

Comment on “A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis”

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A breakthrough article by Ma et al. (2021), published in *Nature*, revealed the neuroanatomical mechanism of the systemic anti-inflammatory effect mediated by the “vagal-adrenal axis” induced by electroacupuncture [1]. Based on previous research, they demonstrated that prokineticin receptor 2 (PROKR2)^{Cre}-marked sensory neurons in the dorsal root ganglia are indispensable for the activation of the vagal-adrenal anti-inflammatory pathway induced by low-intensity electroacupuncture stimulation (ES) at Zusanli (ST36) or Shousanli (LI10) acupoints [2]. They found that peripheral fibers of PROKR2^{Cre}-marked neurons were abundantly distributed in the deep fascia of the ST36 and LI10 regions but sparsely in the superficial tissue of the corresponding sites, as well as other acupoints such as Chengjin (BL56) and the abdominal Tianshu (ST25). This differential distribution of PROKR2^{Cre} nerve fibers provides tangible neuroanatomical evidence for the relative specificity of the local tissue structure at different acupoints, thereby facilitating the prediction of anti-inflammatory effects induced by low-intensity ES at different acupoints. This crucial research finding allows us to explore its impact on the development of traditional Chinese medicine (TCM) as a whole instead of being limited to the acupuncture field.

The scientific value and efficacy of TCM have long been questioned [3, 4]. The research regarding TCM is not groundless but based on a reliable preliminary research foundation combined with scientifically advanced bioengineering technology. The experimental phenomenon that surface stimuli, such as acupuncture, elicit somatosensory autonomic reflexes has a long history [5–7]. During as early as 1979, researchers discovered that stimulating different parts of the skin of rats could affect neural reflexes and gastric motility [5]. Subsequent studies further demonstrated that acupuncture could affect autonomic reflexes represented by gastric motility by stimulating specific afferent fibers [6, 7]. Furthermore, communication between the nervous and immune systems has gradually been revealed in recent years [8–10]. For example, an article published in *Nature Medicine* in 2014 pointed out that ES at the ST36 acupoint can effectively relieve the endotoxemia caused by lipopolysaccharide and multi-bacterial infectious peritonitis caused by cecum ligation and puncture. This somatic autonomic reflex is accomplished via the release of dopamine following activation of the vagal-adrenal axis [8]. It is worth mentioning that ST36 is also the key acupoint in this present study by Ma et al., and it has widely been accepted to play an important role in regulating the digestive system, boosting immunity, and relieving extremity pain [11]. The fundamental research and background factors provide a theoretical basis and a critical research idea for the hypothesis of Ma et al. This hypothesis can be summarized as a pathway as follows: “ES at acupoint-afferent nerves-autonomic nerves-immune regulation”, wherein the identification of afferent nerves is the most important aspect for the elucidation of the scientific importance of acupoints. In fact, based on this hypothesis, Ma et al. (2020) published a similar study in *Neuron*, wherein they discovered that stimulating the ST25 acupoint can activate the sympathetic nerve connecting the spinal cord and spleen to produce norepinephrine, which can further inhibit inflammation by acting on specific receptors in the spleen [2]. However, none of these previous studies have identified the sensory neuron that receives the signal of ES at acupoint. The most important innovation of this study is that the authors pioneered the identification of PROKR2^{Cre}-marked neurons as the neuroanatomical basis for the anti-inflammatory

effects of ES at acupoints. To this end, the authors adopted a series of experimental methods to precisely control PROKR2^{Cre} nerve fibers and carried out detailed logical demonstration.

PROKR2^{Cre}-marked neurons are dorsal root ganglia neurons that persistently or transiently express prokineticin receptor 2 with Cre-mediated recombination marks [1]. These neurons were selected as candidates because they can innervate the deep limb tissues but not the epidermis of the skin, which is consistent with the conclusions of previous studies [2, 12]. The authors verified the differential expression of PROKR2^{adv} neurons using an immunostaining co-localization technique. In addition to intersectional marking and genetic-based PROKR2^{Cre}-specific neuron knockout, researchers have used optogenetic methods to induce expression of the light-sensitive protein *ChR2* in PROKR2^{Cre}-marked neurons. This has allowed scientists to specifically activate these neurons in the deep layer of the acupoint region with blue light to simulate the anti-inflammatory effect of ES at acupoints. This optogenetics-based neuronal modulation technique can be considered the most attractive part of this study, as it directly activates PROKR2^{Cre}-marked neurons to demonstrate the specificity of their relationship with the vagal reflex. All these methods have demonstrated that PROKR2^{Cre}-marked neurons are indispensable for activation of the vagal-adrenal pathway, inhibition of lipopolysaccharide-induced release of pro-inflammatory cytokines, and improvement of the survival rate of model animals after low-intensity ES at ST36 acupoints. Finally, this study verified the structural basis of the anti-inflammatory effect induced by low-intensity ES based on the tissue innervation pattern of PROKR2^{Cre}-labeled sensory nerve fibers. The authors found that in regions with limited PROKR2^{Cre} sensory nerve fiber innervation, low-intensity ES could not achieve the anti-inflammatory effect mediated by the vagal-adrenal axis, while in other areas rich in PROKR2^{Cre} innervation, low-intensity ES could still work, similar to the ST36 acupoint. Another highlight of this study is the overlap of ancient acupuncture concepts with advanced modern bioengineering technology. The findings provide a new perspective for elucidation of the neuroanatomical basis of acupoints and the neurobiological mechanisms of acupuncture.

Acupuncture is gradually being accepted globally as an alternative therapy, and its application ranks first among all traditional medicines [13]. According to a survey by the World Federation of Acupuncture and Moxibustion Societies in 2014, 183 countries and regions worldwide have applied acupuncture, 59 countries and regions have recognized the legal status of acupuncture, and more than 20 countries have enacted legislation on it [14]. Electroacupuncture is an application of acupuncture technology; compared to traditional acupuncture, electroacupuncture has a more reliable curative effect, which is quantifiable owing to the intensity generated via artificial regulation of the current [15]. There is sufficient evidence for the clinical therapeutic effect of acupuncture – anti-inflammatory effects have been reported by several researchers in recent years [2, 8, 13, 16], although the pattern and intensity of ES, stimulated acupoints, and downstream acting molecules are still debated in literature. However, for a medical method to be truly accepted globally, it is critical to elucidate its mechanism clearly and scientifically, in addition to demonstrating its significant clinical effects. According to TCM, the effect of acupuncture is achieved through the conduction of “meridian channels”. Many scholars have

devoted their lives exploring the meridian channels; a recent study has shown that overlapping fluorescent channels along the human pericardial meridian provide strong evidence for the existence of meridian channels [17]. However, currently there is no sufficient evidence to confirm the existence of these channels, which exist independently of blood and lymphatic vessels. Ma et al. (2020) explained the effectiveness of ES at acupoints from a neuroelectrophysiology perspective and performed logical verification from all aspects, i.e., the somatosensory nerves, efferent nerves, and effector organs. The theory of meridian channels is challenged by the autonomic reflex regulation. Nevertheless, this is an exciting phenomenon, as it is at least partially explaining the neuroanatomical basis of acupoints and the scientific mechanism of acupuncture. However, the question still remains that is this the correct training for the revival of acupuncture, even TCM? There is no need to retain all the theories in their entirety. What we should rather strive for is the actual clinical efficacy, based on the exact mechanism elucidated by modern science.

While this study has shed light on many mechanisms, it also leaves some unresolved questions, which may lead to many new ideas. First, for the entire intact autonomic reflex arc, this study lacks validation of an important intermediate part: interneurons. It has been reported that part of the somatosensory afferent signals induced by electroacupuncture is taken over by neurons in the nucleus tractus solitarius to activate parasympathetic preganglionic neurons in the dorsal motor nucleus of the vagus nerve, thus triggering the vagal efferent effect [18]. The role of the nucleus tractus solitarius – dorsal motor nucleus of the vagus relay loop may be important for the generation of vagal reflexes after ES at acupoints, but the exact mechanism remains unclear. Second, PROKR2^{Cre}-marked neurons serve as the anatomical basis for the anti-inflammatory effect of the vagal-adrenal axis; however, the appropriate stimuli sensed by such somatosensory neurons under physiological conditions remain largely unknown. Is there any other biological effect after activation? What intensity and frequency of ES can activate these sensory neurons to enhance the anti-inflammatory effects? These unanswered questions are of great research value and significance for achieving effective clinical translation in the future.

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Competing interests

The authors declare no conflicts of interest.

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Abbreviations

PROKR2, prokineticin receptor 2; ES, electroacupuncture stimulation; TCM, traditional Chinese medicine.

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