consisted of a train of three pulses with duration of 0.3 ms and inter-pulse interval of 3 ms and was applied to the skin directly above the entry zones of the right supraorbital (V1R), infraorbital (V2R) and the mental (V3R) nerve and also the left infraorbital (V2L) nerve [15]. The order of the stimulation was randomized for each session and a template was used in order to keep the same pattern for the electrodes placements. Each session lasted approximately 1.5 hours.

The individual sensory (I_0) and pain thresholds (I_P) to the electrical stimulation were determined for each site before the nBR recordings by the application of an up-down staircase method consisting of 5 series of ascending and descending stimuli (0.2 mA increment rate) [21]. The I_0 was defined as the lowest stimulus intensity that evoked a sensation, whereas the I_P was the lowest intensity that evoked a sharp pin-prick like pain sensation.

For each site, the nBR recordings comprised a total of 6 stimulation blocks with 6 individual sweeps each at an interstimulus interval (ISI) of approximately 15-17 s. The intensities of the blocks were 50, 100, 150, 200, 300 and 400% of I_P and the order of stimulation was randomized for each site and for each session. To avoid contamination with the startle reaction and the related R3 responses, the first stimulus of each block was announced to the participant. Furthermore, the participants were asked to score the stimulus-evoked pain intensity at the end of each block with the aid of a 0-10 numerical rating scale (NRS) with 0 indicating no pain at all and 10 indicating worst pain imaginable.

2.4 Variables

The analyzed outcomes for each block and site were: a) the I_0 (mA); b) the I_P (mA); the EMG records of the R2, quantified as, c) the root mean square (RMS) (μV) and, d) area-under-the-curve (AUC) (μV x ms) of the rectified and averaged sweeps in the time window from 27-87 ms [22]; e) the nBR occurrence (yes/no) and onset latencies (ms) of the R2 responses at 200 and 300% of I_P measured for the averaged sweeps; f) the stimulus-evoked pain intensity (NRS).

2.5 Statistics

Quantitative variables (age, I_0, I_P, RMS, AUC, latency and NRS) were expressed as means and standard deviation (SD), along with a description of the gender distribution and the reflex occurrence. The test-retest, intrarater and interrater reliability was estimated using the intraclass correlation coefficient (ICC) for single measures based on analysis of variance (ANOVA) model for quantitative variables and the Cohen's kappa coefficient (κ) for the nBR occurrence. The intrarater and interrater reliability was estimated only for the nBR occurrence and onset latencies. The magnitude of the ICC was scored as poor reliability (< 0.4), fair (0.4

– 0.59), good (0.6 – 0.75) or excellent (> 0.75) (Rosner, 2006) whereas for κ, the scores were: poor reliability (<0.2), fair (0.21 – 0.4), moderate (0.41 – 0.6), good (0.61 – 0.8) or excellent (> 0.81) [23]. Repeated measures ANOVAs were performed to compare the I_0 and I_P differences between sites (4 levels). Also, multi-way within-subjects ANOVAs was performed as following: a) the factors stimulation site (4 levels) and stimulus intensity (6 levels) were established to compare the pain intensity after $\log_{10}$ transformation; b) the factors stimulation site (4 levels), recording side (2 levels) and stimulus intensity (6 levels) were established to compare RMS and AUC values after $\log_{10}$ transformation; c) the factors stimulation site (4 levels) recording side (2 levels) and stimulus intensity (3 levels) were established to compare the onset latencies of the first session. When appropriate, post hoc analyses were performed using Tukey Honestly Statistical Difference (HSD) with correction for multiple comparisons. Finally, Cochran Q test was used to compare differences regarding the nBR occurrence in the first session. The significance level was set at 5% ($p = 0.050$) and the confidence interval (CI) at 95%. All tests were carried out using the Statistical Package for the Social Sciences (SPSS) v.18.0 (IBM Corp., USA) and STATISTICA, v 12 (StatSoft Inc., USA).

3. Results

3.1 Initial characteristics and differences amongst V branches

Twenty-one participants were evaluated in two sessions by the same examiner (test-retest and intrarater reliability) and two independent examiners evaluated the latencies of the first and second session (interrater reliability). The sample characteristics are presented in Table 1 considering the measurements of the first session made by the first examiner and examples of R2 traces are illustrated in the Figure 1. The mean age (SD) of the participants was 29.3 years (3.7) with a slight female predominance (13 women, 8 men). The I_0 and I_P were similar

among the V1R, V2R and V2L branches of the trigeminal nerve but higher I_0 and I_P values were determined for the V3R compared with, respectively, V1R (Tukey: p = 0.005), V2L (Tukey: p = 0.040) and V1R (Tukey: p = 0.010).

There were main effects of stimulation site (ANOVA: F = 4.69, p = 0.006) and stimulus intensity (ANOVA: F = 193.93, p < 0.001), but without interactions, for the reported pain intensity. The V3R showed lower NRS pain scores compared with V2L (Tukey: p = 0.018) and V1 (Tukey: p = 0.015) and stimulation at the lowest intensity (50% of I_P) induced the lowest pain scores (Tukey: p < 0.001).

There were main effects of stimulation site (ANOVA: F = 22.42, p < 0.001), recording side (ANOVA: F = 57.85, p < 0.001) and stimulus intensity (ANOVA: F = 24.98, p < 0.001) considering the RMS and AUC (stimulation site – ANOVA: F = 24.41, p < 0.001 / recording side – ANOVA: F = 59.63, p < 0.001 / stimulus intensity – ANOVA: F = 23.36, p < 0.001). The V3R (Tukey: p <0.001), the contralateral side (Tukey: p < 0.001) and the intensity at 50% of I_P (Tukey: p < 0.001) showed the lowest values. Also, there were statistically significant interactions between the stimulation site and recording side (ANOVA: F = 3.56, p = 0.020), stimulation site and stimulus intensity (ANOVA: F = 2.71, p < 0.001) and recording side and stimulus intensity (ANOVA: F = 4.84, p < 0.001) considering the RMS. The lowest values were found at 50% of I_P for V3R and for the contralateral sides. This same interaction pattern was also found considering the AUC: between stimulation site and recording side (ANOVA: F = 3.93, p = 0.015), stimulation site and stimulus intensity (ANOVA: F = 2.26, p = 0.005) and between recording side and stimulus intensity (ANOVA: F = 4.65, p = 0.001).

The nBR was not elicited constantly by stimulation of any of the trigeminal nerve branches. The lowest percentage of reflex occurrence was for the stimulation of the V3R at 200% of I_P (p < 0.050), whereas there were no differences in reflex occurrence among the branches considering the intensities of 300 and 400% of I_P (p > 0.050). There was a main effect of

recording side, demonstrating that the latencies of the contralateral side were longer than the ipsilateral (ANOVA: F = 17.01, p = 0.025).

3.2 Reliability

Figure 2 shows the results of the test-retest and intrarater reliability of the nBR considering the four stimulation sites. The overall ICCs showed acceptable values for the test-retest and intrarater reliability of the nBR parameters and the associated psychophysical measures (I_0, I_P and the reported pain intensity). The ICC values of 76% of all measurements were considered fair to excellent (fair: 22%, good: 36% and excellent:18%). Eighty two percent of the psychophysical measures showed fair to excellent reliability (fair: 21%, good: 31%, excellent: 30%). All I_P values presented higher ICCs than I_0 (Figure 2). Also, 75% of nBR parameters showed fair to excellent reliability (fair: 22%, good: 38% and excellent: 15%) and the overall reliability of the ipsilateral values were higher than the contralateral side (Figure 2). The V1R, V2R and V2L had 86% of their ICC values considered fair to excellent, whereas the V3R showed poor reliability in 52% of the measurements (Figure 2). Furthermore, the stimulus intensity at 200 and 300% of I_P showed the highest ICCs, regardless of the stimulation site, with the exception of the V3R, where the RMS and AUC at 100% of I_P showed the highest values. The ICCs for intrarater reliability considering the onset latencies at 200 and 300% of I_P were in the fair to excellent range in 70% of all measurements and the V3R showed the lowest values.

Tables 2-3 show the interrater reliability for the onset latencies at 200 and 300% of I_P, respectively, considering the first and the second sessions and the intra- and interrater reliability of the reflex occurrence. The overall range of ICCs was similar for both sessions and 75% of all measurements were considered fair to excellent (1^{st} session: fair: 44%, good: 25% and excellent: 6% / 2^{nd} session: fair: 55% and good: 20%), even though after the recalibration session, extreme low values were not present. There were similar ICC values

between the two intensities of stimulation and the ipsilateral and contralateral recording sides, although the V1R and V2R presented the highest coefficients (Table 2). All the intra- and interrater kappa values, regardless of the session, showed moderate to excellent reliability with a similar distribution considering the stimulus intensities, recording sides and stimulation sites (Table 3). However, considering the interrater reliability, the scores of the first session were: moderate (37%), good (37%) and excellent (26%), whereas after the recalibration between the two examiners the proportions were: moderate (6%), good (44%) and excellent (50%).

4. Discussion

This study focused on the reliability of the nBR elicited by stimulation of the three branches of the trigeminal nerve and the main findings were: a) the overall test-retest, intrarater and interrater reliability of the nBR and its associated psychophysical measures can be considered acceptable for clinical use and research; b) the stimulation intensities at 200% and 300% of I_P are associated with the highest reliability coefficients; c) the stimulation of the V3R is associated with the lowest reliability coefficients.

The present baseline nBR measurements (RMS and AUC values) are in accordance with previous findings in healthy participants [20]. The nBR elicited by stimulation of the supraorbital nerve in a sample of 104 healthy volunteers showed mean values of I_0 and I_P, respectively, 0.39 and 0.69 mA [20], which are very similar with our mean values, respectively 0.40 and 0.70 mA. However, our mean latencies were shorter when compared with the same healthy population, where the mean onset of the ipsilateral and contralateral responses at a stimulus intensity of 150% of I_P were, respectively, 44.7 (7.3) and 45.4 (7.0) ms [20], whereas the values of the present study were 41.2 (2.7) and 42.8 (1.4) ms at a stimulus intensity of 200% of I_P. The difference in the stimulus intensities could account for

this dissimilarity, in particular the slightly shorter pattern, since higher intensities of stimulation could be associated with more “contamination” of large fiber activity [24]. This concomitant Aβ fiber activity is indeed inferred when compared with the mean onset latencies of the laser-evoked blink reflex (73.2 ms), a valid method to selectively activate only unmyelinated afferents [25].

Direct comparisons between the present and other studies regarding the RMS and AUC values are difficult because of methodological differences, e.g., number of sweeps and multisite stimulations. Yet, the present findings agree with a previous study, where a more pronounced muscle activity at higher intensities was also reported [15]. Finally, comparisons with the stimulation of the other branches are not possible because of the lack of studies testing V2 and V3. To the authors´ knowledge, only a few studies have been published showing the usefulness of measuring the nBR by stimulation of the infraorbital nerve to evaluate the effects of pain disorders affecting the maxillary division of the trigeminal nerve [14, 15]. On the other hand, there is evidence that the BR elicited by stimulation of the mental nerve can produce inconsistent responses and faster habituation when the “standard” ISI (≈ 15 s) is applied [26]. Our results are in accordance with this evidence, considering the low RMS and AUC values from V3 stimulation. There is more substantial habituation of the V3 in comparison with the V1 division when 10 s of ISI is adopted [26]. According to our findings, it seems that adding 5 s on top of that is not long enough to avoid such effect. In fact, it has been reported that stable reflex responses of the V3 division are possible when applying longer ISI (up to 30 s) [27]. Finally, the low reflex occurrence at 200% of I_P also reinforces the evidence that stronger stimulus intensities are required to consistently elicit reflex responses of V3 [27].

Despite the fact that the BR is well established and routinely used and recommended in the clinical setting [28], the electrically elicited so-called nociceptive specific nBR was first

established in 2000 as a new, non-invasive and technically easier method to evaluate trigeminal brainstem nociception [8] compared with the corneal reflex and the laser-evoked blink reflex [25, 29]. However, neither the BR nor the nBR has been systematically tested regarding their reliability properties for all branches of the trigeminal nerve. For this reason, we highlight the relevance of our study for the establishment of the nBR as a sufficiently reliable tool to assess pathophysiologic aspects of orofacial pain conditions. Nonetheless, some particularities regarding the test-retest reliability must be highlighted: 1) the high ICCs at 200 and 300% of I_P indicate that the stimulus intensity to perform the nBR should not be arbitrarily decided. Some low reliability coefficients between 50 and 150% of I_P could be explained by the low variability and amplitude at these intensities, which negatively affect the reliability coefficients [30]. Also, despite the fact that the intensity at 400% of I_P presented good to excellent reliability at some stimulation sites, the choice of this intensity requires caution, considering that the nociceptive specificity of the nBR decreases at very high amplitudes [8]. So, based on the overall reliability results, we recommend the intensity between 200 and 300% of I_P in the nBR measurement. 2) The fact that the V3R presented the lowest coefficients could be associated with the inconsistency of the responses when using similar stimulus parameters as for the V1 and v2. Indeed, the ISI of 15-17 s adopted in this study could also account for the lower reliability coefficients of V3, since there is evidence showing the usefulness of the BR with V3 stimulation in the diagnosis of trigeminal neuropathies [31-34].

The intrarater and interrater reliability of the nBR onset latencies at 200 and 300% of I_P also presented acceptable reliability values. In fact, it was possible to obtain a positive effect of the recalibration session on the reliability coefficients, which means that the onset latencies judgment requires some degree of training and experience between the examiners.

It is also important to note that there are no guidelines on how to accurately measure the onset latencies of the nBR. Also, the low variability of the latencies and, by consequence, the relative homogeneity of the sample could explain some low ICC values, since in such a scenario it is more difficult to distinguish the "true" variability between the participants from the measurement error [18, 30]. So, even though with these possible shortcomings, our results support the adequate reliability of the onset latencies measurements of the nBR.

The reliability values of the associated psychophysical measures also presented acceptable levels to recommend the clinical and research use. The ICCs of I_P presented comparable values with other well-established psychophysical techniques, such as the mechanical pain threshold (MPT) performed in the trigeminal region [35, 36] , which is part of the battery of quantitative sensory testing (QST) [37]. On the other hand, the reliability of the I_0 was generally poor and lower than the I_P. One possible explanation is related to the nociceptive characteristic of the stimulus which brings forth difficulties in properly determining the 'lowest stimulus that evokes a sensation'. However, considering the use of the I_P as the reference for stimulus intensity, the low reliability of I_0 may not have a major impact on the test utility. Furthermore, the pain ratings associated with the nBR presented adequate ICC values and followed the same pattern of the RMS and AUC, i.e., the highest ICCs were presented at highest stimulation intensities and the low variability of the lower intensities could also account for these results.

The strengths of the present study were the use of well-established statistical approaches to estimate the reliability, i.e., ICC and Kappa [38], and the comprehensive assessment of all the trigeminal branches. The study has, on the other hand, some limitations which affect the external validity. The first major point is regarding the methodological particularities of this study. In spite of evidence suggesting optimized parameters for the nBR [20, 39], there is no established standard on how to perform the nBR, e.g., analysis of signal averaging versus

single trials, differences in the stimulus intensity and interstimulus interval and number of the stimulation blocks. Particularly, the difference between analyzing single versus averaged sweeps should be addressed in future studies, inasmuch as averaging measurements can give a more reliable result but also could cause an attenuation of the EMG amplitudes and habituation of the reflex [40]. The ISI can also account for the amplitude decrement and, consequently, for the reflex occurrence judgment. Possibly, this is one of the reasons for the lack of concistent nBR occurrence, mainly in V3, considering that the adopted ISI was not optimal to consistently elicit the reflex. More research is needed to define the ideal ISI and stimulation intensities for the V3 division using the concentric electrode. On the other hand, since we randomized the sites and stimulation intensities for both sessions, other differences among the branches regarding the amount of habituation effect were a result of chance. The second major point is regarding the type of study population. Considering that the reliability is a characteristic of a particular instrument in a particular population and not exclusively from the instrument, reliability values for specific groups of diseases, e.g., neuropathic pain and primary headaches, remains to be established. In this context, the generalization of our results should be made with caution.

5. Conclusion

The nBR and its associated psychophysical measures can be considered a sufficiently reliable test to assess the trigeminal nociceptive function and its use can be recommended as an adjunct examination of the trigeminal nervous system.

Compliance with Ethical Standards

This study was supported by the Danish Dental Association and by the Coordination for the Improvement of Higher Education Personnel (CAPES – Proc. n^{o} BEX 4306/14-7).
None of the authors have potential conflicts of interest to be disclosed. 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.

References

Backonja MM, Attal N, Baron R, Bouhassira D, Drangholt M, Dyck PJ, Edwards RR, Freeman R, Gracely R, Haanpaa MH, Hansson P, Hatem SM, Krumova EK, Jensen TS, Maier C, Mick G, Rice AS, Rolke R, Treede RD, Serra J, Toelle T, Tugnoli V, Walk D, Walalce MS, Ware M, Yarnitsky D and Ziegler D (2013) Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. Pain 154:1807-19

Kersten P, White PJ and Tennant A (2014) Is the pain visual analogue scale linear and responsive to change? An exploration using Rasch analysis. PloS one 9:e99485

Galeotti F, Truini A and Cruccu G (2006) Neurophysiological assessment of craniofacial pain. The journal of headache and pain 7:61-9

Kimura J (1989) The blink reflex. In: Kimura J (ed) Book title, 3rd edn. Oxford University Press, New York

Pearce JM (2008) Observations on the blink reflex. European neurology 59:221-3

Ellrich J and Hopf HC (1996) The R3 component of the blink reflex: normative data and application in spinal lesions. Electroencephalography and clinical neurophysiology 101:349-54

Ellrich J (2000) Brain Stem Reflexes: Probing Human Trigeminal Nociception. News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society 15:94-97

Kaube H, Katsarava Z, Kaufer T, Diener H and Ellrich J (2000) A new method to increase nociception specificity of the human blink reflex. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 111:413-6

de Tommaso M (2013) Reply to "Activating selectively and reliably nociceptive afferents with concentric electrode stimulation: Yes we can! Provided that low stimulus intensities are used!". Clinical Neurophysiology 124:424-425

de Tommaso M, Santostasi R, Devitofrancesco V, Franco G, Vecchio E, Delussi M, Livrea P and Katzarava Z (2011) A comparative study of cortical responses evoked by transcutaneous electrical vs CO(2) laser stimulation. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 122:2482-7

Perchet C, Frot M, Charmarty A, Flores C, Mazza S, Magnin M and Garcia-Larrea L (2012) Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. Pain 153:1244-52

Rehberg B, Baars JH, Kotsch J, Koppe P and von Dincklage F (2012) Comparison of trigeminal and spinal modulation of pain and nociception. The International journal of neuroscience 122:298-304

Giffin NJ, Kowacs F, Libri V, Williams P, Goadsby PJ and Kaube H (2003) Effect of the adenosine A1 receptor agonist GR79236 on trigeminal nociception with blink reflex recordings in healthy human subjects. Cephalalgia : an international journal of headache 23:287-92

Baad-Hansen L, List T, Jensen TS, Leijon G and Svensson P (2005) Blink reflexes in patients with atypical odontalgia. Journal of orofacial pain 19:239-47

Baad-Hansen L, List T, Kaube H, Jensen TS and Svensson P (2006) Blink reflexes in patients with atypical odontalgia and matched healthy controls. Experimental brain research 172:498-506

Kaube H, Katsarava Z, Przywara S, Drepper J, Ellrich J and Diener HC (2002) Acute migraine headache: possible sensitization of neurons in the spinal trigeminal nucleus? Neurology 58:1234-8

Peddireddy A, Wang K, Svensson P and Arendt-Nielsen L (2009) Blink reflexes in chronic tension-type headache patients and healthy controls. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 120:1711-6

Bannigan K and Watson R (2009) Reliability and validity in a nutshell. Journal of clinical nursing 18:3237-43

Mokkink LB, Terwee CB, Patrick DL, Alonso J, Stratford PW, Knol DL, Bouter LM and de Vet HC (2010) The COSMIN study reached international consensus on taxonomy, terminology, and definitions of measurement properties for health-related patient-reported outcomes. Journal of clinical epidemiology 63:737-45

Katsarava Z, Ellrich J, Diener HC and Kaube H (2002) Optimized stimulation and recording parameters of human 'nociception specific' blink reflex recordings. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 113:1932-6

von Dincklage F, Koppe P, Kotsch J, Baars JH and Rehberg B (2010) Investigation of threshold and magnitude criteria of the nociceptive blink reflex. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 121:945-9

Ellrich J and Treede RD (1998) Characterization of blink reflex interneurons by activation of diffuse noxious inhibitory controls in man. Brain research 803:161-8

Landis JR and Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33:159-74

Mouraux A, Iannetti GD and Plaghki L (2010) Low intensity intra-epidermal electrical stimulation can activate Adelta-nociceptors selectively. Pain 150:199-207

Romaniello A, Valls-Sole J, Iannetti GD, Truini A, Manfredi M and Cruccu G (2002) Nociceptive quality of the laser-evoked blink reflex in humans. Journal of neurophysiology 87:1386-94

Jaaskelainen SK (1995) Electrophysiological study of blink reflex in humans: differences in mental and supraorbital nerves. Acta physiologica Scandinavica 154:143-50

Jaaskelainen SK (1995) Blink reflex with stimulation of the mental nerve. Methodology, reference values, and some clinical vignettes. Acta neurologica Scandinavica 91:477-82

Cruccu G, Sommer C, Anand P, Attal N, Baron R, Garcia-Larrea L, Haanpaa M, Jensen TS, Serra J and Treede RD (2010) EFNS guidelines on neuropathic pain assessment: revised 2009. European journal of neurology : the official journal of the European Federation of Neurological Societies 17:1010-8

Ongerboer de Visser BW and Moffie D (1979) Effects of brain-stem and thalamic lesions on the corneal reflex: an electrophysiological and anatomical study. Brain : a journal of neurology 102:595-608

de Vet HCT, C. B.; Mokkink, L. B.; Knol, D. K. (2011) Measurement in Medicine: A Practical Guide. Cambridge University Press, Suffolk

Forssell H, Jaaskelainen S, Tenovuo O and Hinkka S (2002) Sensory dysfunction in burning mouth syndrome. Pain 99:41-7

Forssell H, Tenovuo O, Silvoniemi P and Jaaskelainen SK (2007) Differences and similarities between atypical facial pain and trigeminal neuropathic pain. Neurology 69:1451-9

Teerijoki-Oksa T, Jaaskelainen SK, Forssell K and Forssell H (2004) Recovery of nerve injury after mandibular sagittal split osteotomy. Diagnostic value of clinical and electrophysiologic tests in the follow-up. International journal of oral and maxillofacial surgery 33:134-40

Topka H and Hallett M (1992) Perioral reflexes in orofacial dyskinesia and spasmodic dysphonia. Muscle & nerve 15:1016-22

Baad-Hansen L, Pigg M, Yang G, List T, Svensson P and Drangsholt M (2015) Reliability of intra-oral quantitative sensory testing (QST) in patients with atypical odontalgia and healthy controls - a multicentre study. Journal of oral rehabilitation 42:127-35

Pigg M, Baad-Hansen L, Svensson P, Drangsholt M and List T (2010) Reliability of intraoral quantitative sensory testing (QST). Pain 148:220-6

Rolke R, Baron R, Maier C, Tolle TR, Treede RD, Beyer A, Binder A, Birbaumer N, Birklein F, Botefur IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihofner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M and Wasserka B (2006) Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 123:231-43

Kottner J, Audige L, Brorson S, Donner A, Gajewski BJ, Hrobjartsson A, Roberts C, Shoukri M and Streiner DL (2011) Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. Journal of clinical epidemiology 64:96-106

Giffin NJ, Katsarava Z, Pfundstein A, Ellrich J and Kaube H (2004) The effect of multiple stimuli on the modulation of the 'nociceptive' blink reflex. Pain 108:124-8

Jaaskelainen SK, Forssell H and Tenovuo O (1999) Electrophysiological testing of the trigeminofacial system: aid in the diagnosis of atypical facial pain. Pain 80:191-200

Figure Captions

Fig 1. Examples of R2 traces (average of 6 individual sweeps) of the same participant. V1R = right supraorbital infraorbital nerve, V2R = right infraorbital nerve, V2L = left infraorbital nerve, V3R = right mental nerve, I_P = individual pain threshold and OOc = orbicularis oculi muscle.

Fig 2. Line chart showing the test-retest and intrarater reliability coefficients (ICC) of the "nociceptive" blink reflex (nBR). V1R = right supraorbital nerve, V2R = right infraorbital nerve, V2L = left infraorbital nerve, V3R = right mental nerve, ip = ipsilateral side, cl = contralateral side, I_0 = individual sensory threshold, I_P = individual pain threshold, P = pain intensity (Numerical Rating Scale), R = root mean square (RMS µV), A = area-under-the-curve (AUC, µV x ms), L = latency (ms), 50 = 50% of I_P, 100 = 100% of I_P, 150 = 150% of I_P, 200 = 200% of I_P, 300 = 300% of I_P and 400 = 400% of I_P.

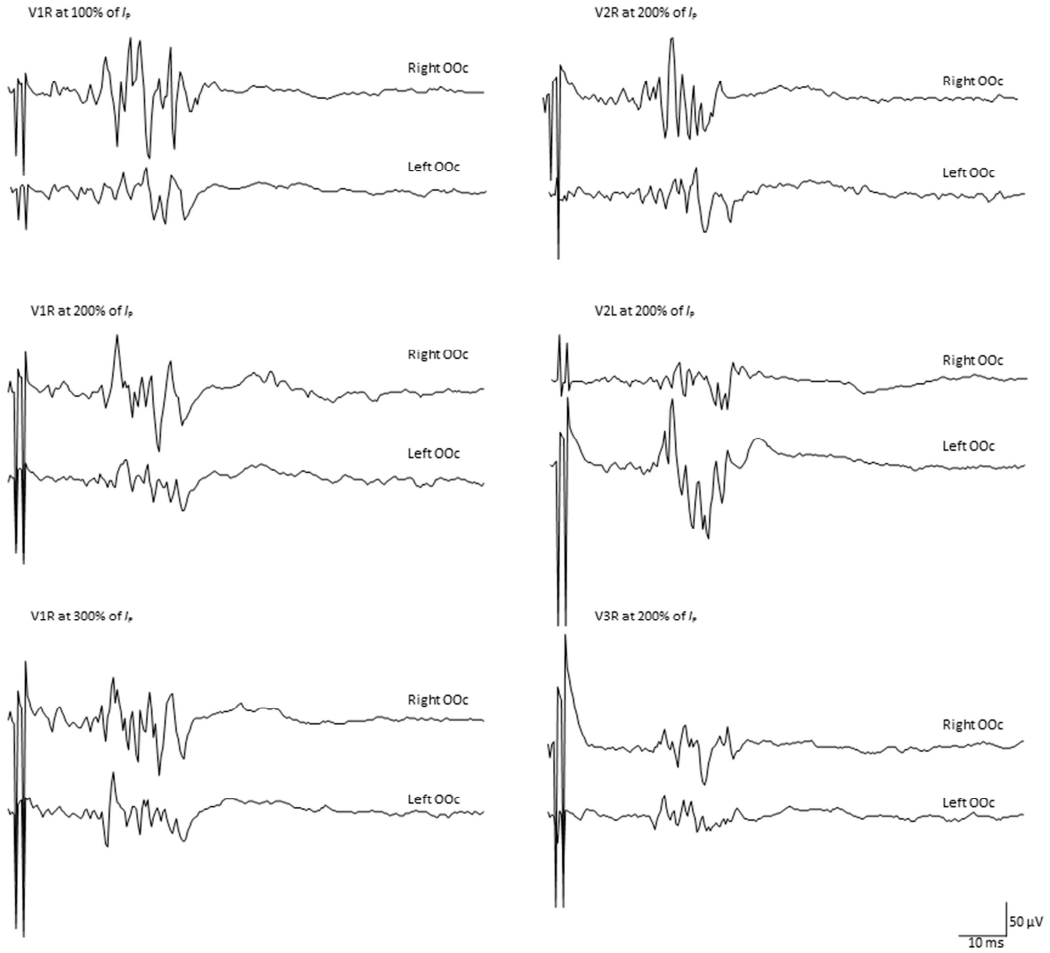
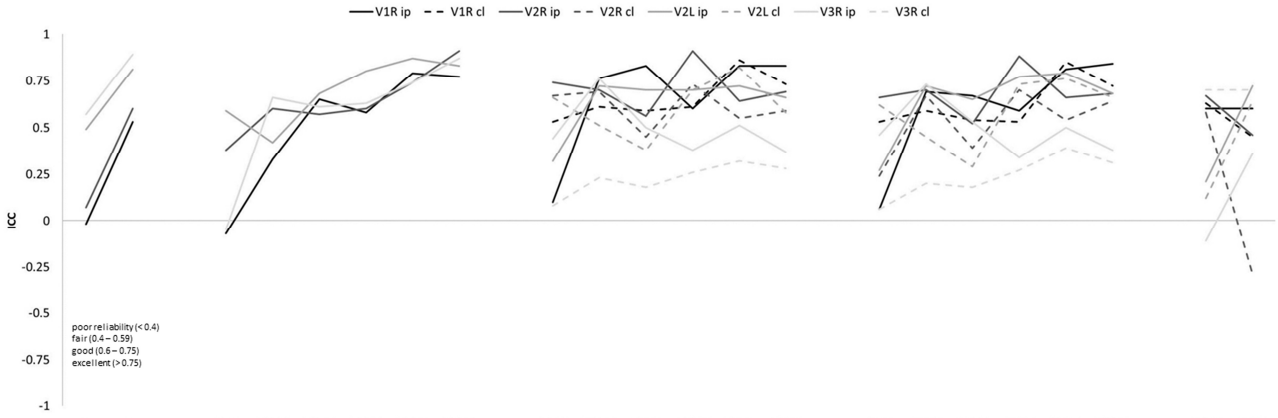

V1R at 100% of I_p
Right OOc
Left OOc
V2R at 200% of I_p
Right OOc
Left OOc
V1R at 200% of I_p
Right OOc
Left OOc
V2L at 200% of I_p
Right OOc
Left OOc
V1R at 300% of I_p
Right OOc
Left OOc
V3R at 200% of I_p
Right OOc
Left OOc
50 µV
10 ms

Figure 1



P-values	I_0	I_P	P50	P100	P150	P200	P300	P400	R50	R100	R150	R200	R300	R400	A50	A100	A150	A200	A300	A400	L200	L300
									Ipsilateral / Contralateral													
V1R	0.500	0.006	0.006	0.050	<0.001	0.001	<0.001	<0.001	0.003 / 0.004	<0.001 / 0.001	<0.001 / 0.002	0.001 / 0.001	<0.001 / <0.001	<0.001 / <0.001	0.300 / 0.005	<0.001 / 0.001	<0.001 / 0.005	0.001 / 0.004	<0.001 / 0.001	<0.001 / 0.001	0.020 / 0.060	0.030 / 0.089
V2R	0.360	0.001	0.032	0.001	0.002	0.001	<0.001	<0.001	<0.001 / <0.001	<0.001 / <0.001	0.002 / 0.010	<0.001 / <0.001	<0.001 / 0.005	<0.001 / 0.004	<0.001 / 0.001	<0.001 / <0.001	0.003 / 0.030	<0.001 / <0.001	<0.001 / 0.004	<0.001 / 0.002	0.009 / 0.020	0.030 / 0.800
V2L	0.010	<0.001	0.001	0.020	<0.001	<0.001	<0.001	<0.001	0.050 / <0.001	<0.001 / 0.004	<0.001 / 0.040	<0.001 / <0.001	<0.001 / <0.001	0.002 / 0.010	0.090 / 0.001	<0.001 / 0.008	0.001 / 0.090	<0.001 / <0.001	<0.001 / <0.001	0.002 / 0.003	0.100 / 0.347	0.002 / 0.014
V3R	0.002	<0.001	0.500	0.001	0.002	0.001	<0.001	<0.001	0.020 / 0.300	<0.001 / 0.001	0.010 / 0.200	0.030 / 0.100	0.010 / 0.070	0.090 / 0.100	0.010 / 0.300	<0.001 / 0.010	0.007 / 0.200	0.050 / 0.100	0.010 / 0.040	0.080 / 0.100	0.500 / 0.014	0.248 / 0.075

Figure 2

Tables

Table 1. Psychophysical parameters and amplitude measures of the R2 response of the nBR. I_0: electrical sensory threshold, I_P: electrical pinprick threshold, V1R: right supraorbital nerve, V2R: right infraorbital nerve, V2L: left infraorbital nerve, V3R: right mental nerve, NRS: numerical rating scale, RMS: root mean square, AUC: area-under-the-curve.

Measurements	Mean (SD) or n (%)[a]			
Stimulation Threshold (mA)				
I_0	0.4 (0.1)	0.5 (0.1)	0.4 (0.1)	0.6 (0.1)
I_P	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)	0.9 (0.3)
Pain Intensity (NRS) – % of I_P				
50%	0.0 (0.1)	0.1 (0.3)	0.1 (0.3)	0.0 (0.1)
100%	0.9 (0.9)	1.0 (1.2)	1.2 (1.0)	0.9 (1.0)
150%	2.1 (1.2)	2.2 (1.8)	2.1 (1.7)	2.2 (2.2)
200%	3.4 (1.4)	3.5 (1.8)	3.6 (1.9)	3.0 (2.1)
300%	4.3 (1.7)	4.5 (1.9)	4.9 (1.8)	3.9 (2.0)
400%	5.3 (1.9)	4.8 (1.7)	5.6 (2.0)	4.6 (1.9)
RMS (µV) – % of I_P				
50%	5.2 (6.9) / 3.8 (2.9)	5.5 (4.7) / 3.3 (2.3)	3.7 (2.0) / 3.3 (2.4)	4.0 (3.6) / 3.0 (2.2)
100%	7.7 (7.1) / 4.3 (3.5)	8.2 (6.0) / 6.1 (5.7)	8.8 (10.4) / 6.4 (5.1)	3.6 (3.3) / 2.6 (1.9)
150%	8.2 (7.2) / 5.0 (3.4)	11.8 (10.5) / 7.5 (8.9)	10.7 (10.8) / 6.7 (5.8)	5.9 (6.7) / 4.1 (3.9)
200%	10.4 (8.6) / 5.8 (5.3)	12.0 (10.8) / 7.7 (7.7)	13.6 (10.7) / 7.3 (7.0)	7.2 (6.8) / 4.9 (4.5)
300%	10.9 (8.7) / 6.5 (6.2)	17.0 (14.1) / 10.5 (8.6)	18.4 (14.2) / 9.6 (8.7)	7.5 (8.1) / 6.5 (6.9)
400%	13.6 (9.3) / 7.3 (6.3)	19.5 (11.3) / 12.1 (9.8)	19.4 (11.4) / 8.8 (6.1)	6.9 (7.7) / 5.7 (6.6)
AUC (µV x ms) – % of I_P				
50%	264.9 (377.3) / 185.3 (154.0)	264.2 (202.5) / 196.2 (166.7)	197.4 (114.2) / 165.1 (126.5	186.9 (161.1) / 143,8 (106,7)
100%	361.3 (318.3) / 199.7 (169.4)	385.6 (284.9) / 287.9 (227.9)	391.8 (432.5) / 297.1 (231.1)	166.9 (149.9) / 122.0 (88.4)
150%	349.6 (250.3) / 230.3 (155.2)	540.9 (474.1) / 322.2 (373.2)	502.8 (447.9) / 278.3 (244.8)	277.3 (311.0) / 193.8 (179.6)
200%	446.7 (340.7) / 264.1 (224.0)	552.9 (488.3) / 332.9 (311.2)	609.2 (479.6) / 317.8 (280.4)	328.9 (323.0) / 226.2 (191.5)
300%	492.8 (376.3) / 295.9 (282.3)	758.7 (613.3) / 463.0 (363.0)	817.8 (620.6) / 400.7 (323.7)	336.0 (353.1) / 281.0 (280.5)
400%	598.9 (409.3) / 325.6 (267.9)	862.8 (506.6) / 516.2 (418.2)	884.6 (546.3) / 388.7 (258.8)	309.6 (336.4) / 241.9 (255.2)
Latency				
Reflex occurrence 200% of I_P	16 (76.2%) / 15 (71.4%)	14 (66.7%) / 14 (66.7%)	17 (80.9%) / 16 (76.2%)	11 (52.3%) / 11 (52.3%)
Onset 200% of I_P (ms)	41.2 (2.4) / 42.8 (1.7)	41.3 (2.5) / 42.1 (2.3)	41.1 (1.9) / 43.2 (2.0)	40.8 (1.8) / 42.3 (2.2)
Reflex occurrence 300% of I_P	13 (61.9%) 13 (61.9%)	17 (85%) / 17 (85%)	16 (80%) / 15 (75%)	13 (65%) / 13 (65%)
Onset 300 % of I_P (ms)	40.6 (2.7) / 41.9 (3.4)	40.6 (2.0) / 41.8 (1.9)	40.5 (1.9) / 42.2 (1.9)	40.0 (2.0) / 42.0 (2.1)
Reflex occurence 400% of I_P	15 (93.7%) / 15 (93.7%)	14 (93.3%) / 15 (100%)	15 (93.7%) / 15 (93.7%)	13 (92.8%) / 14 (100%)
Onset 400% of I_P	40.6 (1.6) / 41.4 (1.75)	41.5 (1.4) / 42.5 (1.6)	41.3 (1.5) / 42.2 (1.6)	41.8 (1.7) / 42.4 (1.7)

[a] Missing data were not taking into consideration to calculate the %.

Table 2. Interrater reliability of the R2 onset latency of the nBR. I_P: electrical pinprick threshold, ICC: intra-class correlation coefficient, V1R: right supraorbital nerve, V2R: right infraorbital nerve, V2L: left infraorbital nerve, V3R: right mental nerve, RMS: root mean square, AUC: area-under-the-curve.

Measurements	ICC	ICC - 95% CI	P- Value
V1R			
200% of I_P	0.57 / 0.57	0.05 – 0.86 / -0.11 – 0.91	0.010 / 0.008
300 % of I_P	0.60 / 0.73	0.05 – 0.88 / 0.06 – 0.93	0.021 / 0.001
V2R			
200% of I_P	0.43 / 0.77	-0.23 – 0.81 / 0.35 – 0.93	0.090 / 0.002
300 % of I_P	0.45 / 0.44	-0.01 – 0.77 / -0.06 – 0.79	0.023 / 0.030
V2L			
200% of I_P	0.08 / 0.11	-0.21 – 0.50 / -0.20 – 0.56	0.330 / 0.280
300 % of I_P	0.61 / 0.64	0.14 – 0.86 / 0.14 – 0.88	0.009 / 0.009
V3R			
200% of I_P	0.42 / 0.06	-0.52 – 0.89 / -0.58 – 0.78	0.180 / 0.495
300 % of I_P	-0.56 / 0.43	-0.90 – 0.18 / -0.14 – 0.83	0.965 / 0.043
V1R			
200% of I_P	0.39 / 0.50	-0.24 – 0.75 / -0.07 – 0.85	0.040 / 0.110
300 % of I_P	0.74 / 0.40	0.36 – 0.90 / -0.08 – 0.75	0.001 / 0.056
V2R			
200% of I_P	0.70 / 0.64	0.34 – 0.88 / 0.21 – 0.86	0.001 / 0.003
300 % of I_P	0.49 / 0.56	0.02 – 0.80 / -0.08 – 0.7	0.028 / 0.010
V2L			
200% of I_P	0.46 / 0.30	0.06 – 0.77 / -0.15 – 0.69	0.020 / 0.090
300 % of I_P	0.51 / 0.28	0.04 – 0.80 / -0.11 – 0.65	0.010 / 0.050
V3R			
200% of I_P	0.39 / 0.44	-0.28 – 0.79 / -0.33 – 0.84	0.110 / 0.110
300 % of I_P	0.57 / 0.42	-0.11 – 0.89 / -0.15 – 0.85	0.054 / 0.052

Table 3. Intra and interrater reliability of the R2 reflex occurrence (yes/no) (Kappa) of the nBR. I_P: electrical pinprick threshold, V1R: right supraorbital nerve, V2R: right infraorbital nerve, V2L: left infraorbital nerve, V3R: right mental nerve, RMS: root mean square, AUC: area-under-the-curve.

Measurements	Kappa	P-Value
V1R		
200% of I_P	0.63 / 0.50	0.002 / 0.020
300 % of I_P	0.40 / 0.71	0.060 / 0.001
V2R		
200% of I_P	0.70 / 0.70	0.001 / 0.001
300 % of I_P	0.63 / 0.80	0.002 / < 0.001
V2L		
200% of I_P	0.53 / 0.42	0.010 / 0.040
300 % of I_P	0.62 / 0.68	0.005 / 0.002
V3R		
200% of I_P	0.60 / 0.43	0.003 / 0.050
300 % of I_P	0.30 / 0.36	0.120 / 0.100
V1R		
200% of I_P	0.70 / 0.52	0.001 / 0.010
300 % of I_P	0.81 / 0.90	< 0.001 / < 0.001
V2R		
200% of I_P	0.90 / 0.81	< 0.001 / < 0.001
300 % of I_P	0.57 / 0.78	0.01 / < 0.001
V2L		
200% of I_P	0.44 / 0.50	0.04 / 0.020
300 % of I_P	0.47 / 0.68	0.02 / 0.002
V3R		
200% of I_P	0.69 / 0.49	0.001 / 0.010
300 % of I_P	0.80 / 0.80	< 0.001 / < 0.001
V1R		
200% of I_P	0.67 / 0.52	0.001 / 0.010
300 % of I_P	0.63 / 0.88	0.004 / < 0.001
V2R		
200% of I_P	1.00 / 0.76	< 0.001 / < 0.001
300 % of I_P	0.88 / 0.89	< 0.001 / < 0.001
V2L		
200% of I_P	0.82 / 0.63	< 0.001 / 0.002
300 % of I_P	0.85 / 0.70	< 0.001 / < 0.001
V3R		
200% of I_P	0.89 / 0.89	< 0.001 / < 0.001
300 % of I_P	0.70 / 0.79	0.001 / < 0.001

2.2. ARTICLE 2

Is the nociceptive blink reflex associated with psychological factors in healthy participants?

Authors: Yuri Martins Costa[* a,b,c], Lene Baad-Hansen [a,b], Leonardo Rigoldi Bonjardim [d], Paulo César Rodrigues Conti [c], Peter Svensson [a,b,e].

[a] Section of Orofacial Pain and Jaw Function, Department of Dentistry, Aarhus University, Aarhus, Denmark.

[b] Scandinavian Center for Orofacial Neurosciences (SCON).

[c] Department of Prosthodontics, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

[d] Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

[e] Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden.

Abstract

Background: The aim of this study was to evaluate the possible association between the nociceptive blink reflex (nBR) and various pain-related psychological measures: the Anxiety Sensitivity Index-3 (ASI-3), the Fear of Pain Questionnaire III (FPQ-III), the Pain Vigilance and Awareness Questionnaire (PVAQ), the Somatosensory Amplification Scale (SSAS), the Pain Catastrophizing Scale (PCS) and the Situational Pain Catastrophizing Scale (S-PCS).

Methods: The nBR was evaluated in 21 healthy participants and was elicited by a "nociceptive-specific" electrode placed over the entry zone of the right supraorbital, infraorbital and the mental nerve and left infraorbital nerve. The outcomes were 1) nBR measurements: a) individual electrical sensory (I_0) and pain thresholds (I_P), b) root mean

square (RMS), area-under-the-curve (AUC) and onset latencies of R2 responses, c) stimulus-evoked pain on a 0-10 numerical rating scale; and 2) ASI-3, FPQ-III, PVAQ, SSAS, PCS and S-PCS. Pearson correlation coefficient was used to associate the average of nBR measurements among all sites and the questionnaires. The significance level was set up after a Bonferroni correction (adjusted α=.8%).

Results: There was no correlation for any pair of variables considering the adjusted significance level (p>.008) and only a single significant correlation considering the standard significance level (p < .05), where the pain intensity (NRS) at 50% of I_P presented a positive and small to moderate correlation with the PCS (r = 0.43, p = .047).

Conclusion: It seems that the nBR and its associated psychophysical measures are not associated with psychological factors in healthy participants.

Keywords: Nociceptive Blink Reflex, Pain-related Anxiety, Pain Vigilance, Somatosensory Amplification, Pain Catastrophizing.

1. Introduction

The development of the biopsychosocial medical model and the identification of motivational and cognitive aspects of the pain can be regarded as important milestones in the understanding of the pain mechanisms and the pain experience in all its complexity.[1, 2] Many studies have highlighted the crucial role of psychological factors for pain perception.[3-5] In brief, psychological characteristics are of significant importance for the etiology,[6] diagnosis,[7] treatment,[8] and prognosis [9] of pain disorders and also for experimental pain outcomes. [10, 11] Hence, it is essential to consider the psychological factors when dealing with pain in the clinical and research field.

The nociceptive blink reflex (nBR) is designed to almost selectively activate the nociceptive afferents.[12] This electrophysiological test has been used to elucidate aspects of the pain mechanisms e.g., modulation of the pain perception and alterations in pain processing, and is regarded as a valid method to assess trigeminal nociceptive function.[13-15] The physiology and anatomical distribution of the nBR can be described as follows: a) the peripheral pathways consist of trigeminal cutaneous fibers, responsible for the afferent limb and a group of motor fibers of the facial nerve, which constitute the efferent limb; b) the central pathways consist of interneurons located at the main and spinal trigeminal and the facial nucleus. Therefore, considering its anatomo-physiological arrangement, the nBR may help in the diagnostic of lesions that could affect the afferent and / or efferent limbs [16] Although there is no consensus about the complete validity of the nBR to activate only small nociceptive fibers, mainly because of lack of neurophysiological evidence regarding the selective small fiber activity, pain mechanisms of primary headaches and chronic orofacial pain have been addressed using the nBR paradigm.[17-20] Furthermore, important studies have been published concerning the

technical aspects of the nBR, e.g., stimulation parameters [21] and the description and properties of the electromyography (EMG) recordings.[22] However, to the best of our knowledge, there are no studies about the possible association with psychological factors for the nBR.

Taking into account the painful characteristic of the nBR test, an association between the nBR and psychological variables might be expected. Furthermore, considering that the nBR can be influenced by the startle response,[23] this presumed association is also a plausible hypothesis. There is evidence that the startle response can be influenced by psychological factors, since high levels of anxiety sensitivity are associated with heightened startle reactivity. [24] Finally, there is also evidence towards the influence of attention in the perceptual processing of BR responses. [25]

Based on that, the aim of this study was to evaluate the correlation between the nBR and psychological factors related to pain-related anxiety, vigilance to pain, somatosensory amplification or trait and situational pain catastrophizing. According to the objective, the overall hypothesis was: the nBR is, indeed, associated with psychological factors.

2. Materials and Methods

2.1 Participants

Healthy participants of both genders were recruited for this study using the convenience sampling method. All participants were staff members at Aarhus University and from the local community. Inclusion criteria were: age > 18 years old and good health without any orofacial pain complaints or headache disorders. On the other hand, the exclusion criteria were: serious dental or medical illness, self-reported psychiatric or personality disorders and inability to communicate or reading in English.

This study was performed in accordance with the Helsinki Declaration II and had the approval from the Central Denmark Region Ethics Committee. All participants gave their voluntary consent after a full explanation of the experiment procedures.

2.2 Variables

The outcomes of this study were the nBR test and the following questionnaires: a) the Anxiety Sensitivity Index-3 (ASI-3);[26] b) the Fear of Pain Questionnaire III (FPQ-III);[27] c) the Pain Vigilance and Awareness Questionnaire (PVAQ);[28] d) the Somatosensory Amplification Scale (SSAS);[29] e) the Pain Catastrophizing Scale (PCS);[30] and f) the Situational Pain Catastrophizing Scale (S-PCS).[31]

2.2.1 nBR

The nBR was performed in a quiet and acclimatized room (20^{o}C). Two surface self-adhesive EMG electrodes (Neuroline 720, Ambu ®, Denmark) were placed on both orbicularis oculi muscles regions to record the muscle activity and the ground electrode was attached to the wrist.[17, 18] The recorded signals were amplified and band-pass filtered between 20-1000 Hz with a sampling rate of 2000 Hz (Nicolet Viking™, Natus Medical Inc., USA). A custom built planar concentric electrode consisting of a central metal cathode and external anode ring with a diameter of, respectively, with 0.5 and 5 mm was used to elicit the nBR by stimulation of all three branches of the trigeminal nerve.[12] Each sweep comprised a train of three pulses with duration of 0.3 ms and inter-pulse interval of 3 ms and was applied to the skin directly above the entry zones of the right supraorbital (V1R), infraorbital (V2R) and the mental (V3R) nerve and also the left infraorbital (V2L) nerve.[18] The stimulation order among the branches was previously randomized by a computer-generated sequence.

The individual sensory (I_0) and pain thresholds (I_P) to the electrical stimulation were determined prior to the nBR recordings using an up-down staircase method consisting of 5 series of ascending and descending stimuli (0.2 mA increment rate) [21]. The I_0 was defined as the lowest stimulus intensity that evoked a sensation, whereas the I_P was the lowest intensity that evoked a sharp pin-prick like pain sensation.[17, 18]

For each site, the nBR recordings consisted of 6 stimulation blocks consisting of 6 individual sweeps with an inter-stimulus interval of 15-17 s to minimize habituation.[16] The intensities of the blocks were 50, 100, 150, 200, 300 and 400% of I_P and the order was also randomized by a computer-generated sequence. To avoid the overlapping with the startle reaction and the related R3 responses, the first stimulus of each block was clearly announced to the participant. Furthermore, the participants were asked to score the stimulus-evoked pain intensity at the end of each block with the aid of a 0-10 numerical rating scale (NRS) with 0 indicating no pain at all and 10 indicating worst pain imaginable.[17, 18]

The analyzed outcomes for the nBR considering each site were: a) the I_0 (mA); b) the I_P (mA); the EMG recordings of the R2 responses at each stimulus intensity, quantified as, c) the root mean square (RMS) (μV) and, d) area-under-the-curve (AUC) (μV x ms) of the rectified and averaged sweeps in the time window from 27-87 ms;[22] e) the onset latencies (ms) of the R2 responses at 200 and 300% of I_P measured for the averaged sweeps; f) the stimulus-evoked pain intensity (NRS) at each stimulus intensity.

2.2.2 ASI-3

The ASI-3 is s self-report questionnaire consisting of 18 items and it is used to measure the anxiety sensitivity, i.e., fear of anxiety-related sensations considering that they have adverse

consequences.[26] The items are rated on a 5-point scale ranging from 0 (agree very little) to 4 (agree very much) and the final score is the sum of all individual items.[26] Its psychometric properties have shown acceptable values for factorial validity (comparative fit index = 0.97) and reliability (Cronbach's alpha > 0.75).[26]

2.2.3 FPQ-III

The FPQ-III is a self-report questionnaire consisting of 30 items and it is used to measure the fear related to specific stimulus situations, e.g., fear related to severe pain (breaking your leg).[27] The items are rated on a 5-point scale ranging from 1 (not at all) to 5 (extreme) and the final score is the sum of all individual items.[27] Its psychometric properties have shown acceptable values for factorial validity (robust-comparative fit index = 0.91) and reliability (Cronbach's alpha = 0.93).[32]

2.2.4 PVAQ

The PVAQ is s self-report questionnaire consisting of 16 items and it is used to measure the attention to pain.[28] The items are rated on a 6-point scale ranging from 0 (never) to 5 (always) and the final score is the sum of all individual items.[28] Its psychometric properties have shown acceptable values for retention (corrected item-total score correlations ranging from 0.36 to 0.76) and reliability (Cronbach's alpha = 0.92).[33]

2.2.5 SSAS

The SSAS is a self-report questionnaire consisting of 10 items and it is used to measure the sensitivity to unpleasant bodily experiences.[29] The items are rated on a 5-point scale ranging from 1 (not at all true) to 5 (extremely true) and the final score is the sum of all individual items.[29] Its psychometric properties have shown acceptable values for concurrent validity

(linear regression coefficient ranging from 0.20 to 0.63) and reliability (Cronbach's alpha = 0.71).[29]

2.2.6. PCS

The PCS is a self-report questionnaire consisting of 13 items and it is used to measure the impact of catastrophic thoughts on past painful experiences.[30] The items are rated on a 5-point scale ranging from 0 (not at all) to 4 (all the time) and the final score is the sum of all individual items.[30] Its psychometric properties have shown acceptable values for factorial validity (robust-comparative fit index = 0.98) and reliability (Cronbach's alpha = 0.95).[34]

2.2.7 S-PCS

The S-PCS is a self-report questionnaire consisting of 6 items adapted from the PCS questionnaire and it is used to measure the thoughts or feelings experienced during laboratory procedures (e.g. the nBR test).[31] The items are rated on a 5-point scale ranging from 0 (not at all) to 4 (all the time) and the final score is the sum of all individual items.[31] Its psychometric properties have shown acceptable values for reliability (Cronbach's alpha = 0.87).[31]

2.3 Design

One examiner evaluated the nBR in a single session lasting approximately 1.5 hours and all questionnaires were applied immediately after the measurements of the nBR.

2.4 Statistics

The descriptive analysis of the nBR measurements (I_0, I_P, RMS, AUC, latency and NRS), expressed as means and standard deviation (SD) along with a description of the reflex occurrence can be found elsewhere.[35] The questionnaire outcomes were expressed as mean

(SD), range (maximum and minimum) and the 95% confidence interval (CI) for the mean was also described. All the quantitative variables were assessed for normal distribution using the Kolmogorov-Smirnov test and a log 10 transformation was performed when the test results were significant, considering an alpha level of 5% ($p < .05$).

The Pearson product-moment correlation coefficient was used to associate the average of nBR measurements among all sites as an overall measure of the nBR elicited by electrical stimulation of the trigeminal nerve, regardless of the branch, and all the questionnaires: a) ASI-3; b) FPQ-III; c) PVAQ; d) SSAS; e) PCS; f) S-PCS. The strength of correlation was evaluated based on the r coefficient, and the following score system was used to interpret the results: small ($r = 0.3$), moderate ($r = 0.5$) or strong ($r = 0.7$) correlation.[36] The sample size in this study was insufficient for use of regression models. Accordingly, in order to adjust for multiple comparisons, a prior planned Bonferroni correction lowered the significance level to 0.8% ($p = .008$) as the cut-off point to establish the statistical significance considering the correlation between the average of nBR measurements among all sites and the questionnaires. Each nBR measurement was considered as a family of comparisons, regardless of the stimulation intensity, and all the questionnaires were regarded as another family. Therefore, the familywise error rate was established considering 6 multiple comparisons and, according to the Bonferroni formula (.05 / k, where k = number of comparisons), an alpha level as $P = .008$ was set up. All tests were carried out using the software STATISTICA, v 12 (StatSoft Inc., USA).

3. Results

This study included twenty-one healthy participants with a mean age (SD) of 29.3 years (3.7) and the following gender distribution: 62 % (n = 13) female and 38% (n = 8) male. The

description of the questionnaires scores is presented in Table 1. None of the participants rated the maximum score in any particular questionnaire and the mean of all, except FPQ-III and SSAS, were below 50% of the maximum score. Also, 4.7% of the sample rated the minimum score for the ASI-3, 9.5% for the PCS and the 14.2% for the S-PCS.

There was no significant correlation for any pair of variables after Bonferroni correction ($p > .008$). However, there was a significant correlation considering the standard significance level ($p < .05$), where the pain intensity (NRS) at 50% of I_P presented a positive and small to moderate correlation with the PCS ($r = 0.43$, $p = .047$) (Table 2).

4. Discussion

The results of this study did not support the hypothesis of association between psychological factors and the nBR measurements and the main findings can be summarized as follows: a) after the correction for multiple comparisons, no significant correlations were found between the nBR measurements and the psychological factors.

Considering that healthy participants comprised the sample one could expect the psychological assessment outcomes to be within a range of "normal" values. In fact, the mean results in this sample for the ASI-3 indicated normative forms of anxiety sensitivity (score < 13).[24] Furthermore, the values of FPQ-III and PVAQ were also similar to clinical non-pain samples.[32, 33] SAS scores over 30 could reflect high somatization and the normal values ranged from 24-29,[37, 38] which agreed with the study mean score of 27.9. Finally, the PCS scores were also similar with non-patient populations.[34] However, the mean values of the S-PCS were lower in comparison with the S-PCS scores of healthy participants under

laboratory-induced pain tests, e.g., heat pain tolerance.[31] This could be explained by differences in the applied procedures. Since the S-PCS is closely related with the painful experience, lower values could be expected for lesser painful tests. Finally, it could be speculate that previous pain experiences might have affected the participants' responses, even though none of them reported to have painful conditions and considering that all questionnaires have previously been successfully applied in the general population. [26, 29, 31-34]

The lack of significant associations between the nBR and the associated psychophysical measurements after the adjustment for multiple comparisons was an interesting finding. Considering the nociceptive characteristic of the test and the battery of stimulations intensities, one would expect significant associations, at least between the reported pain intensities associated with the nBR and some of the questionnaires. For instance, there is evidence that the PCS is significantly correlated with cold pressor-induced pain intensity [39] and the S-PCS is strongly associated with experimental pain outcomes and predicted pain threshold in healthy participants.[40] Also, healthy participants with high levels of anxiety sensitivity present short detection latency for electrical stimuli.[41] The normal level profile regarding anxiety sensitivity presented by the sample could account for the results of no association between I_0 and I_P and the ASI-3. The relationship between the perception of experimental pain and the FPQ-III, which also measures pain-related anxiety constructs, is influenced by gender differences, being associated with low pain tolerance levels to heat pain only in healthy women.[42] So, considering that pain-related anxiety could be affected by gender differences,[42] the gender distribution pattern of this study can partially account for the findings of no association. Furthermore, vigilance to pain was related to sensitivity to heat pain in experimental models.[43] It is plausible that the association between psychological factors measured by these instruments and experimental pain outcomes is test-dependent and

could not be regarded as a general association with experimental pain experiences. Finally, there is no evidence about the relationship between the SAS and experimental pain conditions, although patients with a history of myofascial pain report high levels of somatosensory amplification.[44]

Not only were there no significant correlations after correcting for multiple comparisons, but even considering the standard significance level of 5%, there was only one significant association, which was potentially caused by chance given the high number of computed correlations. Therefore, it is possible to assert that the nBR test seems not to be strongly related with psychological factors and it may be less prone to bias regarding the psychological confounders. These confounders are important to control for when considering other tests used in pain research, such as quantitative sensory testing (QST).[45]

This study has several limitations that must be highlighted. First, the sample size was considered insufficient for use of regression models which is the best approach to analyze multiple correlations considering a single dependent variable. Second, the high number of questionnaires could be considered unnecessary and somehow confusing given the sample size. Nevertheless, it is important to state that the objective was to perform a potentially hypothesis-generating screening and the option for the specified questionnaires was based on their potential to be associated with pain tests, as already described. Furthermore, the possible influence of other psychological aspects not properly assessed with the applied questionnaires, such as attentional bias, depression symptoms and trait anxiety, could not be ruled out and warrant future research. One technical aspect that deserves to be mentioned is regarding the placement of the stimulation electrode. Even though such attachment was defined according to previous reports on the same technique, [12, 17, 18] other areas with high

concentration of nerve fibers, e.g. lips, could provide a more effective painful stimulus. Future studies are warranted to address this topic. Finally, the results should be generalized with caution, considering that in a patient population with chronic pain disorders, the relationship between psychological factors and the nBR may be different.

5. Conclusion

It seems that the nBR and its associated psychophysical measures may not be significantly associated with psychological factors related to pain-related anxiety, vigilance to pain, somatosensory amplification or trait and situational pain catastrophizing. However, sound conclusions remain to be confirmed and further investigations are required in pain populations with more pain-related psychological distress.

Acknowledgements

This study was supported by the Danish Dental Association and by the Coordination for the Improvement of Higher Education Personnel (CAPES – Proc. n^{o} BEX 4306/14-7).

Conflict of Interest Statement

None of the authors have potential conflicts of interest to be disclosed.

References

1. Engel GL. The need for a new medical model: a challenge for biomedicine. Science 1977;196:129-136.

2. Melzack R, Casey KL. Sensory, motivational, and central control determinants of pain. In: Kenshalo DR (ed). The Skin Senses. Springfield: Charles C Thomas, 1968:423-439.

3. Barr WB. Differential diagnosis of psychological factors evoked by pain presentations. Clin Neuropsychol 2013;27:17-29.

4. Lumley MA, Cohen JL, Borszcz GS, Cano A, Radcliffe AM, Porter LS, et al. Pain and emotion: a biopsychosocial review of recent research. J Clin Psychol 2011;67:942-968.

5. McGrath PA. Psychological aspects of pain perception. Arch Oral Biol 1994;39 Suppl:55S-62S.

6. Phyomaung PP, Dubowitz J, Cicuttini FM, Fernando S, Wluka AE, Raaijmaakers P, et al. Are depression, anxiety and poor mental health risk factors for knee pain? A systematic review. BMC Musculoskelet Disord 2014;15:10.

7. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Groupdagger. J Oral Facial Pain Headache 2014;28:6-27.

8. Costa YM, Porporatti AL, Stuginski-Barbosa J, Bonjardim LR, Conti PC. Additional effect of occlusal splints on the improvement of psychological aspects in temporomandibular disorder subjects: A randomized controlled trial. Arch Oral Biol 2015;60:738-744.

9. Fuss I, Angst F, Lehmann S, Michel BA, Aeschlimann A. Prognostic factors for pain relief and functional improvement in chronic pain after inpatient rehabilitation. Clin J Pain 2014;30:279-285.

10. Arntz A, Claassens L. The meaning of pain influences its experienced intensity. Pain 2004;109:20-25.

11. Watkinson P, Wood AM, Lloyd DM, Brown GD. Pain ratings reflect cognitive context: a range frequency model of pain perception. Pain 2013;154:743-749.

12. Kaube H, Katsarava Z, Kaufer T, Diener H, Ellrich J. A new method to increase nociception specificity of the human blink reflex. Clin Neurophysiol 2000;111:413-416.

13. Giffin NJ, Kowacs F, Libri V, Williams P, Goadsby PJ, Kaube H. Effect of the adenosine A1 receptor agonist GR79236 on trigeminal nociception with blink reflex recordings in healthy human subjects. Cephalalgia 2003;23:287-292.

14. Rehberg B, Baars JH, Kotsch J, Koppe P, von Dincklage F. Comparison of trigeminal and spinal modulation of pain and nociception. Int J Neurosci 2012;122:298-304.

15. Perchet C, Frot M, Charmarty A, Flores C, Mazza S, Magnin M, et al. Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. Pain 2012;153:1244-1252.

16. Esteban A. A neurophysiological approach to brainstem reflexes. Blink reflex. Neurophysiol Clin 1999;29:7-38.

17. Baad-Hansen L, List T, Jensen TS, Leijon G, Svensson P. Blink reflexes in patients with atypical odontalgia. J Orofac Pain 2005;19:239-247.

18. Baad-Hansen L, List T, Kaube H, Jensen TS, Svensson P. Blink reflexes in patients with atypical odontalgia and matched healthy controls. Exp Brain Res 2006;172:498-506.

19. Katsarava Z, Lehnerdt G, Duda B, Ellrich J, Diener HC, Kaube H. Sensitization of trigeminal nociception specific for migraine but not pain of sinusitis. Neurology 2002;59:1450-1453.

20. Peddireddy A, Wang K, Svensson P, Arendt-Nielsen L. Blink reflexes in chronic tension-type headache patients and healthy controls. Clin Neurophysiol 2009;120:1711-1716.

21. von Dincklage F, Koppe P, Kotsch J, Baars JH, Rehberg B. Investigation of threshold and magnitude criteria of the nociceptive blink reflex. Clin Neurophysiol 2010;121:945-949.

22. Ellrich J, Treede RD. Characterization of blink reflex interneurons by activation of diffuse noxious inhibitory controls in man. Brain Res 1998;803:161-168.

23. Ellrich J, Katsarava Z, Przywara S, Kaube H. Is the R3 component of the human blink reflex nociceptive in origin? Pain 2001;91:389-395.

24. McMillan KA, Asmundson GJ, Zvolensky MJ, Carleton RN. Startle response and anxiety sensitivity: subcortical indices of physiologic arousal and fear responding. Emotion 2012;12:1264-1272.

25. Anthony BJ, Graham FK. Blink reflex modification by selective attention: evidence for the modulation of 'automatic' processing. Biol Psychol 1985;21:43-59.

26. Taylor S, Zvolensky MJ, Cox BJ, Deacon B, Heimberg RG, Ledley DR, et al. Robust dimensions of anxiety sensitivity: development and initial validation of the Anxiety Sensitivity Index-3. Psychol Assess 2007;19:176-188.

27. McNeil DW, Rainwater AJ, 3rd. Development of the Fear of Pain Questionnaire--III. J Behav Med 1998;21:389-410.

28. Roelofs J, Peters ML, McCracken L, Vlaeyen JW. The pain vigilance and awareness questionnaire (PVAQ): further psychometric evaluation in fibromyalgia and other chronic pain syndromes. Pain 2003;101:299-306.

29. Speckens AE, Spinhoven P, Sloekers PP, Bolk JH, van Hemert AM. A validation study of the Whitely Index, the Illness Attitude Scales, and the Somatosensory Amplification Scale in general medical and general practice patients. J Psychosom Res 1996;40:95-104.

30. Sullivan MJL, Bishop SC, Pivik J. The Pain Catastrophizing Scale: development and validation. Psycho Assess 1995;7:524-532.

31. Edwards RR, Smith MT, Stonerock G, Haythornthwaite JA. Pain-related catastrophizing in healthy women is associated with greater temporal summation of and reduced habituation to thermal pain. Clin J Pain 2006;22:730-737.

32. Osman A, Breitenstein JL, Barrios FX, Gutierrez PM, Kopper BA. The Fear of Pain Questionnaire-III: further reliability and validity with nonclinical samples. J Behav Med 2002;25:155-173.

33. McWilliams LA, Asmundson GJ. Assessing individual differences in attention to pain: psychometric properties of the Pain Vigilance and Awareness Questionnaire modified for a non-clinical pain sample. Pers Individ Dif 2001;31:239-246.

34. Osman A, Barrios FX, Gutierrez PM, Kopper BA, Merrifield T, Grittmann L. The Pain Catastrophizing Scale: further psychometric evaluation with adult samples. J Behav Med 2000;23:351-365.

35. Costa YM, Baad-Hansen L, Bonjardim LR, Conti PC, Svensson P. Reliability of the nociceptive blink reflex evoked by electrical stimulation of the trigeminal nerve. Clin Neurophysiol 2015;Submitted manuscript.

36. da Costa DR, de Lima Ferreira AP, Pereira TA, Porporatti AL, Conti PC, Costa YM, et al. Neck disability is associated with masticatory myofascial pain and regional muscle sensitivity. Arch Oral Biol 2015;60:745-752.

37. Nakao M, Barsky AJ. Clinical application of somatosensory amplification in psychosomatic medicine. Biopsychosoc Med 2007;1:17.

38. Nakao M, Tamiya N, Yano E. Gender and somatosensory amplification in relation to perceived work stress and social support in Japanese workers. Women Health 2005;42:41-54.

39. Sullivan MJ, Thorn B, Rodgers W, Ward LC. Path model of psychological antecedents to pain experience: experimental and clinical findings. Clin J Pain 2004;20:164-173.

40. Campbell CM, Kronfli T, Buenaver LF, Smith MT, Berna C, Haythornthwaite JA, et al. Situational versus dispositional measurement of catastrophizing: associations with pain responses in multiple samples. J Pain 2010;11:443-453 e442.

41. Esteve MR, Camacho L. Anxiety sensitivity, body vigilance and fear of pain. Behav Res Ther 2008;46:715-727.

42. Thibodeau MA, Welch PG, Katz J, Asmundson GJ. Pain-related anxiety influences pain perception differently in men and women: a quantitative sensory test across thermal pain modalities. Pain 2013;154:419-426.

43. Baum C, Huber C, Schneider R, Lautenbacher S. Prediction of experimental pain sensitivity by attention to pain-related stimuli in healthy individuals. Percept Mot Skills 2011;112:926-946.

44. Raphael KG, Marbach JJ, Gallagher RM. Somatosensory amplification and affective inhibition are elevated in myofascial face pain. Pain Med 2000;1:247-253.

45. Backonja MM, Attal N, Baron R, Bouhassira D, Drangholt M, Dyck PJ, et al. Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. Pain 2013;154:1807-1819.

Tables

Table 1. Psychological characteristics of the sample. ASI-3: Anxiety Sensitivity Index-3. FPQ-III: Fear of Pain Questionnaire III. PVAQ: Pain Vigilance and Awareness Questionnaire. SSAS: Somatosensory Amplification Scale. PCS: Pain Catastrophizing Scale. S-PCS: Situational Pain Catastrophizing Scale.

Questionnaire	Mean (SD)	95% CI of Mean	Range	Population Mean (SD)[1]
ASI-3	12.0 (9.1)	7.9 – 16.2	0 – 41	12.8 (10.6)
FPQ-III	81.9 (23.7)	71.0 – 92.7	37 – 133	78.2 (18.1)
PVAQ	38.2 (14.7)	31.5 – 45.0	9 – 67	33.54 (13.18)
SSAS	27.9 (6.1)	25.0 – 30.7	16 – 37	26.1 (7.0)
PCS	16.7 (10.7)	11.8 – 21.6	0 – 35	13.87 (10.11)
S-PCS	3.0 (2.6)	1.8 – 4.2	0 – 10	4.93 (3.89)[2]

[1] These values are described in the following references: #26, #32, #33, #39, #33 and #40.
[2] Values based on supra threshold heat stimuli and cold pressor tests (Reference #40).

Table 2. Pearson product moment correlations between the Anxiety Sensitivity Index-3 (ASI-3), the Fear of Pain Questionnaire III (FPQ-III), the Pain Vigilance and Awareness Questionnaire (PVAQ), the Somatosensory Amplification Scale (SSAS), the Pain Catastrophizing Scale (PCS) and the Situational Pain Catastrophizing Scale (S-PCS) and the nociceptive blink reflex (nBR) measurements. I_0 = individual sensory threshold, I_P = individual pain threshold, NRS = Numerical Rating Scale, RMS = root mean square (RMS µV), AUC = area-under-the-curve (AUC, µV x ms).

nBR measurements	**r / p-value**					
	ASI-3	**FPQ-III**	**PVAQ**	**SAS**	**PCS**	**S-PCS**
Thresholds (mA)						
I_0	0.33 / .13	0.18 / .42	0.16 / .48	0.41 / .06	0.20 / .38	0.19 / .39
I_P	0.25 / .26	0.29 / .18	0.05 / .82	0.43 / .05	0.16 / .47	0.24 / .29
Pain Intensity (NRS) – % of I_P						
50%	0.17 / .44	-0.20 / .37	0.10 / .64	0.16 / .46	0.43 / .04	0.27 / .22
100%	-0.00 / .99	0.10 / .65	0.20 / .36	-0.06 / .77	0.20 / .37	0.29 / .19
150%	0.05 / .80	0.25 / .27	0.31 / .16	-0.01 / .95	0.24 / .28	0.31 / .17
200%	-0.05 / .82	0.28 / .20	0.16 / .46	-0.15 / .50	0.03 / .87	0.35 / .11
300%	-0.07 / .73	0.29 / .19	0.15 / .49	-0.13 / .55	0.04 / .83	0.35 / .10
400%	0.04 / .86	0.17 / .51	-0.09 / .73	-0.17 / .52	-0.06 / .80	0.36 / .15
RMS (µV) – % of I_P						
50%	-0.18 / .42	0.34 / .13	0.03 / .88	0.04 / .84	0.18 / .41	0.19 / .40
100%	0.21 / .33	0.26 / .23	-0.03 / .88	0.27 / .22	0.08 / .72	0.29 / .19
150%	0.28 / .21	0.27 / .21	-0.05 / .82	0.34 / .12	-0.04 / .85	0.30 / .08
200%	0.42 / .05	0.29 / .19	0.05 / .82	0.39 / .07	0.04 / .84	0.34 / .12
300%	0.41 / .06	0.17 / .43	-0.11 / .62	0.40 / .07	-0.08 / .72	0.41 / .06
400%	0.43 / .09	0.26 / .38	-0.03 / .88	0.25 / .33	0.20 / .43	0.44 / .08
AUC (µV x ms) – % of I_P						
50%	-0.18 / .41	0.35 / .11	0.05 / .82	0.03 / .87	0.20 / .36	0.19 / .39
100%	0.20 / .37	0.28 / .20	-0.00 / .98	0.27 / .22	0.11 / .62	0.30 / .17
150%	0.27 / .23	0.28 / .21	-0.03 / .89	0.32 / .15	-0.02 / .90	0.38 / .08
200%	0.42 / .05	0.31 / .16	0.09 / .67	0.41 / .06	0.07 / .75	0.34 / .12
300%	0.39 / .07	0.18 / .41	-0.09 / .66	0.41 / .06	-0.04 / .83	0.41 / .06
400%	0.41 / .12	0.25 / .34	-0.04 / .87	0.23 / .38	0.20 / .44	0.43 / .08
Latency (ms)						
200% Ip	-0.30 / .22	0.18 / .47	-0.18 / .46	-0.32 / .19	-0.32 / .18	-0.18 / .47
300% Ip	-0.41 / .08	-0.03 / .88	-0.01 / .94	-0.24 / .33	0.07 / .75	-0.16 / .51

3 Discussion

3 DISCUSSION

The main findings of this systematic evaluation of the nBR were: a) the ICCs and Kappa values of the nBR are acceptable for clinical and research application; b) the stimulation intensities at 200 and 300% of I_P showed the highest coefficients whereas the stimulations on the V3R presented the lowest ones; c) there were no significant correlations between the nBR measurements and the psychological factors even though some positive correlations between the nBR measurements of RMS and AUC at 200 (1) and 300% (2) of I_P, the ASI-3 (3), SSAS (4) and S-PCS (5) almost reached the the statistical significance level of 5%. (Table 2, Article 2).

Reliability analysis

The first article of this study focused on the estimation of reliability parameters of the nBR. Despite its widespread use, only two studies have presented some reliability measurements (KATSARAVA et al., 2002; VON DINCKLAGE et al., 2010), but considering that their main purposes were not the estimation of this important aspect of medical tests, so far, informative and systematic results were lacking. Therefore, our findings provide evidences for the quality and suitability of the nBR test and the associated psychophysical measurements and it stresses its value for clinical neurophysiologic practice. Furthermore, the distribution of the ICCs calls attention to the technical aspects of the nBR measurements. First, the fair to excellent reliability coefficients for the I_P indicated that it is possible to establish a reproducible electrical stimulation pain threshold using the specific design of a concentric surface electrode. One can argue that the psychophysical aspect of the nBR, which defines the stimulation intensities of the nBR recordings, could be considered a shortcoming, since the patient cooperation and attention account for the results. However, our ICCs showed the individualized threshold determination as an acceptable method. Nonetheless, the low inter-individual variation of the I_P values indicates that it can be plausible to use absolute and standardized intensities of stimulation, which may reduce the subjectivity of the test. Further research is required to explore this important aspect in detail. Another interesting detail regarding the stimulation intensity was the high coefficients for the nBR measurements considering the intensities at 200 and 300% of I_P, which indicate more stable responses within these amplitudes. Finally, there is no established guideline on how to perform the nBR, e.g., analysis of signal averaging versus single trials, differences in the stimulus intensity and ISI,

number of the stimulation blocks and which EMG parameters to use. Although there is evidence pointing out a better performance of some technical aspects (KATSARAVA et al., 2002; VON DINCKLAGE et al., 2010), there is no published protocol summarizing all the methodological details with scientific support.

The different ICCs among the trigeminal branches, in particular the poor reliability for the V3 division is also an interesting finding. The inconsistency of nBR responses from this branch could be associated with the short ISI (15-17 s) adopted in this study that could contribute to an accentuated habituation. In fact, there is evidence showing that the BR can be consistently elicited from V3 division only with long ISIs (around 30 s) (JAASKELAINEN, 1995). Future research should address if different ISIs can affect the reliability not only for the V3R, but also for the other trigeminal nerve branches. We cannot rule out a cumulative habituation effect considering the sequence of stimulation in 4 different sites of the same nerve (trigeminal nerve). Nevertheless, since we randomized the sites and stimulation intensities, any claimed cumulative habituation effect was randomly distributed.

The onset latency is a subjective part of the nBR test, because it is dependent upon the examiner experience and expertise. Also, the time framing for the latency recording allows only a small variability for the values, which means that in this specific situation it is difficult to distinguish the "true" variability from the measurement error (BANNIGAN; WATSON, 2009). All of that could explain the "less than optimal" reliability parameters for the latency assessment. Furthermore, we can see a "learning" effect, or at least a "convergent" evaluation when comparing the readings of the 1st trial (after one calibration session) with the 2nd trial (after a recalibration session). The examiner qualifications, degree of training and clinical background are important factors for the reliability estimation (KOTTNER et al., 2011). In such scenario it is important to highlight the differences in the clinical expertise regarding the nBR test between the two examiners of this study. The first examiner did not have any previous experience in the nBR assessment, whereas the second examiner had previous experience in the nBR test. Probably, different examiners with a daily contact and long experience with the nBR test could achieve higher coefficients. On the other hand, we cannot rule out the assessment bias of the examiners or the Hawthorne effect (MCCAMBRIDGE; WITTON; ELBOURNE, 2014), considering that both examiners were aware that their readings would be compared each other. All in all, these factors should be taken into consideration to evaluate not only our results, but also any reliability study.

Psychological factors analysis

By definition, the nBR is a painful procedure (KAUBE et al., 2000). Accordingly, it would be expected some association between psychological factors and the nBR test, mainly, with the associated psychophysical measurements. We tested the liner correlation between psychological factors related to pain-related anxiety, vigilance to pain, somatosensory amplification or trait and situational pain catastrophizing with the results of the 1st trial of the nBR and we could not find any association. To some extent, it was an unexpected result, considering that these psychological factors, i.e., pain-related anxiety, vigilance to pain, somatosensory amplification or trait and situational pain catastrophizing, are associated with other pain-related tests, such as, cold-pressor pain, heat pain threshold and pressure pain threshold (SULLIVAN et al., 2004; CAMPBELL et al., 2010; BAUM et al., 2011).

Although our findings suggest that the psychological factors could not affect the nBR measurements, sound conclusions remain to be established considering the limitations for this specific part of the study. First, we reported the results of multiple comparisons with a small sample size. So, the risk for type II error is higher than the standard 20% established for hypothesis testing. The report of possible associations between psychological factors and the nBR was a secondary aim for this study and the sample size was not calculated taking into account the specific design of multiple linear regression, where, as general rule, requires between 15-20 subjects for each independent variable to be included in the model. Also, controlling for sex differences was not suitable also because of the small sample. There is evidence that psychological factors are differently associated with the pain-related events when comparing males and females (THIBODEAU et al., 2013). On the other hand, the trends toward statistical significance for some associations might not be totally neglected (Table 1, Annex). For instance, the probability values for the correlation between the ASI-3 and the RMS and AUC at 200 and 300% of I_P could require further investigation with a sample size where is it is possible to analyze the effect of high and low anxiety sensitivity. This distinction can also be applied for the S-PCS, considering that a high pain-related catastrophizing might influence the nBR outcomes. A large sample size is required to properly perform such sub-group analysis.

Other psychological aspects, not properly assessed with the questionnaires applied for this study, such as, attentional bias, depression symptoms and trait anxiety, could be

associated with the nBR. Taking into account the biopsychosocial model, it is presumable and generally accepted that psychological factors are important in any painful event (ENGEL, 1977). However, the tools used to assess these psychological factors sometimes are not straightforward, which is partially explained by the nature of the measured phenomenon, i.e., subjective constructs. Accordingly, studies with questionnaires should be analyzed with caution, since it not possible to rely on their information as a comprehensive psychological assessment. Finally, the results of non-linear correlation do not rule out other possible types of association, such as, curvilinear or exponential. The need for further clarification on this regard considering a bigger sample size and other instruments can also be justified by various trends towards statistical association found in our study. Interestingly, the majority of these trends were related to nBR measurements within the intensity interval between 200 and 300% of I_P, which also presented the highest reliability coefficients. Therefore, the technical aspects of the nBR also might account for a possible association with psychological factors.

4 Conclusions

4 CONCLUSIONS

Based on the studies described in this thesis, we conclude that:

1. The nBR and its associated psychophysical measures can be considered an adequately reliable test to evaluate the trigeminal nociceptive function.

2. The nBR and its associated psychophysical measures seem not linear nor significantly associated with psychological factors related to pain-related anxiety, vigilance to pain, somatosensory amplification or trait and situational pain catastrophizing.

References

REFERENCES

Baad-Hansen L, List T, Jensen TS, Leijon G, Svensson P. Blink reflexes in patients with atypical odontalgia. Journal of orofacial pain. 2005 Summer;19(3):239-47.

Baad-Hansen L, List T, Kaube H, Jensen TS, Svensson P. Blink reflexes in patients with atypical odontalgia and matched healthy controls. Experimental brain research. 2006 Jul;172(4):498-506.

Bannigan K, Watson R. Reliability and validity in a nutshell. Journal of clinical nursing. 2009 Dec;18(23):3237-43.

Baum C, Huber C, Schneider R, Lautenbacher S. Prediction of experimental pain sensitivity by attention to pain-related stimuli in healthy individuals. Percept Mot Skills. 2011 Jun;112(3):926-46.

Campbell CM, Kronfli T, Buenaver LF, Smith MT, Berna C, Haythornthwaite JA, et al. Situational versus dispositional measurement of catastrophizing: associations with pain responses in multiple samples. The journal of pain : official journal of the American Pain Society. 2010 May;11(5):443-53 e2.

Duncan G. Mind-body dualism and the biopsychosocial model of pain: what did Descartes really say? J Med Philos. 2000 Aug;25(4):485-513.

Ellrich J. Brain Stem Reflexes: Probing Human Trigeminal Nociception. News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society. 2000 Apr;15(94-7.

Ellrich J, Hopf HC. The R3 component of the blink reflex: normative data and application in spinal lesions. Electroencephalography and clinical neurophysiology. 1996 Aug;101(4):349-54.

Engel GL. The need for a new medical model: a challenge for biomedicine. Science. 1977 Apr 8;196(4286):129-36.

Esteban A. A neurophysiological approach to brainstem reflexes. Blink reflex. Neurophysiol Clin. 1999 Feb;29(1):7-38.

Fine EJ, Sentz L, Soria E. The history of the blink reflex. Neurology. 1992 Feb;42(2):450-4.

Jaaskelainen SK. Blink reflex with stimulation of the mental nerve. Methodology, reference values, and some clinical vignettes. Acta Neurol Scand. 1995 Jun;91(6):477-82.

Katsarava Z, Ellrich J, Diener HC, Kaube H. Optimized stimulation and recording parameters of human 'nociception specific' blink reflex recordings. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2002 Dec;113(12):1932-6.

Kaube H, Katsarava Z, Kaufer T, Diener H, Ellrich J. A new method to increase nociception specificity of the human blink reflex. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2000 Mar;111(3):413-6.

Kaube H, Katsarava Z, Przywara S, Drepper J, Ellrich J, Diener HC. Acute migraine headache: possible sensitization of neurons in the spinal trigeminal nucleus? Neurology. 2002 Apr 23;58(8):1234-8.

Kottner J, Audige L, Brorson S, Donner A, Gajewski BJ, Hrobjartsson A, et al. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. Journal of clinical epidemiology. 2011 Jan;64(1):96-106.

Kugelberg E. [Facial reflexes]. Brain : a journal of neurology. 1952 Sep;75(3):385-96.

McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. Journal of clinical epidemiology. 2014 Mar;67(3):267-77.

Mokkink LB, Terwee CB, Patrick DL, Alonso J, Stratford PW, Knol DL, et al. The COSMIN study reached international consensus on taxonomy, terminology, and definitions of measurement properties for health-related patient-reported outcomes. Journal of clinical epidemiology. 2010 Jul;63(7):737-45.

Peddireddy A, Wang K, Svensson P, Arendt-Nielsen L. Blink reflexes in chronic tension-type headache patients and healthy controls. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2009 Sep;120(9):1711-6.

Sullivan MJ, Thorn B, Rodgers W, Ward LC. Path model of psychological antecedents to pain experience: experimental and clinical findings. Clin J Pain. 2004 May-Jun;20(3):164-73.

Thibodeau MA, Welch PG, Katz J, Asmundson GJ. Pain-related anxiety influences pain perception differently in men and women: a quantitative sensory test across thermal pain modalities. Pain. 2013 Mar;154(3):419-26.

Valls-Sole J. Neurophysiological assessment of trigeminal nerve reflexes in disorders of central and peripheral nervous system. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2005 Oct;116(10):2255-65.

von Dincklage F, Koppe P, Kotsch J, Baars JH, Rehberg B. Investigation of threshold and magnitude criteria of the nociceptive blink reflex. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2010 Jun;121(6):945-9.

Annexes

Annex 1

Submission letter to the journal *Clinical Oral Investigations*.

Clinical Oral Investigations October 6, 2015 at 12:08 PM

To: Yuri Martins Costa Inbox - Yahoo!

CLOI: A manuscript number has been assigned to Reliability of the nociceptive blink reflex evoked by electrical stimulation of the trigeminal nerve

Dear Dr. Costa,

Your submission entitled "Reliability of the nociceptive blink reflex evoked by electrical stimulation of the trigeminal nerve" has been assigned the following manuscript number: CLOI-D-15-00941.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author.
The URL is http://cloi.edmgr.com/.

Thank you for submitting your work to this journal.

Kind regards,

Editorial Office
Clinical Oral Investigations

Annex 2

Acceptance letter of the journal *Journal of Oral and Facial Pain and Headache*.

Journal of Oral and Facial Pain and Headache
To: Yuri Martins Costa, jop@manuscriptmanager.com
manuscript 1598 - Decision - Journal of Oral and Facial Pain and Headache

October 29, 2015 at 1:48 PM
Inbox - Yahoo!

JO

Manuscript title: Is the nociceptive blink reflex associated with psychological factors in healthy participants?

Dear Dr Costa,

Associate Editor Dr. Greg Murray informed me that your revised manuscript has been reviewed and recommended its acceptance. I have also reviewed the manuscript and agree with this recommendation. Therefore, the manuscript will be forwarded to Quintessence Publishing Company and should appear in the Journal of Oral & Facial Pain and Headache in the near future.

Please note that there may be some points and queries that you will need to address at the proof stage.

Thank you again for submission of this important manuscript to the Journal of Oral & Facial Pain and Headache.

Yours sincerely,
Barry J. Sessle
Editor-in-Chief

Annex 3

Ethical approval letter.

Regionshuset
Viborg
Regionsssekretariatet
Juridisk kontor
De Videnskabsetiske Komiteer
For Region Midtjylland
Skottenborg 26
DK-8800 Viborg
Tel. +45 7841 0183
komite@rm.dk
www.komite.rm.dk

Peter Svensson, Professor, dr.odont.
Aarhus Universitetshospital
Tand-, Mund- og Kæbekirurgisk Afdeling O
Nørrebrogade 44
8000 Aarhus C

midt
regionmidtjylland

Projekt: Sensorisk og elektrofysiologisk undersøgelse af patienter med persisterende idiopatiske orofaciale smerter samt raske forsøgspersoner.

Dato 02-10-2013
Sagsbehandler Anne-Marie Eybye
komite@rm.dk
Tel. +45 7841 0183
Sagsnr. 1-10-72-257-13

De Videnskabsetiske Komitéer for Region Midtjylland har behandlet de anmeldte ændringer modtaget med mail den 7. august 2013 til ovenstående projekt.

Komité II har godkendt tillæg 1 med anmeldelsesnr. 39544. Godkendelsen omfatter de anmeldte ændringer og følgende dokumenter:

- Tillægsprotokol 1, version og dato ikke angivet, fil navngivet V2-300813, fremsendt med mail af 30. august 2013.
- Deltagerinformation til patienter, version 3, fremsendt med mail af 7. august 2013.
- Deltagerinformation til raske forsøgspersoner, version 3, fremsendt med mail af 7. august 2013.
- Samtykkeerklæring (S4), version 4, fil navngivet V4-300813, fremsendt med mail af 30. august 2013.

Projektet er nu registreret med ny afslutningsdato 31. december 2015.

Komitéen vil samtidig minde om forskers forpligtigelser jf. komitéloven.

Komitéloven foreskriver, at den forsøgsansvarlige eller sponsor én gang årligt i hele forsøgsperioden skal indsende en liste til komitéen over alle de alvorlige bivirkninger og alvorlige hændelser, som er indtruffet i perioden, samt give oplysning om forsøgspersonernes sikkerhed. Har der ikke været alvorlige bivirkninger og hændelser skal dette ligeledes indberettes.

Ovennævnte lister og oplysninger skal sendes som pdf-filer til komitéernes mailadresse *komite@rm.dk*. Ved indberetning kan anvendes et skema, der findes på *www.dnvk.*dk.

Senest 90 dage efter projektets planlagte afslutning, skal du underrette komitéen om afslutningen. Afsluttende rapport eller publikation skal du fremsende, når den foreligger.

Afbryder du projektet tidligere end planlagt, skal du meddele afbrydelsesdato og årsag til komitéen inden for 15 dage.

Alle henvendelser vedrørende projektet bedes rettet til komitéernes sekretariat med henvisning til sagsnr.

midt
regionmidtjylland

Venlig hilsen

Anne-Marie Eybye

Anne-Marie Eybye
Sekretær

Kopi til:

- Lektor Lene Baad-Hansen, Aarhus Universitet