THE VALUE OF QUANTITATIVE MEASUREMENT OF PAIN SENSATION

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ABSTRACT

Objective: The value of quantitative measurement of pain sensation after C nociceptor activation with transdermal sinusoidal current wave stimulation protocol and skin biopsy.

Methods: 30 healthy volunteers aged 20-30 years (17 females 18 males) participated after having given their informed consent. Half-sine wave pulses of 0.5sec duration (1Hz) were generated at intensities of 0.2 to 1 mA by a constant current stimulator. Apart from half-sine wave stimulation, sine wave pulses of 60sec duration (4Hz) were generated at intensity of 0.2mA also by the constant current stimulator (Digitimer Ltd, Welwyn Garden City, UK) controlled by DAPSYS 8 (www.dapsys.net). Moreover, we performed 3-mm skin punch biopsies 10 cm above the lateral malleolus of the leg and in the middle of the volar side of the forearm to the volunteer's group.

Results: We demonstrate that delivering transdermal sinusoidal half sine wave when trying to stimulate mechano-sensitive C fibers, when the amplitude of the delivered wave is increased from 0.2mA to 1mA our protocol, pain sensation is also increased following the same scheme. If we observe a different scheme of activation in C fibers, this could be a sign of neuropathic pain. Considering the mechano-insensitive C fibers of pain, when trying to stimulate them we expect increasing pain sensation and then familiarization, desensitization and reduction of pain sensation. As a result, if this scheme isn't observed when sine wave is delivered transdermal with 1 min of duration, and we observe a different scheme, a C fiber neuropathy and neuropathic pain could be involved. Regarding the skin biopsies, a correlation between pain sensation of sine wave (delivered transdermal to stimulate mechano-insensitive C fibers of pain on the forearm), and the nerve fiber density was observed. A correlation between the bifurcated fibers of the biopsy site and the pain sensation was observed when mechanosensitive and mechano-insensitive fibers are stimulated, which needs further investigation. Also, a correlation between the remnant nerve fibers of subepidermal nerve plexus and mechano-insensitive nerve fibers of pain is observed that also needs further investigation.

Conlclusion: Skin biopsy and transdermal electrical stimulation are very promising available tools of diagnosing C fiber neuropathies and assessing neuropathic pain.

Keywords: neuropathic pain, painful neuropathy, peripheral neuropathy, chronic pain, nerve fibers, sinusoidal transdermal stimulation, skin biopsy

Η ΣΗΜΑΣΙΑ ΤΗΣ ΠΟΙΟΤΙΚΗΣ ΕΚΤΙΜΗΣΗΣ ΤΗΣ ΑΙΣΘΗΣΗΣ ΤΟΥ ΠΟΝΟΥ

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Περίληψη

Στόχος: Η σημασία της ποιοτικής εκτίμησης της αίσθησης πόνου μετά την ενεργοποίηση των C ινών με πρωτόκολλο διαδερμικής ημιτονοειδούς διέγερσης και η αξιολόγηση των αποτελεσμάτων με βιοψία δέρματος.

Μέθοδοι: Συμμετείχαν 30 υγιείς εθελοντές ηλικίας 20-30 ετών (17 γυναίκες και 18 άντρες) μετά από

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ

ενημέρωση και έγγραφη συγκατάθεση. Χορηγήθηκαν παλμοί ημιτονοειδούς κύματος διάρκειας 0,5 δευτερολέπτων (1 Hz) σε αυξανόμενες εντάσεις από 0,2 έως 1 mA από έναν διεγέρτη σταθερού ρεύματος. Εκτός από τη διέγερση ημιτονοειδούς κύματος, παλμοί ημιτονοειδούς κύματος διάρκειας 60 δευτερολέπτων (4Hz) χορηγήθηκαν σε ένταση 0,2 mA επίσης από τον διεγέρτη σταθερού ρεύματος (Digitimer Ltd, Welwyn Garden City, UK) που ελέγχεται από το DAPSYS 8 (www.dapsys.net). Επιπλέον, πραγματοποιήθηκαν βιοψίες δέρματος πάχους 3 mm, 10 cm πάνω από τον δεξιό έξω σφυρό και στη μεσότητα του δεξιού αντιβραχίου.

Αποτελέσματα: Αποδεικνύεται ότι η χορήγηση διαδερμικού ημιτονοειδούς ρεύματος κατά την προσπάθεια διέγερσης μηχανοευαίσθητων ινών C, όταν η ένταση του χορηγούμενου ρεύματος αυξάνεται, αυξάνει και η αίσθηση πόνου ακολουθώντας το ίδιο πρότυπο. Εάν παρατηρήσουμε διαφορετικό σχήμα ενεργοποίησης στις ίνες C, μπορεί να είναι ένδειξη νευροπαθητικού πόνου. Όσον αφορά τις μηχανο-ευαίσθητες ίνες C του πόνου, όταν προσπαθούμε να τις διεγείρουμε τις ίνες αυτές αναμένουμε αυξανόμενη αίσθηση πόνου και στη συνέχεια εξοικείωση, απευαισθητοποίηση και μείωση της αίσθησης πόνου. Ως αποτέλεσμα, εάν αυτό το σχήμα δεν παρατηρηθεί όταν το ημιτονοειδές κύμα χορηγείται διαδερμικά με διάρκεια 1 λεπτό και παρατηρήσουμε ένα διαφορετικό σχήμα, μπορεί να εμπλέκεται νευροπάθεια ινών C και νευροπαθητικός πόνος. Όσον αφορά τις βιοψίες δέρματος, παρατηρήθηκε συσχέτιση μεταξύ της αίσθησης πόνου, του ημιτονοειδούς κύματος (που χορηγήθηκε διαδερμικά για την διέγερση των μηχανο-ευαίσθητων C ινών πόνου στο αντιβράχιο), και της πυκνότητας των νευρικών ινών. Παρατηρήθηκε συσχέτιση μεταξύ των δικασμένων ινών της θέσης βιοψίας και της αίσθησης πόνου όταν διεγείρονται μηχανοευαίσθητες και μηχανοευαίσθητες ίνες, κάτι που χρήζει περαιτέρω διερεύνησης. Επίσης, παρατηρείται συσχέτιση μεταξύ των υπολειμμάτων νευρικών ινών του υποεπιδερμικού νευρικού πλέγματος και των μηχανο-ευαίσθητων νευρικών ινών πόνου που χρήζει επίσης περαιτέρω διερεύνησης.

Συμπέρασμα: Η βιοψία δέρματος και η διαδερμική ηλεκτρική διέγερση είναι πολλά υποσχόμενα διαθέσιμα εργαλεία για τη διάγνωση των νευροπαθειών των ινών C και την αξιολόγηση του νευροπαθητικού πόνου.

Λέξεις κλειδιά: Νευροπαθητικός πόνος, επώδυνες νευροπάθειες, περιφερική νευροπάθεια, χρόνιος πόνος, νευρικές ίνες, διαδερμική ηλεκτρική διέγερση, βιοψία δέρματος

INTRODUCTION

Chronic pain disorders are characterized of malfunctioning in one or several mechanisms underlying the nociceptive function (Mendell 2011; Sandkühler 2009; Woolf 2011). Pain sensation disorders are a special type of peripheral neuropathy that affects the sensory C fibers and autonomic nervous system fibers. (Lan Zhou 2009). Conditions with chronic pain expression are diabetes mellitus, alcoholism, amyloidosis, hereditary neuropathies; mononeuropathies such as trigeminal or glossopharyngeal. (Woolf 2004; Horowich et al. 2007; Borchers and Gershwin 2014; Devigili et al. 2008; Kehlet et al. 2006)

Nociceptors innervating superficial and deep somatic tissues are primary sensory neurons impacted by external and internal stimuli. (Gold et al. 2020). Based on their responsiveness to mechanical forces, heat, and exogenous irritant chemicals they have been classified into three main groups, mechano-nociceptors (responsive only to mechanical forces initially)_polymodal nociceptors (responsive to mechanical or chemical stimuli), and silent nociceptors_(Aδ or C fibers unresponsive or with very high threshold to mechanical stimuli when it comes to sustained pressure or because of inflammation(Belmonte C., 2009).

In general, Aδ fibers are the first that respond to a stimulus and they are responsible for the sharp pricking pain experienced when a punctuate mechanical stimulus is applied. In contrast, C polymodal nociceptor fibers initiate a persisting pain sensation. (Belmonte C., 2009). It should be noted that unmyelinated polymodal and silent C-nociceptors have higher activation thresholds for rectangular pulses when compared to myelinated Aδ fibers. Jonas et al. indicated C-nociceptors could be stimulated without Aδ initial fiber activation with sinusoidal transdermal stimulation(Jonas et al. 2018). Thus, we followed a transcutaneous half-sinusoidal stimulation paradigm for selective C mechanosensitive nociceptor activation and a sinusoidal one for mechano-insensitive C fiber activation, protocol, to healthy human volunteers.

Furthermore, skin biopsy enables the quantification of the intraepidermal nerve fibers, thus providing concrete evidence of small-fiber loss as well as assessing in general epidermal innervation. As a result, it is truly regarding as the most reliable tool for diagnosing small-fiber neuropathies. (Truini et al. 2014; Tesfaye et al. 2010, Lauria et al. 2010; Nolano et al. 2013) We compared the results of the half sine

protocol, as well as the sine protocol, with the skin biopsy results to assess the qualitative assessment of pain and small fiber loss.

EXPERIMENTAL PROCEDURE

Subjects

In the experimental protocols 30 healthy volunteers aged 20-30 years (17 females 18 males) participated from November 2019 to March 2020, after having given their informed consent. All subjects were familiar with the principles of the method and the general intention of the study. All control subjects were healthy volunteers without history of diabetes mellitus, neuromuscular disorders, or autonomic dysfunction and without any regular medication. Exclusion criteria were every disease connected to neuropathic pain that could possibly affect the research results such as angiitis, diabetes mellitus and polyneuropathy. The study was approved by the local ethics committee in Attica (Ethics committee of National and Kapodistrian University of Athens). The volunteers were comfortably seated on a reclining chair and their right arm or right foot was placed on the chair's cushion with the volar side up. The limb was stabilized to keep it in the same relaxed position during the whole experiment. The experiment was applied at the following sites: at the right upper extremity: to the volar forearm, to the thenar and to the index finger to the base of distal phalanx, and then at the right lower extremity, to the biopsy site of the distal leg (10cm above the external malleolus), to the foot dorsal, to the foot plantar and to the base of the distal phalanx of the great toe. Experimental sessions lasted about one hour in total..

Electrical stimulation

Initially, half-sine wave pulses of 0.5sec duration (1Hz) were generated at intensities of 0.2 to 1 mA by a constant current stimulator (Digitimer Ltd, Welwyn Garden City, UK) controlled by DAPSYS 8 (www.dapsys.net). Stimuli were delivered by a pair of L-shaped blunted bipolar platinum–iridium electrodes placed on a length of 3 mm onto the skin surface within the innervation territory of characterized C fibers. Then, amplitudes of 0.2, 0.4, 0.6, 0.8, and 1 mA were applied with one repetition for each stimulation intensity and interstimulus intervals of 10 seconds between the stimuli. After every stimulation, the subject was asked to assess the pain sensation.

Apart from half-sine wave stimulation, sine wave pulses of 60sec duration (4Hz) were generated at intensity of 0.2mA also by the constant current stimulator (Digitimer Ltd, Welwyn Garden City, UK) controlled by DAPSYS 8 (www.dapsys.net). Stimuli were delivered as above by a pair of L-shaped blunted bipolar platinum–iridium electrodes placed on a length of 3 mm onto the skin surface within the innervation territory of characterized C fibers. Each 15sec, the subject was asked to assess pain sensation.

Termination of participation

The participation of the subjects at the research could be terminated any time and for many reasons such as, severe no compliance with the protocol as estimated by the researcher, intentional termination by the subject who has the right to cease at any time his participation to the research, induce of severe pain or situations that could interfere with the results of the research as estimated by the researcher always in regard with the Local Ethics Protocol. Each subject that terminated his participation would always be asked for the reason, which would be noted. Nevertheless, nobody terminated the study, but only 19 (11 females and 8 males) of the participants acceoted to undergo skin biopsy because of the invasive part of the procedure.

Psychophysics in controls

The subjects were asked to rate the pain sensation induced by electrical stimuli on a numeric scale from 0 to 10(NRS scale), in which the value of 0 should indicate no pain and 10 should be assigned to the maximum pain the subject have ever felt, and accordingly 1 should be the least pain.

As described above, half-sine wave pulses were delivered through transcutaneous bipolar electrodes at intensities of 0.2-, 0.4-, 0.8-, and 1-mA amplitude to the sites described, to the skin of 16 male and 19 female healthy volunteers (20-30 years), and pain sensation was asked to be rated after every pulse during the interstimulus intervals. Then, during the sine wave pulse of 60sec duration delivered through transcutaneous bipolar electrodes at intensity of 0.2mA, also to the sites described, to the same subjects, pain sensation was asked to be rated every 15 seconds.

Subjects were also asked to describe the quality and the nature of the sensation in their own terms.

Skin biopsy

Moreover, we performed 3-mm skin punch biopsies 10 cm above the lateral malleolus of the leg as described earlier and in the middle of the volar side of the forearm to the volunteer's group. Biopsy specimens were consolidated with 4% buffered paraformaldehyde. Then they were washed in phosphate buffer solution for 3 times with interval time of 10 minutes and subsequently stored in 10% sucrose with 0.1 M phosphate buffer solution when finally, could be cryoprotected.

Statistics

Statistical tests were performed in STATISTICA 7.0 (StatSoft Inc, Tulsa, OK). Responses to half-sine and sine waves were compared between the two genders by one-way analysis of variance (one way ANOVA). T-tests were used for analysis comparing the pain sensation that was induced between the sites, for the same amplitude. The IENFD was estimated according to the European Federation of Neurological Societies guidelines, as number of fibers penetrating the dermal–epidermal junction, expressed as fibers/mm. Then, the IENFD (fibers/mm) that was calculated with the biopsy was compared by one-way analysis of variance (ANOVA) with the pain sensation for the same sites that the subjects noted during the transdermal electrical stimulation.

RESULTS

Psychophysics in Healthy Human Subjects

Skin nociceptor activation was performed upon transcutaneous sine and half sine wave stimuli in 35 human subjects and was quantified psychophysically. Pain sensation was found to be independent of sex ($p > 0.05$, one way ANOVA) in both the sine and the half sine wave stimuli, except from the thenar site, where p value was $p=.03 < .05$ for the half sine wave stimulation.

Intensity response relations.

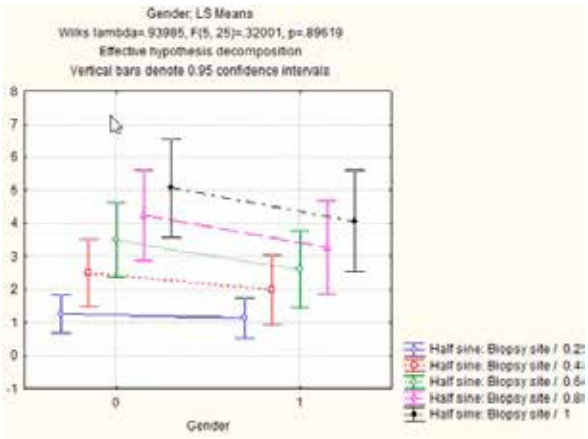

Figure 1Intensity response relations. The zero is for the group of women, wihle the 1 is for the group of men. We observe that increasing the intensity of the transcutaneous stimulation, the pain sensation is also increased.

Mechanosensitive nociceptors

Comparing the mean between the groups, using t test for independent values, for the half sine wave, it was shown that increasing the intensity of the transcutaneous stimulation, the pain sensation increase as well(Figure 1). A statistically significant difference ($p < .05$) was found between the intensity of the pain at the thenar and the intensity between all the other sites, and the pain on the thenar was found to be less than the other sites, especially in men, as we noticed with ANOVA and 2D scatterplot. Similarly, a statistically significant difference ($p < .05$) was found between the values between the index and the toe. Most of the controls described the pain as 'sharp' and 'pricking' pain sensation.

Mechano-insensitive nociceptors

Comparing the mean between the groups by using t test for independent samples, for the sine wave stimulation with 1 min duration, there was found a gradually reduction to the pain sensation with the passage of time. Statistically significant differences were found in the following points: The intensity of pain at the index site was found to have a statistically significant difference with the intensity of pain on the forearm and index ($p < .05$), throughout the recording time of 15, 30, 45 and 60 seconds, because the model of desensitization, and reduction of pain is not observed at the index site. Also, at the index area at 45 seconds, the difference in pain intensity was found to be statistically significant ($p < .05$) compared to the forearm area, with NRS 2 ± 1 higher pain values in the index.

In addition, at 60 seconds there is a statistically significant difference between the pain on the biopsy site and the dorsal surface of the foot. Finally, at 15 seconds there is a statistically significant difference between the intensity of the pain at the toe and the dorsal surface of the foot.

Therefore, peripherally in the upper extremity, we observe a greater sensation of pain at the same intensity of stimulation compared to both centrally with the upper extremity and peripherally with the lower extremity.

Then, we searched if there is statistically significant ($p<.05$) correlation (Correlation dialog) between the pain sensation to each site, which was found statistically significant at the maximum amplitude of 1mA of the halfsine wave stimuli when comparing the pain sensation between each site, which was found statistically significant between the sites.

Time course

Moreover, regarding the sine wave stimulation, when comparing the correlation (Correlation dialog) for the same chronical moment, there was found statistically significant correlation (moderate positive correlation) between the pain sensation except from the following cases. Comparing the intensity of pain in the forearm with the area of the thenar around 15 seconds, no statistically significant correlation

(p> .05) was found between the ratings of pain intensity(Figure 2). In fact, the volunteer's ratings are more sensitive with NRS levels around 4 ± 1 compared to the pain at the thenar site. In addition, no statistically significant correlation (p> .05) was found between the pain in the thenar and the biopsy site after the first 15 seconds, as the volunteers showed greater sensitivity and pain intensity at the biopsy site with levels of NRS scale 3 ± 1 higher than the thenar, while also no statistically significant correlation(p> .05) was found between the biopsy site and the toe for 15 and 30 seconds, as at the biopsy site is more sensitive in relation to the toe. Finally, comparing the sensation of pain between the index and the toe, no statistically significant correlation was found (p> .05) for 15 and 60 seconds, as higher pain intensity appears at the index with levels NRS 2 ± 1 than the toe.

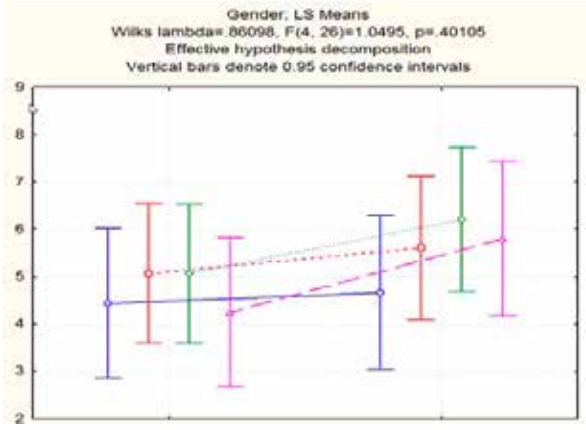

Figure 2 Time Course. Here we compare for the site of skin biopsy again the pain sensation for 1 minute, first diagram is for women while the second is for men. Using t test for independent samples, for the sine wave stimulation with 1 min duration, there was found a gradually reduction to the pain sensation with the passage of time.

Skin biopsy

We compared the pain ratings of the skin punch biopsy sites of the volunteers (to the middle of forearm and 10 cm above the lateral malleolus) with the IENFD (fibers/mm), ratio REM (ratio of remnants of subepidermal nerve plexus) and ratio BIF (ratio of bifurcation of nerve fibers) when searching for a correlation between them, using Correlation dialog.

There were found no differences between the two genders. There was found a statistically significant correlation p=0.048 concerning the pain rating to the forearm and the biopsy results, between the IENFD Arm and the forearm pain rating with sine wave stimulation around 60seconds. There wasn't any correlation between the IENFD Foot at the biopsy site and the pain rating at the same site.

Also, the ratio Rem and the pain rating to the forearm around 45 and 60 seconds was statistically significantly correlated with p=.004 and p=.002 correspondingly. Concerning the biopsy site there was found a correlation between the ratio BIF and the rating with half sine stimulation at the amplitude of 0.4mA(p=.002), and with sine wave stimulation around 45seconds of stimulation(p=.04).

DISCUSSION

We demonstrated that delivering transdermal sinusoidal half sine wave when trying to stimulate mechano-sensitive C fibers, when the amplitude of the delivered wave is increased from 0.2mA to 1mA considering our protocol, pain sensation is also increased following the same scheme. Consequently, if we deliver half sine wave transdermal stimulation, we expect to observe the same scheme of activation of mechanosensitive nerve fibers. If we observe a different scheme of activation in C fibers, may be a sign of neuropathic pain. Also, if the character of pain is as expected "sharp" or "pricking" C fiber neuropathies should be searched.

Also, concerning the mechano-insensitive C fibers of pain, when trying to stimulate them we expect increasing pain sensation and then familiarization, desensitization and reduction of pain sensation. As a result, if this scheme isn't observed when sine wave is delivered transdermal with 1 min of duration, and we observe a different scheme there may be a involved a C fiber neuropathy and neuropathic pain.

Concerning the skin biopsies, a correlation between pain sensation, of sine wave (delivered transdermal to stimulate mechano-insensitive C fibers of pain on the forearm), was observed and the nerve fiber density. A correlation between the bifurcated fibers of the biopsy site and the pain sensation was observed when mechanosensitive and mechano-insensitive fibers are stimulated, which needs further investigation. Also, a correlation between the remnant nerve fibers of subepidermal nerve plexus and mechano-insensitive nerve fibers of pain is observed that also needs further investigation.

The numeric scale from 0 to 10(NRS scale) is a qualitative way of measuring neuropathic pain on neuropathies and it's broadly used (Haefeli et al, 2005) NRS scale has shown high correlation as a pain-assessment tools in several studies and its feasibility compliance have also been proven. (Closs et al, 2004. Jensen MP et al, 1986). As a result, this quantitative sensory test for functional assessment of nerve-fiber density is a tool to diagnose and quantify small fiber neuropathy(Sauerstein et al, 2018. Jonas et al, 2018). It has been reported before that axonal hyperexcitability may contribute to neuropathic pain in a subpopulation of patients with

neuropathy, and assessing axonal excitability with transdermal wave stimulation might be a clinically useful marker to identify subgroups of patients with painful small fiber neuropathy. (Jonas et al, 2018). The transdermal electrical stimulation proposes a new way of estimating pain as, it's a more tangible test. Even if the number of the sample can't lead us to remarkable conclusions, this test can be used for assessing the efficacy of neuropathic medical treatment on diagnosed neuropathies and can be a supplementary test on diagnosing neuropathies.

CONCLUSION

In conclusion, skin biopsy and transdermal electrical stimulation are very promising available tools of diagnosing C fiber neuropathies and assessing neuropathic pain. Even if the number of the sample can't lead to remarkable conclusions, this neuropsychological test is a very promising method on assessing neuropathic pain on diagnosed neuropathies. It can be a method of measuring the efficacy of prescribed drugs on neuropathies medical treatment.

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