# Endocannabinoid System-Mediated Mechanisms of Acupuncture for Pain, Pruritus and Comorbid Neuropsychiatric Symptoms: A Systematic Review

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**Abstract.** In chronic pain, pruritus, anxiety, and depression—highly comorbid neurological disorders characterized by neuroinflammation, central sensitization, and circuit dysfunction—the endocannabinoid system (ECS, comprising CB1/CB2/GPR55 receptors, endogenous ligands AEA/2-AG, and metabolic enzymes FAAH/MAGL) serves as a key regulatory hub modulating inflammatory processes, synaptic plasticity, and neuronal homeostasis. Here, we present a systematic review of preclinical studies from the past three years (2023–2026), integrating multi-level evidence of ECS-mediated acupuncture for the above disorders. Preclinical evidence has established that acupuncture precisely modulates the ECS at peripheral (activating fibroblast CB2R to inhibit mast cell degranulation and inflammatory cytokines; upregulating sensory neuronal CB1R to suppress Nav1.8/COX-2 pathways), spinal (CB2R promoting microglial M2 polarization to alleviate neuroinflammation; CB1R inhibiting glial TLR4/MyD88/TRAF6 cascades), and supraspinal levels (cell-type-specific CB1R in vlPAG/RVM/ACC bidirectionally regulating pain and itch; hippocampal CB2R/AEA and GPR55 pathways mediating antidepressant and anxiolytic effects). Notably, the ECS exhibits dynamic compensatory crosstalk with the opioid system—when TRPM8 is deleted, analgesia shifts to exclusive ECS reliance; moreover, the hypothalamic OX1R-CB1R pathway activated by PC6 (Neiguan) stimulation provides an opioid-independent, tolerance-free analgesic strategy. Current challenges include lack of standardized acupuncture parameters, species differences, and insufficient clinical translational evidence. This review synthesizes molecular, cellular, and circuit-level evidence to support the ECS as a core integrative target for acupuncture in pain, pruritus, anxiety, and depression, and calls for advancing standardized acupuncture regimens and clinical translation of ECS-targeted therapies.

**Keywords:** Acupuncture, Endocannabinoid system, Pain, pruritus and neuropsychiatric disorders, Cell-type-specific mechanism.

## 1. Introduction

Chronic pain, pruritus, anxiety, and depression are highly prevalent comorbid neurological disorders sharing pathological underpinnings such as neuroinflammation, central sensitization, and circuit dysfunction. Current pharmacotherapies are limited by significant adverse effects and the opioid crisis, necessitating non-pharmacological alternatives [1-2]. The endocannabinoid system (ECS), comprising CB1/CB2/GPR55 receptors, ligands (AEA, 2-AG), and metabolic enzymes (FAAH, MAGL), is critically dysregulated across these conditions and serves as the key endogenous bridge for acupuncture's multi-target effects [3-4]. Preclinical evidence indicates that acupuncture modulates the ECS at peripheral (fibroblast CB2R, sensory neuronal CB1R), spinal (microglial polarization, glial inhibition), and supraspinal levels (cell-type-specific CB1R in vlPAG/RVM/ACC, hippocampal CB2R/GPR55), while engaging dynamic crosstalk with opioid and TRPV1 pathways [5-12].This systematic review synthesizes evidence from the past three years to outline ECS-mediated mechanisms of acupuncture for pain, pruritus, anxiety, and depression, assess translational prospects, and identify future directions [6,13].

Literature retrieval was performed across PubMed, Web of Science, CNKI and CQVIP. Search keywords included: "acupuncture", "electroacupuncture", "endocannabinoid system", "CB1 receptor", "CB2 receptor". Time limit: January 2023 -- December 2026. Inclusion criteria: (1) preclinical in vivo/in vitro experiments focusing on acupuncture-regulated ECS; (2) complete data of acupoint selection, stimulation parameters, animal models and molecular verification; (3) peer-reviewed original articles. Exclusion criteria: reviews, case reports, clinical trials without animal mechanistic exploration, duplicate publications, studies lacking genetic/pharmacological validation of ECS pathways. All retrieved literatures were screened by two independent researchers, with discrepancies resolved via group discussion.

## 2. Overview of the Endocannabinoid System

ECS exerts pleiotropic biological functions via multiple receptor subtypes, metabolic enzymes and endogenous ligands, with distinct expression patterns and physiological functions distributed in central and peripheral tissues.

### 2.1. Classification, Distribution and Function of Cannabinoid Receptors

#### 2.1.1. Classic Receptors CB1R and CB2R

CB1R is highly enriched in the central nervous system, widely distributed in nociceptive regulatory brain regions and neuronal axon terminals of peripheral primary sensory neurons, serving as a vital endogenous inhibitory brake against pain hypersensitivity. Conditional knockout of CB1 in primary sensory neurons (CB1cKO) induces persistent hyperalgesia in mice, which confirms the indispensable tonic analgesic function of CB1R in peripheral afferent nerves [3]. Transcriptomic sequencing of dorsal root ganglia (DRG) reveals dynamic adaptive changes after CB1 deletion: early differential genes concentrate on energy metabolism and complement-coagulation pathways reflecting acute cellular stress; late altered transcripts mainly involve pain conduction circuits, representing maladaptive remodeling of nociceptive pathways. After nerve injury, CB2R exhibits robust compensatory upregulation in CB1cKO mice. However, excessive CB2R activation aggravates astrocyte overactivation and neuroinflammation, while CB2 antagonists can relieve neuropathic pain, indicating complex mutual regulation between CB1R and CB2R [14].

Different from neuron-dominant CB1R, CB2R is predominantly expressed on peripheral immune cells (mast cells, T cells, macrophages, fibroblasts), with minimal expression in normal central neurons, thus mediating anti-inflammatory effects without central psychotropic side effects. CB2R acts as a core therapeutic target for various inflammatory lesions including arthritis, enteritis and dermatitis.

#### 2.1.2. Atypical Receptor GPR55

GPR55 is a non-canonical cannabinoid receptor independent of CB1R/CB2R signaling, highly expressed in the hippocampus and closely associated with stress-induced neuropsychiatric disorders. In chronic social defeat stress (CSDS) mouse models, hippocampal GPR55 protein expression (rather than mRNA) is markedly reduced, accompanied by severe neuroinflammation and impaired hippocampal neurogenesis. The selective GPR55 agonist O-1602 reverses these pathological alterations, whereas GPR55 antagonists completely eliminate EA's antidepressant effects [4]. This evidence verifies that hippocampal GPR55 modulates stress resilience via post-transcriptional regulatory mechanisms and acts as an exclusive molecular target for acupuncture's anti-depression efficacy, separated from classic CB1/CB2 pathways.

### 2.2. Endocannabinoid Ligands and Metabolic Enzymes

AEA and 2-AG are two primary endogenous ligands of ECS, whose bioavailability is strictly controlled by two rate-limiting metabolic enzymes: FAAH and MAGL. FAAH mainly degrades AEA, while MAGL dominates 2-AG catabolism; imbalance between ligand synthesis and degradation leads

to ECS dysfunction, which participates in the pathological progression of visceral pain, depression and drug addiction.

In TNBS-induced visceral hypersensitivity models, intestinal inflammation suppresses CB1R expression in the ileum, spinal cord and pain-regulating brain regions, alongside elevated FAAH and MAGL levels. This dual disorder of receptor downregulation and ligand accelerated degradation causes systemic endocannabinoid deficiency and aggravated visceral hyperalgesia. EA intervention reverses this pathological phenotype: it restores CB1R expression and inhibits FAAH/MAGL overexpression, thus elevating endogenous ligand concentration to relieve visceral pain [15].

The FAAH--AEA--CB1R axis also participates in neuronal structural plasticity related to substance addiction. Cocaine exposure reduces hippocampal dorsal dentate gyrus FAAH activity, triggering AEA accumulation and excessive CB1R activation. Overactivated CB1R initiates downstream cascades to induce abnormal dendritic remodeling of granule neurons, which mediates addictive memory formation. Pharmacological FAAH inhibition reproduces cocaine-associated memory disorders, while CB1R blockade reverses all neuronal structural damage induced by cocaine exposure [16].

Collectively, ligand-metabolizing enzymes FAAH and MAGL serve as master regulators of ECS homeostasis, and targeted modulation of these enzymes is a universal strategy for treating diseases featuring endocannabinoid insufficiency or overactivation.

## 3. Peripheral ECS Mechanisms Underlying Acupuncture Analgesia and Anti-Inflammation

Peripheral tissues contain abundant CB1R and CB2R distributed in immune cells, fibroblasts and sensory nerve terminals. Acupuncture mechanical stimulation activates peripheral ECS to block nociceptive signal initiation and restrain local inflammatory cascade before pain signals transmit to the spinal cord, avoiding central nervous system adverse reactions.

### 3.1. CB2R-Mediated Peripheral Anti-Inflammatory and Analgesic Effects

CB2R is the universal peripheral anti-inflammatory effector molecule activated by acupuncture across multiple systemic diseases, with consistent verified efficacy in articular disorders, gynecological pain, dermatitis, enteritis and systemic endotoxemia models.

#### 3.1.1. Rheumatoid Arthritis and Knee Osteoarthritis

In adjuvant-induced rheumatoid arthritis mouse models, manual acupuncture (MA) primarily targets fascial fibroblasts rather than bone and cartilage tissue to improve pathological injury. Mechanical stimulation of acupoints initiates mechanotransduction cascades (Piezo1/RhoA/YAP1), upregulates ECM synthetic genes (fibromodulin, Col-I, HAS2) and fibroblast marker CB2R. CB2R upregulation acts as a core anti-inflammatory switch to suppress local inflammatory response and mast cell degranulation. Selective ablation of acupoint fibroblasts completely abolishes MA's analgesic and tissue repair effects, confirming fascial fibroblasts as indispensable peripheral effector cells of acupuncture via CB2R signaling [5].

For knee osteoarthritis (KOA), EA at optimized parameters (2 Hz, 1 mA) upregulates meniscus fibroblast CB2R expression and inhibits pro-inflammatory IL-1β secretion. In CB2 knockout mice, all therapeutic phenotypes of EA disappear entirely. EA elevates mechanical pain threshold, thermal pain latency, limb weight-bearing capacity and OARSI histological scores only in wild-type animals, which confirms that CB2R activation is an essential non-redundant pathway for EA to alleviate IL-1β-driven chronic joint pain [17].

#### 3.1.2. Primary Dysmenorrhea

EA relieves writhing responses, alleviates uterine pathological damage and normalizes serum PGF2α/PGE2 ratio in primary dysmenorrhea (PDM) rat model. EA simultaneously downregulates spinal pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and uterine/spinal TRPV1/iNOS, while

increasing anti-inflammatory mediators IL-10, Arg1 and $PGE_2$. Mechanistically, EA upregulates CB2R in uterine and spinal tissues, promotes M2 microglial polarization (Arg1 as biomarker), and suppresses peripheral-central cross-talking neuroinflammation to alleviate dysmenorrhea pain and uterine spasm [18].

#### 3.1.3. Inflammatory Skin Diseases (Atopic Dermatitis, Allergic Contact Dermatitis)

In chronic itch and atopic dermatitis models, EA suppresses mast cell and $CD4^+$ T cell proliferation, downregulates skin lesion pruritic inflammatory factors IL-4, IL-13, IL-31 and their DRG-expressed receptors. EA upregulates CB2R and modulates ECS metabolic enzymes; CB2 knockout eliminates EA's anti-pruritic and anti-inflammatory capacity and reverses EA-induced ERK phosphorylation inhibition. This demonstrates that CB2R activation inhibits ERK inflammatory kinase signaling to restrain immune cell overproliferation and cutaneous inflammation [6].

For DNFB-induced allergic contact dermatitis, EA inhibits ear swelling, IgE production, mast cell infiltration and degranulation, alongside reduced p38 MAPK phosphorylation. CB2 antagonist AM630 fully reverses all EA therapeutic effects, while CB1 antagonist exerts no interference, verifying that EA's anti-dermatitis activity specifically depends on mast cell CB2R-mediated p38 MAPK suppression [19].

#### 3.1.4. Inflammatory Bowel Disease and Systemic Endotoxemia

EA at BL25 attenuates visceral hypersensitivity and mechanical allodynia in TNBS-induced inflammatory bowel disease (IBD) mice. EA activates colonic macrophage CB2R to inhibit macrophage overactivation and IL-1β/iNOS expression. The protective effects are completely blocked by CB2 antagonist AM630 or global CB2 knockout, proving that CB2R-mediated macrophage inflammatory inhibition is the core peripheral mechanism of EA for IBD treatment [20].

Seven-day EA pretreatment at ST36 suppresses LPS-induced systemic inflammation in rats: it reduces serum TNF-α, IL-1β, IL-6, enhances tissue CB2R expression, blocks $Ca^{2+}$ influx and inactivates TLR4/NF-κB inflammatory cascades. AM630 reverses all above changes, positioning CB2R as the core intersection of calcium signaling and TLR4/NF-κB transcriptional regulation for EA's broad-spectrum anti-inflammatory function [21].

### 3.2. CB1R-Mediated Peripheral Analgesic Mechanisms

Distinct from immune cell-located CB2R, CB1R abundantly distributes on peripheral sensory nerve terminals and DRG neurons to directly suppress nociceptor excitability before pain signals transmit to the spinal cord, exerting independent peripheral analgesic effects.

In complete Freund's adjuvant (CFA)-induced inflammatory pain models, EA elevates CB1R expression on peripheral nerve terminals, which co-localizes with Nav1.8 tetrodotoxin-resistant sodium channels. Activated CB1R directly inhibits Nav1.8 channel activity to reduce neuronal hyperexcitability and pain signal generation; meanwhile, EA downregulates COX-2 to cut prostaglandin synthesis and alleviate peripheral nociceptor sensitization. CB1R antagonist AM251 completely abolishes EA analgesia, though it cannot reverse EA-induced CB1R upregulation and Nav1.8/COX-2 downregulation. This indicates that EA induces stable biochemical remodeling of sensory neurons, while complete CB1R signaling is mandatory to translate these molecular changes into pain relief [7].

Peripheral CB1R also mediates EA's therapeutic efficacy for migraine. Trigeminal ganglion electrical stimulation elevates serum CGRP, PGE2 and trigeminal IL-1β, COX-2, PPE to mimic migraine neuroinflammatory microenvironment. EA reverses all pathological upregulation, and CB1R antagonist fully eliminates EA's anti-migraine anti-inflammatory activity, confirming peripheral CB1R as the essential mediator of EA against migraine peripheral neuroinflammation [22].

Pharmacological blocking experiments further verify the exclusive peripheral analgesic role of CB1R: EA-induced sustained anti-nociception can be eliminated by CB1R antagonist AM251, without any response to CB2R antagonist AM630. Inhibitors of endocannabinoid degradation and

reuptake potentiate EA analgesia, proving that prolonging endogenous ligand availability to activate peripheral CB1R strengthens acupuncture pain relief [23].

## 4. Spinal ECS Mechanisms Underlying Acupuncture Analgesia

The spinal dorsal horn acts as the primary synaptic relay station for ascending nociceptive signals, where ECS modulates microglial polarization, glial inflammation and synaptic transmission to realize spinal pain gating, jointly controlled by CB1R and CB2R with distinct functional divisions.

### 4.1. Spinal CB2R: Anti-inflammatory and Analgesic Effects

In primary dysmenorrhea rat models, EA elevates spinal CB2R and Arg1 (M2 microglia marker), reduces spinal IL-1β, TNF-α, IL-6 and iNOS. Spinal CB2R activation shifts microglia from pro-inflammatory M1 phenotype to reparative M2 phenotype, suppressing central neuroinflammation induced by peripheral uterine lesions and reducing spinal nociceptive signal amplification [18]. This mechanism is widely replicated in enteritis, arthritis and neuropathic pain models, establishing spinal CB2R as the core receptor mediating anti-neuroinflammatory effects via microglial phenotypic transformation.

IVDD is the primary cause of chronic low back pain, featured by nucleus pulposus cell (NPC) senescence, ECM degradation and matrix metalloproteinase overexpression. CB2R expression markedly declines in senescent NPCs and degenerative disc tissue, alongside elevated senescence marker p16INK4a and SASP factors (MMP9, MMP13, HMGB1). EA activates CB2R to initiate downstream AMPK/GSK3β signaling, exerting comprehensive anti-senescence effects: reducing SA-β-gal positive senescent cells, suppressing p16INK4a and SASP secretion, upregulating ECM anabolic proteins Col-II and SOX9, inhibiting hypertrophic differentiation marker Col-X, and restoring disc matrix metabolic homeostasis. AMPK pharmacological inhibition fully reverses CB2R-mediated protective effects, verifying AMPK/GSK3β as the mandatory downstream effector of CB2R anti-senescence function [24].

Selective CB2 agonist JWH133 reproduces all protective phenotypes of EA: it alleviates NPC oxidative stress, inhibits inflammatory cytokine and MMP secretion, preserves Col-II and aggrecan in vitro; in needle-puncture IVDD rat models, JWH133 elevates disc height index (DHI), improves disc histological morphology and slows degenerative progression in vivo [25].

In summary, CB2R activation is a core therapeutic strategy for IVDD, targeting cellular senescence, oxidative stress and ECM imbalance via the AMPK/GSK3β cascade, providing a novel senomorphic intervention target for degenerative musculoskeletal disorders [24-25].

### 4.2. Spinal CB1R: Suppressing Glial Reactivity and Synergizing with the Opioid System

In fibromyalgia mouse models, EA upregulates spinal CB1R to restrain glial overactivation and block TLR4--MyD88--TRAF6 inflammatory cascades. CB1 agonists simulate EA analgesia, while CB1 antagonists fully eliminate EA efficacy, verifying spinal CB1R's independent anti-inflammatory and analgesic capacity [11].

For neuropathic pain, single-acupoint EA (ST36) only activates the endogenous opioid system to weakly inhibit spinal synaptic transmission, whereas combined multi-acupoint EA (ST36+GB30) simultaneously recruits both opioid and ECS pathways to achieve stronger analgesia. Only co-administration of opioid and cannabinoid receptor antagonists can completely block multi-acupoint EA's pain relief, which confirms that spinal ECS acts as a compensatory synergistic pathway to amplify acupuncture analgesia when multiple acupoints are matched [26].

Visceral inflammation induces universal spinal CB1R downregulation alongside elevated FAAH/MAGL to break spinal ECS homeostasis. EA restores spinal CB1R expression and inhibits metabolic enzyme overexpression, rebuilding spinal endogenous pain-suppressive capacity to relieve visceral hypersensitivity [15].

## 5. Supraspinal ECS Mechanisms of Acupuncture Analgesia

Multiple brainstem and cortical regions form an integrated ECS-mediated descending pain regulatory network, dominated by cell-type-specific CB1R functions in vlPAG, RVM and ACC.

### 5.1. vlPAG CB1R: Cell-Type-Specific Bidirectional Regulation of Pain and Itch

vlPAG is the core hub of descending pain/itch modulation, and CB1R's biological effect completely depends on whether it localizes to GABAergic or glutamatergic neurons, presenting opposite regulatory phenotypes in chronic pain versus chronic itch.

#### 5.1.1. Chronic Pain Models (KOA)

EA upregulates vlPAG 2-AG and CB1R expression, selectively activating CB1R on GABAergic neurons. Activated CB1R inhibits GABA release to relieve tonic GABAergic suppression of descending 5-HT inhibitory pathways, amplifying spinal nociceptive signal suppression via the 2-AG--CB1R--GABA--5-HT cascade. Intra-vlPAG CB1 antagonist injection eliminates EA analgesia and blocks diffuse noxious inhibitory controls (DNIC), confirming GABAergic neuronal CB1R as the core analgesic target of vlPAG in chronic pain [8].

#### 5.1.2. Chronic Itch Models

In dry-skin chronic itch models, vlPAG CB1R expression abnormally rises under pathological status. Electroacupuncture selectively activates CB1 receptors on glutamatergic neurons, thereby suppressing the activity of these neurons, which in turn inhibits downstream serotonin release in the medulla oblongata and gastrin-releasing peptide receptor (GRPR) signaling in the spinal cord, ultimately reducing scratching behavior.. Cell-type-specific knockout experiments verify that knocking out glutamatergic neuronal CB1R fully abolishes EA's anti-pruritic effect, while GABAergic neuronal CB1R knockout alleviates itch, revealing reverse functional roles of two neuronal subtypes in itch regulation [9].

#### 5.1.3. Opioid-Independent OX1R--CB1R Pathway in vlPAG

Repeated median nerve stimulation at PC6 (MNS-PC6) activates hypothalamic orexin neurons to release orexin A into vlPAG, binding OX1R to trigger local 2-AG synthesis. Newly generated 2-AG activates CB1R to induce retrograde disinhibition of GABAergic transmission, enhancing descending pain inhibition to produce potent analgesia. This entire signaling cascade is unaffected by opioid antagonists and does not induce drug tolerance, remaining fully functional even in morphine-tolerant animals, which provides a unique non-opioid therapeutic target for intractable pain [2,12].

### 5.2. RVM CB1R on GABAergic Neurons Mediates EA Analgesia

RVM is the final common relay station transmitting supraspinal pain signals to the spinal dorsal horn, and GABAergic neuronal activity determines the overall tone of descending pain inhibition. In CFA inflammatory pain and chronic constriction injury of the sciatic nerve (CCI) neuropathic pain models, EA at ST36 reduces RVM GABAergic neuron calcium activity, firing frequency and c-Fos expression to suppress GABAergic tonic inhibition of descending analgesic pathways. Chemogenetic inhibition of RVM GABAergic neurons simulates EA analgesia, whereas chemogenetic activation eliminates EA pain relief. Selective CB1R knockdown on RVM GABAergic neurons completely abrogates EA efficacy, confirming that EA activates RVM GABAergic CB1R to suppress neuronal excitability, disinhibit descending pain-suppressive circuits and realize spinal analgesic gating [10].

### 5.3. ACC CB1R: Regulating Pyramidal Neuron Overactivity for Pain-Affective Relief

ACC is the core brain region processing pain's emotional and cognitive components, and CB1R dysfunction in ACC causes pyramidal neuron hyperactivity to amplify pain unpleasantness in fibromyalgia and chronic inflammatory pain.

In intermittent cold stress (ICS) fibromyalgia models, ACC CB1R expression is significantly downregulated, accompanied by upregulated pain-related kinases and TRPV1. EA at 2 Hz restores ACC CB1R and endogenous AEA/2-AG levels [27]. Activated CB1R recruits HINT1 protein to inhibit NMDAR NR1 subunit function, directly reducing pyramidal neuron overexcitation to relieve persistent pain perception and pain-associated negative emotion. CB1R agonists mimic EA's cortical analgesic effect, while CB1R antagonists block all EA-induced molecular and behavioral improvements [3].

The hypothalamic paraventricular nucleus (PVN) acts as an upstream stress sensor regulating ACC CB1R signaling: chemogenetic PVN activation suppresses ACC CB1R expression and triggers widespread pain sensitization, whereas PVN inhibition elevates cortical CB1R to relieve pain, forming a complete PVN--CB1--TRPV1 pain regulatory axis spanning hypothalamus and cortex [28].

## 6. ECS Mechanisms Underlying Acupuncture Treatment of Non-Analgesic Disorders

Beyond pain and inflammation, ECS mediates acupuncture's therapeutic efficacy against neuropsychiatric disorders (anxiety, depression) and degenerative musculoskeletal diseases (intervertebral disc degeneration) via region- and cell-specific receptor signaling.

### 6.1. Medial Prefrontal Cortex (mPFC): Glutamatergic Neuronal CB1R Relieves Anxiety

Chronic restraint stress (CRS) induces anxiety-like behaviors and ECS deficiency in mPFC. EA selectively elevates CB1R expression on mPFC glutamatergic excitatory neurons without altering GABAergic neuronal CB1R. Knocking out glutamatergic CB1R alone induces severe anxiety and eliminates EA's anxiolytic capacity, whereas GABAergic CB1 knockout exerts no influence. Mechanistically, activated glutamatergic CB1R suppresses GR/SGK1 stress signaling cascades to reverse maladaptive stress responses, establishing mPFC glutamatergic CB1R as the exclusive molecular target of EA's anti-anxiety effect [29].

### 6.2. Hippocampus: CB2R/AEA and GPR55 Mediate Antidepressant Effects

Social defeat stress reduces hippocampal CB2R and AEA levels to trigger depressive phenotypes. EA upregulates hippocampal CB2R and AEA, and CB2 antagonist AM630 completely blocks EA's antidepressant function, confirming the CB2R/AEA axis as a core anti-depression pathway [11]. Hippocampal GPR55 acts as an independent auxiliary target: CSDS downregulates hippocampal GPR55 protein (post-transcriptional modification) to induce neuroinflammation and impaired neurogenesis; GPR55 agonists reverse depressive behaviors, while GPR55 antagonists eliminate EA's antidepressant efficacy via suppressing anti-inflammatory and neuroprotective effects [4].

### 6.3. Ventral Hippocampus (vHPC): Differential CB1R Modulation Separates Anxiolysis from Analgesia

In IBD combined anxiety models, EA relieves visceral pain and anxiety simultaneously through distinct vHPC CB1R regulatory pathways independent of each other. EA downregulates CB1R on vHPC GABAergic neurons and upregulates CB1R on glutamatergic neurons. Cell-type knockout verifies that GABAergic CB1 deletion mimics anti-anxiety effects, while glutamatergic CB1 deletion induces severe anxiety and abolishes EA anxiolysis; neither manipulation alters visceral pain, proving EA's anxiolytic function is mediated by vHPC CB1R without relying on analgesic effects [30].

## 7. Crosstalk Between ECS and Other Endogenous Signaling Pathways

ECS does not function independently; it forms synergistic, compensatory and exclusive regulatory loops with the opioid system, TRPV1 ion channel and orexin system to jointly mediate acupuncture analgesia.

### 7.1. Synergy and Compensation Between ECS and the Opioid System

#### 7.1.1. Synergistic Co-Activation Under Normal Physiological Status

In wild-type CFA inflammatory pain mice, EA at GB34/LR3 simultaneously activates opioid and ECS analgesic pathways. Separate administration of opioid antagonist naloxone or CB1R antagonist AM251 only partially weakens EA analgesia; combined co-blockade completely eliminates pain relief, confirming the two systems work in parallel to synergistically amplify acupuncture efficacy under normal conditions [1].

#### 7.1.2. Compensatory ECS Activation When Opioid Pathways Are Impaired

TRPM8 ion channel is required for acupuncture to mobilize the opioid system. In TRPM8 knockout mice or wild-type animals with TRPM8 pharmacological inhibition, EA cannot recruit opioid pathways and fully relies on ECS to produce analgesia. CB1 agonist WIN exerts stronger analgesic effects in TRPM8$^{-/-}$ mice, illustrating that ECS serves as a functional compensatory backup when opioid signaling is blocked [1].

#### 7.1.3. Fully Opioid-Independent OX1R--CB1R Analgesic Cascade

PC6 peripheral nerve stimulation initiates the hypothalamic orexin--vlPAG CB1R axis without any opioid participation: MNS-PC6 activates hypothalamic orexin neurons → orexin A release in vlPAG → OX1R activation → 2-AG synthesis → CB1R retrograde disinhibition of GABAergic transmission → enhanced descending pain inhibition. Naloxone cannot interfere with this pathway, which maintains full efficacy in morphine-tolerant animals and never induces analgesic tolerance, representing a breakthrough therapeutic target for opioid-refractory chronic pain [2,12].

### 7.2. Regulatory Axis of CB1R--TRPV1

TRPV1 is a core nociceptive ion channel mediating inflammatory pain sensitization, whose expression and function are negatively controlled by CB1R across peripheral nerves, spinal cord and supraspinal PVN-ACC circuits.

In ICS fibromyalgia models, chronic stress suppresses CB1R expression while upregulating TRPV1 in DRG, spinal cord, PAG and hypothalamus to induce widespread mechanical and thermal hyperalgesia. EA elevates multi-region CB1 levels to restrain TRPV1 overexpression and overactivation. CB1R agonists mimic EA-induced pain relief, while CB1R antagonists abolish EA's inhibitory effect on TRPV1. TRPV1 knockout mice display baseline pain relief without EA intervention, which directly verifies that EA alleviates fibromyalgia hypersensitivity mainly through CB1-mediated TRPV1 suppression.

The PVN acts as the upstream master switch of this regulatory axis. Chemogenetic activation of PVN inhibits global CB1 expression and boosts TRPV1 signaling to trigger pain-like behaviors; targeted inhibition of PVN reverses this process, upregulating CB1 and downregulating TRPV1 to ease hyperalgesia. The complete PVN--CB1--TRPV1 cascade forms a stress-sensitive pain regulatory loop, which is the key molecular pathway through which EA relieves stress-induced chronic pain such as fibromyalgia [28].

## 8. Research Limitations, Future Directions and Translational Prospects

### 8.1. Existing Limitations of Current Preclinical Evidence

1. All eligible studies included in this review are animal and in vitro preclinical experiments, with several prominent limitations restricting clinical transformation:

2. Species difference of ECS expression: CB1/CB2/GPR55 distribution, ligand metabolic levels and downstream signaling have notable discrepancies between rodents and humans; conclusions drawn from mice/rats cannot be directly replicated in human tissue.

3. Lack of standardized acupuncture protocols: Stimulation frequency, current intensity, acupoint combinations and intervention courses vary widely across different research teams, leading to poor reproducibility of experimental results and difficulty in horizontal comparison.

4. Insufficient stratified research: Few studies explore gender, age, menstrual cycle differences in ECS response; aging, menopause-related ECS remodeling remains poorly characterized.

5. Limitations of molecular tools: Highly selective, cell-specific CB2 agonists and FAAH/MAGL inhibitors with low systemic toxicity are still scarce; most pharmacological reagents have off-target effects that interfere with result interpretation.

6. Short-term observation only: The majority of experiments adopt short intervention cycles (days to weeks); long-term safety and steady-state changes of ECS after repeated acupuncture are rarely reported.

7. Ignored atypical cannabinoid receptors: Research on GPR55 is still preliminary, and its tissue-specific functions and crosstalk with CB1/CB2 remain unclear.

### 8.2. Basic Research Future Directions

1. Cell-targeted mechanism exploration: Utilize cell-specific conditional knockout mice to separately clarify GABAergic/glutamatergic CB1 functions in vlPAG, RVM, ACC, vHPC, to fully dissect cell-type differentiated regulatory effects.

2. Atypical receptor research: Deeply uncover GPR55's post-transcriptional regulatory mode in the hippocampus, and develop GPR55 selective agonists/antagonists to verify its independent anti-depression pathway.

3. Sex and age stratification experiments: Compare ECS signaling differences between male/female, young/aged animals, to reveal personalized regulatory patterns of acupuncture.

4. Long-term intervention observation: Set long-course EA groups to record lasting ECS homeostasis alterations and potential adverse reactions after repeated stimulation.

5. Multi-omics integrated analysis: Combine transcriptomics, proteomics and lipidomics to systematically map dynamic changes of AEA, 2-AG, receptor and metabolic enzymes under acupuncture intervention.

### 8.3. Pharmaceutical Development & Acupuncture Regimen Optimization

1. Drug development priority: Design tissue-restricted CB2 agonists and peripheral FAAH/MAGL inhibitors that cannot cross the blood-brain barrier, avoiding central psychotropic side effects caused by CB1 activation.

2. Precise acupuncture scheme design:

3. Inflammatory skin, joint and intestinal diseases: Prioritize acupoints activating peripheral CB2 to suppress local inflammation.

4. Opioid-resistant neuropathic pain: Adopt low-frequency PC6 electrostimulation to trigger OX1R--CB1 non-opioid analgesic pathway.

5. Fibromyalgia and stress pain: Combine limb and scalp acupoints to simultaneously upregulate PVN-ACC CB1 and inhibit TRPV1.

6. Depression/anxiety: Select hippocampus/mPFC-regulating acupoints to target CB2, GPR55 and glutamatergic CB1.

7. Combined therapy strategy: Low-dose ECS-targeted medicines plus standardized acupuncture, to lower drug dosage and reduce single-agent side effects while maintaining curative effect.

### 8.4. Clinical Transformation Research Outlook

1. Identify peripheral ECS biomarkers: Detect serum AEA, 2-AG, FAAH and peripheral blood mononuclear cell CB2 expression to realize objective typing of chronic pain, depression patients and predict acupuncture efficacy.

2. Launch large-sample randomized controlled trials (RCTs): Stratify patients by pain type, medication history (opioid use/non-use), test optimized acupuncture regimens, and evaluate long-term safety, recurrence rate and quality of life improvement.

3. Develop cell-targeted neuromodulation equipment: Improve electroacupuncture instruments with adjustable frequency and current targeting peripheral immune nerve or descending pain brain circuits.

4. Establish translational research chains: Link animal mechanistic findings to clinical biomarker testing and patient cohort verification, shorten the cycle from preclinical discovery to clinical application.

## 9. Core Conclusion of this Review

Over the three years from 2023 to 2026, abundant preclinical studies have systematically clarified the multi-layer ECS mechanisms behind manual and electroacupuncture treatment. As a whole regulatory network spanning peripheral immune tissue, spinal nociceptive relay and supraspinal emotional/pain-modulating brain regions, ECS exerts pleiotropic therapeutic effects through cell-type-specific CB1/CB2/GPR55 signaling, coordinated metabolic enzyme regulation, and dynamic crosstalk with opioid, orexin and TRPV1 pathways.

The ECS not only mediates acupuncture's analgesic and antipruritic effects across diverse pain states (inflammatory, neuropathic, and fibromyalgia) but also participates in alleviating anxiety, depression, and disc degeneration through independent molecular mechanisms. The fully opioid-independent OX1R–CB1 axis discovered in recent studies represents a paradigm-shifting answer to the opioid crisis and opioid-resistant chronic pain.

Current research still faces obstacles including species gaps, non-standardized acupuncture protocols and insufficient clinical evidence. Further interdisciplinary integration of molecular biology, neuroimaging and clinical acupuncture research is required to translate ECS mechanistic discoveries into standardized acupuncture regimens and safe, selective ECS-targeted drugs, so as to promote the wider integration of traditional acupuncture into modern precision pain and neuropsychiatric disease management systems.

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