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Manuscript Draft

Manuscript Number: ARCHIVES-PMR-D-10-00769R2

Title: Spinal Cord Mechanism Involving the Remote Effects of Dry Needling on the Irritability of Myofascial Trigger Spots in Rabbit Skeletal Muscle

Article Type: Original Article

Keywords: Neural pathway; Dry needling; Myofascial trigger spot; Electromyography; Remote effect

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Abstract: Objective: To elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit skeletal muscle myofascial trigger spots (MTrSs) via analyses of their endplate noise (EPN) recordings.
Design: Experimental animal controlled trial.
Setting: An animal laboratory of a university.
Animals: Ninety-six male New Zealand rabbits (body weight: 2.5-3.0 kg, age: 16-20 weeks).
Intervention: Animals received no intervention for neural interruption in Group I, transection of the tibial nerve in Group II, transection of L5 and L6 spinal cord in Group III, and transection of the T1 and T2 spinal cord in Group IV. Each group was further divided into 4 subgroups: animals received ipsilateral dry needling (IDN), contralateral dry needling (CDN), ipsilateral sham needling (ISN), or contralateral sham needling (CSN), of gastrocnemius (GAS) MTrSs.
Main Outcome Measures: EPN amplitudes of biceps femoris (BF) MTrSs.
Results: BF MTrS mean EPN amplitudes significantly increased ($P < .05$) initially after GAS verum needling, but reduced to a level significantly lower ($P < .05$) than the pre-needling level in Groups I and IV with IDN or CDN, and in Group II with CDN (but not IDN). No significant EPN amplitude changes were observed in BF MTrS in Group III or in the control animals receiving superficial needling (sham).
Conclusion: This remote effect of dry needling depends on an intact afferent pathway from the stimulating site to the spinal cord and a normal spinal cord function at the levels corresponding to the innervation of the proximally affected muscle.

November 14, 2010

RE: **ARCHIVES-PMR-D-10-00769R1**

Dear Dr. Basford:

Thank you very much again for further reviewing our manuscript entitled "**Spinal Cord Mechanism Involving the Remote Effects of Dry Needling on the Irritability of Myofascial Trigger Spots in Rabbit Skeletal Muscle**". I deeply appreciate the suggestion about editing and English correction. Dr. Hsieh and I have tried our best to make revision following the comments and suggestions from you and the reviewer.

Please find the following items accompanied with this submission:

1. A list of our **responses to reviewer's comments** - highlighted in yellow.
2. A **marked copy of the revised manuscript** - highlighted on the revised portion in yellow.
3. A **clean copy of the revised manuscript**.
4. Two copies of new **Figure 2** (black and white)- pdf and power-point files.
5. Four copies (one for each author) of the **signed "Disclosure Statements and Copyright Assignment form"**

We are looking forward to hearing from you about the final decision. Thank you again.

With regards

Chang-Zern Hong, M.D.
Research Professor
Hung-Kuang University
Sa-Lu, Tai-Chung
TAIWAN
and
Recalled Professor
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RESPONSES TO REVIEWERS COMMENTS

-Highlighted in yellow

Ms. Ref. No.: **ARCHIVES-PMR-D-10-00769R1**
Title: **Spinal Cord Mechanism Involving the Remote Effects of Dry Needling on the Irritability of Myofascial Trigger Spots in Rabbit Skeletal Muscle**

Comments from Editor-in-Chief

Presentation. While your revisions have led to an improved paper, there remain a number of areas where it is diffucult to follow your thoughts. This is most likely in part due to the changes you have made. A number of specific suggestions appear below, but I again suggest that the revised paper undergo a final editing with attention to word flow and clarity.

Abstract. Objective. Delete the first six words and begin with "To.."
Response:
We have modified this sentence according to the reviewer's suggestion (see **Comments from Reviewer** below).
It has been changed into "To elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit skeletal muscle myofascial trigger spots (MTrSs) via analyses of their endplate noise (EPN) recordings".

Lines 55-57. This material in the first sentence is difficult to follow and may be more detailed than necessary. Would the substitution of something on the order of "Myofascial pain is one of the most common sources of musculoskeletal pain and has as its hallmark the presence of taut bands and small hyperirritable regions referred to as myofascial trigger points (MTrPs).
Response:
We have corrected this sentence according to your suggestion.

Line 63. Perhaps "Dry needling has a well established role in the treatment of myofascial pain.
Response:
We have modified this sentence according to your suggestion.

Line 66. Delete "either..located."
Response:
We have modified this portion according to the reviewer's suggestion (see **Comments**

from Reviewer below).

It has been changed into "Clinical studies have demonstrated that dry needling MTrPs at the pain site suppresses their activity resulting in pain reduction, but also that those MTrPs can also be suppressed through dry needling distant MTrPs that are anatomically proximal or distal to the site of clinical pain.[8-12] Acupuncture studies have demonstrated that the Traditional Chinese Medicine principle that pain conditions can be improved by stimulating acupuncture points distant from the site of pain.[4,13-14]".

Line 111. "Carefully" might be deleted.

Response:

We have deleted the word "Carefully".

Lines 452-465. See reviewer comments about deleting this section.

Response:

We try to emphasize the importance of this special technique (to elicit LTRs). If the experiment is not carried with this technique, we would not be able to find the remote effects of needling. Furthermore, both LTRs and neural control of needling are related to the spinal cord mechanism. The spinal cord mechanism of dry needling can change EPN activity and achieve the remote needling effect. Therefore, we prefer to keep this section.

Figure 2. Please convert to black and white.

Response:

We have converted this figure 2 into black and white.

A Disclosure Statements and Copyright Assignment form **will be emailed to you shortly.** We require ALL coauthors to complete, sign, and submit the form at this time. These forms replace those provided at original submission. Please return the forms by EES upload or fax. Editors must have these documents in hand for each member of the author group before proceeding with manuscript evaluation. Again, if you submitted these forms online, you must resubmit them to the Editorial Office now.

Response:

Four copies (one for each author) of the signed "Disclosure Statements and Copyright Assignment form" have been sent accompanied with this submission.

When you submit the revised manuscript, please include, in a file separate from your cover letter, an itemized response to each of the suggested revisions and any other changes made. Use consecutive line numbering and cite line numbers for each change. In addition, highlight each change in the revised manuscript. You must return the revised manuscript by **Nov 30 2010 12:00AM.**

Response:

We have sent the following items accompanied with this submission:

1. A **marked copy of the revised manuscript** - highlighted on the revised portion in yellow.
2. A **clean copy of the revised manuscript**.
3. Two copies of new **Figure 2** (black and white)- pdf and power-point files.
4. Four copies (one for each author) of the **signed "Disclosure Statements and Copyright Assignment form"**

Reviewer comment excerpts:

General Issues

Is a MTrS really any different than a MTrP? Perhaps MTrP use across the paper might be less confusing for the reader if they are clinically equivalent and this could just be notated in the abstract

Response:

We used this term for all animal-study papers published previously, since late Dr. David Simons insisted that we could not measure the subjective pain In animal (like human) and should use the term "MTrS" for animal. As far as we can define the term clearly (as listed in the "Acronyms" in this paper), the reader should not be confused.

Abstract

In the Objective section, "The purpose of this study is to investigate the possible neural pathway for the remote effects of dry needling based on the assessment of endplate noise (EPN) recorded for the myofascial trigger spot (MTrS) in rabbit skeletal muscle" might be better worded "The purpose of this study is to elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit skeletal muscle myofascial trigger spots (MTrSs) via analyses of their endplate noise (EPN) recordings."

Response:

We have made a revision according to your suggestion.

In the Intervention section line 24, "on the MTrS of the gastrocnemius" would be better worded "of gastrocnemius (GAS) MTrSs"

Response:

We have made a revision according to your suggestion.

In the Main Outcome Measures section lines 25-26, "Amplitudes of EPN on the MTrS region of biceps femoris" would be better worded "EPN amplitudes of biceps femoris (BF)

MTrSs"

Response:

We have made a revision according to your suggestion.

In the Results section, "Mean EPN amplitudes significantly increased (P< .05) initially, but reduced to a level significantly lower (P< .05) than the pre-needling level in Group I with IDN or CDN, Group II with CDN 29 (but not IDN), and Group IV with IDN or CDN, but no such changes were observed in Group III. There were no significant changes (P> .05) in EPN amplitudes in all control animals" could be more succinctly and perhaps better worded as "BF MTrS mean EPN amplitudes significantly increased (P< .05) initially after GAS verum needling, but reduced to a level significantly lower (P< .05) than the pre-needling level in Groups I and IV with IDN or CDN, and in Group II with CDN (but not IDN). No significant EPN amplitude changes were observed in BF MTrS in Group III or in the control animals receiving superficial needling (sham)."

Response:

We have revised the above two sentences according to your suggestion.

In Conclusions section, line 34, "level corresponding to the innervations of the proximally affected muscle" would be better worded "levels corresponding to the innervation of the proximally affected muscle"

Response:

We had modified it as your suggestion.

Introduction

First paragraph, "Myofascial pain is one of the most common musculoskeletal pain associated with myofascial trigger points (MTrPs) which are hyperirritable spots in taut bands of skeletal muscle fibers due to accumulation of unique hypersensitive loci.1,2 Clinically, an MTrP is characterized with a typical referred pain pattern and a local twitch response (LTR) in response to snapping palpation" could be better worded and is not quite accurate as LTR are no longer considered a diagnostic criterion to diagnose MTrPs. A more accurate alternative wording might be "Myofascial pain syndrome, a common source of musculoskeletal pain, is associated with hypersensitive loci in taut bands of skeletal muscle fibers which are termed myofascial trigger points (MTrPs).1,2 Clinically, a given MTrP has a characteristic referred pain pattern, and may be associated with a local twitch response (LTR) in response to snapping palpation"

Response:

The first sentence ""Myofascial pain is one…… of unique hypersensitive loci.1,2 " has been changed (as suggested by the Editor-in-Chief) into "Myofascial pain is one of the most

common sources of musculoskeletal pain and has as its hallmark the presence of taut bands and small hyperirritable regions referred to as myofascial trigger points (MTrPs).".
The second sentence "Clinically, an MTrP is characterized …..to snapping palpation" has also been changed into "Clinically, a given MTrP has a characteristic referred pain pattern, and may be associated with a local twitch response (LTR) in response to snapping palpation".

line 66, the reference "8,9-12" could just be "8-12" since the references are consecutive

Response:

We have made a revision according to your suggestion.

lines 64-66, "In addition to direct needling of the painful MTrP, clinical studies have demonstrated suppressive effect on this MTrP with dry needling at a remote MTrP, either proximally or distally located.8,9-12 Similar remote effectiveness in pain control has also been documented in acupuncture therapy.4, 13. 14" is somewhat awkwardly worded and might better be worded "Clinical studies have demonstrated that dry needling MTrPs at the pain site suppresses their activity resulting in pain reduction, but also that those MTrPs can also be suppressed through dry needling distant MTrPs that are anatomically proximal or distal to the site of clinical pain.8-12 Acupuncture studies have demonstrated that the Traditional Chinese Medicine principle that pain conditions can be improved by stimulating acupuncture points distant from the site of pain. 4,13-14"

Response:

We have revised these two sentences according to your suggestion.

As per the recommendations for alteration to purpose noted in the abstract, perhaps the last paragraph of the introduction might be better worded "The purpose of this study is to confirm the remote effects of dry needling on trigger points and to elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit skeletal muscle myofascial trigger spots (MTrSs) via analyses of their endplate noise (EPN) recordings."

Response:

We have made a revision based on your suggestion. This sentence has been changed into: "The purpose of this study is to confirm the remote effects of dry needling on trigger points and to elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit myofascial trigger spots (MTrSs, equivalent to human MTrPs) in the gastrocnemius muscle (GAS)[17, 18] via analyses of EPN recordings from the biceps femoris (BF).".

Materials and Methods

Line 82 "age" should be "ages"

Response:

We have made a revision according to your suggestion.

Line 83, "cared" should be "cared for"

Response:

We have made a revision according to your suggestion.

Line 110, consider using "MTrP" instead of "MTrS" throughout paper

Response:

As mentioned above, We used this term for all animal-study papers published previously, since late Dr. David Simons insisted that we could not measure the subjective pain In animal (like human) and should use the term "MTrS" for animal. As far as we can define the term clearly (as listed in the "Acronyms" in this paper), the reader should not be confused.

Line 115, should put the manufacturer data in instead of the "a"

Response:

According to the “Instruction for Authors” (listed below), the suppliers should not be put and cited directly in the manuscript. Therefore, we did not make correction on this issue. Additionally, we have provided more information (**mailing address of suppliers**) in the “suppliers” listed after “References”.

Supplier: After the References section, provide a Suppliers list with contact information (names and complete mailing addresses) for manufacturers of devices and other nondrug products used directly in a study (ie, do not provide such information for products not directly used in your research but mentioned in studies you cite). Identify equipment and/or materials in text, tables, and legends by superscript lower case letters. List suppliers consecutively in the order they are mentioned in the text.

Lines 128-9, should mention which manufacturer's needle was used and then similar to correction on line 15 the authors can get rid of "b" superscript

Response:

According to the “Instruction for Authors” (listed below), the suppliers should not be put and cited directly in the manuscript. Therefore, we did not make correction on this issue.

Line 163 need to also cross out (eliminate) the "in Group IV animals"

Response:

We have deleted these words according to your suggestion.

Line 172, put manufacturer information in and eliminate the "c"

Response:

According to the “Instruction for Authors” (listed below), the suppliers should not be put and

cited directly in the manuscript. Therefore, we did not make correction on this issue.

Line 201, how was the needle fixed to the skin

Response:

It was carefully and firmly taped on the skin. This sentence has been changed into "Then the needle was fixed in place **(carefully and firmly taped on the skin)** to ensure that this EPN can run continuously on the recording screen with constant amplitudes."

Lines 210-213, "Five randomly selected samples of EPN recordings (10 ms each) were taken before, during, and 3 min after the completion of the needling treatment for all groups, and also taken before and 30 minutes after surgery for Group II animals, and every 30 minutes up to 120 minutes after surgery for Group III and IV animals" would be better worded "Five randomly selected samples of EPN recordings (10 ms each) were taken before, during, and 3 min after the completion of the needling treatment for all groups; before and 30 minutes after surgery for Group II animals; and every 30 minutes up to 120 minutes after surgery for Group III and IV animals."

Response:

We have revised the above statements according to your suggestion.

Line 246, put manufacturer in and eliminate the "d"

Response:

According to the "Instruction for Authors" (listed below), the suppliers should not be put and cited directly in the manuscript. Therefore, we did not make correction on this issue.

Line 265, should also remove "treated differently"

Response:

We had removed these two words according to your suggestion.

Line 276-280 sentence-not sure it adds much new information that isn't already presented in prior sentence and Figure 3, and same for sentence lines 282-4

Response:

We would like to emphasize the important findings of "the initial increase followed by the subsequent decrease in EPN amplitudes after IDN or CDN, but not after ISN or CSN", although these findings could be identified in Figure 3. Therefore, we prefer to keep these statements.

Information in sentence lines 284-7 could have been incorporated in lines 279-80 to make this result section more succinct

Response:

Based on your suggestion, these sentences have been merged as listed below:

"There were significant differences in EPN amplitudes recorded either during or after needling between IDN and ISN, or between CDN and CSN subgroups (Bonferroni post-hoc test, *P*< .05), but not between IDN and IDN subgroups (Bonferroni post-hoc test, *P*> .05)**".**

Lines 324-327 sentence-again seems to be repeating same information as prior sentences in a slightly different way

Response:

These statements have been merged to the previous sentence as listed below:

" There were significant differences in EPN amplitudes among those recorded before, during, and after needling (repeated measures ANOVA, F= 80.77, *P*< .05) in CDN subgroup (**similar to the changes in Group I, Bonferroni post-hoc test, *P*< .05)**, but not in IDN subgroup (repeated measures ANOVA, F= 2.89, *P*> .05)**.".**

Information in lines 334-336 could have been incorporated in the 329-332 sentence to make this results section more succinct

Response:

These statements have been merged to the previous sentence as listed below:

"There were significant differences in mean EPN amplitudes between CDN and its comparable CSN subgroups, **but not between IDN and ISN subgroups (Bonferroni post-hoc test, *P*> .05)**, at the time during (Bonferroni post-hoc test, *P*< .05) and after (Bonferroni post-hoc test, *P*< .05) needling"

Lines 360-363 sentence-I don't think it is necessary information to report and does not add to the results

Response:

Regarding the result of "During the 2 h period after transection, the mean EPN amplitudes were significantly lower than the pre-transected levels**", we have made discussion about this issue in the section of " Electrophysiological Findings of the Remote Effect after Interruption of Certain Neural Circuits " and considered that this could be related to spinal shock. Therefore, we prefer to keep this.**

Lines 376-381 - sentence information could be merged to be more succinct

Response:

These statements have been merged as listed below:

"There were no significant differences in each time-dependent alterations of EPN amplitude between IDN and ISN subgroups, **between IDN and CDN subgroups,** or between CDN and CSN

subgroups (Bonferroni post-hoc test, **all** *P*> .05).".

Lines 399-406 sentences the information could be merged to reflect the EPN amplitudes normalized 2 hours post cord transaction to be similar for all groups

Response:

These statements have been merged as listed below:

". . . **but recovered to pre-transection level after 90 min (Bonferroni post-hoc tests, all *P*> .05), and normalized to be similar for all subgroups by 120 min after transaction (two-way ANOVA, F=0.09, P> .05).**".

Line 421-423-is this information necessary

Response:

This sentence has been deleted.

Lines 423-425 information could be incorporated in lines 416-417

Response:

This statement has been merged to the previous sentence as listed below:

" . . . but there were no significant differences in these changes between IDN and CDN subgroups (Bonferroni post-hoc test, *P*> .05). ".

Lines 452-465-not sure how much this information adds to purpose of paper, consider deleting

Response:

We try to emphasize the importance of this special technique (to elicit LTRs). If the experiment is not carried with this technique, we would not be able to find the remote effects of needling. Furthermore, both LTRs and neural control of needling are related to the spinal cord mechanism. The spinal cord mechanism of dry needling can change EPN activity and achieve the remote needling effect. Therefore, we prefer to keep this section.

Lines 467-468-don't need to put subtitle in, and same for lines 481-482 and 514

Response:

We have deleted those subtitles per your suggestion.

ORIGINAL ARTICLE

Spinal Cord Mechanism Involving the Remote Effects of Dry Needling on the Irritability of Myofascial Trigger Spots in Rabbit Skeletal Muscle

Yueh-Ling Hsieh, PT, PhD, Li-Wei Chou, MD, MSc, Yie-San Joe, MD, Chang-Zern Hong, MD

Chou LW contributed equally to the first author in this work.

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Word counts: Abstract : 245, Text: 4088.

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Running title: NEURAL MECHANISM FOR REMOTE EFFECT OF DRY NEEDLING

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No commercial party having a direct financial interest in the results of the research supporting this article has or will confer a benefit upon the authors or upon any organization with which the authors are associated.

Devices

a. **Thermometer:** Physitemp Instruments, Inc. 154, Huron Avenue, Clifton, New Jersey, USA.

b. **Digital EMG machine:** Neuro-EMG-Micro; Neurosoft, 5, Voronin Str, Ivanovo, Russia.

ORIGINAL ARTICLE

Spinal Cord Mechanism Involving the Remote Effects of Dry Needling on the Irritability of Myofascial Trigger Spots in Rabbit Skeletal Muscle

Running title: NEURAL MECHANISM FOR REMOTE EFFECT OF DRY NEEDLING

ABSTRACT

Objective: ~~The purpose of this study is to investigate the possible neural pathway for the remote effects of dry needling based on the assessment of endplate noise (EPN) recorded for the myofascial trigger spot (MTrS) in rabbit skeletal muscle.~~ To elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit skeletal muscle myofascial trigger spots (MTrSs) via analyses of their endplate noise (EPN) recordings.

Design: Experimental animal controlled trial.

Setting: An animal laboratory of a university.

Animals: Ninety-six male New Zealand rabbits (body weight: 2.5–3.0 kg, age: 16–20 weeks).

Intervention: Animals received no intervention for neural interruption in Group I, transection of the tibial nerve in Group II, transection of L5 and L6 spinal cord in Group III, and transection of the T1 and T2 spinal cord in Group IV. Each group was further divided into 4 subgroups: animals received ipsilateral dry needling (IDN), contralateral dry needling (CDN), ipsilateral sham needling (ISN), or contralateral sham needling (CSN), ~~on the MTrS of the gastrocnemius~~ of gastrocnemius (GAS) MTrSs.

Main Outcome Measures: ~~Amplitudes of EPN amplitudes on the MTrS region of biceps femoris (BF) MTrSs~~ EPN amplitudes of biceps femoris (BF) MTrSs.

Results: ~~Mean EPN amplitudes significantly increased (*P*< .05) initially, but reduced to a level significantly lower (*P*< .05) than the pre-needling level in Group I with IDN or CDN, Group II with CDN (but not IDN), and Group IV with IDN or CDN, but no such changes were observed in Group III. There were no significant changes (*P*> .05) in EPN amplitudes in all control animals.~~ BF MTrS mean EPN amplitudes significantly increased (P< .05) initially after GAS verum needling, but reduced to a level significantly lower (P< .05) than the pre-needling level in Groups I and IV with IDN or CDN, and in Group II with CDN (but not IDN). No significant EPN amplitude changes were observed in BF MTrS in Group III or in the control animals receiving superficial needling (sham).

Conclusion: This remote effect of dry needling depends on an intact afferent pathway from the stimulating site to the spinal cord and a normal spinal cord function at the levels corresponding to the innervations of the proximally affected muscle.

Key Words: Neural pathway; Dry needling; Myofascial trigger spot; Electromyography; Remote effect.

DEFINITION OF ACRONYMS

BF = biceps femoris muscle;

CDN = contralateral dry needling;

CSN = contralateral sham needling;

EMG = electromyographic;

EPN = endplate noise;

GAS = gastrocnemius muscle;

IDN = ipsilateral dry needling;

ISN = ipsilateral sham needling;

LTR = local twitch response;

MTrP = myofascial trigger point (human);

MTrS = myofascial trigger spot (rabbit)

INTRODUCTION

~~Myofascial pain is one of the most common musculoskeletal pain associated with myofascial trigger points (MTrPs) which are hyperirritable spots in taut bands of skeletal muscle fibers due to accumulation of unique hypersensitive loci.~~ Myofascial pain is one of the most common sources of musculoskeletal pain and has as its hallmark the presence of taut bands and small hyperirritable regions referred to as myofascial trigger points (MTrPs).[1,2] ~~Clinically, an MTrP is characterized with a typical referred pain pattern and a local twitch response (LTR) in response to snapping palpation.~~ Clinically, a given MTrP has a characteristic referred pain pattern, and may be associated with a local twitch response (LTR) in response to snapping palpation.[2] In the MTrP region, electromyographic (EMG) activity of endplate noise (EPN) can be recorded, and both prevalence and amplitude of EPN can be used as indicators to assess the irritability of MTrP.[2-5]

~~Dry needling of MTrP to alleviate myofascial pain has long been established and widely used in treating patients.~~ Dry needling has a well-established role in the treatment of myofascial pain.[6-9] ~~In addition to direct needling of the painful MTrP, clinical studies have demonstrated suppressive effect on this MTrP with dry needling at a remote MTrP, either proximally or distally located.[8,9-12] Similar remote effectiveness in pain control has also been documented in acupuncture therapy.[4, 13. 14]~~

Clinical studies have demonstrated that dry needling MTrPs at the pain site suppresses their activity resulting in pain reduction, but also that those MTrPs can also be suppressed through dry needling distant MTrPs that are anatomically proximal or distal to the site of clinical pain.[8-12] Acupuncture studies have demonstrated that the Traditional Chinese Medicine principle that pain conditions can be improved by stimulating acupuncture points distant from the site of pain.[4,13-14] The effects of acupuncture may also spread to the contralateral side.[15] Studies of acupuncture needle stimulation in anesthetized animals have identified a wide variety of reflex responses in remote modification of various organ functions.[16] However, its underlying neuronal control mechanism remains unclear.

~~The purpose of this study is to confirm the remote effects of dry needling and to investigate the possible neural pathway for the remote effects of dry needling of the myofascial trigger spot (MTrS) in the rabbit gastrocnemius (GAS) muscle based on the assessment of EPN recorded for the MTrS in the biceps femoris (BF) muscle. The animal model with myofascial trigger spots (MTrS, equivalent to human MTrP) has been established previously,[17, 18] and was used in our study.~~

The purpose of this study is to confirm the remote effects of dry needling on trigger points and to elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit myofascial trigger spots (MTrSs, equivalent to

human MTrPs) in the gastrocnemius muscle (GAS)[17, 18] via analyses of EPN recordings from the biceps femoris (BF).

MATERIALS AND METHODS

Animals

The experiments were performed on adult male New Zealand rabbits (~~age~~ ages from 16 to 20 weeks, body weight of 2.5–3.0 kg). Each animal was housed and cared for following the ethical guidelines of the International Association for Study of Pain in animals were followed.[19] Effort was made to minimize discomfort and to reduce the number of animals used. All animal experiments were conducted with the procedure approved by the Animal Care and Use Committee of a university in accordance with the Guidelines for Animal Experimentation.

Ninety-six rabbits were divided randomly into four groups (fig 1) based on the procedure performed. Group I (n=24) animals received no surgical intervention (intact neural pathway), Group II animals (n=24) received transection of tibial nerve in the electrophysiologically investigated side (peripheral sensory pathway), Group III animals (n = 24) received transection of L5-L6 spinal cord (BF innervation level), and Group IV animals (n=24) received transection of T1-T2 spinal cord (supra-segment of BF innervation). For the EPN amplitude variable, a sample size of 24 subjects in each group was sufficient to give statistical power of 97.06% with a significance level of $P < .05$. Animals in each group were randomly divided further into four subgroups based on the condition of treatment on GAS: experimental animals with ipsilateral dry

needling (IDN, n=8), or contralateral dry needling (CDN, n=8), control animals with ipsilateral sham needling (ISN, n=4), or contralateral sham needling (CSN, n=4), on the MTrS of the gastrocnemius. Fewer animals were studied in the control group since no significant changes were observed in all animals treated with sham needling. Regarding the assignment of groups or subgroups, animals were selected from the first available litter, and subsequently from the next litter, and so forth according to the sequence in a random table.

Animal Preparation

Before anesthesia, the tenderest spots (i.e., MTrS) of BF and GAS were identified by finger pinching. The animal responded to pinch stimulation with withdrawal of the lower limb, turning its head, screaming, etc, only when the most painful spot was pinched, and this most tender spot was confirmed as the MTrS. [2,17,18,20-23] These painful regions were marked on the skin ~~carefully~~ with an indelible marker and were designated for electrophysiological assessment or dry needling. The animals were anesthetized with 2% isoflurane in oxygen flow for induction followed by a 0.5% maintenance dose.[24] Body temperature was monitored by a thermistor probe of a thermometer [a] in the rectum and maintained at approximately 37.5 °C using a body temperature control system consisting of thermostatically regulated DC current heating pad and an infrared lamp. The hind limbs of anesthetized rabbits were shaved

and cleaned with povidone-iodine solution. The skin of the lateral thigh in one randomly selected side was incised to expose the BF, which served as an EPN recording site. The marked spot region in the BF muscle was grasped between two fingers from behind the muscle and the muscle palpated by gently rubbing (rolling) it between the fingers to discover a taut band. A taut band felt like a clearly delineated "rope" of muscle fibers and was roughly 2–3 mm or more in diameter. The fibers of the taut band were unmistakably firmer in consistency than the surrounding muscle.

Needling of Gastrocnemius Muscle

All needling procedures were performed by the same investigator who was blinded to the group assignment regarding to surgical intervention on neural pathway. Dry needling stimulation was performed with a disposable 30G acupuncture needle (300 μm in diameter, 1.5 inches in length)[b] at ipsilateral or contralateral GAS (fig 2). The technique of dry needling was similar to that suggested by Hong [18, 20,25-27] with multiple needle insertions to elicit rabbit-LTRs as much as possible. For needling in MTrS of GAS, the needle was first inserted through the skin perpendicularly at the center of the marked spot and advanced slowly and gently into the muscle until the needle tip touched the bone surface to estimate the thickness of the muscle. The needle was withdrawn back to the subcutaneous layer, and rapidly moved in and out for insertion of multiple sites in different directions (in a cone shape with the center at

158 the initial needle insertion of a perpendicular direction, and the angle of the cone
159 margin was about 20°). For each needle insertion, the needle was advanced into the
160 depth near the bone surface. Simultaneous needle rotation was performed to facilitate
161 fast "in-and-out" needle movement as suggested by Chou et al.[4] in order to elicit as
162 many LTRs as possible. For sham needling, the needle was inserted into the
163 subcutaneous layer of the marked MTrP region at a depth approximately 1-2 mm from
164 the skin surface. After insertion, the needle stayed there without further movement.
165

166 ## Transection Operations

167 **1. Transection of tibial nerve**

168 During anesthesia for the animals in Group II, the incision was made over the
169 posterior aspect of one thigh ipsilaterally to the EPN recording side. Under the
170 operating microscope, the sciatic nerve was exposed, and the tibial nerve isolated and
171 transected at the site about 1 cm from its insertion into the GAS.

172 **2. Transection of spinal cord**

173 After completing laminectomy and making a slit in the dorsal portion of the dura
174 mater, the cord was transected by a knife and then aspirated by suction at about 2 mm
175 caudal and rostral to the level of transection, at L5-L6 levels of the spinal cord for
176 animals in Group III, or at T1-T2 levels of the spinal cord for Group IV animals.

Gelfoam was placed into the empty vertebral column to seal the empty vertebral cavity and reduce bleeding. In a previous study [20] and in our preliminary data, about 2½ h after surgery, the rabbits would have almost completely recovered from spinal shock~~ in Group IV animals~~. The animal would then be ready for the needling study.

Recording of Endplate Noise

1. Electromyography setting

For EPN assessment, a two-channel digital EMG machine[c] and monopolar needle electrodes (37 mm disposable Teflon-coated model) were used. The gain was set at 20μV per division for recordings from both channels. Low-cut frequency filter was set at 100 Hz and the high-cut at 1,000 Hz. Sweep speed was 10 ms per division. The search needle for EPN recording was inserted into the MTrS region and connected to the first channel of the EMG machine. The control needle was inserted into the non-taut band region near the MTrS in the same muscle and connected to the second channel. A common reference needle electrode for each channel was placed on the incised skin and connected to both channels via a y-connector.

2. Search for endplate noise

This procedure was performed by an investigator who was blind to the group assignment. The search needle was inserted into the MTrS region in a direction

196 parallel to the muscle fibers at an angle of approximately 60° to the surface of the
197 muscle. After initial insertion just short of the depth of the MTrS or to a comparable
198 depth in the case of control sites, the needle was advanced very slowly with
199 simultaneous slow rotation to prevent it from 'grabbing' and releasing the tissue
200 suddenly to advance in a large jump. Each advance was of minimal distance (~1 mm).
201 When the needle approached an active locus (EPN locus), the continuous distant
202 electrical activity, i.e., EPN, can be heard. As soon as EPN with amplitude higher than
203 10 μV could be recorded, the examiner stopped advancing the needle, but minimally
204 moving the needle gently to different direction, trying to obtain EPN with highest
205 amplitude. If this was impossible, the needle was advanced to another site until an
206 EPN with optimal amplitude (usually higher than 30μV) could be recorded. Then the
207 needle was fixed in place (carefully and firmly taped on the skin) to ensure that this
208 EPN can run continuously on the recording screen with constant amplitudes.
209 Continuous EPN tracing was recorded throughout the entire course of the experiment,
210 and provided the opportunity for continuous visual observation of EPN changes on
211 the EMG screen. If the EPN could not be sustained, the searching needle would be
212 moved to another site until a satisfactory EPN tracing could be obtained. The entire
213 EPN tracing found in MTrS of BF were recorded for the analysis of amplitude
214 changes.

3. Measurement of the amplitude of endplate noise

Five randomly selected samples of EPN recordings (10 ms each) were taken before, during, and 3 min after the completion of the needling treatment for all groups; ~~and also taken~~ before and 30 minutes after surgery for Group II animals; and every 30 minutes up to 120 minutes after surgery for Group III and IV animals. The mean amplitude of EPN of 5 random samples was analyzed and calculated through the embedded software in the Neuro-EMG-Micro equipment, and was recorded as the value for a certain measurement point for each animal.

Data Analysis

Data of EPN amplitudes in different measurement points for different groups or subgroups were expressed as the mean ± standard error of the mean (SEM) for further statistical analysis. The Shapiro-Wilk's normality test was conducted to determine whether the data fit a normal distribution prior to subsequent analyses, and showed all measures of EPN amplitude were normally distributed. Tests of 'homogeneity' or 'baseline balance' on covariates including body weight, age, and anesthesia condition were measured and equivalent before the needling treatment in all animals. The differences in EPN amplitude across measurement points in each group were carried out using repeated measures ANOVA, and later further analyzed by a Bonferroni

post-hoc method. The differences in EPM amplitudes within each of the subgroups (IDN, CDN, ISN and CSN) and across measurement points (before, during, and after needling) were analyzed using two-way ANOVA (side × time) followed by a Bonferroni post-hoc analysis for each group. The differences in EPN amplitude within measurement point (before, during, and after needling) across subgroups (IDN, CDN, ISN and CSN) were tested by paired t-test. A p value of <0.05 was considered to be statistically significant. All data was analyzed using SPSS version 10.0 for Windows.[d]

RESULTS

Effects of Dry Needling of Distal MTrS in Intact Rabbits (Group I)

The serial alterations of the mean EPN amplitude before, during, and after dry needling at ipsilateral and contralateral GAS for Group I are demonstrated in figure 3. Before needling treatment, there was no significant difference among the four subgroups ~~treated differently~~ (two-way ANOVA, F=0.10, $P > .05$).

The mean amplitudes of EPN before, during, and after needling were 18.20±0.70μV, 27.71±0.47μV, and 13.15±0.59μV, respectively in IDN subgroup, and 17.96±0.69μV, 24.66±1.47μV, and 14.01±0.86μV, respectively in CDN subgroup. In either IDN or CDN subgroup, the amplitudes at different times were significantly different (repeated measures ANOVA: F=45.99 and $P < .05$ for IDN, F=113.98 and $P < .05$ for CDN). Compared with the data in the pre-needling level, the EPN amplitudes were significantly increased during the dry needling (Bonferroni post-hoc test, $P < .05$), and then significantly decreased to a much lower level after completion of the needling treatment (Bonferroni post-hoc test, $P < .05$) for either IDN or CDN subgroup as shown in figure 3. However, these serial alterations of EPN amplitudes were not found in the comparable subgroup ISN or CSN (repeated measures ANOVA, $P > .05$). There were significant differences in EPN amplitudes recorded either during or after needling between IDN and ISN, or between CDN and CSN subgroups

(Bonferroni post-hoc test, $P < .05$), but not between IDN and IDN subgroups ~~In addition, alterations in EPN amplitudes in IDN subgroup were similar to those in CDN subgroup. The magnitude or time-dependent alteration of EPN amplitude after CDN was not significantly different from that after IDN~~ (Bonferroni post-hoc test, $P > .05$).

Effects of Dry Needling of Distal MTrS in Rabbits With Tibial Nerve Transection (Group II)

The serial alterations of the mean EPN amplitude (±SEM) throughout the entire experiment in Group II are demonstrated in figure 4. The mean amplitude of EPN had no significant changes before, during, and after ipsilateral tibial nerve transection (i.e., GAS denervation) (repeated measures ANOVA, F=0.06, $P > .05$). Before the needling treatment, there was no significant difference in EPN amplitude among the subgroups treated with dry or sham needling at ipsilateral or contralateral side (two-way ANOVA, F=0.68, $P > .05$).

The mean amplitudes (±SEM) of EPN recorded from BF before, during, and after needling were 16.72±0.34μV, 16.64±0.37μV, and 15.46±0.50μV, respectively in IDN subgroup, and were16.90±0.38μV, 21.63±0.91μV, and 12.40±0.36μV, respectively in CDN subgroup. There were significant differences in EPN amplitudes among those recorded before, during, and after needling (repeated measures ANOVA, F= 80.77,

$P < .05$) in CDN subgroup (similar to the changes in Group I, Bonferroni post-hoc test, $P < .05$), but not in IDN subgroup (repeated measures ANOVA, F= 2.89, $P > .05$). ~~In the CDN subgroup, the EPN amplitudes were increased during needling (Bonferroni post-hoc test, $P < .05$). However, 3 min later and after cessation of needling, it was reduced significantly to a level less than that before needling (Bonferroni post-hoc test, $P < .05$).~~ The EPN amplitudes also had no significant changes after ISN (repeated measures ANOVA, F= 0.10, $P > .05$) or after CSN (repeated measures ANOVA, F=0.02, $P > .05$). There were significant differences in mean EPN amplitudes between CDN and its comparable CSN subgroups, but not between IDN and ISN subgroups (Bonferroni post-hoc test, $P > .05$), at the time during (Bonferroni post-hoc test, $P < .05$) and after (Bonferroni post-hoc test, $P < .05$) needling. Moreover, there were significant differences in the magnitude or time-dependent alterations of EPN amplitude between CDN and CSN subgroups (Bonferroni post-hoc test, $P < .05$). ~~However, there were no significant differences between IDN and ISN subgroups at the time course either during (Bonferroni post-hoc test, $P > .05$) or after (Bonferroni post-hoc test, $P > .05$) treatment at ipsilateral GAS.~~

Effects of Dry Needling of Distal MTrS in Rabbits With Lumbar Cord Transection (Group III)

The serial alterations of the mean EPN amplitude (±SEM) throughout the entire experiment for Group III are shown in figure 5. There were significant differences in EPN amplitude among those recorded before, immediately, and 30, 60, 90, and 120 min after L5-L6 transection (repeated measures ANOVA, F=29.81, $P < .05$). During the 2 h period after transection, the mean EPN amplitudes were significantly lower than the pre-transected levels (Bonferroni post-hoc test, all were $P < .05$ at 30, 60, 90, and 120 h). There was no significant difference among the four subgroups treated with dry or sham needling at the ipsilateral or contralateral side regardless of the time before (two-way ANOVA, F=0.23, $P > .05$), during (F=1.45, $P > .05$), or after (F=1.72, $P > .05$) needling treatments.

The mean amplitudes (±SEM) of EPN before, during, and after needling were 11.56±0.36μV, 11.47±0.43μV, and 11.28±0.47μV, respectively in IDN subgroup, and were 11.67±0.45μV, 12.32±0.46μV, and 12.33±0.46μV, respectively in CDN subgroup. There was no significant difference in EPN amplitude among those recorded before, during, and after IDN (repeated measures ANOVA, F=0.63, $P > .05$), CDN (repeated measures ANOVA, F=1.17, $P > .05$), ISN treatment (repeated measures ANOVA, F=0.23, $P > .05$), or CSN (repeated measures ANOVA, F=0.52, $P > .05$). There were no significant differences in each time-dependent alterations of EPN amplitude between IDN and ISN subgroups, between IDN and CDN subgroups, or between CDN

and CSN subgroups (Bonferroni post-hoc test, all $P > .05$). ~~There were also no significant differences in each time-dependent alteration of EPN amplitude between IDN subgroup and CDN subgroup (Bonferroni post-hoc test, all were $P > .05$ at each recording time).~~

Effects of Dry Needling of Distal MTrS in Rabbits With Thoracic Cord Transection (Group IV)

The serial alterations of the mean EPN amplitude (±SEM) throughout the entire experiment for Group IV are shown in figure 6. The EPN amplitude recorded at 30 and 60 min after T1-T2 transection was significantly reduced when compared to the pre-transection level (Bonferroni post-hoc test, $P < .05$), but recovered to pre-transection level after 90 min (Bonferroni post-hoc tests, all $P > .05$), and normalized to be similar for all subgroups by 120 min after transaction (two-way ANOVA, F=0.09, P> .05). ~~The EPN amplitude recorded at 90 and 120 min recovered to the level with no significant differences from pre-transection level (Bonferroni post-hoc tests, all were $P > .05$ at 90 and 120 min recording time). Before needling treatment, there was no significant difference among all four subgroups treated with dry or sham needling at the ipsilateral or contralateral side (two-way ANOVA, F=0.09, P> .05).~~

The mean amplitudes (±SEM) of EPN recorded from BF before, during, and after needling were 18.17±0.36μV, 26.88±0.43μV, and 15.74±0.26μV, respectively in IDN subgroup, and were 18.28±0.45μV, 27.72±0.47μV, and 16.20±0.22μV respectively in CDN subgroup. There were significant differences in EPN amplitudes among those recorded before, during, and after IDN (repeated measures ANOVA, F= 264.29, $P < .05$) or CDN (repeated measures ANOVA, F= 243.11, $P < .05$). The mean EPN amplitudes were significantly increased during IDN or CDN (Bonferroni post-hoc test, $P < .05$), but after cessation of needling, they reduced significantly to a level less than that before needling (Bonferroni post-hoc test, $P < .05$), but there were no significant differences in these changes between IDN and CDN subgroups (Bonferroni post-hoc test, $P > .05$). These serial alterations of EPN amplitudes were not found in either ISN (repeated measures ANOVA, F=1.02, $P > .05$) or CSN subgroup (repeated measures ANOVA, F=2.02, $P > .05$). ~~There were significant differences between dry needling and its comparable sham needling subgroups (Bonferroni post-hoc test, $P < .05$). Moreover, there were no significant differences in the magnitude or time-dependent alterations of EPN amplitude between CDN and IDN subgroups (Bonferroni post-hoc test, $P < .05$).~~

DISCUSSION

To our knowledge, the present study is the first animal study to investigate the neural mechanism of the remote effects of dry needling. In this study, we found that an intact afferent nerve from the remote stimulation site and normal spinal cord segments corresponding to the innervation of the affected proximal muscle are essential for the remote effect from either ipsilateral or contralateral stimulation.

~~Technical Issues on Dry Needling~~

The dry needling used in this study is a technique of MTrP injection with multiple high-speed needle insertions into different sensitive loci in an MTrP region suggested by Hong.[25-27] High speed needling can provide high-pressure stimulation to the sensitive loci in the MTrP region to elicit LTRs. It is essential to elicit LTRs during needling of an MTrP in order to obtain immediate and complete pain relief.[6, 25-29] Dry needling at the MTrS was effective in diminishing spontaneous electrical activity (i.e., EPN) of MTrS of rabbit skeletal muscle if LTRs were elicited.[30] After several LTRs had been elicited by the needling of an MTrS of rabbit skeletal muscle, no more LTRs could be elicited from the same region [18] and the irritability of the MTrS could be suppressed. [30] Needling-elicited LTRs are involuntary discharges of muscle fiber mediated through the nervous system and integrated at the spinal cord level.[18, 20] Therefore, it is important to apply this needling technique to achieve the

best needling effect or remote needling effect for the study on the neural mechanism.

~~Electrophysiological Confirmation of the Remote Effect in Normal Neural Circuits~~

Changes in the EPN amplitude in the MTrS were found during and after dry needling at the distal MTrSs in animals with intact neural circuits (Group I). It appears that either IDN or CDN to the distal MTrS could initially increase the irritability of the proximal MTrS, followed by a suppression effect after cessation of needling. Fernandez-Camero, et al. [31] also found an increase in spontaneous electrical activity at an MTrP region during a persistent noxious stimulation at another distant MTrP, followed by a suppression of electrophysiological irritability after cessation of needling. The two findings above strongly support clinical observations related to the interaction between one MTrP and another MTrP located in the region of the referred pain (referred zone) of that MTrP.[2,6,9-12,26-29]

~~Electrophysiological Findings of the Remote Effect after Interruption of Certain Neural Circuits~~

In the study on Group II rabbits with ipsilateral denervation of GAS, the remote effect disappeared after IDN, but persisted after CDN. These results demonstrated the importance of an intact afferent pathway to the spinal cord in the remote modulation

of EPN amplitudes. After destruction of spinal cord corresponding to the level of BF (Group III), the remote effect disappeared after either IDN or CDN treatment. This finding suggested the existence of intraspinal connections between the GAS afferents and BF spinal interneurons. The partial suppression of the EPN amplitude after spinal cord transection at L5-L6 is possibly related to the influence of the spinal shock. After interruption of upper motor neuron and supra-sensory connections (Group IV), the remote effect persisted, either with IDN or CDN, but smaller than that in intact animals (Group I). It may imply the possible influences from supraspinal centers, such as descending pain inhibitory systems. These influences are anticipated to be minimal after transection at higher spinal levels. Loss of inputs to this system would weaken the inhibition on the pain level,[32] which could lead to the EPN amplitude being less suppressed. Therefore, the recruitment of the diffuse noxious inhibitory control (DNIC) system may be also elicited by dry needling treatment on regions remote to the stimulation site.

~~Possible Neural Control for the Remote Effect of Dry Needling~~

The neural pathway for the remote effect appears to be a spinal reflex, probably similar to that mediating the referred pain[26, 29] and local twitch response.[18, 20,26,29] Hong has hypothesized a corresponding "MTrP circuit" for each MTrP, which can modulate the pain, referred pain, and local twitch response elicited by stimulating the

MTrP.[6, 29, 33] The neural connection in the spinal cord responsible for this remote effectiveness is probably similar to that for the referred pain.[26, 29]

The initial increase in EPN with remote dry needling followed by a reduction in EPN after local twitch responses are elicited indicating inactivation of the remote MTrS. Strong stimulation from continuous dry needling of an MTrS can activate the sensitized nociceptors and generate strong impulses propagating to the spinal cord to activate the corresponding motoneurons (including those in the same segment corresponding to the needling muscle and other segments corresponding to the remote muscles) to fire reflexively, thereby causing increased EPN amplitude in MTrSs not only at the needling muscle but also at other remote muscles. These strongly activated motoneurons are also controlled by recurrent inhibitions. As the firing rate of motoneuron increases, the amount of recurrent inhibitions will also increase, subsequently limiting and suppressing the firing rate of the efferents. In this way, these impulses elicited by dry needling eventually breaks the vicious cycle of the neural circuits (i.e. MTrP circuits [29, 33]) responsible for MTrSs through spatial and temporal interactions in the spinal cord. Thus the EPN amplitude is suppressed after dry needling. Possibly, there are certain neural connections among the inhibitory interneuron and descending pain control system in the spinal cord that can modulate the irritability of MTrPs when a remote painful dry needling stimulation is applied

(fig 7). The initial increase in EPN is consistent with suppression of the DNIC system, and the subsequent reduction in EPN is consistent with activation or enhancement of the DNIC system. Therefore, the physiological basis for the remote effects of dry needling may be related to an inactivation of MTrS[29, 33] and activation of DNIC [34, 35] induced by noxious stimulation applied at the painful region (such as trigger point needling) or at a remote site (such as in remote dry needling). This is probably the mechanism of remote pain control by dry needling which is similar to hyperstimulation analgesia in acupuncture.[32,36]

Limitations of the Study

The difficulty in confirming the correlation between the alterations of EPN amplitude and pain intensity in rabbit may be criticized. However, a conclusion based on the human study[4] may be reasonably applied on rabbits, because there are plenty of similarities between the human MTrP and rabbit MTrS.[18, 26, 28, 29] Lack of follow-up assessments for the long-term remote effect is another deficiency of this study. However, we rarely see the long-term effects of dry needling if the underlying pathology of MTrP activation is not treated appropriately.[6, 26, 27, 28, 33] Another problem is that out sham needling (similar to superficial dry needling) may not be appropriate as a control. Fortunately, we see no significant changes after treatment with sham

needling. However, this could be related to the small sample size. In our clinical practice, we have observed much less effectiveness of superficial needling than that of deep dry needling with our multiple quick insertion technique. In addition, considering the individual differences in the motoneuron excitability and the supraspinal control of spinal inhibitory interneurons, just based on electrophysiological study, we are unable to distinguish the relative contribution from each inhibitory mechanism for motoneuronal excitability to the changes in MTrP irritability due to remote dry needling. All the above factors should be taken into consideration for data interpretation.

CONCLUSION

We have demonstrated that an intact afferent from the stimulating site to the spinal cord and a normal function of spinal cord corresponding the innervation of the remotely affected muscles are essential for this remote effectiveness. This study may help in the understanding of the mechanism for beneficial effects of dry needling at remote MTrPs for myofascial pain control.

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Suppliers

a. Physitemp Instruments, Inc. 154, Huron Avenue, Clifton, New Jersey, USA.

b. Yu-Kuang Industrial Co., Ltd., 5F-6, NO. 20, Lane 609, Sec.5, Chung-shing Rd. San-Chung City, Taipei, Taiwan.

c. Neuro-EMG-Micro; Neurosoft, 5, Voronin Str, Ivanovo, Russia.

d. Statistical Package for the Social Sciences version 10.0 for Windows; SPSS Inc. Headquarters, 233 S. Wacker Dr, 11th Fl, Chicago, IL 60606.

Figure Legends

Fig 1. Study flow diagram. BF: biceps femoris; Contra: contralateral; EPN: endplate noise; GAS: gastrocnemius; Ipsi: ipsilateral; MTrS: myofascial trigger spot.

Fig 2. Sites of EPN recording or dry needling for all animals, and area receiving surgical transection for animals in Groups II, III, and IV.

Fig 3. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before, during, and after dry needling manipulation at gastrocnemius (GAS) in the Group I. (A) Time course of EPN amplitude. (B) Sample recordings of EPN responses in two rabbits of Group I.

†: $P < .05$, showed significant differences among the four subgroups. *: $P < .05$ showed the significant differences compared to the values at pre-needling level in subgroups with dry needling at ipsilateral and contralateral GAS.

Fig 4. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before and after tibial nerve transection surgery, and then, before, during and after dry needling manipulation at gastrocnemius (GAS) in Group II. (A) Time course of EPN

amplitude. (B) Sample recordings of EPN responses in two rabbits from Group II.

†: $P < .05$, showed significant differences among the four subgroups. *: $P < .05$, showed the significant differences compared with the values at pre-needling level in subgroups with dry needling at ipsilateral and contralateral GAS.

Fig 5. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before and 30–120 min after lumbar transection surgery, as well as before, during, and after dry needling manipulation at gastrocnemius (GAS) in Group III. (A) Time course of EPN amplitude. (B) Sample recordings of EPN responses in two rabbits from Group III.

#: $P < .05$, showed the significant differences when compared the values at pre-transected levels.

Fig 6. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before and 30–120 min after thoracic transection surgery, as well as before, during, and after dry needling manipulation at gastrocnemius (GAS) in Group IV. (A) Time course of EPN amplitude. (B) Sample recordings of EPN responses in two rabbits from Group IV.

†: $P < .05$, showed significant differences among the four subgroups. *: $P < .05$,

showed the significant differences compared with the values at pre-needling level in subgroups with dry needling at ipsilateral and contralateral GAS; #: $P < .05$, showed significant differences when compared with the values at pre- transected levels.

Fig 7. Schematic drawing of the proposed neural mechanisms for remote effect on proximal MTrS in response to dry needling at distal MTrS.

1. Strong irritation to nociceptors in the MTrS by dry needling at gastrocnemius. 2. Afferent input from gastrocnemius to dorsal horn (L6-S2 sensory neuron) probably in the MTrS circuit. 3. Ascending projection to upper (L5-L6) sensory neurons probably in another MTrS circuit (in dorsal horn). 4. Impulse via interneuron to L5-L6 motoneuron (anterior horn) corresponding to biceps femoris. 5. Increase efferent output to neuromuscular junction in the biceps femoris. 6. Increase EPN amplitude. 7. Strongly activated motoneuron also activates inhibitory interneuron to increase recurrent inhibition on firing rate. 8. Suppress efferent output from motoneuron. 9. Depress the EPN amplitude. 10. The excitability of inhibitory interneuron can also be influenced by descending inputs, thereby altering the overall excitability of the motoneuron pool and efferent as well as the irritability of MTrS at biceps femoris.

ORIGINAL ARTICLE

Spinal Cord Mechanism Involving the Remote Effects of Dry Needling on the Irritability of Myofascial Trigger Spots in Rabbit Skeletal Muscle

Running title: NEURAL MECHANISM FOR REMOTE EFFECT OF DRY NEEDLING

ABSTRACT

Objective: To elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit skeletal muscle myofascial trigger spots (MTrSs) via analyses of their endplate noise (EPN) recordings.

Design: Experimental animal controlled trial.

Setting: An animal laboratory of a university.

Animals: Ninety-six male New Zealand rabbits (body weight: 2.5–3.0 kg, age: 16–20 weeks).

Intervention: Animals received no intervention for neural interruption in Group I, transection of the tibial nerve in Group II, transection of L5 and L6 spinal cord in Group III, and transection of the T1 and T2 spinal cord in Group IV. Each group was further divided into 4 subgroups: animals received ipsilateral dry needling (IDN), contralateral dry needling (CDN), ipsilateral sham needling (ISN), or contralateral sham needling (CSN), of gastrocnemius (GAS) MTrSs.

Main Outcome Measures: EPN amplitudes of biceps femoris (BF) MTrSs.

Results: BF MTrS mean EPN amplitudes significantly increased ($P < .05$) initially after GAS verum needling, but reduced to a level significantly lower ($P < .05$) than the pre-needling level in Groups I and IV with IDN or CDN, and in Group II with CDN

(but not IDN). No significant EPN amplitude changes were observed in BF MTrS in Group III or in the control animals receiving superficial needling (sham).

Conclusion: This remote effect of dry needling depends on an intact afferent pathway from the stimulating site to the spinal cord and a normal spinal cord function at the levels corresponding to the innervation of the proximally affected muscle.

Key Words: Neural pathway; Dry needling; Myofascial trigger spot; Electromyography; Remote effect.

DEFINITION OF ACRONYMS

BF = biceps femoris muscle;

CDN = contralateral dry needling;

CSN = contralateral sham needling;

EMG = electromyographic;

EPN = endplate noise;

GAS = gastrocnemius muscle;

IDN = ipsilateral dry needling;

ISN = ipsilateral sham needling;

LTR = local twitch response;

MTrP = myofascial trigger point (human);

MTrS = myofascial trigger spot (rabbit)

INTRODUCTION

Myofascial pain is one of the most common sources of musculoskeletal pain and has as its hallmark the presence of taut bands and small hyperirritable regions referred to as myofascial trigger points (MTrPs).[1,2] Clinically, a given MTrP has a characteristic referred pain pattern, and may be associated with a local twitch response (LTR) in response to snapping palpation.[2] In the MTrP region, electromyographic (EMG) activity of endplate noise (EPN) can be recorded, and both prevalence and amplitude of EPN can be used as indicators to assess the irritability of MTrP.[2-5]

Dry needling has a well-established role in the treatment of myofascial pain.[6-9] Clinical studies have demonstrated that dry needling MTrPs at the pain site suppresses their activity resulting in pain reduction, but also that those MTrPs can also be suppressed through dry needling distant MTrPs that are anatomically proximal or distal to the site of clinical pain.[8-12] Acupuncture studies have demonstrated that the Traditional Chinese Medicine principle that pain conditions can be improved by stimulating acupuncture points distant from the site of pain.[4,13-14] The effects of acupuncture may also spread to the contralateral side.[15] Studies of acupuncture needle stimulation in anesthetized animals have identified a wide variety of reflex responses in remote modification of various organ functions.[16] However, its underlying neuronal

control mechanism remains unclear.

The purpose of this study is to confirm the remote effects of dry needling on trigger points and to elucidate the neural mechanisms underlying the remote effects produced by dry needling rabbit myofascial trigger spots (MTrSs, equivalent to human MTrPs) in the gastrocnemius muscle (GAS)[17, 18] via analyses of EPN recordings from the biceps femoris (BF).

MATERIALS AND METHODS

Animals

The experiments were performed on adult male New Zealand rabbits (ages from 16 to 20 weeks, body weight of 2.5–3.0 kg). Each animal was housed and cared for following the ethical guidelines of the International Association for Study of Pain in animals were followed.[19] Effort was made to minimize discomfort and to reduce the number of animals used. All animal experiments were conducted with the procedure approved by the Animal Care and Use Committee of a university in accordance with the Guidelines for Animal Experimentation.

Ninety-six rabbits were divided randomly into four groups (fig 1) based on the procedure performed. Group I (n=24) animals received no surgical intervention (intact neural pathway), Group II animals (n=24) received transection of tibial nerve in the electrophysiologically investigated side (peripheral sensory pathway), Group III animals (n = 24) received transection of L5-L6 spinal cord (BF innervation level), and Group IV animals (n=24) received transection of T1-T2 spinal cord (supra-segment of BF innervation). For the EPN amplitude variable, a sample size of 24 subjects in each group was sufficient to give statistical power of 97.06% with a significance level of $P < .05$. Animals in each group were randomly divided further into four subgroups based on the condition of treatment on GAS: experimental animals with ipsilateral dry

needling (IDN, n=8), or contralateral dry needling (CDN, n=8), control animals with ipsilateral sham needling (ISN, n=4), or contralateral sham needling (CSN, n=4), on the MTrS of the gastrocnemius. Fewer animals were studied in the control group since no significant changes were observed in all animals treated with sham needling. Regarding the assignment of groups or subgroups, animals were selected from the first available litter, and subsequently from the next litter, and so forth according to the sequence in a random table.

Animal Preparation

Before anesthesia, the tenderest spots (i.e., MTrS) of BF and GAS were identified by finger pinching. The animal responded to pinch stimulation with withdrawal of the lower limb, turning its head, screaming, etc, only when the most painful spot was pinched, and this most tender spot was confirmed as the MTrS. [2,17,18,20-23] These painful regions were marked on the skin with an indelible marker and were designated for electrophysiological assessment or dry needling. The animals were anesthetized with 2% isoflurane in oxygen flow for induction followed by a 0.5% maintenance dose.[24] Body temperature was monitored by a thermistor probe of a thermometer [a] in the rectum and maintained at approximately 37.5 °C using a body temperature control system consisting of thermostatically regulated DC current heating pad and an infrared lamp. The hind limbs of anesthetized rabbits were shaved

and cleaned with povidone-iodine solution. The skin of the lateral thigh in one randomly selected side was incised to expose the BF, which served as an EPN recording site. The marked spot region in the BF muscle was grasped between two fingers from behind the muscle and the muscle palpated by gently rubbing (rolling) it between the fingers to discover a taut band. A taut band felt like a clearly delineated "rope" of muscle fibers and was roughly 2–3 mm or more in diameter. The fibers of the taut band were unmistakably firmer in consistency than the surrounding muscle.

Needling of Gastrocnemius Muscle

All needling procedures were performed by the same investigator who was blinded to the group assignment regarding to surgical intervention on neural pathway. Dry needling stimulation was performed with a disposable 30G acupuncture needle (300 μm in diameter, 1.5 inches in length) [b] at ipsilateral or contralateral GAS (fig 2). The technique of dry needling was similar to that suggested by Hong [18, 20,25-27] with multiple needle insertions to elicit rabbit-LTRs as much as possible. For needling in MTrS of GAS, the needle was first inserted through the skin perpendicularly at the center of the marked spot and advanced slowly and gently into the muscle until the needle tip touched the bone surface to estimate the thickness of the muscle. The needle was withdrawn back to the subcutaneous layer, and rapidly moved in and out for insertion of multiple sites in different directions (in a cone shape with the center at

the initial needle insertion of a perpendicular direction, and the angle of the cone margin was about 20°). For each needle insertion, the needle was advanced into the depth near the bone surface. Simultaneous needle rotation was performed to facilitate fast "in-and-out" needle movement as suggested by Chou et al.[4] in order to elicit as many LTRs as possible. For sham needling, the needle was inserted into the subcutaneous layer of the marked MTrP region at a depth approximately 1-2 mm from the skin surface. After insertion, the needle stayed there without further movement.

Transection Operations

1. Transection of tibial nerve

During anesthesia for the animals in Group II, the incision was made over the posterior aspect of one thigh ipsilaterally to the EPN recording side. Under the operating microscope, the sciatic nerve was exposed, and the tibial nerve isolated and transected at the site about 1 cm from its insertion into the GAS.

2. Transection of spinal cord

After completing laminectomy and making a slit in the dorsal portion of the dura mater, the cord was transected by a knife and then aspirated by suction at about 2 mm caudal and rostral to the level of transection, at L5-L6 levels of the spinal cord for animals in Group III, or at T1-T2 levels of the spinal cord for Group IV animals.

Gelfoam was placed into the empty vertebral column to seal the empty vertebral cavity and reduce bleeding. In a previous study [20] and in our preliminary data, about 2½ h after surgery, the rabbits would have almost completely recovered from spinal shock. The animal would then be ready for the needling study.

Recording of Endplate Noise

1. Electromyography setting

For EPN assessment, a two-channel digital EMG machine[c] and monopolar needle electrodes (37 mm disposable Teflon-coated model) were used. The gain was set at 20μV per division for recordings from both channels. Low-cut frequency filter was set at 100 Hz and the high-cut at 1,000 Hz. Sweep speed was 10 ms per division. The search needle for EPN recording was inserted into the MTrS region and connected to the first channel of the EMG machine. The control needle was inserted into the non-taut band region near the MTrS in the same muscle and connected to the second channel. A common reference needle electrode for each channel was placed on the incised skin and connected to both channels via a y-connector.

2. Search for endplate noise

This procedure was performed by an investigator who was blind to the group assignment. The search needle was inserted into the MTrS region in a direction

parallel to the muscle fibers at an angle of approximately 60° to the surface of the muscle. After initial insertion just short of the depth of the MTrS or to a comparable depth in the case of control sites, the needle was advanced very slowly with simultaneous slow rotation to prevent it from 'grabbing' and releasing the tissue suddenly to advance in a large jump. Each advance was of minimal distance (~1 mm). When the needle approached an active locus (EPN locus), the continuous distant electrical activity, i.e., EPN, can be heard. As soon as EPN with amplitude higher than 10 μV could be recorded, the examiner stopped advancing the needle, but minimally moving the needle gently to different direction, trying to obtain EPN with highest amplitude. If this was impossible, the needle was advanced to another site until an EPN with optimal amplitude (usually higher than 30μV) could be recorded. Then the needle was fixed in place (carefully and firmly taped on the skin) to ensure that this EPN can run continuously on the recording screen with constant amplitudes. Continuous EPN tracing was recorded throughout the entire course of the experiment, and provided the opportunity for continuous visual observation of EPN changes on the EMG screen. If the EPN could not be sustained, the searching needle would be moved to another site until a satisfactory EPN tracing could be obtained. The entire EPN tracing found in MTrS of BF were recorded for the analysis of amplitude changes.

3. Measurement of the amplitude of endplate noise

Five randomly selected samples of EPN recordings (10 ms each) were taken before, during, and 3 min after the completion of the needling treatment for all groups; before and 30 minutes after surgery for Group II animals; and every 30 minutes up to 120 minutes after surgery for Group III and IV animals. The mean amplitude of EPN of 5 random samples was analyzed and calculated through the embedded software in the Neuro-EMG-Micro equipment, and was recorded as the value for a certain measurement point for each animal.

Data Analysis

Data of EPN amplitudes in different measurement points for different groups or subgroups were expressed as the mean ± standard error of the mean (SEM) for further statistical analysis. The Shapiro-Wilk's normality test was conducted to determine whether the data fit a normal distribution prior to subsequent analyses, and showed all measures of EPN amplitude were normally distributed. Tests of 'homogeneity' or 'baseline balance' on covariates including body weight, age, and anesthesia condition were measured and equivalent before the needling treatment in all animals. The differences in EPN amplitude across measurement points in each group were carried out using repeated measures ANOVA, and later further analyzed by a Bonferroni

post-hoc method. The differences in EPM amplitudes within each of the subgroups (IDN, CDN, ISN and CSN) and across measurement points (before, during, and after needling) were analyzed using two-way ANOVA (side × time) followed by a Bonferroni post-hoc analysis for each group. The differences in EPN amplitude within measurement point (before, during, and after needling) across subgroups (IDN, CDN, ISN and CSN) were tested by paired t-test. A p value of <0.05 was considered to be statistically significant. All data was analyzed using SPSS version 10.0 for Windows.[d]

RESULTS

Effects of Dry Needling of Distal MTrS in Intact Rabbits (Group I)

The serial alterations of the mean EPN amplitude before, during, and after dry needling at ipsilateral and contralateral GAS for Group I are demonstrated in figure 3. Before needling treatment, there was no significant difference among the four subgroups (two-way ANOVA, F=0.10, $P > .05$).

The mean amplitudes of EPN before, during, and after needling were 18.20±0.70μV, 27.71±0.47μV, and 13.15±0.59μV, respectively in IDN subgroup, and 17.96±0.69μV, 24.66±1.47μV, and 14.01±0.86μV, respectively in CDN subgroup. In either IDN or CDN subgroup, the amplitudes at different times were significantly different (repeated measures ANOVA: F=45.99 and $P < .05$ for IDN, F=113.98 and $P < .05$ for CDN). Compared with the data in the pre-needling level, the EPN amplitudes were significantly increased during the dry needling (Bonferroni post-hoc test, $P < .05$), and then significantly decreased to a much lower level after completion of the needling treatment (Bonferroni post-hoc test, $P < .05$) for either IDN or CDN subgroup as shown in figure 3. However, these serial alterations of EPN amplitudes were not found in the comparable subgroup ISN or CSN (repeated measures ANOVA, $P > .05$). There were significant differences in EPN amplitudes recorded either during or after needling between IDN and ISN, or between CDN and CSN subgroups

(Bonferroni post-hoc test, $P < .05$), but not between IDN and IDN subgroups (Bonferroni post-hoc test, $P > .05$).

Effects of Dry Needling of Distal MTrS in Rabbits With Tibial Nerve Transection (Group II)

The serial alterations of the mean EPN amplitude (±SEM) throughout the entire experiment in Group II are demonstrated in figure 4. The mean amplitude of EPN had no significant changes before, during, and after ipsilateral tibial nerve transection (i.e., GAS denervation) (repeated measures ANOVA, F=0.06, $P > .05$). Before the needling treatment, there was no significant difference in EPN amplitude among the subgroups treated with dry or sham needling at ipsilateral or contralateral side (two-way ANOVA, F=0.68, $P > .05$).

The mean amplitudes (±SEM) of EPN recorded from BF before, during, and after needling were 16.72±0.34μV, 16.64±0.37μV, and 15.46±0.50μV, respectively in IDN subgroup, and were16.90±0.38μV, 21.63±0.91μV, and 12.40±0.36μV, respectively in CDN subgroup. There were significant differences in EPN amplitudes among those recorded before, during, and after needling (repeated measures ANOVA, F= 80.77, $P < .05$) in CDN subgroup (similar to the changes in Group I, Bonferroni post-hoc test, $P < .05$), but not in IDN subgroup (repeated measures ANOVA, F= 2.89, $P > .05$).

There were significant differences in mean EPN amplitudes between CDN and its comparable CSN subgroups, but not between IDN and ISN subgroups (Bonferroni post-hoc test, $P > .05$), at the time during (Bonferroni post-hoc test, $P < .05$) and after (Bonferroni post-hoc test, $P < .05$) needling. Moreover, there were significant differences in the magnitude or time-dependent alterations of EPN amplitude between CDN and CSN subgroups (Bonferroni post-hoc test, $P < .05$).

Effects of Dry Needling of Distal MTrS in Rabbits With Lumbar Cord Transection (Group III)

The serial alterations of the mean EPN amplitude (±SEM) throughout the entire experiment for Group III are shown in figure 5. There were significant differences in EPN amplitude among those recorded before, immediately, and 30, 60, 90, and 120 min after L5-L6 transection (repeated measures ANOVA, F=29.81, $P < .05$). During the 2 h period after transection, the mean EPN amplitudes were significantly lower than the pre-transected levels (Bonferroni post-hoc test, all were $P < .05$ at 30, 60, 90, and 120 h). There was no significant difference among the four subgroups treated with dry or sham needling at the ipsilateral or contralateral side regardless of the time before (two-way ANOVA, F=0.23, $P > .05$), during (F=1.45, $P > .05$), or after (F=1.72, $P > .05$) needling treatments.

The mean amplitudes (±SEM) of EPN before, during, and after needling were 11.56±0.36μV, 11.47±0.43μV, and 11.28±0.47μV, respectively in IDN subgroup, and were 11.67±0.45μV, 12.32±0.46μV, and 12.33±0.46μV, respectively in CDN subgroup. There was no significant difference in EPN amplitude among those recorded before, during, and after IDN (repeated measures ANOVA, F=0.63, $P > .05$), CDN (repeated measures ANOVA, F=1.17, $P > .05$), ISN treatment (repeated measures ANOVA, F=0.23, $P > .05$), or CSN (repeated measures ANOVA, F=0.52, $P > .05$). There were no significant differences in each time-dependent alterations of EPN amplitude between IDN and ISN subgroups, between IDN and CDN subgroups, or between CDN and CSN subgroups (Bonferroni post-hoc test, all $P > .05$).

Effects of Dry Needling of Distal MTrS in Rabbits With Thoracic Cord Transection (Group IV)

The serial alterations of the mean EPN amplitude (±SEM) throughout the entire experiment for Group IV are shown in figure 6. The EPN amplitude recorded at 30 and 60 min after T1-T2 transection was significantly reduced when compared to the pre-transection level (Bonferroni post-hoc test, $P < .05$), but recovered to pre-transection level after 90 min (Bonferroni post-hoc tests, all $P > .05$), and normalized to be similar for all subgroups by 120 min after transaction (two-way

ANOVA, F=0.09, P> .05).

The mean amplitudes (±SEM) of EPN recorded from BF before, during, and after needling were 18.17±0.36μV, 26.88±0.43μV, and 15.74±0.26μV, respectively in IDN subgroup, and were 18.28±0.45μV, 27.72±0.47μV, and 16.20±0.22μV respectively in CDN subgroup. There were significant differences in EPN amplitudes among those recorded before, during, and after IDN (repeated measures ANOVA, F= 264.29, $P< .05$) or CDN (repeated measures ANOVA, F= 243.11, $P< .05$). The mean EPN amplitudes were significantly increased during IDN or CDN (Bonferroni post-hoc test, $P< .05$), but after cessation of needling, they reduced significantly to a level less than that before needling (Bonferroni post-hoc test, $P< .05$), but there were no significant differences in these changes between IDN and CDN subgroups (Bonferroni post-hoc test, $P> .05$). These serial alterations of EPN amplitudes were not found in either ISN (repeated measures ANOVA, F=1.02, $P> .05$) or CSN subgroup (repeated measures ANOVA, F=2.02, $P> .05$).

DISCUSSION

To our knowledge, the present study is the first animal study to investigate the neural mechanism of the remote effects of dry needling. In this study, we found that an intact afferent nerve from the remote stimulation site and normal spinal cord segments corresponding to the innervation of the affected proximal muscle are essential for the remote effect from either ipsilateral or contralateral stimulation.

The dry needling used in this study is a technique of MTrP injection with multiple high-speed needle insertions into different sensitive loci in an MTrP region suggested by Hong.[25-27] High speed needling can provide high-pressure stimulation to the sensitive loci in the MTrP region to elicit LTRs. It is essential to elicit LTRs during needling of an MTrP in order to obtain immediate and complete pain relief.[6, 25-29] Dry needling at the MTrS was effective in diminishing spontaneous electrical activity (i.e., EPN) of MTrS of rabbit skeletal muscle if LTRs were elicited.[30] After several LTRs had been elicited by the needling of an MTrS of rabbit skeletal muscle, no more LTRs could be elicited from the same region [18] and the irritability of the MTrS could be suppressed. [30] Needling-elicited LTRs are involuntary discharges of muscle fiber mediated through the nervous system and integrated at the spinal cord level.[18, 20] Therefore, it is important to apply this needling technique to achieve the best needling effect or remote needling effect for the study on the neural mechanism.

Changes in the EPN amplitude in the MTrS were found during and after dry needling at the distal MTrSs in animals with intact neural circuits (Group I). It appears that either IDN or CDN to the distal MTrS could initially increase the irritability of the proximal MTrS, followed by a suppression effect after cessation of needling. Fernandez-Camero, et al. [31] also found an increase in spontaneous electrical activity at an MTrP region during a persistent noxious stimulation at another distant MTrP, followed by a suppression of electrophysiological irritability after cessation of needling. The two findings above strongly support clinical observations related to the interaction between one MTrP and another MTrP located in the region of the referred pain (referred zone) of that MTrP.[2,6,9-12,26-29]

In the study on Group II rabbits with ipsilateral denervation of GAS, the remote effect disappeared after IDN, but persisted after CDN. These results demonstrated the importance of an intact afferent pathway to the spinal cord in the remote modulation of EPN amplitudes. After destruction of spinal cord corresponding to the level of BF (Group III), the remote effect disappeared after either IDN or CDN treatment. This finding suggested the existence of intraspinal connections between the GAS afferents and BF spinal interneurons. The partial suppression of the EPN amplitude after spinal cord transection at L5-L6 is possibly related to the influence of the spinal shock. After interruption of upper motor neuron and supra-sensory connections (Group IV), the

remote effect persisted, either with IDN or CDN, but smaller than that in intact animals (Group I). It may imply the possible influences from supraspinal centers, such as descending pain inhibitory systems. These influences are anticipated to be minimal after transection at higher spinal levels. Loss of inputs to this system would weaken the inhibition on the pain level,[32] which could lead to the EPN amplitude being less suppressed. Therefore, the recruitment of the diffuse noxious inhibitory control (DNIC) system may be also elicited by dry needling treatment on regions remote to the stimulation site.

The neural pathway for the remote effect appears to be a spinal reflex, probably similar to that mediating the referred pain[26, 29] and local twitch response.[18, 20,26,29] Hong has hypothesized a corresponding "MTrP circuit" for each MTrP, which can modulate the pain, referred pain, and local twitch response elicited by stimulating the MTrP.[6, 29, 33] The neural connection in the spinal cord responsible for this remote effectiveness is probably similar to that for the referred pain.[26, 29]

The initial increase in EPN with remote dry needling followed by a reduction in EPN after local twitch responses are elicited indicating inactivation of the remote MTrS. Strong stimulation from continuous dry needling of an MTrS can activate the sensitized nociceptors and generate strong impulses propagating to the spinal cord to activate the corresponding motoneurons (including those in the same segment

corresponding to the needling muscle and other segments corresponding to the remote muscles) to fire reflexively, thereby causing increased EPN amplitude in MTrSs not only at the needling muscle but also at other remote muscles. These strongly activated motoneurons are also controlled by recurrent inhibitions. As the firing rate of motoneuron increases, the amount of recurrent inhibitions will also increase, subsequently limiting and suppressing the firing rate of the efferents. In this way, these impulses elicited by dry needling eventually breaks the vicious cycle of the neural circuits (i.e. MTrP circuits [29, 33]) responsible for MTrSs through spatial and temporal interactions in the spinal cord. Thus the EPN amplitude is suppressed after dry needling. Possibly, there are certain neural connections among the inhibitory interneuron and descending pain control system in the spinal cord that can modulate the irritability of MTrPs when a remote painful dry needling stimulation is applied (fig 7). The initial increase in EPN is consistent with suppression of the DNIC system, and the subsequent reduction in EPN is consistent with activation or enhancement of the DNIC system. Therefore, the physiological basis for the remote effects of dry needling may be related to an inactivation of MTrS[29, 33] and activation of DNIC [34, 35] induced by noxious stimulation applied at the painful region (such as trigger point needling) or at a remote site (such as in remote dry needling). This is probably the mechanism of remote pain control by dry needling which is similar to

hyperstimulation analgesia in acupuncture.[32,36]

Limitations of the Study

The difficulty in confirming the correlation between the alterations of EPN amplitude and pain intensity in rabbit may be criticized. However, a conclusion based on the human study[4] may be reasonably applied on rabbits, because there are plenty of similarities between the human MTrP and rabbit MTrS.[18, 26, 28, 29] Lack of follow-up assessments for the long-term remote effect is another deficiency of this study. However, we rarely see the long-term effects of dry needling if the underlying pathology of MTrP activation is not treated appropriately.[6, 26, 27, 28, 33] Another problem is that out sham needling (similar to superficial dry needling) may not be appropriate as a control. Fortunately, we see no significant changes after treatment with sham needling. However, this could be related to the small sample size. In our clinical practice, we have observed much less effectiveness of superficial needling than that of deep dry needling with our multiple quick insertion technique. In addition, considering the individual differences in the motoneuron excitability and the supraspinal control of spinal inhibitory interneurons, just based on electrophysiological study, we are unable to distinguish the relative contribution from each inhibitory mechanism for motoneuronal excitability to the changes in MTrP

404 irritability due to remote dry needling. All the above factors should be taken into

405 consideration for data interpretation.

CONCLUSION

We have demonstrated that an intact afferent from the stimulating site to the spinal cord and a normal function of spinal cord corresponding the innervation of the remotely affected muscles are essential for this remote effectiveness. This study may help in the understanding of the mechanism for beneficial effects of dry needling at remote MTrPs for myofascial pain control.

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Fig 1. Study flow diagram. BF: biceps femoris; Contra: contralateral; EPN: endplate noise; GAS: gastrocnemius; Ipsi: ipsilateral; MTrS: myofascial trigger spot.

Fig 2. Sites of EPN recording, dry needling for all animals, and area receiving surgical transection for animals in Groups II, III, and IV.

Fig 3. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before, during, and after dry needling manipulation at gastrocnemius (GAS) in the Group I. (A) Time course of EPN amplitude. (B) Sample recordings of EPN responses in two rabbits of Group I.

†: $P < .05$, showed significant differences among the four subgroups. *: $P < .05$ showed the significant differences compared to the values at pre-needling level in subgroups with dry needling at ipsilateral and contralateral GAS.

Fig 4. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before and after tibial nerve transection surgery, and then, before, during and after dry needling manipulation at gastrocnemius (GAS) in Group II. (A) Time course of EPN

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Fig 5. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before and 30–120 min after lumbar transection surgery, as well as before, during, and after dry needling manipulation at gastrocnemius (GAS) in Group III. (A) Time course of EPN amplitude. (B) Sample recordings of EPN responses in two rabbits from Group III.

#: $P < .05$, showed the significant differences when compared the values at pre-transected levels.

Fig 6. A series of changes in the EPN amplitude measured at MTrS of biceps femoris before and 30–120 min after thoracic transection surgery, as well as before, during, and after dry needling manipulation at gastrocnemius (GAS) in Group IV. (A) Time course of EPN amplitude. (B) Sample recordings of EPN responses in two rabbits from Group IV.

†: $P < .05$, showed significant differences among the four subgroups. *: $P < .05$,

showed the significant differences compared with the values at pre-needling level in subgroups with dry needling at ipsilateral and contralateral GAS; #: $P < .05$, showed significant differences when compared with the values at pre- transected levels.

Fig 7. Schematic drawing of the proposed neural mechanisms for remote effect on proximal MTrS in response to dry needling at distal MTrS.

1. Strong irritation to nociceptors in the MTrS by dry needling at gastrocnemius. 2. Afferent input from gastrocnemius to dorsal horn (L6-S2 sensory neuron) probably in the MTrS circuit. 3. Ascending projection to upper (L5-L6) sensory neurons probably in another MTrS circuit (in dorsal horn). 4. Impulse via interneuron to L5-L6 motoneuron (anterior horn) corresponding to biceps femoris. 5. Increase efferent output to neuromuscular junction in the biceps femoris. 6. Increase EPN amplitude. 7. Strongly activated motoneuron also activates inhibitory interneuron to increase recurrent inhibition on firing rate. 8. Suppress efferent output from motoneuron. 9. Depress the EPN amplitude. 10. The excitability of inhibitory interneuron can also be influenced by descending inputs, thereby altering the overall excitability of the motoneuron pool and efferent as well as the irritability of MTrS at biceps femoris.

Figure 1

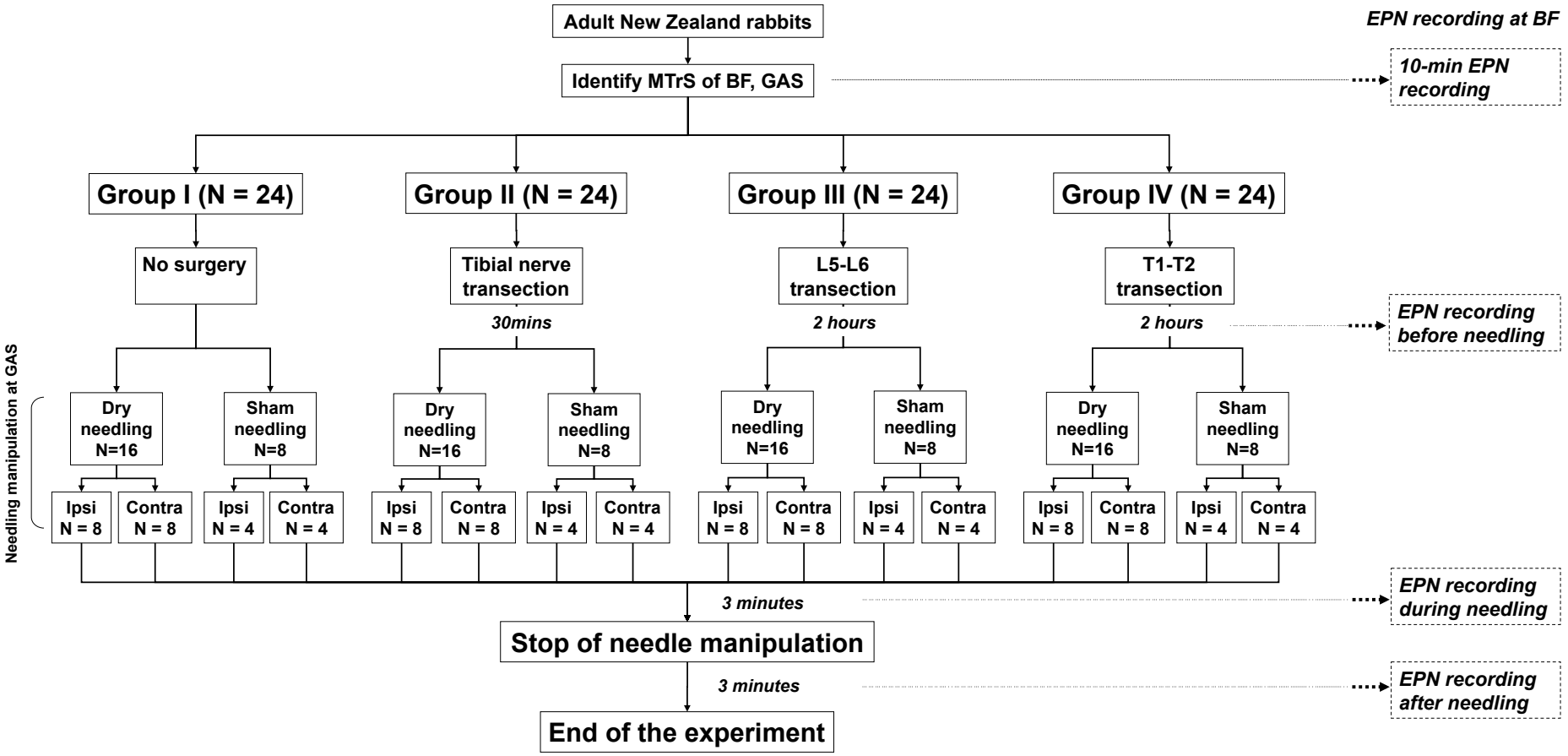

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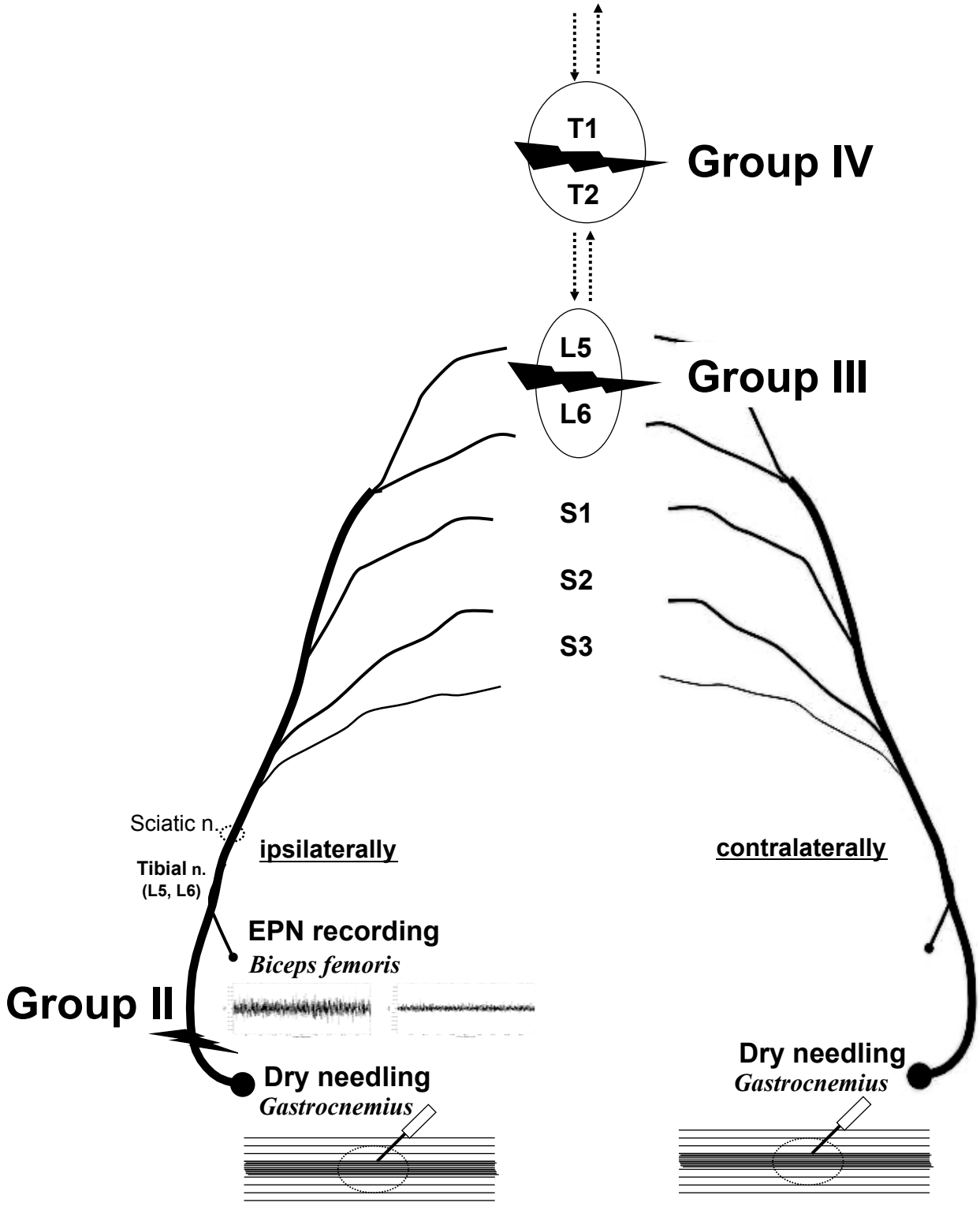
Figure 2



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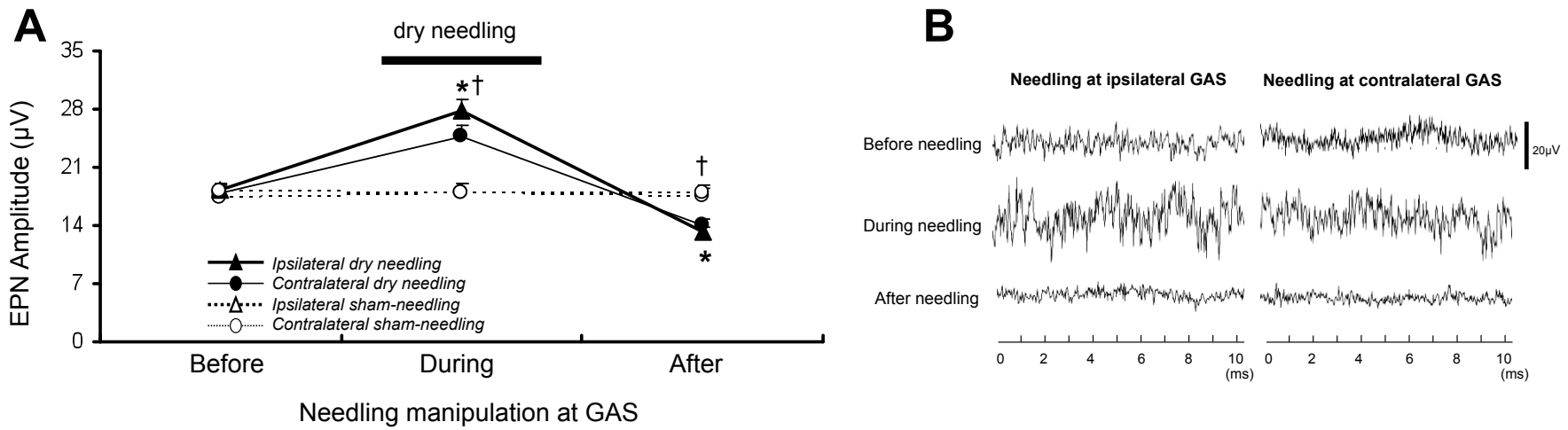

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Figure 4

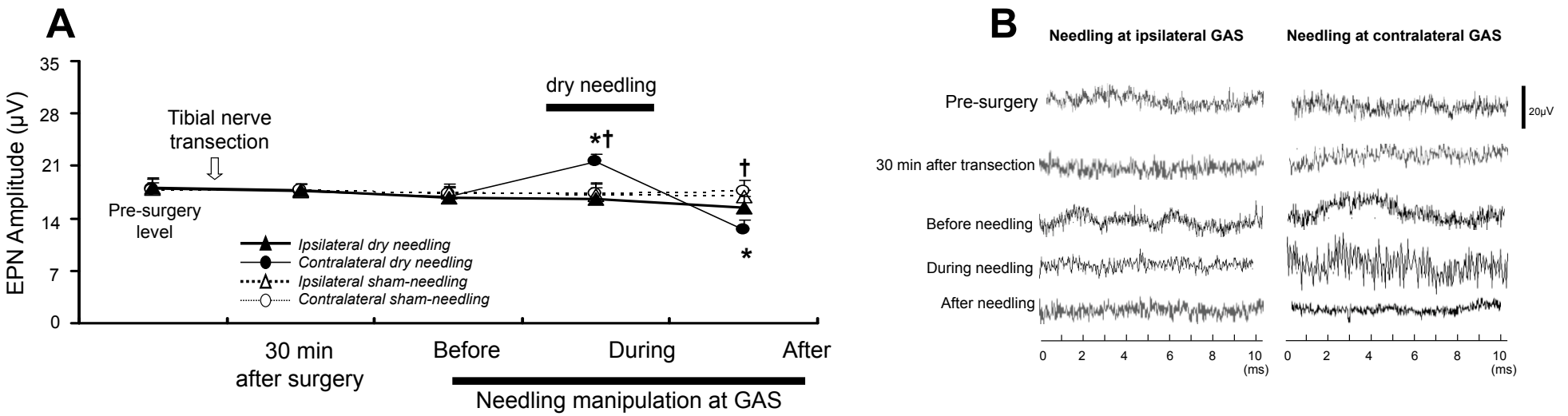

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Figure 5

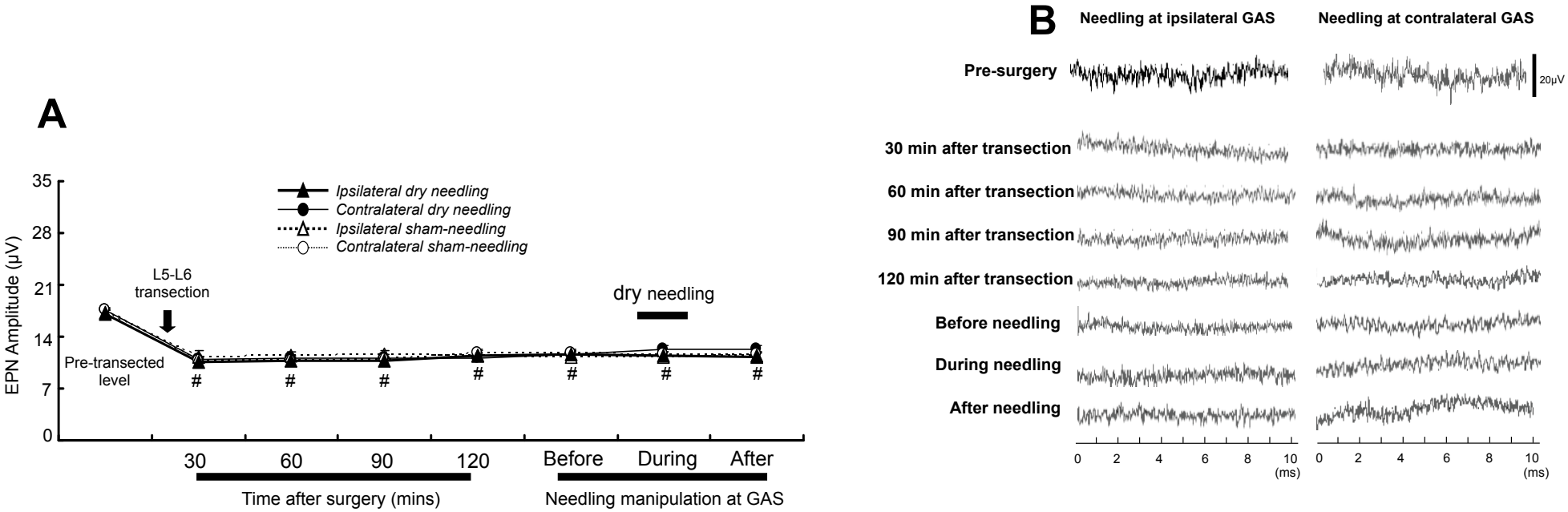

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Figure 6

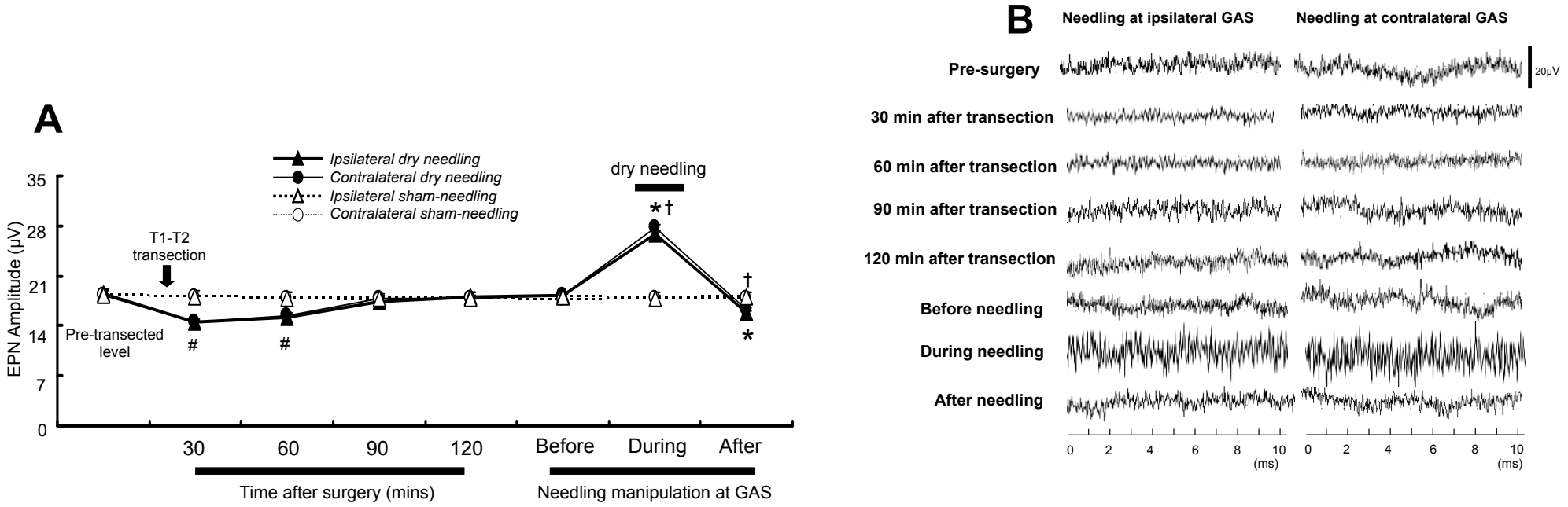

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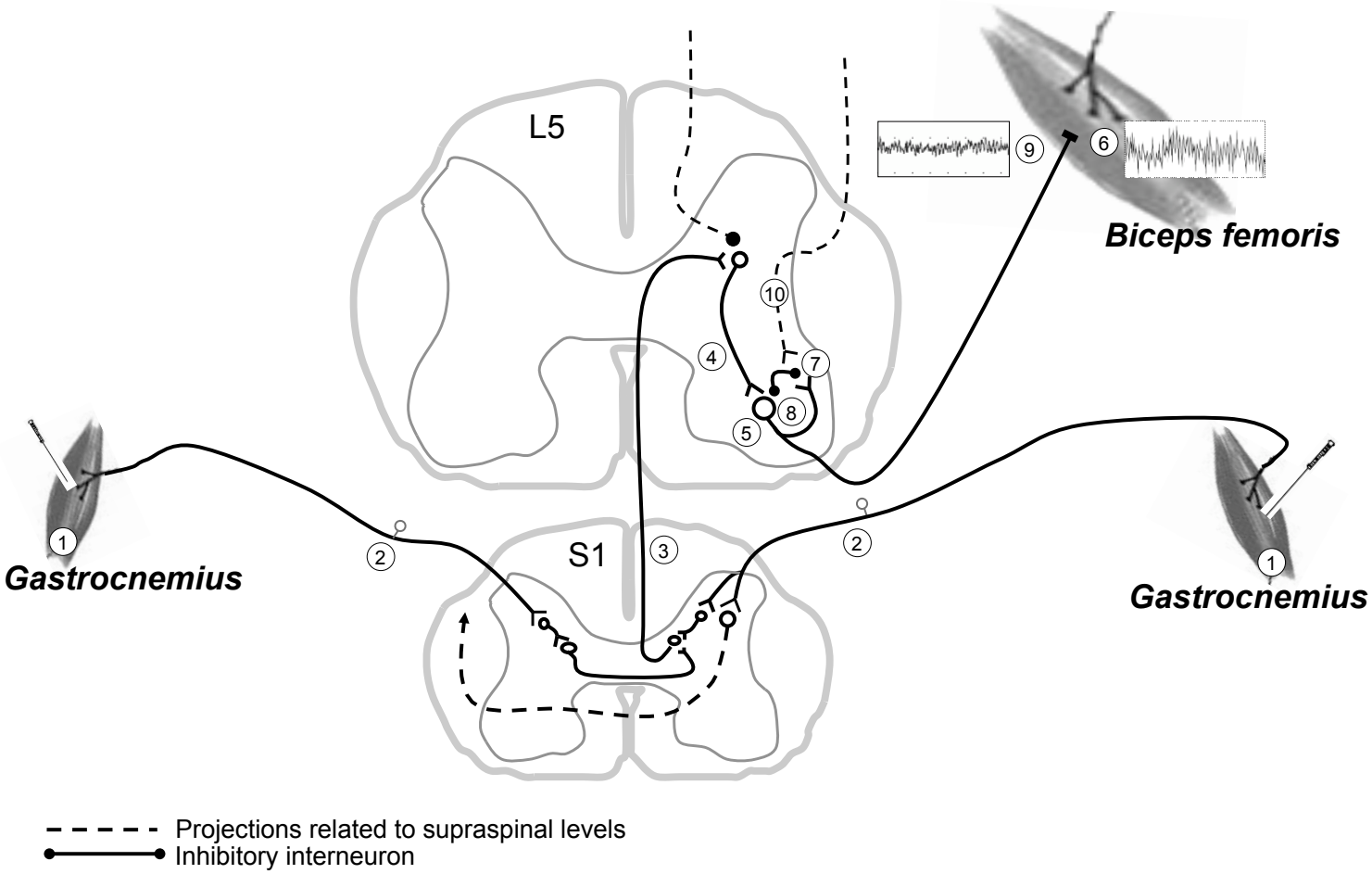
Figure 7



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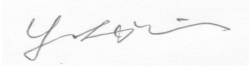
Signature: 

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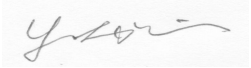
Signature: 

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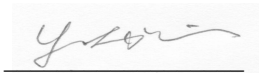
Signature: 

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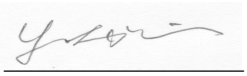
Signature: 

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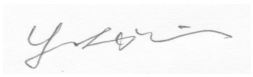

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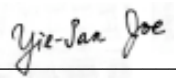
Signature: 

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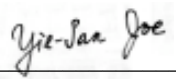
Signature: 

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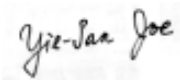

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