Peer Review File

Article information: https://dx.doi.org/10.21037/lcm-21-48

Reviewer A

Comment 1 Quite a few of the same examples are repeat in the argument. Body of text can be streamlined. The scientific and literature rational is strong.

Seeing the thesis is that neurological explanation is the only mechanism that can explain the effect of acupuncture--from MRI to pain, to visceral, and even sham needle effects--it is advise that the present title be changed to more clearly express the thesis.

Current title: The neuroanatomic and neurophysiologic basis of acupuncture anatomy and physiology

Suggested title: Evidence support neurological mechanism as the sole basis for acupuncture anatomy and physiology.

Consider using stronger language to this effect in the title to emphasis that there can be no other mechanisms that can explain the therapeutic effect of acupuncture.

Reply 1 Thank you for these thoughtful comments- will change the title accordingly and will streamline the text as possible

Changes in text 1 title changed: Acupuncture's Neuroanatomic and Neurophysiologic Basis

Reviewer B

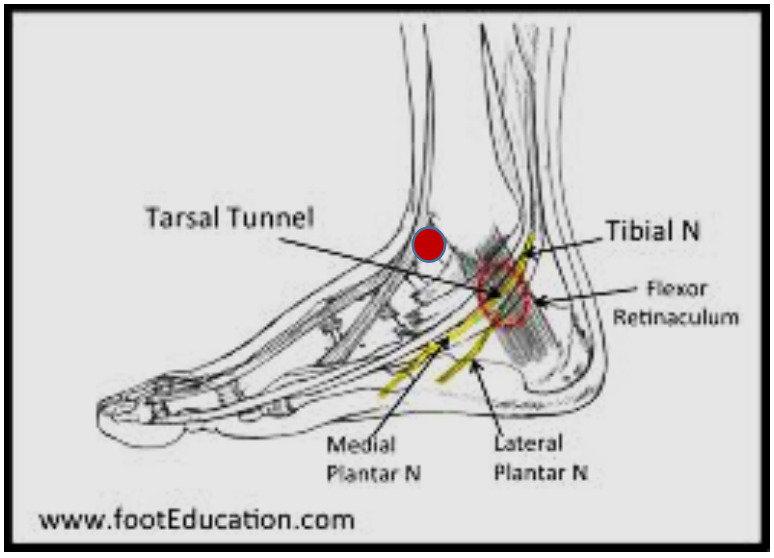
Comment 1 Lines 88-90

This is incorrect. The saphenous nerve is just 1 nerve along the course of the leg region when comparing it to the spleen meridian. Deep to this area also lies the tibial nerve in the tarsal tunnel. At the ankle is too generic. I suggest that the author make a specific distinction between the distal aspect of the spleen meridian and saphenous nerve. Also the saphenous does not correspond to the more proximal parts of the spleen meridian and can add some confusion.

Reply 1 The reviewer's anatomical assessment described above is not correct and does not fully reflect the manuscript's text and imaging.

Document lines 88-90 clearly indicate that just the distal Spleen Channel is being examined in this portion of the manuscript to compare to the lower leg and foot saphenous nerve distribution and to the Neijing description of the visibility of the Spleen Channel near the medial malleolus as per line 78-80, where the saphenous vein and its accompanying saphenous nerve is visible anteromedial to the medial malleolus as shown in Figure 1 anatomic illustration and on an actual leg. The tarsal tunnel is anatomically posterior and inferior to the medial malleolus per anatomic references, so no Spleen acupoint (including SP-5) will enter the tarsal tunnel anatomically- the recommended transverse insertion 0.3-0.5 cun per Deadman and O'Connor and Bensky acupuncture references will not enter the tarsal tunnel but may

impact the medial malleolus with deeper insertion (confirmed also Chen's cross-sectional acupoint anatomy atlas). The other recommended insertion direction (transverse insertion at SP-5 towards ST-41) by these acupuncture references similarly passes above and anterior to the anatomic location of the tarsal tunnel as illustrated below with SP-5 approximate location shown in red.



The Spleen Channel distribution mirrors the distribution of the saphenous nerve throughout the lower leg as shown in Figure 1 as well as Figure 4b from 3 different print and 3d digital anatomy resources. The saphenous nerve is the terminal sensory branch of the femoral nerve, and the femoral nerve distribution mirrors that of the Spleen Channel from the groin to the distal thigh as shown in Figure 4b and as discussed in the manuscript from line 223-233 in the pdf the reviewer is referencing- the multiple directional changes of the Spleen Channel in the lower extremity mirrors those of the femoral/saphenous nerves. I do not see that Figure 4b or lines 223-233 were considered in the review, which covered the more proximal portions of the leg as well as the entire thigh region as suggested by the review

Changes in text 1 Will change the wording in the 88-90 line region to specify Figure 1 is examining only the distal lower leg for clarity

Comment 2 Lines 169-181 and figures 2 & 3

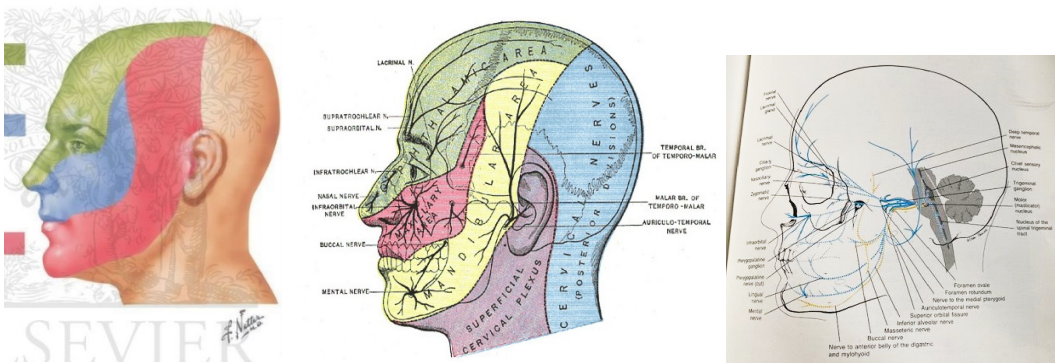
The description between acupoints, meridians, and anatomical structures are being superimposed and assumed. The only citation that is listed is in Chinese and 4 pages of description. There is an assumption as to how these points are located in relation to the anatomy, but there is not mention of anatomical variation. For example, the facial nerve has a wide degree of anatomical variation that can span more that one particular acupoint. This is also true for the infraorbital, supraorbital, and mental nerves. As an example of variation of structure, please see:

Martínez Pascual P, Maranillo E, Vázquez T, Simon de Blas C, Lasso JM, Sañudo JR. Extracranial Course of the Facial Nerve Revisited. Anat Rec (Hoboken). 2019 Apr;302(4):599-608. doi: 10.1002/ar.23825. Epub 2018 Apr 24. PMID: 29659175.

It would be very useful if the author cited examples of actual data regarding anatomical correlation and or variation of acupoints than just superimposing a generic anatomy atlases This has led to a lot of confusion in the field and a wide range of assumptions.

Reply 2 The purpose of the neuroanatomic correlation portion of this paper is to help clear the confusion in the field re acupoint anatomy. The comment that only one citation is listed and in Chinese is not accurate. The reference 39 in your pdf is "Cross-Sectional Anatomy of Acupoints" by Eachou Chen, which was developed in cooperation with the Shanghai College of Traditional Medicine, and provides dissection based cross-sectional anatomy data on 378 acupuncture points including all 361 Classical acupuncture points including the nerve branches those 378 acupoints influence- it is not in Chinese and am not sure what that "Chinese" reference is being referred to. I have added to that section data from the Shanghai College of Traditional Medicine text that on page 111 documents their study showing 323/324 acupoints studies were supplied by nerves both in terms of gross anatomy as well as under histologic examination- this reference also notes a "particularly close relationship between the paths of the channels on the limbs and the pathways of peripheral nerves", which is consistent with this manuscript's hypothesis and as seen in figures 1,2, and 4 of this manuscript. Those two references alone cover the demonstrated cadaveric cross-sectional anatomy of all Classical acupoints to peripheral nerves plus I have added Chiang's groups' (references 31, 48 and 53) and Anatomy for Acupuncture (43) references in applicable sections. These changes will include lines 137-150, 156-159, 167-168, and 198-201 in the revised manuscript.

None of the anatomic correlations are to the facial nerve but rather the trigeminal nerve. The variations described by the reference provided by the reviewer are specific to the facial nerve and are not generalizable to the trigeminal nerve distribution, as these nerves have drastically different anatomy. There is not the same degree of anatomic variation in distribution of the trigeminal nerve- the supraorbital nerve exits the supraorbital foramen, the infraorbital nerve the infraorbital foramen and mental nerve the mental foramen, as examples. All widely accepted anatomic atlases including Gray to Grant to Clemente to Netter show similar distributions of the trigeminal nerve and its branches. The following imaging documents this including Wilson-Pauwels cranial nerve anatomy reference. These are not "generic" references but authoritative anatomic references that are based in anatomy/dissections



Netter Gray's Anatomy Wilson-Pauwels: Cranial Nerves

Thus there is multiple lines of evidence anatomically from Shanghai College of Traditional Medicine studies, Eachou Chen's cross-sectional anatomy atlas, and the 36 head region acupoints studied by Meltz of the close anatomic relationship of Classical acupoints to neural structures. Any modest anatomical variations of any of the trigeminal nerve branches shown/correlated will not affect anatomic correlations noted in Figure 2. Even if there are anatomic variations, acupoint descriptions in reference texts are not rigid fixed measures, but rather guidelines to approximate locations of acupoints to account for anatomic variations (*Shanghai College of Traditional Medicine, 1981, p 124 "the purpose of proportional measurements is to facilitate the finding of the approximate locations of points over gross distances on the body, rather than a single absolute standard for making fine measurements")*

Further evidence that these acupoint to trigeminal nerve relationships are not just "assumed" is shown in Figure 2 that documents the anastomosis of the supratrochlear and greater occipital nerve consistent with the Bladder channel distribution in the head and neck from the orbit to the upper cervical spine. Similarly the Gallbladder channel distribution is in the supraorbital nerve distribution and the Stomach channel near the orbit in the infraorbital nerve distribution. The reviewer did not address/contest these apparent distribution overlaps.

Changes in text 2 Extensive, dissection based anatomic verification of acupoint-nerve relationships has been added as requested by the reviewer- discussion of all anatomic variations is not possible in the scope of this manuscript due to length and image number considerations, further it would not change these anatomic correlations of acupoints and nerves

Comment 3 Figure 187 -201

This section makes another broad assumption about anatomy and the location of Urinary Bladder acupoints.

I suggest the author look at: Umemoto K, Saito T, Naito M, Hayashi S, Yakura T, Steinke H, Nakano T. Anatomical relationship between BL23 and the posterior ramus of the L2 spinal nerve. Acupunct Med. 2016 Apr;34(2):95-100. doi: 10.1136/acupmed-2015-010847. Epub 2015 Oct 27. PMID: 26508662.

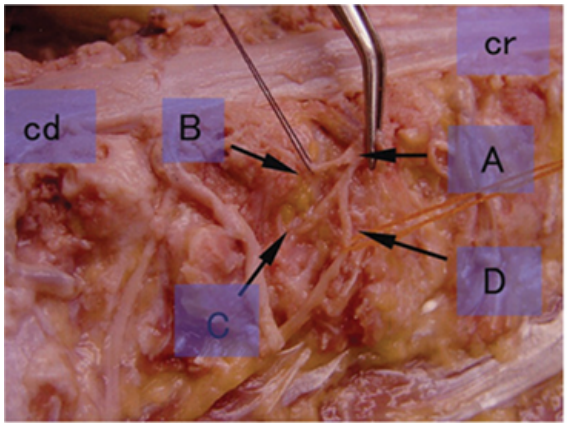
Generally, the author is making a number of assumptions on the anatomy without discussing the distribution of the nerves. Anecdotal statements about the correlation of acupoints with atlases are not enough. The author needs to look at the anatomical variation or at the very least discuss the anatomical variation that occurs. Anatomical variation is taken into account for different types of surgical procedures and screening methods. This should not be broadly ignored in a review such as this.

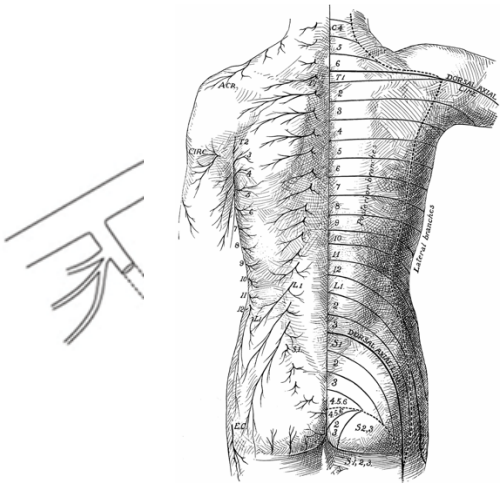
Digital 3D modeling has its limitation based on resolution where smaller structures can easily be missed. The limitation of these imaging techniques should be mentioned.

Reply 3 While anatomic variations are important for surgical procedures that may transect/injure nerves, acupuncture does not traumatize nerves or cause tissue damage in practice, of course.

Anatomic variations of nerves in terms of acupuncture practice as previously discussed are accounted for in acupoint localization techniques- acupuncture point localizations are not rigid measurements, only approximate locations to allow for these anatomic variations to be accounted for when localizing acupoints clinically

The distributions of the spinal nerves per Gray, Grant, Clemente, Netter and other authoritative anatomic resources do not show any large variation in the dermatomal/myotomal distribution of the spinal nerves in their ventral or dorsal branches. This is illustrated in Figures 3 and 5, as well Gray's anatomy below. The digital anatomic references further confirm these anatomic references' described spinal nerve anatomy, as does Chen's cross sectional anatomy atlas





From Saito Anesthesiology January 2013, Vol. 118, 88–94. Gray's anatomy B medial, c intermediate, d lateral branch dorsal ramus-note C+D come off common trunk lateral to medial branchof dorsal ramus

Further, prior work by Saito 2006 showed that "intermediate" and "lateral" branches of the dorsal ramus may come off same trunk, and Bogduk 1982 also demonstrated that sometimes there are only two branches of the dorsal ramus in the lumbar spine. These "anatomic variations" of the spinal nerves do not generalize to their overall segmental distributions however- the important point being shown in Figures 3 and 5 is that the Bladder Classical acupoints influence/stimulate *differing* branches of the dorsal ramus of the spinal nerves (along dermatomes/myotomes), whether it is the medial, intermediate, or lateral branch of the dorsal ramus stimulated. The reference supplied by the reviewer also documents the sympathetic autonomic innervation of those dorsal branches, which follows from the fact that 20% or more of spinal nerve are SANS fibers (reference 68)- this will have relevance in subsequent reviewer concerns re Back Shu points relationship to the segmental spinal autonomic innervation of the organs.

The manuscript shows the anatomic correlations of Classical acupoint locations to the various branches of the ventral and dorsal rami of the spinal nerves in Figure 3, whose dermatomal/myotomal distributions are similar for all the widely accepted anatomic atlases- these points are accurately placed and can be proofed/validated by the reader without difficulty

Changes in text 3 The manuscript was changed to add "intermediate" branch to the dorsal ramus discussion per the reviewer's reference. Discussion of all anatomic variations of every nerve is beyond the scope of this paper as is the accuracy of 3d digital anatomy resources, but will mention this issue in manuscript

Comment 4 Lines 244-248

This section should be expanded on. There is a lot more anatomical evidence for acupoints than is being portrayed in this review. I would even suggest moving this section before the general anatomy section in order to set up the general concepts of correlation and anatomical variation.

Reply 4 and changes in text 4 This was done as requested in the revised manuscript, as previously outline

Comment 5 Lines 324-322

If the author is going to make such a bold description in the correlation, there needs to be a citation with recent anatomical evidence with correlation. Bonica and Beal review the actual anatomy and do not provide any new data in the realm of acupoints. This is a far over reach in interpretation and association. The articles are also over thirty years old.

Reply 5 The reviewer requests "recent" anatomical data that simply does not exist, as the segmental autonomic innervation of the organs as outline years ago by the Bonica and Beal references are based not only in synthesis of multiple prior anatomic studies of autonomic innervation but also functional evidence through spinal nerve root, axial, and sympathetic block procedures. Any "recent" literature I have seen invariably refers to these references or anatomic studies by Gray, which are much older than 30 years old. That segmental autonomic anatomic innervation anatomy and physiology is uniformly accepted by researchers and to my knowledge has not been questioned in terms of accuracy/veracity in the past decades, and the anatomy and physiology of this in humans is not changed since those studies. As discussed previously, 20% or more of spinal nerve roots are composed of SANS fibers consistent with the sympathetic innervation of the dorsal rami branches discussed in the AIM reference provided by the reviewer

The Back Shu points, that directly affect organ function, likewise are well accepted by acupuncturists in terms of their anatomic spinal levels for insertion.

Thus it is unclear to me what "over-reach" exists in correlating these Back Shu point anatomic spinal levels to the known/accepted anatomic/physiologic spinal segmental sympathetic autonomic innervation to the various organs described by Bonica and Beal, with Bonica's article and text being the authoritative reference for anesthesiologists and pain management practitioners.

That 9/12 of the Back Shu points happen to influence organ function consistent with the accepted thoracolumbar SANS segmental autonomic innervation to that organ is a straightforward *correlation* that statistically is extremely unlikely to be by chance as

the manuscript documents- the Back Shu levels similarly fundamentally overlap with the spinal levels manual medicine practitioners manipulate to alter that same organ function again consistent with the known SANS segmental innervation of the organs. This is more clinical evidence that this is very unlikely due to chance- the Back Shu points, spinal manipulation levels of manual medicine, and SANS segmental organ innervation all likely are expressing their shared neural innervation

Changes in text 5 No changes. I feel the reader should have the opportunity to see this data and decide for themselves if they concur with these findings

Comment 6 Line 360. Since there are several subtopics associated with the physiology of acupoints, the term 'non-pain effects' is confusing. I would suggest stating what they actually are: neuro-metabolic or neuro-endocrine.

Reply 6 It seems more logical to keep categories broad, as there are so many non-pain effects of acupuncture from anti-emesis, to organ function change, allergy reduction, etc that it would create too many subcategories. The important point is that the neuroanatomic/neurophysiologic effect of acupoints affects more than just pain conditions and affects immune, endocrine, vascular, and all organ systems through one mechanism- neural stimulation

Changes in text 6 none

Comment 7 Figure 6 and proposed model. Although the proposed model may seem like a good model. The author neglects the vascular implications at various acupoint anatomy. The level of analgesia seen in some of the clinical studies on acupuncture has been associated with vascular beds at selected acupoints such as ST36. Also, the author fails to show which clinical trials are the most accurate. There is an established protocol for clinical trial reporting. See MacPherson H, Altman DG, Hammerschlag R, et al. Revised STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA): extending the CONSORT statement. PLoS Med 2010; 7: e1000261

Reply 7 Actually, the manuscript does not neglect the vascular system in terms of acupoint anatomy or physiology, but rather as per lines 108 -114 (reference 14-16), the acupuncture basic science literature clearly demonstrates that at least for pain studies, there is no contribution of vascular or humoral afferent signaling. A tourniquet in human and animal models has no effect on acupuncture analgesic response, even as it abolishes vascular/humoral signaling into the general circulation from acupoints. Isolation of an acupoint's vascular supply from the general circulation similarly has no effect on analgesia again confirming the lack of vascular system influence on acupuncture analgesia. Thus the ST36 findings of "correlation" of vascular beds to analgesic does not imply causality and is not compatible with those basic science acupuncture studies.

The request to "show which clinical trials are the most accurate" not within the scope of this review paper and creates an unclear request- the Haake GERAC lumbar pain study and Cherkin's RCT of acupuncture for back pain are well known studies with

widely accepted results- is the reviewer questioning the methodology and veracity of the results of these trials so that I should not accept/use their results including their sham intervention results? Please clarify. These studies used non-penetrating and minimally penetrating needling for sham needling, and the unexpected positive clinical results from the "sham" intervention are likely explainable by the neuroanatomic model proposed, and consistent with Ots' recent publication in AIM

Per journal submission guidelines stricta or consort does not apply to a review article

Changes in text 7 none

Comment 8 Overall the manuscript need major reworking. A number of the publications being cited are from over thirty years or more ago. Also, there is a statement that there is no conflict of interest with the authors; however, the author is citing a commercially available product as a viable source for acupoint imagining that he authored. I find this to be a big conflict of interest in the arguments made in this review. (Dorsher PT, Cummings M. Anatomy for Acupuncture [DVD]. Primal Pictures: 696 London, UK, 2006. ISBN 9781904369707 available through Primal Anatomy.TV).

Reply 8 I have modified the manuscript to address the reviewers issues where possible. I have submitted my conflict of interest statement previously and correctly report it as negative- I have never received any monies from that project in 2006 or subsequently. Please clarify what this reviewer sees as a "big conflict of interest"

Changes in text 8 done previously

Comment 9 If this is a true review of the neuroanatomical structures, then there has to be a review of actual data, not textbooks, websites, or other reviews done by the author.

Reply 9 Only a small portion of the paper covers neuroanatomy and the preponderance fmri, neuroendocrinologic, neuroimmunologic considerations, The data had been there already through Eachou Chen's cross-sectional acupoint anatomy but have added further data as previously described. Authoritative anatomy atlases (Gray, Grant, Clemente, Netter, Bonica, Chen) used are appropriate accepted resources by all major journals, whether or not they are "textbooks" or available only digitally on "websites" like some of the 3d anatomic resources. The authors are cited in only 7 of 107 references and do not form the basis of the correlations.