NEUROSCIENTIFIC UPDATE ON THE MECHANISMS OF ACTION OF ACUPUNCTURE IN CHRONIC PAIN

Atualização Neurocientífica dos Mecanismos de Ação da Acupuntura em Dor Crônica

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ABSTRACT

Acupuncture is a treatment method that emerged in Ancient China according to specific philosophical concepts of Traditional Chinese Medicine, which has its own systems of diagnosis and treatment of diseases. Since the use of acupuncture spread throughout the West, theories and concepts have emerged to explain its effects, including the possible role of physiological mechanisms. For instance, one of these theories states that acupuncture promotes neuromodulation by applying stimuli to the so-called acupoints, which are transmitted throughout the peripheral and central nervous systems by vast neural networks. One of the clinical applications of acupuncture is the treatment of chronic pain, but its mechanisms of action are not completely understood. Recently, however, studies have contributed to deepening the knowledge about the topic, suggesting that central neural pathways and molecular and humoral mechanisms are involved in the perception of chronic pain and in its treatment using acupuncture. These studies suggest, among other mechanisms of action, possible neurochemical effects of acupuncture on central and peripheral nervous systems, as well as modulation of somatosensory, affective and cognitive areas of the brain, observed in neuroimaging studies. This review aims to provide an overview of the effects and mechanisms of action of acupuncture in chronic pain, based on recent neuroscientific evidence.

Keywords: acupuncture, chronic pain, analgesia.

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INTRODUCTION

Acupuncture is a complementary or integrative treatment method that emerged in Ancient China according to specific philosophical concepts of Traditional Chinese Medicine (TCM), which has its own systems of diagnosis and treatment of diseases[1]. As acupuncture spread throughout the world, theories and ideas emerged in the West to explain its effects, following the introduction of modern principles of anatomy and physiology, and advances in biochemistry and biophysics[1,2].

The practice involves the insertion of fine needles at specific points on the body (classical acupuncture) or by applying electrical stimuli conducted by the needles at variable frequencies (electroacupuncture)[2]. Both techniques have been increasingly used as a complementary therapy in the treatment of pain[3].

Acupuncture analgesia – which is the main purpose for which the technique has been widely studied – represents the effects of integrative processes at different levels of the nervous system,

including the peripheral, central, somatic and autonomic nervous systems[4]. Several studies have reported that acupuncture exerts reproducible neurobiological effects in animal models[5-8]. In recent years, electroneuromyography and neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), have been used to study the neural mechanisms involved in the clinical outcomes of acupuncture[3,9]. In parallel, interest in clinical research has grown in recent decades, as well as the strength of evidence regarding the efficacy of acupuncture in pain management has increased as a result of randomized controlled trials (RCTs)[10].

This study presents an integrative review of the evidence on the various mechanisms of action of acupuncture analgesia, including neurophysiological and molecular effects. This review also provides a brief historical context of this technique and presents the clinical evidence of acupuncture in pain management. Finally, we present the main research gaps identified in the literature, aiming to guide further studies, and conclude with the authors' considerations on the subject. Due to the heterogeneous methods used in the reviewed studies, this study did not attempt to perform a meta-analysis. Instead, we intend to describe and discuss the evolution of the knowledge about the mechanisms suggested for the effects of acupuncture on pain modulation and analgesia, based on experimental studies.

BRIEF HISTORY OF ACUPUNCTURE

The development of acupuncture dates back to at least a few thousand years ago. The theory and practice of acupuncture originated in Ancient China, and gradually, the technique became one of the standard treatments in TCM, as well as massage, diet, herbs, and moxibustion[2,11,12]. It is believed that the specific points where the needles are inserted – the so-called acupoints – are distributed along the body and can be pierced by a needle or heated by burning the herb Artemisia vulgaris (better known as moxa, which is a type of mugwort, burned in a technique known as moxibustion). Cupping can also stimulate them, as well as pressure (acupressure), electrical stimulation (electroacupuncture) and, more recently, laser[1].

TCM understands human physiology and mental health through the concepts of Qi (vital energy), correlating it with physical and mental processes and also with emotional states. Diseases would appear due to interference in the circulation of Qi, which normally flows through energy channels known as meridians. They form a network-like system that connects different parts of the body and ensures the circulation of Qi[1]. Chinese medicine associates each meridian to a mental, physical or emotional function, and acupuncture is believed to restore the integrity of these elements. However, despite these beliefs having guided the practice of acupuncture for over thousands of years, there is currently no scientific evidence to support these concepts, and research has not yet been able to find any explanation according to current medical knowledge[13].

Nevertheless, acupuncture has become popular in recent decades, mainly for its effect on relieving acute or chronic pain of various origins, which is one of the reasons that have led to the interest in research on the mechanisms behind such analgesic effects[14]. In this regard, acupuncture research may be important not only to unravel the phenomena associated with its mechanisms of action, but also to explore new directions in human physiology that may not have been systematically studied yet.

EVOLUTION OF BASIC RESEARCH IN ACUPUNCTURE

Several questions about the mechanisms that mediate acupuncture analgesia have emerged in recent decades, especially regarding those involved in chronic pain. Initial studies, especially those using animal models, suggested the involvement of endogenous opioids in the nervous system, autonomic inhibition, axonal and medullary reflexes, and the release of non-opioid mediators and neurotransmitters, such as glutamate, serotonin and adenosine[4,15]. Other hormones, such as cholecystokinin (CCK), and immune mediators, such as inflammatory cytokines, appear to contribute to the mediation of acupuncture analgesia. In the latter case, they cause activation or inhibition of signal transduction pathways in target-cells, modulating mitogen-activated protein-kinase cascades (p38, ERK1/2, JNK). More recently, experiments regarding the anti-hyperalgesia effects of acupuncture have evaluated the control of inflammatory pain via inhibition of NMDA and AMPA receptors, neuroglial activation, and diffuse noxious inhibitory control (DNIC)[4,12,15].

In recent years, with the development of fMRI and positron emission computed tomography (PET/CT), the possible modulation of brain activity and pain pathways by acupuncture has attracted attention from the scientific community[9,12,16]. These new studies evaluated brain function during resting state, comparing it with the pattern of brain activity after performing acupuncture and electroacupuncture for painful stimuli. This technique allows the neuromodulatory effects of acupuncture on the brain to be identified, including the deactivation of the so-called default mode network and the modulation of regions linked to affective and pain processing and memory[17].

Therefore, this review divides the current knowledge gathered about the main mechanisms involved in acupuncture analgesia into two major types: neural (direct action on nervous tissue) and humoral/molecular.

REVIEW OF THE EVIDENCE ON THE MECHANISMS OF ANALGESIA ASSOCIATED WITH ACUPUNCTURE

Methods

An integrative literature review was carried out through bibliographic searches in the MEDLINE/PubMed, Latin American and Caribbean Health Sciences (LILACS) and EMBASE databases, including studies published from 2000 to 2021. The searches were performed in March and April 2021, and were limited to full-text articles published in English in peer-reviewed journals. Health descriptors and Medical Subject Headings (MeSH) were used to answer the question: "What are the possible mechanisms of acupuncture for the treatment of chronic pain?", using the keywords "analgesia", "pain", "mechanisms" and "acupuncture", with the addition of the Boolean operator AND. A descriptive analysis of the articles was then performed.

The analysis of the studies was descriptively carried out in order to answer the research question, taking into account ethical aspects, and respecting the authorship of the ideas, concepts and definitions present in the included articles. The titles and abstracts of the articles were initially evaluated to refine the sample, highlighting those that responded to the question of this review. Subsequently, each selected publication was thoroughly read, providing elements to discuss the neurophysiology and mechanisms of action of acupuncture, aiming to identify relevant aspects replicated or highlighted among studies. Articles were then organized to retrieve data to perform this integrative review.

Neural Mechanisms in Acupuncture

The direct effects that acupuncture stimulation causes on the nervous system seem to vary, and several neural mechanisms appear to mediate its analgesic properties. Studies suggest that acupuncture targets several areas distributed across the autonomic, peripheral and central nervous systems (CNS)[15,17]. In addition, it has also been suggested that the effects of acupuncture probably encompass extensive neural networks associated with affective, cognitive and somatosensory processing[9]. Furthermore, it has been demonstrated that the effects of acupuncture on the nervous system can continue even after the needle is removed, which suggests the procedure may have a long-term action[16].

The neural pathways related to acupuncture analgesia seem to be intertwined with the pathways involved in transmitting and detecting pain[4]. Moreover, it has already been observed that acupuncture not only acts on pathways responsible for somatic pain, but also on those involved in visceral, inflammatory, cancer and other types of pain[18,19]. Studies also suggest that acupuncture can attenuate central pain sensitization, which is often observed in states of hyperalgesia or allodynia[20,21]. A possible explanation for this diversity in neural mechanisms lies in the premise that the effects of acupuncture may vary according to stimulation parameters, such as its depth (dermatome, myotome, sclerotome, neurotome), intensity (weak, medium, strong, electrical), and anatomical location (motor, trigger, pain, skin or reflex points)[15].

Effects of classical acupuncture on sensory (afferent) pathways

Although initial studies indicate that acupuncture selectively stimulates primary afferent nerves, recent research has revealed that it can stimulate both myelinated and unmyelinated afferent fibers that innervate the skin and muscles[22,23]. In addition, acupuncture needle insertion has been shown to stimulate all four types of somatic afferent fibers: Group 1 (Aα type), Group 2 (Aβ type), Group 3 (Aδ type), and Group 4 (C type)24. However, analgesic the effects seem to be primarily mediated by fibers belonging to Groups 2, 3 and 4 (Aβ, Aδ, and C)[25].

Furthermore, studies suggest that acupuncture stimulation on somatic afferent pathways produces perceptible effects in three dimensions: 1) inhibition of nociceptive inputs resulting in analgesia, which could therefore justify the clinical use of acupuncture in pain syndromes; 2) regulation of visceral function via the reflex arc composed of somatic afferent pathways and autonomous visceral efferent pathways, also known as somatic-autonomic reflexes, which could justify the clinical use of acupuncture in non-painful visceral disorders; and 3) decreased skeletal muscle tone, determined by the somatic motor reflex, thus justifying the clinical use of acupuncture in conditions of sustained muscle tone[24].

Modulation of afferent signal processing in spinal cord pain induced by acupuncture

Studies suggest that spinal cord multireceptive neurons, especially those found in the dorsal horn (posterior column; laminae I-VI) and in the dorsal root ganglion, play an essential role in acupuncture analgesia[18,20,26-28]. Furthermore, modulation of several molecular pathways involved in pain processing in the spinal cord has been associated with acupuncture analgesia in pain models (Table 1). In particular, although the modulation of the opioid system remains the mechanism most widely associated with the action of acupuncture at the spinal level, the role of several other molecular mechanisms, including other neurotransmitters and protein kinases, has recently been reported[3,19,29]. Table 1 presents these molecular mechanisms, which will be further discussed below.

TABLE 1. Proposed mechanisms for the analgesic effects of acupuncture targeting the spinal cord

MECHANISM	PAIN TYPE
BDNF[19]	Inflammatory, neuropathic
C-FOS gene[3]	Visceral
CCK-8[4,15]	Inflammatory
Dopamine[3,4]	Inflammatory
EPHB3, EPHB6 and Ephrin-B genes[27]	Neuropathic
GABA[13,18]	Inflammatory, neuropathic
GDNF[15]	Neuropathic
Glial cells[15]	Inflammatory, neuropathic
NMDA glutamatergic receptors[3,15,44]	Visceral, inflammatory
Nociceptin / Orphanin FQ[4]	Inflammatory
Norepinephrine[4]	Inflammatory, neuropathic
p38 MAPK[3,15]	Visceral, inflammatory, neuropathic
Serotonin[3,4]	Visceral, Inflammatory, neuropathic
Substance P[3,15]	Inflammatory, neuropathic
μ, δ e κ opioid receptors[4]	Visceral, inflammatory, neuropathic, oncologic

BDNF: brain-derived neurotrophic factor; CCK-8: cholecystokinin octapeptide 8; EPHB: ephrin type-B receptor; GABA: gamma-aminobutyric acid; GDNF: glial cell line-derived neurotrophic factor; p38 MAPK: p38 mitogen-activated protein kinase

Acupuncture analgesia mechanisms related to the brainstem

Brainstem nuclei and their ascending pathways have been increasingly associated with chronic pain. For example, abnormalities in neuronal activity in the ventromedial rostral bulb and trigeminocervical complex have been implicated in migraine and may be attenuated by acupuncture, an effect that is attributed, as suggested by some studies, to calcitonin gene-related peptide (CGRP)[30]. Similarly, the dorsal column gracilis nucleus in the brainstem has been implicated in chronic pain states, and nitrous oxide has been suggested as the key molecular mechanism[28]. Furthermore, neurons in the dorsal periaqueductal gray matter (PAG) in the midbrain have been associated with acupuncture analgesia and seem to be modulated mainly by opioids and CCK-8[18,28]. Also, the reticular formation and other brainstem nuclei involved in pain pathways, which will be described below, play an important role in mediating acupuncture analgesia[31].

Acupuncture analgesia mechanisms related to the diencephalon

The targets of acupuncture analgesia in the diencephalon include the posterior and anterior hypothalamus, in addition to the centromedian nuclei of the thalamus, which connect to cortical regions involved in pain discrimination and perception, having an integrative role[4,28,32]. Furthermore, the suggested effect of acupuncture on thalamic nuclei, such as the parafascicular nucleus and lateral central nucleus, had already been described more than 50 years ago[32]. This effect on the thalamus was also found in a recent fMRI study, which suggests a role of thalamic nuclei in the habituation observed after repeated and prolonged acupuncture stimulation[33]. Another study using PET/CT suggested that the hypothalamus also mediates acupuncture analgesia[34].

Descending pain inhibitory pathways: central regulators of acupuncture analgesia

One of the methods used to explain the neural mechanisms of acupuncture classifies them as local, segmental (distal), and general or whole-body[35]. According to this model, descending pain inhibitory pathways, predominantly located in the medulla and midbrain, seem to be the main modulators of chronic pain, central hyperexcitability to pain, and acupuncture analgesia. A variety of neurotransmitters appear involved in these pathways, including acetylcholine, gamma-aminobutyric acid (GABA), serotonin, opioids, dopamine, and norepinephrine[20,35-40].

Since the 1970s, several nuclei related to pain pathways have been associated with acupuncture analgesia[20,38-40]. Those that make up the descending pain inhibitory pathways most strongly associated with acupuncture analgesia are listed in Table 2. More recently, the expression of the P2X3 receptor was found in the PAG, which is believed to undergo a modulatory effect by electroacupuncture, leading to analgesic effects in neuropathic pain[36]. Furthermore, studies have suggested that brain-derived neurotrophic factor (BDNF) is involved in the effect of acupuncture on the PAG and on the rostral ventromedial bulb, likely leading to increased central pain sensitization[20].

TABLE 2. Descending pain pathway nuclei implicated in acupuncture analgesia

Posterior arcuate nucleus[27,28]
Ventromedial hypothalamic nucleus[27,28]
Locus ceruleus[13]
Periaqueductal gray matter[3]
Nucleus raphe magnus[27,28,30]
Paragigantocellularis nucleus[27]
Gigantocellular reticular nucleus[27]
Rostral ventromedial medulla[3,27,28]
Nucleus tractus solitarii[3,28]
Anterior cingulate cortex[3,28]

Acupuncture mechanisms related to diffuse noxious inhibitory control

For many decades, it was believed that acupuncture itself was a noxious stimulus that produced pain, which, in turn, would activate a mechanism called DNIC through type Aδ and C afferent fibers4. Excitatory-inhibitory neurons in the raphe nuclei possibly regulate these mechanisms, and may be affected by descending pain pathways[4,40,41]. Extra-segmental acupuncture (which consists of inserting needles at sites distant from the original pain focus) could induce this type of diffuse inhibitory effect, causing distant analgesia[41]. In animal models, short-term DNIC can be observed through high-intensity electrical stimulation of acupoints[42,43]. However, a recent study, carried out with healthy volunteers, was not able to reproduce this effect using low-intensity stimulation. Even so, the authors noted that this does not exclude the possibility that acupuncture can exert this effect in a scenario of chronic pain or high-intensity stimulation[42]. Other authors have also suggested that an adequate intensity of stimulation for each individual may be necessary for this effect to completely manifest[43].

Neuroimaging studies and the brain mechanisms of acupuncture analgesia

Neuroimaging studies have revealed that pain processing and regulation are complex functions involving several areas of the brain. The sensation of pain seems to be mediated by several integrated and diffuse neural networks involving somatosensory (S1, S2 and thalamus), affective (hippocampus and amygdala) and cognitive (cingulate and insular cortex) areas[44,45]. Furthermore, abnormal plasticity has been observed in brain neural networks during pain states[45-47].

Regarding the brain mechanisms of acupuncture in chronic pain, interesting findings have been reported by neuroimaging studies[9,16,48,49]. A systematic review and meta-analysis of studies using fMRI revealed that acupuncture stimulated vast brain networks, including areas involved in somatosensory, affective and cognitive processing[9]. Another systematic review of several neuroimaging techniques reported that acupuncture can reverse the abnormal neural plasticity associated with chronic pain and restore normal plasticity, thus improving clinical symptoms[48]. Furthermore, acupuncture may induce several other neural responses, such as improvements in neural network efficiency and connectivity, activation of specific areas, as well as temporal changes, affecting the somatosensory, affective and cognitive areas of the brain. Also, changes in blood oxygenation levels in critical regions of the brain, including the limbic

system, brainstem, and various areas of the cerebellum, have also been observed after acupuncture stimulation[49].

HUMORAL AND MOLECULAR MECHANISMS OF ACUPUNCTURE ANALGESIA

Recently, studies have suggested that needle acupuncture may affect the body's neuroendocrine-immune regulatory network via molecular and humoral mechanisms shared between the two systems, including neuropeptides and neurotransmitters, as well as cytokines and hormones[4,50].

In addition to evidence suggesting that acupuncture can directly modulate the secretion of these mediators through stimulation of the nervous system, studies also suggest it can indirectly modulate the production of several cytokines and hormones through, respectively, the inflammation pathways related to the local injury at the acupoints and secretion of releasing hormones by the hypothalamus[50,51]. Consistently, studies have also proposed that the humoral mechanisms of acupuncture may be largely responsible for the therapeutic benefits in cases of inflammatory, visceral, and cancer pain, and that the use of acupuncture may also alleviate other associated symptoms, such as anxiety or depression[19, 52-54].

Many theories also discuss the effects of connective tissue manipulation by acupuncture needles, suggesting that this could be an important factor that induces humoral responses via mechanotransduction. This could be especially important considering that the acupoints are thought to be located in anatomical areas rich in loose connective tissue and intermuscular fascia[55]. To investigate this hypothesis, a study analyzed mast cell activation after mechanical stimulation of peripheral nerves, revealing that these cells release adenosine triphosphate (ATP) after the stimulation[56]. Likewise, the response of mechanically stimulated fibroblasts has also been studied, revealing that the cytoskeleton of these cells responds to the stimulation by the acupuncture needle[56,57].

Role of endogenous opioid peptide activity in acupuncture analgesia

Central and peripheral release of endogenous opioid peptides (β-endorphin, enkephalin, and endomorphin-1 via stimulation of δ- and -μ-opioid receptors, and dynorphin via stimulation of κ-opioid receptor) is the best-established molecular mechanism thought to mediate analgesia by acupuncture[4,58,59]. Since the 1970s, it has been demonstrated that the modulation of chronic pain induced by acupuncture is mediated by the action of opioid neurotransmitters in different points of several peripheral, spinal and supraspinal neural pathways, in models of inflammatory, oncologic, neuropathic and visceral pain[4,18,59].

Of all endogenous opioids, it is believed that β-endorphin is the predominant peptide in acupuncture analgesia, while enkephalins seem to be responsible for other effects, such as anti-anxiety and antidepressive[31,59]. Acupuncture stimulation may also induce short- and long-term increases in μ-opioid receptor binding potentials in brain regions that process pain, such as the cingulate cortex, caudate nucleus, and the thalamus[60].

The characteristics of opioid system stimulation, such as the profile of endogenous opioid peptide release and receptor activation, may depend on the intensity of the stimulus produced by acupuncture[4,61]. Recently, the growing awareness of the effects of acupuncture on endogenous opioids has resulted in studies regarding its possible effects on the consumption of exogenous opioids, including clinical scenarios such as analgesia and addiction to these substances. An interesting finding reported by one of these studies was a reduction of the need to use exogenous opioids in chronic pain syndrome after stimulation of endogenous opioids by acupuncture[62]. In addition, acupuncture has shown potential benefits in some cases of addiction to opioids[63].

Role of monoamines (serotonin, dopamine and noradrenaline) in acupuncture analgesia

Several studies indicate that monoamines seem involved in the analgesic effects of acupuncture[64,65]. For instance, evidence indicates that acupuncture regulates the activity of the serotonergic and dopaminergic neurotransmission systems in the central nervous system[4,59,66]. Several serotonin receptors, such as 5-HT1, 5-HT2, 5-HT3, 5-HT7, appear related to the analgesic effects of acupuncture, acting predominantly through pain inhibitory pathways located in the nucleus raphe magnus and in the dorsal horn of the spinal cord[67-70]. Likewise, acupuncture action on peripheral and cortical structures may also contribute to serotonin-mediated analgesic effects[4,58,67]. In addition to the effects on serotonergic transmission, it was observed that acupuncture also seems to cause an increase in dopaminergic activity via D1 and D2 receptors in the nucleus accumbens, associated with its analgesic effects[4,71,72].

Although the role of norepinephrine in acupuncture analgesia has been known for a long time, especially regarding the activity of locus coeruleus neurons and the stimulation of the descending pain inhibitory pathway[73], the exact mechanisms are not yet fully understood. Both the increase and decrease in noradrenergic activity after acupuncture have been reported[4,74]. Studies have also shown that the α2A adrenergic receptor can mediate the analgesic effect of acupuncture in cases of inflammatory pain[75], although its expression levels in the brain and spinal cord after electroacupuncture vary 76.

Acupuncture mechanisms related to inhibition of microglial activation

The maintenance of neuronal homeostasis is considered a fundamental mechanism of acupuncture[4]. Moreover, there is increasing evidence that acupuncture attenuates neuropathic pain caused by peripheral nerve injury. In the nervous system, glial cells – including microglia, astrocytes and oligodendrocytes (in the peripheral nervous system, Schwann cells only) – surround neurons and regulate and maintain their homeostasis and cellular function, in addition to participating in responses after acute and chronic injuries[25,27]. Because of this, great interest has arisen regarding the possible role of these cells in the analgesic effects of acupuncture.

Hence, studies have shown that microglia and astrocytes in the spinal dorsal horn are involved in the maintenance and onset of pathological pain by releasing pro-inflammatory cytokines and chemokines, in addition to other substances and factors that control pain signaling, such as glutamate and CGRP[3]. Microglia inhibition in rats is associated with a reduction in inflammation-induced mecha-

nical allodynia and with an enhancement of the analgesic effects of electroacupuncture in inflammation-induced pain[77].

Humoral and molecular mechanisms of electroacupuncture analgesia

Electroacupuncture has been the preferred modality in studies investigating the effects of acupuncture on chronic pain due to its known advantages, which include the possibility of standardizing and controlling the stimulus frequency, voltage, and waveform[78]. Many of these studies suggest that several neurotransmitters and their receptors, such as endogenous opioids (μ, δ, and κ), noradrenaline (α2), serotonin (5-HT1 and 5-HT3), acetylcholine (M1), glutamate (NMDA) and GABA (GABAA and GABAB), play a role as mediators of the analgesic effects of electroacupuncture. It is believed that these neurotransmitters act in structures such as the anterolateral tracts of the spinal cord and in the descending pain inhibitory pathways[79,80]. The release of cytokines by astrocytes in rats, as well as other molecules such as adenosine, has been suggested as another mechanism involved in electroacupuncture analgesia[81,82].

Furthermore, it has been observed that differences in the frequency of electrical stimulation affect the analgesic effects of electroacupuncture and its mechanisms, such as the activation profile of the opioid system. For example, it has been suggested that low-frequency electroacupuncture stimulates both μ- and -δ-opioid receptors and serotonergic pathways, while high-frequency acupuncture stimulates κ-opioid receptors[78].

Recent studies have also shown that electroacupuncture may play a role in modulating the signaling network of the endocannabinoid system, regulating numerous physiological and cognitive processes. One of the findings was the activation of cannabinoid receptors 1 (CB1) in the ventrolateral area of the PAG, which seems essential for the central antinociceptive effect of electroacupuncture. The activation of cannabinoid receptors 2 (CB2) in the periphery, on the other hand, seems related to its anti-inflammatory effects. Activation of both CB1 and CB2 would therefore alleviate behavioral responses to inflammatory and neuropathic pain[83]. Furthermore, other studies in rats have shown an increase in the levels of endocannabinoids AEA and 2-AG in the brain[84] after electroacupuncture.

RESEARCH GAPS

In recent years, large randomized clinical trials and systematic reviews have reported good qualitative and quantitative results of acupuncture[85]. On the other hand, some recent high-quality studies comparing acupuncture with sham acupuncture (sham) have found small differences in clinical outcomes[86,87].

Despite recent advances, there is still a significant gap in acupuncture research, especially regarding the translation of experimental evidence into clinical practice3. Likewise, there are several methodological challenges and major inconsistencies in clinical research on acupuncture for pain, starting with the clinical condition studied, which is broad and complex. Other methodological challenges include difficulties to ensure adequate blinding and to define the best clinical trial design. For instance, consensus regarding the best comparator for acupuncture still lacks, such as no treatment, waitlist assignment, pharmacological treatment, and sham acupuncture; the ideal follow-up time; and the most clinically relevant outcomes86. Added to this is the lack of standardization of treatment protocols, which differ both in randomized clinical trials and in clinical practice, given that the TCM diagnostic method is complex and individualized[88,89]. This can include variability in acupuncture point locations, as well as differences in point selection (fixed or variable point approach) and in the duration, frequency, and intensity of acupuncture or electroacupuncture. As a result, the technique varies among practitioners, making its replication more difficult and challenging[90].

Moreover, despite the possible analgesic mechanisms attributed to acupuncture, the main target of the procedure has not yet been clarified, including transmission, perception or modulation of pain. Multiple mechanisms are likely to occur, with many overlapping effects, resulting in a complexity that poses additional challenges to acupuncture research. Isolating different mechanisms is also challenging, especially considering that the use of simulated or minimal needling in clinical research is controversial, as even minimal superficial sensory inputs may be sufficient to stimulate some of the same neural pathways thought to mediate the analgesic effects of acupuncture. As a result, sham acupuncture could not be considered completely physiologically inert, as traditional placebo pills[91].

Furthermore, recent research has shown that acupuncture analgesia is likely due, in part, to integrative processes at different levels in the peripheral and central nervous systems. Thus, the concept of neuromodulation, which is also associated with a wide range of cellular and molecular inflammatory pathways, may play a central role in acupuncture analgesia and should therefore be further investigated. Finally, much of the current knowledge about possible mechanisms of acupuncture analgesia results from studies that used animal models, which are difficult to translate to the clinical setting. Nevertheless, despite these remaining gaps regarding the applicability of the results of preclinical experiments in humans, acupuncture analgesia is a promising strategy for several pain disorders. Therefore, the main goal of future prospective research should be to translate the findings of mechanistic studies into clinical applications.

CONCLUSION

Although acupuncture has been used in TCM for the treatment of pain for thousands of years and has gained significant popularity in medical practice around the world, there has been a strong impetus in recent decades towards studying the neurobiological mechanisms underlying the analgesic effects of the technique. Understanding these mechanisms is crucial for the general acceptance and consolidation of acupuncture in the treatment of chronic pain.

Despite the great advances in the methodology used in acupuncture research, the mechanisms of its analgesic effects are not yet fully understood. Based on the most recent findings reported in the literature, our review suggests two types of mechanisms possibly associated with these effects: neural and humoral/molecular. The first includes inhibition of pain sensory pathways (primarily targeting Aβ, Aδ, and C afferent fibers); modulation of pain signal processing in the spinal

cord (in posterior gray matter columns and anterolateral tracts); changes in pain processing in the brainstem, diencephalon (hypothalamic and thalamic nuclei) and cerebral cortex (cognitive, affective and somatosensory areas); and activation of descending pain inhibitory pathways and of the DNIC. Changes in connectivity and brain activity were observed in studies with neuroimaging, which allowed to identify possible effects of acupuncture on the brain. Humoral and molecular mechanisms, in turn, involve modulation of the endogenous opioid system and several other neurotransmitters and their receptors, including monoamines and neuropeptides involved in pain processing. At the cellular level, microglial activation seems also involved. These neurobiological mechanisms probably underlie the effects of acupuncture on different types of pain, such as somatic, visceral, oncologic and inflammatory, and are likely concurrent.

As with other pain models, most knowledge regarding acupuncture analgesia comes from experiments with animal models, in which the effects and mechanisms described may not be applicable to humans. Important research gaps, including differences in acupuncture techniques and study design, as well as other issues regarding research methodology in the area, which are not yet consensual, must be discussed and addressed. Future studies on the mechanisms of acupuncture should consider these issues, so that it will be possible to translate most of these basic and preclinical research findings to relevant clinical applications.

CONFLICTS OF INTERESTS

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