## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Detection for peripheral and central sensitization at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study |
| **AUTHORS** | Yan, Chao-qun; Zhang, Shuai; Li, Qian-Qian; Zhang, Liwen; Wang, Xue-Rui; Fu, Qing-Nan; Shi, Guangxia; LIU, Cunzhi |

## VERSION 1 - REVIEW

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| **REVIEWER** | Rueda JC<br>Universidad Católica de Murcia (UCAM)<br>SPAIN |
| **REVIEW RETURNED** | 30-Oct-2016 |

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| **GENERAL COMMENTS** | Inclusion criteria for Healthy participants is not so clear.<br>It seems difficult to reproduce the values of PPT in acupoint and non-acupoint.<br>With respect to the non-acupoint only 2 cm to Tianzong, today we now the effect too close to acuponint, and possible stimulation. |

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| **REVIEWER** | Dr Zhen Zheng<br>RMIT University<br>Australia |
| **REVIEW RETURNED** | 08-Nov-2016 |

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| **GENERAL COMMENTS** | The existence of peripheral and central sensitization at acupoints in patients with unilateral shoulder pain<br><br>Authors of this exploratory study examined pressure pain threshold (PPT) at 5 sites among 30 patients with unilateral shoulder pain and 30 gender- and age- matched pain free volunteers. The found that PPTs on acupoints were lower on the painful shoulder when compared with non-painful, contralateral shoulder; and PPTs in the patients with shoulder pain were lower when compared with the health controls. PPT on one non-acupoint did neither differ between painful and non-painful shoulders, nor between patients with chronic pain and the healthy control. The authors further devised the concept of peripheral sensitisation index and central sensitisation index to classify patients. The paper concluded that 1) peripheral and central sensitisation present at the acupoints in patients with unilateral shoulder pain; 2) this was particularly true in three key points commonly used for shoulder pain.<br><br>Overall the study is well-designed, and well-conducted and the report is adequate. Lower PPT on the painful shoulder in patients with unilateral shoulder pain has been reported before. The novel |

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| | aspects of this paper are 1) PPTs were measured at acupoints; 2) the concept of peripheral sensitisation index and central sensitisation index. I agree that peripheral sensitisation presented at the acupoints; but I am not convinced with the current data the central sensitisation also presented at the acupoints.<br><br>Major concerns<br>I suggest the authors reconsidering the conclusion.<br>1. Interpretation of central sensitisation: The authors considered a lower PPT on the pain-free side of the shoulder reflecting a status of central sensitisation. This could be an approach. However the common indicator of central sensitisation is reduced PPTs at the site distal or remote to the pain site. For instance reduced PPTs on the leg in patients with shoulder pain (Paul et al 2012). This needs to be addressed in the discussion.<br>2. Acupoints specific: The evidence supporting this conclusion was from a) PPTs measured at acupoints reduced; 2) PPT on one non-acupoint did not reduce. These data are not sufficient. Ideally to ensure sensitisation is acupoint specific, one has to test more than one acupoints. Furthermore, Ashi points are common in shoulder pain and are commonly used in acupuncture treatment. How will Ashi points be defined in this instance?<br><br>3. Please explain why the two sensitisation indexes were calculated in this particular manner. Please provide reference to support this decision.<br>4. A large part of the Discussion was dedicated to explain the sensitisation status of acupoints. I expect to see some discussion on the internal validity. The blinding of the participants of the purpose of the study; the knowledge of acupoints of the assessor and its impact on the assessment; the reliability of testing PPT over a soft area such SI9; the selection of non-acupoints….<br>Minor comments:<br>• Why BDI cut off points was 4 in this study? The common cut off point is 9.<br>• Page 4, line 13: please cite the previous study<br>• Page 4, line 23, lower PPT is hyperalgesia, which could be explained by peripheral central sensitisation or central sensitisation. Lower PPT itself is not peripheral sensitisation.<br>• Page 4, line 25, central sensitisation needs to be explained here<br>• Page 4: the aims need to be better articulated<br>• Page 4, line 42, add Ethics approval number<br>• Page 4, line 54, it is difficult to imagine the types of shoulder pain that do not have areas overlapping the four acupoints. Examples here will be very helpful.<br>• Page 5, line 1: were those with pain in the thoracic area excluded?<br>• Page 7, line 46, it should be “paired t-tests”;<br>• Page 7, line 51, this should be “independent t-tests”.<br>• Page 10, line 30, Is Pearson r adequate for examining association between binary data (sex) and continuous data?<br>Additional comments:<br>I am interested to see if PSI and CSI could be used to classify patients and if the three groups (PSI, CSI, and PSI and CSI) differ in any demographic data or pain features.<br>References<br>Paul et al (2012) Central Hypersensitivity in Patients With Subacromial Impingement Syndrome. Archives of Physical Medicine and Rehabilitation 93(12): 2206–2209. http://dx.doi.org/10.1016/j.apmr.2012.06.026 |

| REVIEWER | Ying Lu<br>Stanford University, USA |
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| REVIEW RETURNED | 01-Dec-2016 |

| GENERAL COMMENTS | Review BMJ Open bmjopen-2016-014438<br><br>Major Comments<br><br>This paper reports a cross-sectional case-control observation study to evaluate peripheral and central sensitization of pain at acupoints for patients with unilateral shoulder pain. They found a significantly lower pressure-pain thresholds (PPTs) at acupints on the painful side compared with non-painful side in clinical patients. The PPTs on the non-painful side of clinical patients, although were significantly higher than PPTs on the painful side, were still significantly lower than the ipsilateral side of the matched healthy controls. They observed significant correlations in peripheral and central sensitization indices. They reached the conclusion that there exisits relationship among the acupoints that are usually chosen to treat the shoulder pain, which supports the acupoint selection to treat shoulder pain by acupuncture.<br><br>The study was carefully designed and appeared to be conducted well. The topic of research is interesting to the readers of the journal. I am not able to assess the clinical significance of the findings. The data was presented in reasonable way, although some of the analyses methods need to be modified. The conclusions are expected remaining true. There several major weaknesses for authors to address before it can be published in the journal.<br><br>1. Study Design.<br><br>1.1 The study design is not “a purely observational” study. It appears to be a prospective cross-sectional case-control observational study.<br><br>1.2 It was not clear how patients were screened and enrolled. Who were the first 164 patients that were used as the pool for the selection of the final 30 clinical patients enrolled to the study? Were they consecutive patients seen in the clinics? How many of the 164 clinical patients satisfied study enrollment criteria and how the final patients were selected? Were the controls matched to cases individually or in frequency? It is still necessary to include a consort flow chart.<br><br>1.3 It appears that the paper has authors from multiple institutions, yet the study seems to be conducted in a single institution. It will be helpful to clarify the contribution of co-authors.<br><br>2. Statistical Analysis.<br><br>2.1 Comparisons of experimental pain responses between the painful side and non-painful side of clinical patients should use paired t-test because they came from the same patients and should be correlated. It only improves the statistical significance. The study team used paired t-test to compare the PPTs of non-painful side from patients with the ipsilateral side of the healthy controls. If controls were selected in a matched pair with cases, this is |
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| | appropriate. Please explain in the design stage to show how the cases and controls were matched.<br><br>2.2 The analysis plan suggested that non-parametric statistical tests were used for data that failed test for normal distributions. It was not clear which variables failed the normality assumption. Since all patients should have their VAS above 50, the distribution of VAS may not normally distributed and should not be summarized by mean and standard deviations as shown in Table 1.<br><br>2.3 Sex is a binary variable. The differences in PPTs by gender should not be evaluated by Pearson's correlation coefficient. Either t-test or non-parametric Wilcoxon test should be used to compare PPTs difference between two genders.<br><br>2.4 PPTs of cases were normalized using a Z-scores according to the mean and standard deviations of controls. Peripheral sensitization for the painful side of clinical patients based on the 25th percentiles of z-score of the non-painful side of clinical patients, assuming matching the same acupoints. The central sensitization was based on the 25th percentile of the ipsilateral side of the controls. Actually there seems no need for the z-score transformation because the percentiles should not be affected by normalization transform. Also, Pearson's correlation coefficients were not affected by z-score transformation.<br><br>It will be helpful to present the corresponding cutoff values for all acupoints.<br><br>What was the justification to use 25%tiles as the cutoff value? Does that mean there should be 25% PPTs below the cutoff value in the reference group. In this case, the 95% confidence interval of proportion of patients who were sensitized should be between 10% to 40% by chance alone. The authors should talk to statisticians to evaluate the significance of the observed sensitization rates.<br><br>2.5 There are multiple comparisons for acupoints in the paper. There was no discussions about multiple comparisons. The analysis could be improved by performing multivariate statistical analysis to simultaneously consider PPTs from all acupoints together to reduce the burden of multiple comparisons.<br><br>3. English<br><br>3.1 The paper can benefit from the help of an English editor. Several areas are confusing and some are heard to understand. Professional editor help is needed throughout the manuscript.<br><br>Specific Comments<br><br>Page 2 line 10: A prospective (? Please confirm by the author) cross-sectional matched case-control observational study.<br><br>Page 2 line 27: Please clarify if the controls are patients or healthy participants. They have been characterized in consistently throughout the paper.<br><br>Page 2 line 36: "diminished" is not appropriate here. It means disappeared. The fact that "P < 0.05" means that the PPTs on non-painful side remained significant below than the ipsilateral side of |

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| | controls.<br><br>Page 2 line 50: delete “obvious”<br><br>Page 2 line 51: delete “n”<br><br>Page 3 line 6-8: please justify why this study of shoulder pain supports the generalized conclusion of musculoskeletal pain.<br><br>Page 3 line 35: Please provide reference of WHO report.<br><br>Page 4 line 11: please provide your publication and clinical trial registration number.<br><br>Page4 line 48: All participants were required to sign informed consent form to enter the study.<br><br>Page 5 line 13: Please elaborate your matching algorithm. Was cases and controls matched individually? What is the range for age match? How long did the matched controls enroll to the study after the cases were identified? Does the gap in time affect the measurements and what are the quality control procedures used to assure the experiment consistency?<br><br>Page 5 line 28: please see comment above for page 2 line 10.<br><br>Page 6 line 36-37: Please explain the data base used for the study. Were the data double entered? The analysis was done using SPSS 17.0. Please provide the software vender information. The sentence “The distributed data were used parametric statistical test if it agreed with normal distribution” was not clear. Which criteria was used to determine whether the variables follow normal distributions? If the method is “XX”, you can say that “XX test was used for all study variables to determine if they follow the normal distribution. For variables did not meet the criteria of normality, non-parametric (which) statistic tests were used for comparison between group differences.”<br><br>Page 6 line 47: Independent t-test was used incorrectly. Acupoints from the same patient are correlated and should use paired t-test to compare the affected and non-affected sides.<br><br>Page 6 line 57 to page 7 line 20: please revise the text as they are confusing. If there is literature to support the 25th percentile as a lower limit reference value for enhanced sensitivity, please provide references.<br><br>Page 7 line 40-42: The age means and standard deviations for cases and controls are different from those presented in Table 1.<br><br>Page 8 line 10: please provide percentage of females.<br><br>Page 8 line 15: If VAS did not follow normal distribution, you may want to present median and inter-quartile range.<br><br>Page 8 line 16: please add body mass index in Table 1.<br><br>Page 9 line 13: what were the differences between two sides for healthy participants? Were that measured? If so, can authors provide them in supplemental materials? |

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| | Page 10 line 18: delete “Then”<br><br>Page 10 line 23: Should the “ratio” be proportion?<br><br>Page 10 line 28-31: Wrong statistics were used to evaluate gender difference.<br><br>Page 25 line 40: correlation between Jianliao in PSI and CSI cannot be above 1. |

**VERSION 1 – AUTHOR RESPONSE**

2. Response to Reviewer 1

2.1 Inclusion criteria for Healthy participants is not so clear.

Response: We feel very sorry that we did not describe the inclusion criteria clearly in the manuscript. The healthy controls were matched to the patients with shoulder pain individually. Each healthy control was matched for gender, age (±1 year), ethnicity and dominant hand to one patient. Healthy controls were recruited from the community via posted flyers and general advertisements between May 2014 and September 2014. Healthy controls were eligible if they were not currently performing resistance exercise for the upper extremity. They were excluded based on the following criteria: receiving acupuncture or other analgesic therapies in the preceding month, experiencing neck or shoulder pain, having a history of shoulder surgery or neurological impairments of the upper extremity, a shoulder skin infection, having difficulty in understanding instructions, and taking any pain medication currently.

2.2 It seems difficult to reproduce the values of PPT in acupoint and non-acupoint. With respect to the non-acupoint only 2 cm to Tianzong, today we now the effect too close to acuponint, and possible stimulation.

Response: Thanks for your insightful suggestion. Firstly, the formation of acupoints is actually a creation of the ancient Chinese based on an ambiguous logical concept. And, the size range of an acupoint is also ambiguous. Therefore, it increases the difficulty to choose the non-acupoints. Secondly, in some studies including non-acupoints, we found that the chosen non-acupoints were close to acupoints in distance. For example, the distance was 1 cun (2-3 cm) between the non-acupoints and acupoints in a study and clinical outcome of showed acupoints treatment alleviated symptoms superior to non-acupoints treatment [1]. The measured sites are adjacent in some studies including PPT measured [2, 3]. Thirdly, according to the textbook, about 361 acupoints are founded in humankind. We found the smallest distance between two acupoints is 0.5 cun (Supplement Table 1), and especially 1 cun is common for the distance between two acupoints (Supplement Table 2). In our study, the distance is 2 cm between the non-acupoint and Tianzong (SI 11), which is acceptable. For reducing the stimulation effect, there is an approximately 2 min interval between the repetitions. The probability of a possible stimulation by too close to acupoint is low but it could not be ruled out completely. We have added this lack in the section of Limitation.

Literature cited

1. Liu Z, Yan S, Wu J, et al. Acupuncture for Chronic Severe Functional Constipation: A Randomized Trial. Annals of Internal Medicine. 2016 12:6;165(11):761-769.

2. Hong-You Ge a, Ce ´sar Ferna´ndez-de-las-Pen ˜as b, Pascal Madeleine, et al. Topographical mapping and mechanical pain sensitivity of myofascial trigger points in the infraspinatus muscle. European Journal of Pain. 2008 12: 859–865.

3. Binderup AT, Arendt-Nielsen L, Madeleine P. Pressure pain threshold mapping of the trapezius muscle reveals heterogeneity in the distribution of muscular hyperalgesia after eccentric exercise. European Journal of Pain. 2010;14(7):705-12.

Supplement Table 1. The list of acupoints from another acupoints with the distance of 0.5 cun.
Lingdao HT 4 - Tongli HT 5
Qihai CV 6 - Yingjiao CV 7
Tongli HT 5 - Yinxi HT 6
Lieque LU 7 - Jingqu LU 8
Yinxi HT 6 - Shenmen HT 7
Shangxing GV 23 - Shenting GV 24
Houding GV 19 - Baihui GV 20

Supplement Table 2. The list of acupoints from another acupoints with the distance of 1 cun.
Jingqu LU 8 - Taiyuan LU 9
Juque CV 14 - Zhongting CV 16
Zhongfu LU - 1 Yunmen LU 2
Xialian LI 8 - Shanglian,LI 9
Tianfu LU 3 - Xiabai LU 4
Shanglian,LI 9 - Shousanli LI 10
Burong ST 19 - Chenman, ST 20
Henggu KI 11 - Dahe KI 12
Chenman, ST 20 - Liangmen ST 21
Dahe KI 12 - Qixue KI 13
Liangmen ST 21 - Guanmen ST 22
Qixue KI 13 - Siman KI 14
Guanmen ST 22 - Taiyi ST 23
Siman KI 14 - Zhongzhu KI 15
Taiyi ST 23 - Huairoumen ST 24
Zhongzhu KI 15 - Huangshu KI 16
Huairoumen ST 24 - Tianshu ST 25
Shiguan KI 18 - Yindu KI 19
Tianshu ST 25 - Wailing ST 26
Yindu KI 19 - Futonggu KI 20
Wailing ST 26 - Daju ST 27
Futonggu KI 20 - Youmen KI 21
Daju ST 27 - Shuidao ST 28
Jianshi PC 5 - Neiguan PC 6
Shuidao ST 28 - Guilai ST 29
Waiguan TE 5 - Zhigou TE 6
Guilai ST 29 - Qichong ST 30
Zhigou TE 6 - Sanyangluo TE 8
Yinshi ST 33 - Liangqiu ST 34
Tianjing TE 10 - Qinglengyuan TE 11
Muchuang GB 16 - Zhengying GB 17
Xinghui GV 22 - Shangxing GV 23
Guangming GB 37 - Yangfu GB 38
Zhongji CV 3 - Guanyuan CV 4
Yangfu GB 38 - Xuanzhong GB 39
Guanyuan CV 4 - Shimen CV 5
Xiguan LR 7 - Yanglingqun SP 9
Yingjiao CV 7 - Shenque CV 8
Zuwuli LR 10 - Yinlian LR 11
Shenque CV 8 - Shuifen CV 9

Shuifen CV 9 - Xiawan CV 10
Jianli CV 11 - Zhongwan CV 12
Xiawan CV 10 - Jianli CV 11
Zhongwan CV 12 - Shangwan CV 13
Shangwan CV 13 - Juque CV 14

3. Response to Reviewer 2
Major concerns
3.1 Interpretation of central sensitisation: The authors considered a lower PPT on the pain-free side of the shoulder reflecting a status of central sensitisation. This could be an approach. However the common indicator of central sensitisation is reduced PPTs at the site distal or remote to the pain site. For instance reduced PPTs on the leg in patients with shoulder pain (Paul et al 2012). This needs to be addressed in the discussion.
Response: We thank to the reviewer for the constructive suggestion. Central sensitization (CS) is a central process of the nervous system associated with an enhanced responsiveness of the central neurons. It reflects increased activity of pain facilitation pathways and malfunctioning of descending pain inhibitory pathways [1-5]. Nevertheless, CS is challenging clinically, since no standard assessment exists6. Some studies recommended the use of various modalities for pain sensitivity at local and distal locations [2, 7]. However, other researchers showed that decreased PPTs at the painful and non-painful shoulder, but not at the muscle tibialis anterior [8-10]. According to “Criteria for the Classification of Central Sensitization Pain”, patients with diffuse pain distribution, allodynia, and hyperalgesia are more likely to present with CS. One of the patterns of pain distribution is that patients have bilateral pain/mirror pain [11]. In the patients with shoulder pain, the increased sensitivity to mechanical input in the contralateral shoulder would be interpreted as central sensitization [12]. A large number of studies define central sensitization as pain sensitivity at local and distal locations [13, 14]. We chose bilateral pain to define central sensitization, unlike those studies, and to determine whether there existed patterns of experimental pain responses at shoulder acupoints. We have described the central sensitization afresh, and addressed it in the discussion.

Literature cited
1. Janicki T. Chronic pelvic pain as a form of complex regional pain syndrome. Clinical obstetrics and gynecology. 2003; 46:797-803.
2. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. Manipulation Association of Chartered Physiotherapists. 2010; 15:135-141.
3. Meyer R, Campbell, JN, Raja, SN. Peripheral neural mechanisms of nociception. In: Wall P, Melzack, R (eds). Textbook of Pain. 3rd Ed. Churchill Livingstone, Edinburgh, 1995, pp 13-44.
4. Staud R, Craggs JG, Robinson ME, et al. Brain activity related to temporal summation of C-fiber evoked pain. Pain. 2007; 129:130-142.
5. Meeus M, Nijs J. Central sensitization: A biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. Clinical rheumatology. 2007; 26:465-473.
6. Meeus M, Nijs J, Van de Wauwer N, et al. Diffuse noxious inhibitory control is delayed in chronic fatigue syndrome: An experimental study. Pain. 2008; 139:439-448.
7. Paul TM, Soo Hoo J, Chae J, et al. Central hypersensitivity in patients with subacromial impingement syndrome. Arch Phys Med Rehabil. 2012 Dec;93(12):2206-9.
8. Ge HY, Fernández-de-las-Peñas C, Arendt-Nielsen L. Sympathetic facilitation of hyperalgesia evoked from myofascial tender and trigger points in patients with unilateral shoulder pain. Clinical neurophysiology. 2006;117:1545–1550.
9. Alburquerque-Sendín F, Camargo PR, Vieira A, et al. Bilateral myofascial trigger points and pressure pain thresholds in the shoulder muscles in patients. The Clinical journal of pain. 2013;29(6):478-86.
10. Gwilym SE, Oag HC, Tracey I, et al. Evidence that central sensitisation is present in patients with

shoulder impingement syndrome and influences the outcome after surgery. The Journal of bone and joint surgery. British volume. 2011;93(4):498-502.
11. Nijs J, Torres-Cueco R, van Wilgen CP, et al. Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain. Pain Physician. 2014;17(5):447-57.
12. Borstad J, Woeste C. The role of sensitization in musculoskeletal shoulder pain. Brazilian journal of physical therapy. 2015;19(4):251-7.
13. Hidalgo-Lozano A, Fernández-de-las-Peñas C, Alonso-Blanco C, et al. Muscle trigger points and pressure pain hyperalgesia in the shoulder muscles in patients with unilateral shoulder impingement: a blinded, controlled study. Experimental brain research. 2010;202(4):915-25.
14. Coronado RA1, Kindler LL, Valencia C, et al. Thermal and pressure pain sensitivity in patients with unilateral shoulder pain: comparison of involved and uninvolved sides. The Journal of orthopaedic and sports physical therapy. 2011;41(3):165-73.

3.2 Acupoints specific: The evidence supporting this conclusion was from a) PPTs measured at acupoints reduced; 2) PPT on one non-acupoint did not reduce. These data are not sufficient. Ideally to ensure sensitisation is acupoint specific, one has to test more than one acupoints. Furthermore, Ashi points are common in shoulder pain and are commonly used in acupuncture treatment. How will Ashi points be defined in this instance?
Response: We thank to the reviewer for the constructive suggestion. We measured five sites in this study including four acupoints and one non-acupoint. We should test more acupoints and non-acupoints to ensure acupoints specific of sensitization in subsequent studies.
We have added this lack in the section of Limitation that no Ashi points were measured. Ashi points also named reflexing points or tender spots. They are the phenomenon acupoints or temporary acupoints, which are dissimilar from acupoints of the fourteen meridians or extraordinary points. Generally, Ashi points have no specific names and definite locations, and will vanish after disease recovered. The aim of our study is to investigate the pattern of experimental pain responses at acupoints, which have specific names and definite locations. For that reason, the Ashi points do not take into account in this study. Further work about whether Ashi points exist central sensitization are needed and should be done in the future.

3.3 Please explain why the two sensitisation indexes were calculated in this particular manner. Please provide reference to support this decision.
Response: Thanks very much for your valuable opinion. We have corrected it. According to another reviewer's suggestion, we have deleted the part of calculated z-score and used the 25th percentile as the cutoff value. Because, the percentiles and Pearson's correlation coefficients were not affected by z-score transformation. The decision why choosing the 25th percentile as the cutoff value is on the basis of reference [1]. The study defined the 25th percentile as the lower limit reference value for enhanced sensitivity and the 75th, 90th and 95th percentiles as reference values for pain hyposensitivity.

Literature cited
1. Neziri AY, Scaramozzino P, Andersen OK, et al. Reference values of mechanical and thermal pain tests in a pain-free population. European journal of pain. 2011;15(4):376-83.

3.4 A large part of the Discussion was dedicated to explain the sensitisation status of acupoints. I expect to see some discussion on the internal validity. The blinding of the participants of the purpose of the study; the knowledge of acupoints of the assessor and its impact on the assessment; the reliability of testing PPT over a soft area such SI9; the selection of non-acupoints….
Response: We thank to the reviewer for the constructive suggestions. We have added it in the section of Discussion.
To our knowledge, this is the first study to research peripheral and central sensitization at acupoints in

patients with shoulder pain. One of the advantages of the study is that the measured acupoints and non-acupoint were marked by an acupuncturist with 24 years of experience in clinical acupuncture treatment. The evaluator who measured PPTs also has extensive experience with using the algometer and without basic knowledge of acupoints. The internal validity is increased by blinding the evaluator who did not know whether the measured sites were acupoints or not during testing. In addition, the evaluator was blinded as to whether the test participant was a patient with shoulder pain or a healthy control. The participants were asked to take different positions when different acupoints were measured. For example, the participants were required take a prone position on the examination bed with a suitable pillow under the chest and the arms close to the body when Jianzhen (SI 9) was measured. It increases the reliability of testing PPT over a soft area.
The non-acupoint was chosen as 2 cm down from Tianzong (SI 11) because the shoulder blade is relatively flat and may reduce the measurement errors between acupoints and non-acupoints. In the clinical trial published recently, the distance the distance was 1 cun (2-3 cm) between the non-acupoints and acupoints, and clinical outcome showed that acupoints treatment alleviated symptoms superior to non-acupoints treatment [1]. The measured sites are adjacent in some studies including PPT measured [2, 3]. The distance is 2 cm between the non-acupoint and Tianzong (SI 11), which is acceptable in our study. For reducing the stimulation effect, there is an approximately 2 min interval between the repetitions. The probability of a possible stimulation by too close to acuponint is low but it could not be ruled out completely.

Literature cited
1. Liu Z, Yan S, Wu J, et al. Acupuncture for Chronic Severe Functional Constipation: A Randomized Trial. Annals of Internal Medicine. 2016 12:6;165(11):761-769.
2. Hong-You Ge a, Ce ´sar Ferna´ndez-de-las-Pen ˜as b, Pascal Madeleine, et al. Topographical mapping and mechanical pain sensitivity of myofascial trigger points in the infraspinatus muscle. European Journal of Pain. 2008 12: 859–865.
3. Binderup AT, Arendt-Nielsen L, Madeleine P. Pressure pain threshold mapping of the trapezius muscle reveals heterogeneity in the distribution of muscular hyperalgesia after eccentric exercise. European Journal of Pain. 2010;14(7):705-12.

Minor comments:
• Why BDI cut off points was 4 in this study? The common cut off point is 9.
Response: The Beck Depression Inventory is a 21-item self-administered scale that measuring various symptoms of depression. The norms of Beck Depression Inventory for Chinese were established by the Chinese Scale Cooperative Group [1]. The four point scale indicates the degree of severity, which is follows: 0-4: no depression, 5-7：minimal depression, 8-15: moderate depression, >15: severity depression. We have added reference in the manuscript.

Literature cited
1. Zheng HB, Zheng YP. [The application of Beck Depression Inventory in depression patients]. Chinese Journal of Nervous and Mental Diseases. 1987;13(4):236

• Page 4, line 13: please cite the previous study
Response: We are very sorry that we made a mistake here. The number register of the previous study has been added in the corresponding position.

• Page 4, line 23, lower PPT is hyperalgesia, which could be explained by peripheral central sensitisation or central sensitisation. Lower PPT itself is not peripheral sensitisation.
Response: We are very grateful for your constructive suggestions. Pressure Pain Threshold (PPT) is an estimation of mechanical pain sensitivity. The PPT is the minimal amount of pressure where a sensation of pressure first changes to pain [1]. A lower PPT is indicative of decreased nociceptive thresholds to pain [2]. It could be explained by peripheral or central sensitization.

Literature cited

1. Vanderweeën L, Oostendorp RA, Vaes P, et al. Pressure algometry in manual therapy. Manual therapy. 1996;1:258–265.
2. Borstad J, Woeste C. The role of sensitization in musculoskeletal shoulder pain. Brazilian journal of physical therapy. 2015;19(4):251-7.

• Page 4, line 25, central sensitization needs to be explained here

Response: Central sensitization refers to an amplification of neural signaling within the central nervous system that elicits pain hypersensitivity. It reflects increased activity of pain facilitation pathways and malfunctioning of descending pain inhibitory pathways [1-5]. The explanation of central sensitization has been added in the section of Introduction clearly.

Literature cited

1. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011 March ; 152(3 Suppl): S2–15.
2. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. Manipulation Association of Chartered Physiotherapists. 2010; 15:135-141.
3. Meyer R, Campbell, JN, Raja, SN. Peripheral neural mechanisms of nociception. In: Wall P, Melzack, R (eds). Textbook of Pain. 3rd Ed. Churchill Livingstone, Edinburgh, 1995, pp 13-44.
4. Staud R, Craggs JG, Robinson ME, et al. Brain activity related to temporal summation of C-fiber evoked pain. Pain. 2007; 129:130-142.
5. Meeus M, Nijs J. Central sensitization: A biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. Clinical rheumatology. 2007; 26:465-473.

• Page 4: the aims need to be better articulated

Response: We feel very sorry that we did not describe clearly in the manuscript. We have revised the Introduction section. We hypothesized that peripheral sensitization or central sensitization at acupoints would be present in the patients with unilateral shoulder pain as evidenced by PPTs detection, when compared with healthy controls.

• Page 4, line 42, add Ethics approval number

Response: We are so sorry that we made a mistake here. We have added it in the manuscript.

• Page 4, line 54, it is difficult to imagine the types of shoulder pain that do not have areas overlapping the four acupoints. Examples here will be very helpful.

Response: We have deleted it in the manuscript.

• Page 5, line 1: were those with pain in the thoracic area excluded?

Response: Patients with pain in the thoracic area will be excluded. We have added thoracic area pain in the part of exclusion criteria.

• Page 7, line 46, it should be “paired t-tests”;

Response: We thank to the reviewer for the constructive suggestion. We have revised it in the corresponding part. A paired t-test was used when comparing the experimental pain responses between the painful side and non-painful side of the patients with shoulder pain. And, the results have been changed in the Table 2.

• Page 7, line 51, this should be “independent t-tests”.

Response: We are so sorry that we made a mistake here. We used paired t-test to compare the PPTs between the non-painful side of patients and the ipsilateral side of healthy controls for the reason that the study used a matched-pair design. Each healthy control was matched for gender, age (±1 year),

ethnicity and dominant hand to one patient. And, the PPT values in patients and healthy controls have high correlations (Supplement Table 3). Besides, another reviewer has similar suggestion. He thought that paired t-test was used to compare the PPTs of non-painful side from patients with the ipsilateral side of healthy controls, which was appropriate if healthy controls were selected in a matched pair with patients.

Supplement Tale 3. Correlations between the non-painful side of the shoulder patients and the ipsilateral side of health controls.

| Sits | Pearson's correlation |
|---|---|
| Tianzong (SI 11) | 0.85 |
| Jianliao (SJ 14) | 0.87 |
| Jianyu (LI 15) | 0.55 |
| Jianzhen (SI 9) | 0.79 |
| Non-acupoints | 0.86 |

• Page 10, line 30, Is Pearson r adequate for examining association between binary data (sex) and continuous data?

Response: We are so sorry that we made a mistake here. We have corrected it in the section of Method and Result. According to another reviewer's suggestion, either t-test or non-parametric Wilcoxon test should be used to compare PPTs difference between two genders. However, t-test or non-parametric Wilcoxon test was not performed due to the small sample after dividing groups on the basis of sex. Hence, we deleted the part of analysis for association between PSI or CSI and sex.

4. Response to Reviewer 3

4.1 Study Design.

4.1.1 The study design is not "a purely observational" study. It appears to be a prospective cross-sectional case-control observational study.

Response: Thanks very much for your valuable opinion. This work is quite a cross-sectional matched case-control study. The modification is shown in the Abstract, and the study design is added in the section of Materials and Methods.

4.1.2 It was not clear how patients were screened and enrolled. Who were the first 164 patients that were used as the pool for the selection of the final 30 clinical patients enrolled to the study? Were they consecutive patients seen in the clinics? How many of the 164 clinical patients satisfied study enrollment criteria and how the final patients were selected? Were the controls matched to cases individually or in frequency? It is still necessary to include a consort flow chart.

Response: We feel very sorry that the study design was not described clearly in the original manuscript. The 30 patients were chosen from a multicenter, randomized trial. In total, 164 patients were recruited from three centers in the random trial. During the study, we found the phenomenon that the pain threshold decreased at related acupoints in patients with unilateral shoulder pain was widespread. Hence, we enrolled the final 30 patients from 76 patients that screened in the center of Beijing Hospital of Traditional Chinese Medicine to investigate this phenomenon. This cross-sectional observational study performed before any treatments were dispensed. The healthy controls were matched to the patients individually. We have revised the contents of this part in the revised manuscript, and draw a flow chart as follows. This study is the part in the grey rectangle in Supplement Figure 1.

Supplement Figure 1. Flow chart.

4.1.3 It appears that the paper has authors from multiple institutions, yet the study seems to be conducted in a single institution. It will be helpful to clarify the contribution of co-authors.

Response: Master Li-Wen Zhang is the postgraduate from Shandong University of Traditional Chinese Medicine. Now, she is studying in the Beijing Hospital of Traditional Chinese Medicine until

graduation. She is a student of the professor Cun-Zhi Liu. The study is conducted in a single institution, even though authors come from multiple institutions. And, we have removed the institution of Shandong University of Traditional Chinese Medicine in the manuscript.

4.2 Statistical Analysis.
4.2.1 Comparisons of experimental pain responses between the painful side and non-painful side of clinical patients should use paired t-test because they came from the same patients and should be correlated. It only improves the statistical significance. The study team used paired t-test to compare the PPTs of non-painful side from patients with the ipsilateral side of the healthy participantss. If controls were selected in a matched pair with cases, this is appropriate. Please explain in the design stage to show how the cases and controls were matched.
Response: Thanks for your constructive suggestions. We have made the change in the corresponding part of the Data Analysis section. A paired t-test was used when comparing the experimental pain responses between the painful side and non-painful side of patients. We used paired t-test to compare the PPTs between non-painful side of patients and the ipsilateral side of the healthy controls for the reason that the study used a matched-pair design. Each healthy control was matched for gender, age (±1 year), ethnicity and dominant hand to one patient. And, the PPT values in patients and healthy controls have high correlations (Supplement Table 3). The detail is shown in the Method section.
Supplement Tale.1 Correlations between the non-painful side of patients with shoulder pain and the ipsilateral side of health controls.
Sits Pearson's correlation
Tianzong (SI 11) 0.85
Jianliao (SJ 14) 0.87
Jianyu (LI 15) 0.55
Jianzhen (SI 9) 0.79
Non-acupoints 0.86

4.2.2 The analysis plan suggested that non-parametric statistical tests were used for data that failed test for normal distributions. It was not clear which variables failed the normality assumption. Since all patients should have their VAS above 50, the distribution of VAS may not normally distributed and should not be summarized by mean and standard deviations as shown in Table 1.
Response: We thank to the reviewer for the insightful suggestions. The variable BDI score is not agreed with normal distribution. To compare the BDI scores between the two groups, Wilcoxon's signed rank test was used. We have added it in Table 1. Indeed, the distribution of VAS is not normally distributed and we have represented it as median and interquartile range in Table 1.

4.2.3 Sex is a binary variable. The differences in PPTs by gender should not be evaluated by Pearson's correlation coefficient. Either t-test or non-parametric Wilcoxon test should be used to compare PPTs difference between two genders.
Response: We are so sorry that we made a mistake here. We have corrected it in the section of Method and Result. According to reviewer's suggestion, either t-test or non-parametric Wilcoxon test should be used to compare PPTs difference between two genders. However, t-test or non-parametric Wilcoxon test was not performed due to the small sample after dividing groups on the basis of sex. Hence, we deleted the part of analysis for association between PSI or CSI and sex.

4.2.4 PPTs of cases were normalized using a Z-scores according to the mean and standard deviations of controls. Peripheral sensitization for the painful side of clinical patients based on the 25th percentiles of z-score of the non-painful side of clinical patients, assuming matching the same acupoints. The central sensitization was based on the 25th percentile of the ipsilateral side of the controls. Actually there seems no need for the z-score transformation because the percentiles should not be affected by normalization transform. Also, Pearson's correlation coefficients were not affected

by z-score transformation.
It will be helpful to present the corresponding cutoff values for all acupoints.
What was the justification to use 25%tiles as the cutoff value? Does that mean there should be 25% PPTs below the cutoff value in the reference group. In this case, the 95% confidence interval of proportion of patients who were sensitized should be between 10% to 40% by chance alone. The authors should talk to statisticians to evaluate the significance of the observed sensitization rates.
Response: We thank to the reviewer for the constructive suggestions. We assumed that the percentiles and Pearson's correlation coefficients were not affected by z-score transformation after reanalysis. There is no need for the z-score transformation. We have corrected it in the section of Data Analysis. The justification to use 25th percentile as the cutoff value is on the basis of reference [1]. The study defined the 25th percentile as a lower limit reference value for enhanced sensitivity and the 75th, 90th and 95th percentiles as reference values for pain hyposensitivity.

Literature cited
1. Neziri AY, Scaramozzino P, Andersen OK, et al. Reference values of mechanical and thermal pain tests in a pain-free population. European journal of pain. 2011;15(4):376-83.

4.2.5 There are multiple comparisons for acupoints in the paper. There was no discussions about multiple comparisons. The analysis could be improved by performing multivariate statistical analysis to simultaneously consider PPTs from all acupoints together to reduce the burden of multiple comparisons.
Response: We thank to the reviewer for the constructive suggestions. There are multiple comparisons for acupoints in the manuscript. To adjust for multiple comparisons, an alpha level of 0.025 was used for all pairwise comparisons according to bonferroni's correction.

4.3 English
3.1 The paper can benefit from the help of an English editor. Several areas are confusing and some are heard to understand. Professional editor help is needed throughout the manuscript.
Response: The manuscript has been improved by Marc FISHER, a senior lecturer of neurology at Harvard Medical School.

Specific Comments
Page 2 line 10: A prospective (? Please confirm by the author) cross-sectional matched case-control observational study.
Response: We thank to the reviewer for the suggestion. The mistake has been corrected.

Page 2 line 27: Please clarify if the controls are patients or healthy participants. They have been characterized in consistently throughout the paper.
Response: We feel sorry for the unclear description in the manuscript and have revised that part.

Page 2 line 50: delete "obvious"
Page 2 line 51: delete "n"
Response: We thank to the reviewer for the suggestions and have deleted that section of Abstract.

Page 3 line 6-8: please justify why this study of shoulder pain supports the generalized conclusion of musculoskeletal pain.
Response: We feel so sorry that we made a mistake here. We forget to write the word of "shoulder" in front of musculoskeletal pain and it has already been added.

Page 3 line 35: Please provide reference of WHO report.
Response: The reference has been added in the manuscript.

Page 4 line 11: please provide your publication and clinical trial registration number.
Response: We have added the clinical trial registration number.

Page4 line 48: All participants were required to sign informed consent form to enter the study.
Response: We are so sorry that we made a mistake here. We have mended it.

Page 5 line 13: Please elaborate your matching algorithm. Was cases and controls matched individually? What is the range for age match? How long did the matched controls enroll to the study after the cases were identified? Does the gap in time affect the measurements and what are the quality control procedures used to assure the experiment consistency?
Response: Thank you for your constructive suggestion. The healthy controls were matched to the patients with shoulder pain individually at the ratio of 1:1. Each healthy control was matched for gender, age (±1 year), ethnicity and dominant hand to one patient. We enrolled the final 30 patients from 76 patients screened in the site of Beijing Hospital of Traditional Chinese Medicine between January 2014 and September 2014. The matched healthy controls were enrolled between May 2014 and September 2014. Healthy controls were recruited from the community via posted flyers and general advertisements. Thanks for your suggestion again and we will pay attention to this detail in the future work.

Page 5 line 28: please see comment above for page 2 line 10.
Response: We feel so sorry that we made a mistake here. We have corrected it and the study design is added in the Materials and Methods section.

Page 6 line 36-37: Please explain the data base used for the study. Were the data double entered? The analysis was done using SPSS 17.0. Please provide the software vender information. The sentence “The distributed data were used parametric statistical test if it agreed with normal distribution” was not clear. Which criteria was used to determine whether the variables follow normal distributions? If the method is “XX”, you can say that “XX test was used for all study variables to determine if they follow the normal distribution. For variables did not meet the criteria of normality, non-parametric (which) statistic tests were used for comparison between group differences.”
Response: We are very grateful for your constructive suggestions. The data were double entered with an adequate check in EpiData. SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Shapiro-Wilk test and observation of histograms and normal probability plots were used for all study variables to determine whether they followed a normal distribution.

Page 6 line 47: Independent t-test was used incorrectly. Acupoints from the same patient are correlated and should use paired t-test to compare the affected and non-affected sides.
Response: We thank reviewer for the suggestions. We have used paired t-test to the painful and non-painful sides in the patients with shoulder pain.

Page 6 line 57 to page 7 line 20: please revise the text as they are confusing. If there is literature to support the 25th percentile as a lower limit reference value for enhanced sensitivity, please provide references.
Response: We feel so sorry that we made a mistake here. The reference has been added in the appropriate place.

Page 7 line 40-42: The age means and standard deviations for cases and controls are different from those presented in Table 1.
Response: We thank to the reviewer for the suggestion. The reason for the difference is that the range for age match has one year discrepancy. The patients and healthy controls matched very well, except for one healthy control with one-year older compared with the patient with shoulder pain.

Page 8 line 10: please provide percentage of females.
Page 8 line 15: If VAS did not follow normal distribution, you may want to present median and inter-quartile range.
Page 8 line 16: please add body mass index in Table 1.
Response: Thank the reviewer for the insightful suggestions. The percentage of females and the body mass index have been added in Table 1. The modification of median and inter-quartile range has been changed in Table 1.

Page 9 line 13: what were the differences between two sides for healthy participants? Were that measured? If so, can authors provide them in supplemental materials?
Response: We thank to the reviewer for the constructive suggestion. The data of two sides for healthy controls have been provided in the Supplemental Table 2. No distinct differences of PPT values were found between the two sides in healthy controls ($P > 0.05$).
Supplemental Table 4. Pressure pain threshold values in both sides for health controls.

| Sites | Right | Left | Mean (95% CI) | P value |
|---|---|---|---|---|
| Tianzong (SI 11) | 592.00 ± 154.06 | 604.10 ± 159.42 | 12.10 (-19.65, 43.85) | 0.44 |
| Jianliao (SJ 14) | 665.69 ± 208.37 | 652.13 ± 204.86 | 13.56 (-25.01, 52.12) | 0.48 |
| Jianyu (LI 15) | 591.73 ± 176.90 | 609.81 ± 186.97 | 18.08 (-0.77, 36.93) | 0.06 |
| Jianzhen (SI 9) | 620.08 ± 210.50 | 631.20 ± 208.47 | 11.12 (-0.80, 23.04) | 0.07 |
| Non-acupoint | 560.69 ± 141.41 | 575.23 ± 151.37 | 14.53 (-1.11, 30.18) | 0.07 |

Page 10 line 18: delete “Then”
Page 10 line 23: Should the “ratio” be proportion?
Response: We are so sorry that we made a mistake here. The “Then” word has deleted. The ratio has been replaced by proportion.

Page 10 line 28-31: Wrong statistics were used to evaluate gender difference.
Response: We are so sorry that we made a mistake here. We have corrected it in the part of Method and Result.

Page 25 line 40: correlation between Jianliao in PSI and CSI cannot be above 1.
Response: We are so sorry that we made a mistake here. This is an error, and the number “3.6” should be “0.36”. We have revised it in the Appendix Table 6.

## VERSION 2 – REVIEW

| | |
|---|---|
| **REVIEWER** | JUAN CARLOS RUEDA<br>SABIC<br>SPAIN |
| **REVIEW RETURNED** | 30-Jan-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | I consider that the points of review indicated have been solved in this new revision. |

| | |
|---|---|
| **REVIEWER** | Zhen Zheng<br>RMIT University |
| **REVIEW RETURNED** | 16-Feb-2017 |

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|---|---|
| **GENERAL COMMENTS** | The authors have addressed the questions satisfactorily. |

| | |
|---|---|
| | A couple of more points:<br>1. Please check appendix table 4. The content and what mentioned in the text are not consistent.<br>2. Discussion: Page 62 line 13: “peripheral sensitization was not a prerequisite for the presence of central sensitization at…” This statement needs to be clarified. |

| | |
|---|---|
| **REVIEWER** | Ying Lu<br>Stanford University, USA |
| **REVIEW RETURNED** | 14-Feb-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | Thanks for the revision and carefully addressed most of the comments. The revision has substantially improved the manuscript.<br><br>My main concern is about the PSI and CSI, which are critical for the paper.<br><br>I still not clear about how the PSI and CSI were generated. It may be helpful to add an supplementary description of their calculations.<br><br>Lines 30 and 37 on page 55, should PSI and CSI be the proportions or the ratios? Ratio means two values. If the ratio is based on proportion, such as 77%/25%=3.08, for example, one will need to describe Table 3 in ratios instead of percentage. But in your reports, you always use the number and percentage of patients fell below the 25%tiles.<br><br>By definition, the 25%tile of 30 patients is a value in between 7th and 8th lowest observations. How did you break the ties?<br><br>Table 3 showed the percentages of patients whose PSI and CSI that are below the corresponding 25%tiles of the reference values. Many of such differences did not reach statistical significance. One can use a Fisher’s exact test to compare whether the PSI and CSI proportion reached statistical significance levels. If you use a significance level of 0.05, you will need a sensitivity index above 54% to achieve the significance. If you use 0.025% as the significant level, you will need to a sensitivity index above 57%. Some of the observed sensitivity indices are well below these thresholds.<br><br>Specific comments:<br><br>Line 17 on page 55, (IPQ) should be IQR<br><br>Line 48 on page 55, did you use p-value of 0.025 instead of 0.05 as a significant level?<br><br>Line 3 on page 56, the mean age for control group was 50.60 while the mean in Table 1 (line 12) was 50.63. Please make them consistent.<br><br>Page 60 line 7, please clarify if the Peripheral Sensitization (column 2) means sensitization at peripheral only but not central, and the Central Sensitization (column 3) means sensitization at central only but not peripheral acupoints? Please clarify this as the table gave the impression that each column represents one cell in the 2x2 table of sensitization status. |

## VERSION 2 – AUTHOR RESPONSE

2. Response to Reviewer 2
2.1 Please check appendix table 4. The content and what mentioned in the text are not consistent.
Response: The number of “0.86” should be “0.086”. We have revised it as “0.09” (depicted as two decimal fractions) in Appendix Table 4. And, the text was revised as below:
A distinct and significant association (Appendix Table 4) was observed between Jianliao (SJ 14) and Jianyu (LI 15) ($p < 0.01$), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$) in CSI.

2.2 Discussion: Page 62 line 13: “peripheral sensitization was not a prerequisite for the presence of central sensitization at…” This statement needs to be clarified.
Response: The statement has been clarified afresh at the corresponding part as below:
We determined whether peripheral and central sensitization were more likely to occur together or alone. In Table 3, we describe that 19 patients had central sensitization and 13 patients had peripheral sensitization in Jianyu (LI 15). Previous study showed that long-term peripheral sensitization can lead central nervous system changes occurring and resulting in central sensitization [11]. However, there were 6 patients having only central sensitization without peripheral sensitization. This result indicates that peripheral sensitization is not a prerequisite for the presence of central sensitization at acupoints.

3. Response to Reviewer 3
3.1 I still not clear about how the PSI and CSI were generated. It may be helpful to add an supplementary description of their calculations.
Response: We thank to the reviewer for the constructive suggestion. The detailed presentation is listed below:
Peripheral Sensitization Index (PSI)
Peripheral sensitization is referred to a patient’s response for PPT on the painful side that fell below the 25th percentile when compared to the non-painful side [22]. The PPT value of the 25th percentile is determined by the average value of 7th and 8th lowest observations on the non-painful side. We examined a patient’s proportional response for PPT on the painful side fell below the 25th percentile. Each response PPT is considered for peripheral sensitization index (PSI). For example, the results show the PPT values on the painful side at Jianliao (SJ 14) of 11 patients are below the average value of 7th and 8th lowest observations on the non-painful side, which indicates 11 patients have peripheral sensitization at Jianliao (SJ 14).

Central Sensitization Index (CSI)
Central sensitization indicates that a patient’s response for PPT on the non-painful side fell below the 25th percentile (the average value of 7th and 8th lowest observations) as compared to the ipsilateral side of healthy controls. We computed the proportional responses for PPT on the non-painful side fell below the 25th percentile as compared to the ipsilateral side of healthy controls. Each response PPT is considered for central sensitization index (CSI). For instance, the PPT values of 17 patients on the non-painful side are below the average value of 7th and 8th lowest observations on the ipsilateral side of health control at Jianliao (SJ 14), which indicated 17 patients have central sensitization at Jianliao (SJ 14).

3.2 Lines 30 and 37 on page 55, should PSI and CSI be the proportions or the ratios? Ratio means two values. If the ratio is based on proportion, such as 77%/25%=3.08, for example, one will need to describe Table 3 in ratios instead of percentage. But in your reports, you always use the number and percentage of patients fell below the 25%tiles.
Response: Thanks for your valuable advice. The ratio has been replaced by proportional at the corresponding part.

3.3 By definition, the 25%tile of 30 patients is a value in between 7th and 8th lowest observations.

How did you break the ties?
Response: The 25%tile is determined by the average value of 7th and 8th lowest observations. The detailed description is shown at the answer in the question 3.1.

3.4 Table 3 showed the percentages of patients whose PSI and CSI that are below the corresponding 25%tiles of the reference values. Many of such differences did not reach statistical significance. One can use a Fisher's exact test to compare whether the PSI and CSI proportion reached statistical significance levels. If you use a significance level of 0.05, you will need a sensitivity index above 54% to achieve the significance. If you use 0.025% as the significant level, you will need to a sensitivity index above 57%. Some of the observed sensitivity indices are well below these thresholds.
Response: In Table 3, we just wanted to observe the percentages of patients with peripheral or central sensitization. Statistical significant differences should not be taken into account here. The results show that peripheral sensitization is not a prerequisite for the presence of central sensitization, which has been also reported by Nijs et al [1]. But a close correlation of PSI and CSI should not be ignored. Peripheral sensitization indicates that the expansion of nociception occurs in tissues innervated by the peripheral nervous system. With prolonged peripheral sensitization, central nervous system changes can also occur and result in central sensitization [2-3]. Therefore, whether the proportion difference of peripheral sensitization or central sensitization reached statistical significance levels was not be considered in this study.

Literature cited
1. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. Man Ther. 2010;15(2):135-41.
2. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011;152(Suppl 3):S2-15.
3. Borstad J, Woeste C. The role of sensitization in musculoskeletal shoulder pain. Brazilian journal of physical therapy. 2015;19(4):251-7.

3.5 Line 17 on page 55, (IPQ) should be IQR
Response: The revision is made it in the manuscript as below:
Distributed data were summarized using mean ± standard deviation (SD) or median and interquartile range (IQR).

3.6 Line 48 on page 55, did you use p-value of 0.025 instead of 0.05 as a significant level?
Response: We determine whether patients with shoulder pain demonstrated peripheral, central, a mixed-pattern or no sensitization, and analyzed the association between sensitization subgroups and the relevant baseline characteristics including demographic and clinical variables. Comparisons among the variables were examined by using one way analysis of variance or Chi-square. Multiple comparisons are not used in this section. A P value <0.05 was defined as statistically significant.

3.7 Line 3 on page 56, the mean age for control group was 50.60 while the mean in Table 1 (line 12) was 50.63. Please make them consistent.
Response: We have corrected it.

3.8 Page 60 line 7, please clarify if the Peripheral Sensitization (column 2) means sensitization at peripheral only but not central, and the Central Sensitization (column 3) means sensitization at central only but not peripheral acupoints? Please clarify this as the table gave the impression that each column represents one cell in the 2x2 table of sensitization status.
Response: Peripheral Sensitization (column 2) means that all patients had peripheral sensitization, regardless of whether the patient had central sensitization. Central Sensitization (column 3) means that all patients had central sensitization, no matter if the patients had peripheral sensitization or not.

Peripheral and Central Sensitization (column 4) means all patients had both central sensitization and peripheral sensitization, and if the patients just had central sensitization or peripheral sensitization they were not included in column 4.