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Acupuncture Ameliorates Depressive-Like Behaviors Via SCFA-Mediated TLR4 Signaling in the Gut-Brain Axis

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Abstract. Objective: To explore the possible mechanisms by which acupuncture improves depressive-like behaviors induced by chronic unpredictable mild stress (CUMS). Methods: Forty male C57BL/6J mice were randomly divided into Control, CUMS, CUMS + Acu, and CUMS + Acu + TAK-242 groups. Except for the Control group, the other groups received 4-week CUMS combined with isolation modeling; acupuncture was performed at Baihui, Yinzhang, and bilateral Zusanli points, with continuous intervention using 2 Hz/15 Hz sparse and dense waves; the TAK-242 group was given a TLR4-specific inhibitor in addition to acupuncture. Depression-like phenotypes were evaluated through sugar water preference test, forced swimming test, and tail suspension test. Short-chain fatty acids (SCFAs) in feces, inflammatory factors in the hippocampus, tight junction proteins of the intestinal barrier, and TLR4/NF-κB pathway proteins were detected using GC-MS, ELISA, and Western blot. Results: CUMS significantly reduced the sucrose preference rate, weight gain, acetic acid/propionic acid/butyric acid, and ZO-1/Occludin expression, and increased immobility time, TNF-α, IL-1β, IL-6, TLR4, and p-NF-κB levels. Acupuncture could systematically reverse these changes; after combined with TAK-242, behavioral and neuroinflammatory improvements were further enhanced, while SCFAs did not show corresponding upstream superposition. Conclusion: Acupuncture may improve depressive-like behaviors induced by CUMS by restoring the metabolic ecological niche of SCFAs, repairing the intestinal barrier, and inhibiting the amplification loop of TLR4/NF-κB inflammation in the hippocampus.

Keywords: Acupuncture, Depression-like behavior, Gut-brain axis, Short-chain fatty acids, TLR4/NF-κB

1. Introduction

Depression is not a linear lesion caused by a single deficiency of neurotransmitters. Instead, it is a systemic pathological state resulting from the interweaving of stress, disrupted intestinal ecology, barrier damage, immune activation, and impaired central plasticity. The gut-brain axis research indicates that the intestinal microbiota and its metabolites can regulate the inflammatory homeostasis in the brain through multiple pathways [1, 2]. Among them, short-chain fatty acids (SCFAs) are the core hub connecting the intestinal ecology and emotional behaviors [3, 4].

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Chronic stress reduces the abundance of SCFAs-producing microbiota, disrupts intestinal tight junctions, and promotes the entry of intestinal-origin inflammatory signals into the bloodstream and amplification through the TLR4/MyD88/NF-κB axis, thereby interfering with hippocampal neurogenesis and synaptic plasticity [5-10]. Although acupuncture has the characteristics of multi-target network regulation and shows anti-inflammatory potential in depression models [11-15], previous studies lack a continuous evidence chain regarding the mediation of TLR4 signaling by SCFAs. Using a CUMS mouse model and the TLR4 inhibitor TAK-242, this paper investigated the multi-level mechanisms underlying the antidepressant-like effects of acupuncture.

2. Materials and methods

2.1. Animals and grouping

In this experiment, 40 healthy male C57BL/6J mice aged 6-8 weeks and weighing 20-25 grams were purchased from Beijing Weitai Genomics Life Sciences Co., Ltd.

After the adaptation period, the mice were randomly divided into 4 groups, with n = 10 in each group: the control group (Control), the model group (CUMS), the acupuncture group (CUMS + Acu), and the mechanism reversal group (CUMS + Acu + TAK-242). Except for the control group, the other groups were modeled by combining CUMS with solitary housing for 4 weeks.

2.2. Intervention protocol

The CUMS + Acu group received acupuncture intervention 1 hour before each model establishment. The Baihui, Yinzhang, and bilateral Zusanli acupoints were selected, and they were disinfected routinely. Then, a sterile needle of 0.16 × 13 mm was inserted and connected to the electro-acupuncture device, with a frequency setting of sparse wave at 2 Hz and dense wave at 15 Hz. Each acupuncture treatment lasted for 20 minutes, once a day, for 4 consecutive weeks.

The CUMS + Acu + TAK-242 group received weekly intraperitoneal injections of the TLR4-specific inhibitor TAK-242 twice a week at a dose of 3 mg/kg on top of the above acupuncture intervention.

2.3. Behavioral, biochemical and protein assays

Behavioral evaluations include the sucrose preference test (SPT), the forced swimming test (FST), and the tail suspension test (TST). The SPT is used to reflect the degree of anhedonia, while the FST and TST are used to assess the behavioral despair-like phenotype. The rate of weight change is used to supplement the observation of the overall physiological depletion caused by chronic stress.

The fecal SCFAs were quantified by gas chromatography-mass spectrometry (GC-MS), with a focus on detecting the levels of acetic acid, propionic acid, butyric acid, and total SCFAs. The hippocampal TNF-α, IL-1β, and IL-6 were detected by ELISA. The colon ZO-1, Occludin, and hippocampal TLR4, p-NF-κB/NF-κB were analyzed by Western blot, with GAPDH or total NF-κB as the internal reference.

2.4. Statistical analysis and quantitative formulas

The data are presented as Mean ± SD. In this study, standardized effect index and pathway inhibition rate were introduced for comprehensive interpretation. The formulas are as follows:

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$$SPT\,(\%) = V_{sucrose} / \left(V_{sucrose} + V_{water}\right) \times 100\% \tag{1}$$

$$\Delta BW\,(\%) = \left(BW_{end} - BW_{baseline}\right) / BW_{baseline} \times 100\% \tag{2}$$

$$R_{inhibition}\,(\%) = \left(X_{CUMS} - X_{treatment}\right) / \left(X_{CUMS} - X_{Control}\right) \times 100\% \tag{3}$$

Among them, Vsucrose represents the sugar water intake, Vwater represents the water intake, BW indicates body weight, and X can represent the relative expression levels of inflammatory factors or pathway proteins. This formula is used to assist in explaining the extent to which acupuncture and TAK-242 reduce the abnormal elevated indicators, rather than replacing the original statistical tests.

3. Results

3.1. Acupuncture improved CUMS-induced depression-like behaviors

After the CUMS model was established, as shown in Table 1, chronic stress had resulted in a stable phenotype of anhedonia, behavioral despair, and overall exhaustion. After acupuncture intervention, the sucrose preference rate increased to 74.5 ± 5.5%, the immobility time significantly shortened, and body weight partially recovered. When combined with TAK-242, the behavioral improvement was further enhanced, indicating that the inhibition of TLR4 signaling could continue to reduce the inflammatory-driven behavioral impairments on the basis of acupuncture.

Table 1. Behavioral assessment and body weight change among groups

Experimental Group	Preference Rate for Sweet Water (%)	Stationary Time in FST (s)	Stationary Time in TST (s)	Weight Change Rate (%)
Control	86.4 ± 4.2	82.5 ± 12.4	75.3 ± 10.8	15.2 ± 1.8
CUMS	52.1 ± 6.8$^{###}$	178.6 ± 21.5$^{###}$	165.4 ± 18.2$^{###}$	-4.5 ± 2.1$^{###}$
CUMS+Acu	74.5 ± 5.5***	115.2 ± 15.3***	108.6 ± 14.5***	8.6 ± 1.5***
CUMS+Acu+TAK-242	81.2 ± 4.8$^{\&}$	92.4 ± 11.6$^{\&}$	88.5 ± 12.1$^{\&}$	11.4 ± 1.2$^{\&}$

Note: Mean ± SD; Compared with the Control group, ###p<0.001; Compared with the CUMS group, ***p<0.001; Compared with the CUMS + Acu group, &p<0.05.

3.2. Acupuncture restored fecal SCFAs depleted by chronic stress

As shown in Table 2 and Figure 1, the SCFAs results indicate that chronic stress significantly reduced the levels of acetic acid, propionic acid, butyric acid, and total short-chain fatty acids. This suggests that chronic stress not only alters emotional behaviors but also simultaneously weakens the anti-inflammatory foundation of intestinal fermentation metabolism. This is consistent with reports on the simultaneous occurrence of short-chain fatty acid disorders and inflammatory processes in stress-susceptible animals [7, 8].

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Table 2. Fecal short-chain fatty acid concentrations quantified by GC-MS

Experimental Group	Acetic Acid (μmol/g)	Caproic Acid (μmol/g)	Butyric Acid (μmol/g)	Total SCFAs (μmol/g)
Control	42.5 ± 3.8	18.2 ± 2.1	22.4 ± 2.5	83.1 ± 6.5
CUMS	25.4 ± 2.9$^{###}$	10.5 ± 1.4$^{###}$	8.6 ± 1.2$^{###}$	44.5 ± 4.8$^{###}$
CUMS+Acu	36.8 ± 3.2***	15.8 ± 1.8***	18.5 ± 1.9***	71.1 ± 5.2***
CUMS+Acu+TAK-242	37.5 ± 3.5	16.1 ± 1.5	19.2 ± 2.1	72.8 ± 5.8

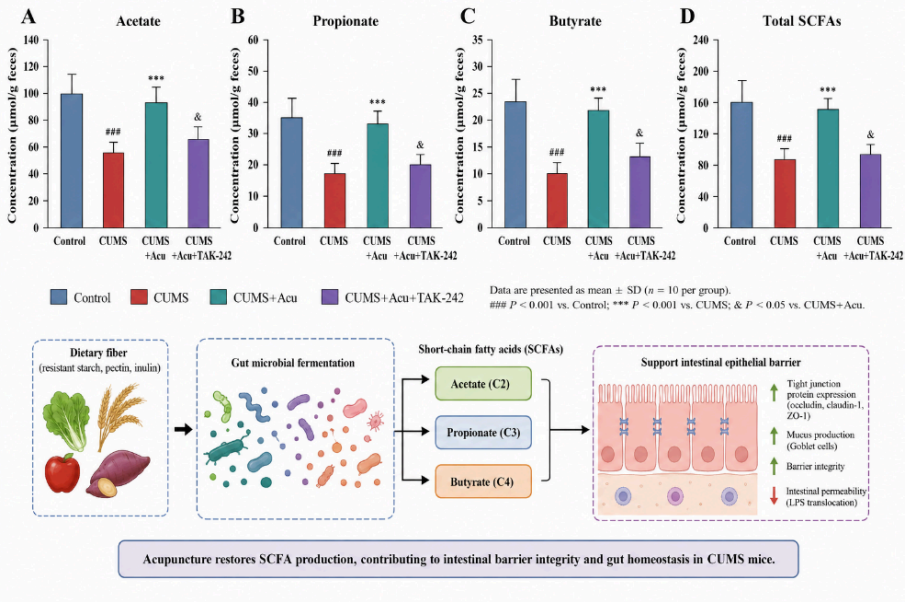


Figure 1. SCFAs reconstruction profile induced by acupuncture

3.3. Acupuncture suppressed hippocampal pro-inflammatory cytokines

As shown in Table 3 and Figure 2, the detection of hippocampal inflammatory factors indicated that in the CUMS group, TNF-α, IL-1β, and IL-6 rose to 112.5 ± 10.8, 95.6 ± 8.4, and 86.4 ± 7.5 pg/mg protein respectively, suggesting that peripheral stress and intestinal inflammatory load might have transformed into a central inflammatory phenotype. The above factors in the acupuncture group decreased significantly; after combined with TAK-242, they further approached the levels of the Control group. This result supports the key relay role of the TLR4 signal in the anti-inflammatory effect of acupuncture at the functional level.

Table 3. Hippocampal pro-inflammatory cytokine levels measured by ELISA

Experimental group division	TNF-α(pg/mg protein)	IL-1β(pg/mg protein)	IL-6(pg/mg protein)
Control	35.6 ± 4.2	28.4 ± 3.5	22.1 ± 2.8
CUMS	112.5 ± 10.8$^{###}$	95.6 ± 8.4$^{###}$	86.4 ± 7.5$^{###}$
CUMS+Acu	65.4 ± 6.5***	54.2 ± 5.1***	48.5 ± 4.2***
CUMS+Acu+TAK-242	42.1 ± 5.2$^{\&\&}$	35.8 ± 4.5$^{\&\&}$	31.2 ± 3.6$^{\&\&}$

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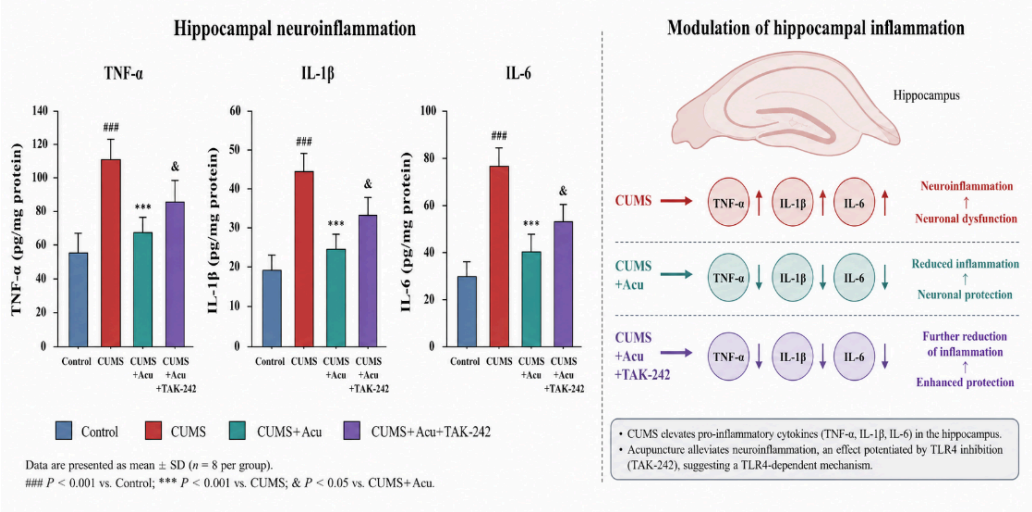


Figure 2. Hippocampal inflammatory cytokine network after CUMS and acupuncture

3.4. Acupuncture repaired intestinal barrier and inhibited hippocampal TLR4/NF-κB signaling

The Western blot results (as shown in Table 4) indicate that CUMS significantly reduces the expression of ZO-1 and Occludin in the colon, while it significantly increases TLR4/GAPDH and p-NF-κB in the hippocampus. This suggests that the disruption of the intestinal barrier is consistent with the activation of the central innate immunity [5, 9]. This result, along with the SCFAs table, indicates that the upstream advantage of acupuncture lies in restoring the homeostasis of the intestinal metabolic barrier, while the advantage of TAK-242 lies in blocking the downstream amplification effect of inflammation [10].

Table 4. Relative protein expression of intestinal barrier markers and hippocampal TLR4/NF-κB signaling

Experimental groups	Colonic ZO-1/GAPDH	Colonic Occludin/GAPDH	Hippocampal TLR4/GAPDH	Hippocampal p-NF-κB/NF-κB
Control	1.15 ± 0.08	1.12 ± 0.10	0.32 ± 0.05	0.28 ± 0.04
CUMS	0.42 ± 0.05$^{###}$	0.48 ± 0.06$^{###}$	1.25 ± 0.12$^{###}$	0.95 ± 0.09$^{###}$
CUMS+Acu	0.88 ± 0.07***	0.92 ± 0.08***	0.68 ± 0.08***	0.52 ± 0.06***
CUMS+Acu+TAK-242	0.95 ± 0.06	0.98 ± 0.07	0.38 ± 0.04$^{\&\&}$	0.31 ± 0.03$^{\&\&}$

4. Discussion

Acupuncture alleviating depressive-like behaviors induced by chronic unpredictable mild stress (CUMS) is a cascading regulatory process centered on "metabolic ecological reconstruction - barrier function repair - inflammation pathway inhibition", which can simultaneously correct the behavioral and metabolic abnormalities caused by CUMS and has the attribute of system homeostasis reconstruction [6-8, 11, 12]. The reduction of short-chain fatty acids (SCFAs) is the key upstream evidence of this mechanism chain. Acupuncture restores the levels of SCFAs and repairs the tight junction proteins in the intestine, blocking the central transmission of intestinal-origin inflammation

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through the TLR4/NF-κB pathway, rather than simply inhibiting a single inflammatory factor [1-5, 9, 11, 13]. Although the combined intervention of TAK-242 can further improve behavioral and inflammatory indicators, it does not affect the accumulation of SCFAs, confirming that TLR4 mainly acts on the downstream amplification loop of the inflammatory pathway, while acupuncture is located at a more upstream position in the "SCFAs - barrier - TLR4" axis [10, 14, 15]. This study still has limitations, lacking microbiome evidence such as 16S rRNA sequencing and experiments such as gene knockout and microbiota transplantation to clarify the specific source and causal relationship of SCFAs changes.

5. Conclusion

This article's conclusion reveals that CUMS can induce typical depressive-like behaviors in mice, accompanied by a decrease in fecal SCFAs, damage to intestinal tight junction proteins, activation of hippocampal TLR4/NF-κB signaling, and an increase in pro-inflammatory cytokines. Acupuncture can achieve consistent improvements at the levels of behavior, metabolism, barrier, and inflammatory signaling. Its mechanism of action may be manifested as follows: by restoring the metabolic niche of intestinal SCFAs, enhancing the barrier integrity mediated by ZO-1 and Occludin, thereby reducing the intensity of intestinal-origin inflammation transmitted to the hippocampal TLR4/NF-κB pathway, and ultimately improving depressive-like behaviors. The additive effect of TAK-242 further suggests that TLR4 is the explanatory downstream inflammatory hub in this mechanism chain.

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