Journal of Inflammation Research

Dovepress
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ORIGINAL RESEARCH

The Effect of Acupuncture on the Morphology and Neural Coding Damage of the Central Amygdala in Mice with Chronic Inflammatory Pain and Depression

Cui Ma*, Ren-Zhen Zhang*, Hong-Yu Lu, Ting-Ting Dou, Li Li, Xing-Ke Yan

School of Acupuncture-Moxibustion and Tuina, Gansu University of Traditional Chinese Medicine, Lanzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xing-Ke Yan, Acupuncture and moxibustion and Massage College of Gansu University of Traditional Chinese Medicine, 35 Dingxi East Road, Lanzhou, People's Republic of China, Email yanxingke@126.com

Purpose: Observing the effects and roles of acupuncture on the morphology and neural coding damage of central amygdala (CeA) neurons in chronic inflammatory pain with depression (CIPD) mice and exploring the central nervous mechanism of acupuncture intervention in CIPD.

Methods: A CIPD model was established by injecting Complete Freund's Adjuvant (CFA) into the left hind foot. Using paw withdrawal latency (PWLs), forced swimming, and open field tests, 40 mice with successfully replicated models were selected and randomly divided into a model group, acupuncture group, and sham acupuncture group, with 12 mice in each group. After treatment, Nissl staining was used to observe the morphology of CeA neurons and the number of Nissl bodies. In vivo multi-channel recordings measured the spontaneous firing frequency, waveform amplitude, inter-spike interval (ISI), and power spectral density (PSD) of CeA neurons.

Results: The PWLs in the model and sham acupuncture groups were shortened, the activity time and distance in the central region were reduced, and the forced swimming immobility time increased. The arrangement of CeA neurons is sparse, neurons are damaged, and the number of Nissl bodies is reduced; CeA neurons exhibit abnormal changes in spatiotemporal patterns, with a decrease in spontaneous discharge frequency, discharge amplitude, PSD, and an increase in ISI. The acupuncture group showed prolonged PWLs, increased activity time and distance in the central region, and decreased immobility during forced swimming. The loss of CeA neurons decreased, the cells were arranged neatly, the nucleoli were evident, and the number of Nissl bodies increased. The damage to CeA neural coding was significantly improved, with increased spontaneous discharge frequency, discharge amplitude, PSD, and shortened ISI.

Conclusion: Acupuncture may alleviate pain and depressive-like behaviors in CIPD mice and reduce neuronal damage, possibly through mechanisms related to improving the spatiotemporal characteristics of neural coding impairments in the CeA brain region.

Keywords: acupuncture, chronic inflammatory pain with depression, central amygdala, neural coding

Introduction

Chronic pain is defined as pain lasting for more than three months. Internationally, 20% of adults (aged 18–65 years) and ≥33% of older adults (>65 years) experience chronic pain.[1] Chronic pain is often accompanied by depressive moods, with a high comorbidity between the two, which is one of the major factors influencing pain treatment efficacy in clinical settings.[2] Studies show that approximately 40% to 60% of patients with chronic pain exhibit significant depressive symptoms, and depression can increase pain sensitivity, creating a vicious cycle.[3] Clinically, chronic inflammatory pain often coexists with depression,[4] and treatment usually involves a combination of analgesics and antidepressants. While these can somewhat alleviate pain symptoms and improve depressive moods, they also come with drawbacks, such as drug dependence and severe gastrointestinal side effects.[5] Acupuncture offers a multidimensional regulatory advantage for treating Chronic Inflammatory Pain with Depression (CIPD), significantly relieving pain symptoms and improving depressive moods.[6] However, the central nervous electrophysiological mechanisms underlying this treatment remain incompletely understood.

Received: 16 October 2024
Accepted: 18 February 2025
Published: 6 March 2025

The amygdala (AMY) plays a crucial role in regulating pain and mental disorders such as depression and anxiety. Within the amygdala, the central amygdala (CeA), often referred to as the "nociceptive AMY", is the primary subnucleus responsible for AMY's information output.[7] It possesses substantial sensory and emotional dimensions, participating in pain modulation, analgesia, and emotional processing.[8] The process by which single or clustered neurons transmit and express information through discrete action potentials and firing sequences is known as neural coding, which is essential for neuronal function.[9] Studies have shown that neurons in mice with chronic inflammatory pain exhibit abnormal firing patterns, potentially impairing their neural information encoding capabilities.[10,11] The multi-channel neuronal microelectrode array (M-NEMEA) technology is an extracellular recording technique that can simultaneously record and observe the firing activity of neuron clusters in specific brain regions of awake, freely moving, or anesthetized animals.[12,13] The electrical activity of neuronal clusters can be represented by parameters such as firing frequency, waveform amplitude, inter-spike interval (ISI), and power spectral density (PSD), which reflect the spatiotemporal patterns of neural coding within complex biological networks.[14] Therefore, this study employs M-NEMEA technology to observe abnormal spatiotemporal patterns and changes in neural coding in the CeA of CIPD mice from frequency and temporal encoding perspectives. This investigation aims to reveal the electrophysiological mechanisms underlying neuronal pathology in the CeA of CIPD mice. Additionally, it explores the impact of acupuncture on changes in neural coding within the CeA of CIPD mice, providing a basis for acupuncture effect studies and clinical applications.

Material and Methods

Experimental Animals

Fifty-two healthy male C57BL/6J mice aged 8–10 weeks, with a body weight of 20–25 g, provided by Beijing Sibeifu Biotechnology Co., Ltd. [SPF grade, license number: SCXK (Jing) 2019–0010]. The mice were housed in separate cages in the school's SPF laboratory, with five mice per cage, and fed and drank water at regular intervals (ethical approval number: SY2023-785). The disposal of the animals throughout the experiment was by the Guiding Opinions on Treating Experimental Animals Well issued by the Ministry of Science and Technology in 2006.[15]

Main Reagents and Instruments

① Reagent: Isoflurane (20231004, Ruipu Tianjin Biopharmaceutical Co., Ltd.); Complete Freund's adjuvant (SLCF1289, Sigma company); Nissl dye solution (G1036, Wuhan Saiweier Biotechnology Co., Ltd.); ② Instrument: Huacheng brand disposable sterile acupuncture and moxibustion needle (0.18mm×13mm, Beijing Keyuanda Medical Supplies Factory); Smart 3.0 video analysis software (Smart 3.0, Shenzhen Ruiwode Life Technology Co., Ltd.); Upright optical microscope (Nikon Eclipse E100, Nikon, Japan); UGO Foot Pain Gauge (37370–001, Ugo Basile, Italy); Multi-channel neural signal recording system (Cerebus, Blackrock Microsystem Inc); Desktop digital brain stereotaxic device (68025, Shenzhen Ruiwode Life Technology Co., Ltd.); Handheld miniature cranial drill (78001, Shenzhen Ruiwode Life Technology Co., Ltd.); 2×8-channel microfilament array electrode (CWMA-2S8, Beijing Kesheng Technology Co., Ltd.).

Establishment of Chronic Inflammatory Pain with Depression Model

After 7 days of adaptive feeding, 12 C57BL/6J mice (n = 52) were randomly selected as a blank group. The remaining 40 mice were injected with complete Freund's adjuvant (CFA) into the left hind foot to replicate the CIPD model:[16,17] The mice were fixed, and the skin of the left hind foot was disinfected with iodine cotton balls. A 1mL syringe was used to extract 50 μL of CFA, and the weakest point of the mouse's left hind foot palm was located. The needle was inserted into the palm at a 45° angle to the skin, and the CFA solution was slowly injected. After the needle is removed, immediately press the needle hole with a dry cotton ball for a moment, place it in a supine position for 3 minutes, and then release the mouse to prevent drug leakage. After 7 days, repeat the injection of 10 μL CFA. Forty-eight hours after the first injection, the mouse model foot showed apparent redness and swelling, and the pain threshold decreased significantly. After 14 d, compared with the blank group, the mice in the modeling group showed depression-like behaviors of increased despair. They decreased exploratory behaviors, such as increased time of forced swimming immobility, decreased time of activity in the central area of the open field, and decreased percentage of the distance of activity in the central area ($P < 0.05$), and

so on, which were regarded as the success of modeling.[18] Thirty-six mice with successfully replicated models were selected and randomly divided into a model group, an acupuncture group, and a sham acupuncture group using a random number table method, with 12 mice in each group.

Intervention Methods

Intervene the day after the model evaluation is completed. Acupuncture group: Based on the relevant literature, acupuncture treatment for chronic inflammatory pain with depression primarily involves the use of acupoints such as "Zusanli (ST36)", "Sanyinjiao (SP6)", "Hegu (LI4)", and "Taichong (LR3)".[17,19] Therefore, this study selected these four acupoints for acupuncture intervention. The acupoint location was referred to in the "Names and Location of Common Acupoints for Experimental Animals Part 3: Mice T/CAAM 0002–2020" issued by the Chinese Acupuncture and Moxibustion Society in 2020.[20] By calculating the ratio of human bones to rat bones, the bone size of rats is estimated to select acupoints. "Zusanli (ST36)" refers to the posterior lateral side of the knee joint, about 2mm below the fibular head; San Yin Jiao (SP6): about 5mm above the tip of the inner ankle of the hind limb; Hegu (LI4): Between the first and second metacarpal bones of the forelimb; Taichong (LR3): The depression between the first and second metatarsal bones on the back of the hind limb. Operation: In the awake state of the mouse, fix it in a prone position with a self-made fixture. Conventional disinfection: "Zusanli" and "Sanyinjiao" needles should be inserted vertically for 2–3mm, "Hegu" needles should be inserted diagonally at a depth of 1–2mm, and "Taichong" needles should be inserted diagonally at a 45° angle to the skin for 2–3mm; the above acupoints are all treated with the technique of tonifying and purging (twisting amplitude of 180°, frequency of 60–80 times/minute), with a duration of 3 minutes per acupoint once a day, and 7 days as one course of treatment, for a total of 2 courses of treatment.[17,18]

The sham acupuncture group: By consulting the literature and combining the analysis of sham acupuncture points in Experimental acupuncture and moxibustion,[21] four non-meridian and non-acupoint acupuncture points near the trunk tail root, 1–2cm near the trunk tail root, 1/3 in the middle of the tail, and the tip of the tail were selected, and acupuncture was carried out by the technique of flat tonicity and flat diarrhea (twisting amplitude of 180°, frequency of 60–80 times/min), and the operation of each point was for 3 min, once/d, and the course of 7 d was one course of treatment, and the treatment was carried out in a total of 2 courses of treatment. The blank and model groups were subjected to the same grasping and fixation as the acupuncture group without any other intervention.

Material Selection

After the behavioral experiment, samples were taken the next day. The mice were anesthetized by inhalation of isoflurane (airflow rate 300–500mL/min, concentration 1–2%), and the chest cavity was opened to expose the heart. The perfusion needle was inserted into the left ventricle, and the right atrial appendage was cut open. One hundred milliliters of 0.9% sodium chloride solution was used for flushing, and 50mL of 4% paraformaldehyde was used for perfusion. After the eyes, muscles, and liver turned white, the brain was quickly removed and soaked in 4% paraformaldehyde for 24 hours. Then, dehydration and paraffin embedding were performed, and slices (thickness of 5um) were made for Nissl staining.

Observation Indicators and Detection Methods

Paw Withdrawal Latency (PWL) Detection

Perform PWLs testing on day 1 before mold making, and on days 1, 7, 14, 21, and 28 after mold making. The mice were pre-adapted to the testing environment for 3 days, with the ambient temperature maintained at (24 ± 1)°C. During the testing, the mice were placed in a transparent plastic box on the testing platform and adapted for 1 hour before starting the experiment. Aim the emission center of the thermal radiation light source at the left hind foot of the mouse, start timing from the start of irradiation, and record the time when the hind limb is first lifted, ie PWLs.[22] Each mouse is tested three times with an interval of no less than 5 minutes. The maximum measurement value is 20 seconds to prevent injury caused by prolonged thermal radiation time. When the time that triggers the animal's foot contraction reflex decreases, it indicates it is experiencing thermal pain sensitization.

Depression Like Behavior Testing

(1) Open field experiment (OFT): Conduct open field experiments on the day after modeling and the day after intervention. Adopting a white inner wall, 50cm*50cm*50cm glue open box. Ensure a quiet experimental environment, place the mice in the central area at the bottom of the box, and use the SMART3.0 behavioral video analysis system to record the trajectory map, activity time, and total distance traveled by the mice in the central area. Calculate the total distance traveled in the open field, activity time in the central area and percentage of activity distance in the central area. After each mouse is tested, clean the open field experimental chamber with alcohol, and wait for the odor to dissipate before testing the next mouse.

(2) Forced swimming test (FST): The open field experiment was conducted on the day after its completion. Water at (23 ± 2)°C was poured into a transparent glass bucket, and the height of the water in the bucket should be such that the hind limbs of the mice could not touch the bottom of the bucket. After each mouse was placed steadily in the water, the SMART3.0 behavioral video analysis system was used to detect the activity time of the mice within 6 minutes, and the floating time of each mouse within 4 minutes was recorded. After each mouse test is completed, wipe it dry, and replace the water in the glass bucket to avoid residual odors affecting the test results of each mouse.

Observation of CeA Tissue Morphology by Nissl Staining

Paraffin sections were deparaffinized to water, stained with toluidine blue for 2–5 minutes, washed with water, transparent with xylene for 10 minutes, and sealed with neutral gum. Observe the number of neurons and Nissl bodies in the CeA brain area under a 400 × 400 optical microscope.

Observation of Hippocampal Tissue Morphology in Rats Using Nissl Staining

After routine dewaxing and hydration treatment, the sample was stained with 2% sulfur at 60 °C for 30 minutes. This was followed by color separation with 95% ethanol, dehydration with anhydrous ethanol, transparency with xylene, and sealing with neutral gum. The morphology of rat hippocampal neurons was observed using a ×400 optical microscope.

CeA Brain Region M-NEMEA Detection

(1) Detection method: ① Administer isoflurane inhalation anesthesia (airflow 300–500mL/min, concentration 1–2%) to mice and fix them on a stereotaxic brain locator. ② Trim the skull fur, bluntly separate the subcutaneous fascia with precision forceps, and disinfect with iodine to fully expose skull landmarks such as the anterior fontanelle and midline. Refer to the "Stereoscopic Localization Atlas of Mouse Brain"[23] to locate the CeA area (ML: −2.40mm; DV: −4.7mm; AP: −1.28mm;). ③ Hold a miniature skull drill and polish the skull until the dura mater blood vessels can be seen. Carefully open the skull with the tip of the syringe, peel off the dura mater, and expose the cerebral cortex. ④ Connect the 8-channel nickel chromium alloy microfilament electrode to the electrode holder and fix it on the brain stereotaxic device. Slowly lower the electrode, and after contacting the pia mater, quickly pierce the pia mater downwards and slowly enter the CeA by 4.7mm. ⑤ The Cerebus multi-channel electrophysiological neural signal acquisition system is used to collect the electrical activity of CeA neurons.

(2) Detection indicators: Import the data into Neuro Explorer neural signal analysis software to analysis of discharge frequency, discharge amplitude, ISI, PSD and other indicators.

Statistical Processing

The data were analyzed using SPSS 22.0 software and compiled into statistical graphs using GraphPad Prism 5.0 software. Quantitative data conforming to a normal distribution are expressed as mean ± standard deviation ($\bar{x} \pm S$), one-way ANOVA is used for inter-group comparisons, while the LSD test is used for pairwise comparisons. The difference is statistically significant with $P < 0.05$. The data collected by M-NEMEA is analyzed using NeuroExplorer neural data analysis software, which can perform online synchronization and offline data analysis.

Results

Acupuncture Can Significantly Alleviate Thermal Hyperalgesia in CIPD Mice

The experimental design process is shown in Figure 1A. The model replication and acupuncture treatment are shown in Figures 1B and C. Compared with the Control group, the PWLs of the Model group mice were significantly shortened at all time points ($P < 0.05$), indicating successful replication of chronic inflammatory pain. Compared with the Model group at the same time point, the PWLs in the AP group were significantly prolonged on days 21 and 28 ($P < 0.05$), indicating that acupuncture can alleviate thermal hyperalgesia in inflammatory pain mice. Compared with the AP group,

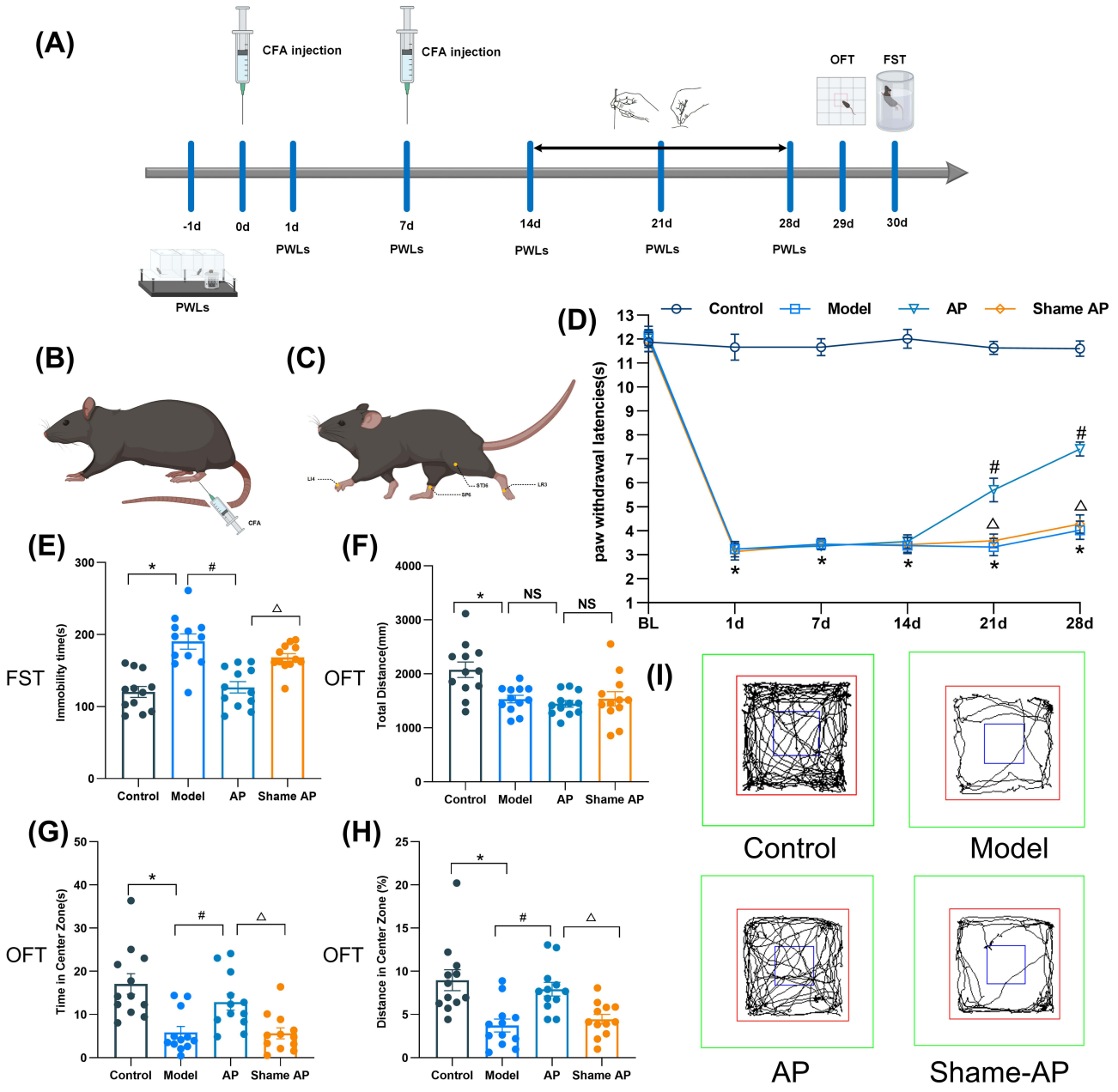

Figure 1 Acupuncture alleviates thermal nociceptive hypersensitivity and depressive-like behavior in CIPD mice. (**A**) Flow chart of the experiment. (**B**) Schematic diagram of CFA model replication. (**C**) Schematic diagram of acupuncture intervention. Acupuncture intervention points are Foot Sanli, Sanyinjiao, Hegu, and Taichong. (**D**) Comparison of PWLs among the four groups. (**E**) Comparison of FST immobilization time among the four groups. (**F**) Comparison of total OFT movement distance in 4 groups. (**G**) Comparison of OFT central region movement time. (**H**) Comparison of the percentage of the central region of OFT active distance in 4 groups of mice. (**I**) Representative movement trajectories in the OFT. Compared with the Control group, *P<0.05; with the Model group, #P<0.05; with the AP group, ΔP<0.05. NS indicates no significant difference. Data are presented as the mean ± SD, n=12 mice/group.

https://doi.org/10.2147/JIR.S501170

the PWLs of the Sham AP group were significantly shortened on days 21 and 28 ($P < 0.05$), indicating that sham acupuncture has no therapeutic effect on inflammatory pain (Figure 1D).

Acupuncture Can Improve Depressive Like Behavior Caused by Chronic Inflammatory Pain

Compared with the Control group, the Model group mice showed a significant increase in forced swimming immobility time ($P < 0.05$), a significant decrease in total open field distance, central area activity time, and percentage of central area activity distance ($P < 0.05$), indicating successful replication of the CIPD model. Compared with the Model group, the AP group showed a significant decrease in forced swimming immobility time ($P < 0.05$), a significant increase in central area activity time and percentage of central area activity distance ($P < 0.05$), and no statistically significant difference in total exercise distance ($P > 0.05$). This suggests that acupuncture can improve depression-like behavior in CIPD mice but has no significant effect on spontaneous activity. Compared with the AP group, the Sham AP group showed a significant increase in forced swimming immobility time ($P < 0.05$), a significant decrease in central area activity time and percentage of central area activity distance ($P < 0.05$), and no statistically significant difference in total exercise distance ($P > 0.05$) (Figure 1E–I).

Acupuncture Can Improve the Morphological Abnormalities of CeA Neurons in CIPD Mice

The structure of CeA neurons in the Control group is intact and arranged neatly, with round nuclei, apparent nucleoli, deeply stained cytoplasm, and many Nissl bodies. Compared with the Control group, the morphology of CeA neurons in the Model group was mostly vacuolar, with indistinct nucleoli and a reduced number of Nissl bodies. Compared with the Model group, the AP group had a more complete cell structure, clearer nucleoli, apparent cytoplasm, increased number of Nissl bodies, and reduced damage to CeA neurons. Compared with the AP group, the morphology of CeA neurons in the Sham AP group was mostly vacuolar, with indistinct nucleoli and a reduced number of Nissl bodies (Figure 2A).

Nissl staining was performed on CeA, and the results showed that compared with the Control group, the gray value of CeA integration in the Model group mice was significantly reduced ($P < 0.05$), indicating a decrease in the number of Nissl bodies in CIPD mice; compared with the Model group, the gray value of CeA score in the AP group mice significantly increased ($P < 0.05$), indicating an increase in the number of Nissl bodies in CIPD mice after acupuncture treatment; compared with the AP group, the gray value of CeA integration in the Sham AP group mice was significantly reduced (Figure 2B).

The Effect of Acupuncture on the Spatiotemporal Encoding Patterns of CeA Neurons

Acupuncture Can Regulate the Firing Frequency of CeA Neurons

Figures 3A and B show an in vivo multi-channel microelectrode array neural signal recording schematic diagrams. The self-discharge frequency of the Control group mice is mainly concentrated within 280 hz, with a high discharge frequency band and a continuous discharge pattern, and there is a significant burst discharge of neuronal intervals. The discharge frequency of the Model group mice is mainly concentrated within 100 hz, with the lowest discharge frequency band and apparent intermittent discharge. The discharge frequency of AP group mice is mainly concentrated within 260 hz, with a moderate discharge frequency band and a continuous discharge mode; The discharge frequency of Sham AP group mice is mainly concentrated within 150 hz, with a low discharge frequency band and a continuous discharge mode (Figure 3C–F).

The analysis of the firing frequency of neurons in each group of mice showed that compared with the Control group, the spontaneous firing frequency of CeA neurons in the Model group mice was significantly reduced ($P < 0.05$), indicating that the firing activity of CIPD mice was inhibited. Compared with the Model group, the spontaneous discharge frequency of CeA neurons in the AP group mice was significantly increased ($P < 0.05$), indicating that acupuncture can enhance the discharge activity of CIPD mice. Compared with the AP group, the CeA spontaneous

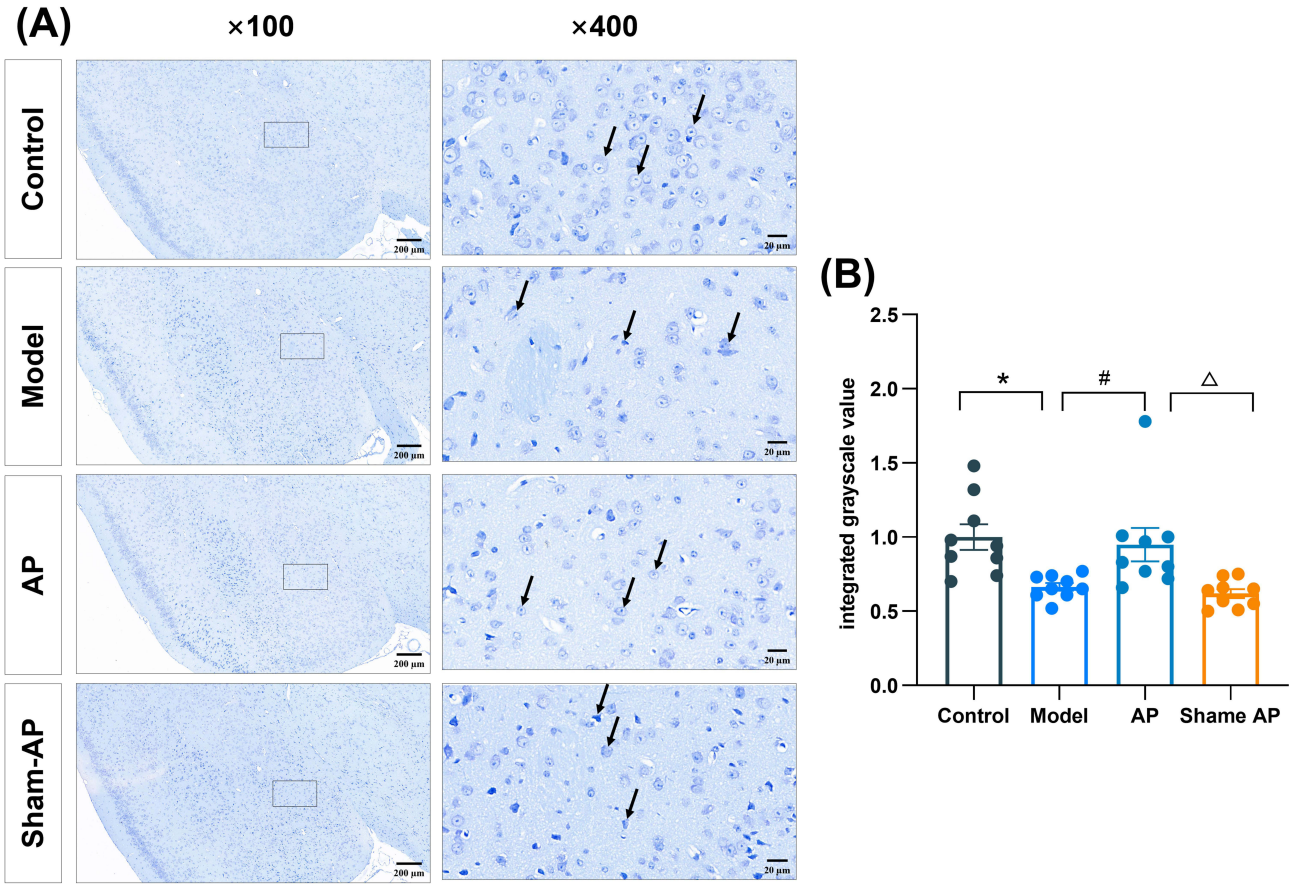

Figure 2 Effect of acupuncture on the pathologic morphology of CeA neurons in chronic inflammatory pain with depression mice. (**A**) Representative graphs of CeA Nysted staining in each group. (**B**) Gray scale values of CeA neuronal cell integral in each group. Compared with the Control group, *P<0.05; with the Model group, #P<0.05; with the AP group, ΔP<0.05. n=3 mice/group, three regions were collected from each Niehl's staining image for statistics.

emission frequency of Sham AP group mice was significantly reduced ($P < 0.05$), indicating that sham acupuncture had no significant effect on the spontaneous emission frequency of mice, consistent with the Model group (Figure 3G).

Acupuncture Can Regulate the Firing Amplitude of CeA Neurons

The shape of CeA firing neurons in Control mice shows regular bidirectional waves, closely following the mean waveform. Many firing neurons have large amplitude, indicating obvious action potential staging. The pattern of CeA firing neurons in Model mice shows bidirectional waves, with scattered waveforms and a significant degree of dispersion from the mean waveform. There are few firing neurons, the amplitude is the smallest, and the action potential staging is not apparent. The discharge neurons of CeA in AP mice exhibit bidirectional waves with a relatively regular waveform and a small degree of dispersion from the mean waveform. More discharge neurons with a small amplitude indicate precise action potential staging. The CeA firing neurons in Shame AP mice exhibit bidirectional waves, with scattered waveforms and a high degree of dispersion from the mean waveform. There are fewer firing neurons, smaller wave amplitudes, and unclear action potential staging (Figure 4A–D).

The analysis of the firing amplitude of neurons in each group of mice showed that compared with the Control group, the firing amplitude of CeA neurons in the Model group was significantly reduced ($P < 0.05$), indicating a decrease in firing intensity in CIPD mice; compared with the Model group, the firing amplitude of CeA neurons in the AP group mice was significantly increased ($P < 0.05$), indicating that acupuncture can enhance the firing intensity of CIPD mice; Compared with the AP group, the discharge amplitude of CeA in the Shame AP group mice was significantly reduced ($P < 0.05$), indicating that sham acupuncture had no significant effect on the discharge intensity of CIPD mice, consistent with the model group (Figure 4E).

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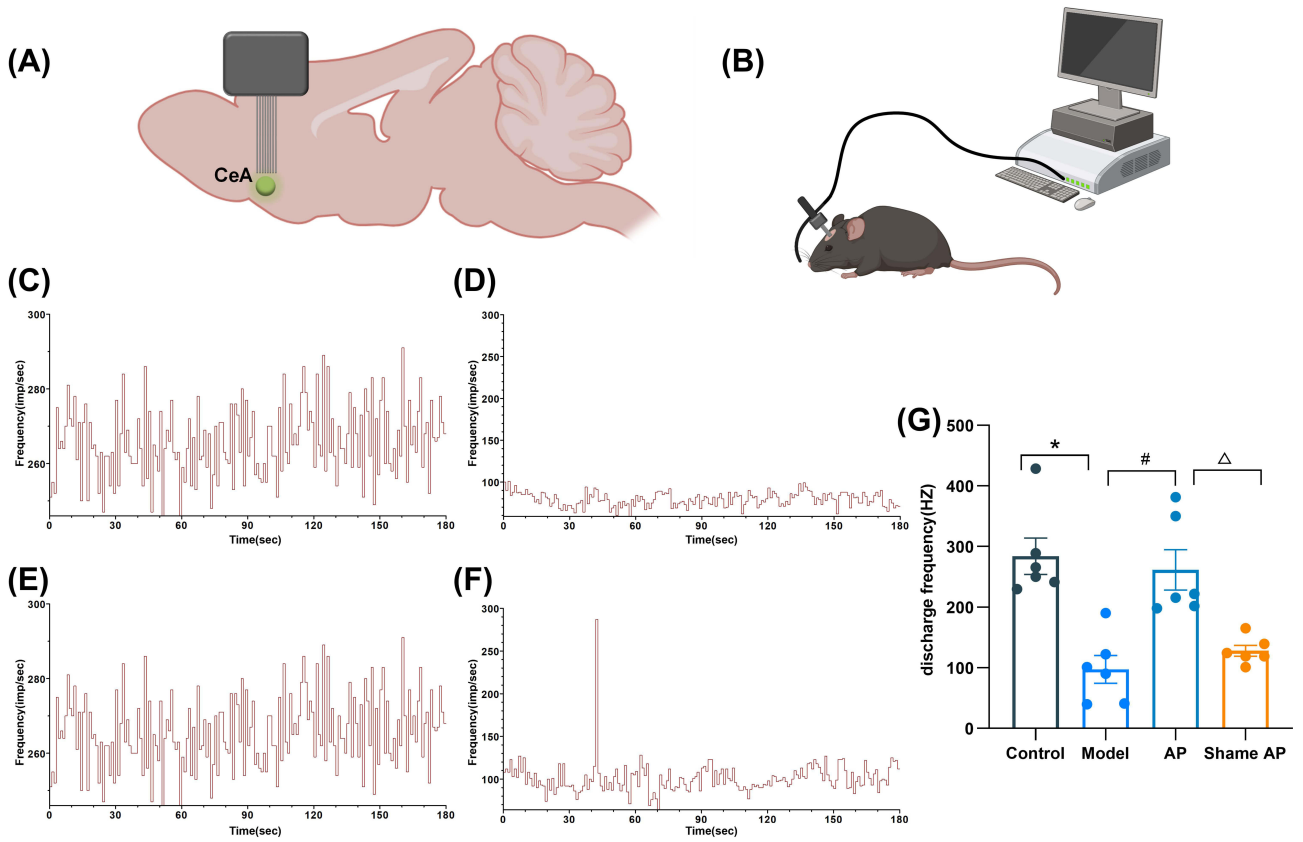

Figure 3 Effect of acupuncture on the firing frequency of CeA neurons: (**A-B**) Schematic diagrams of in vivo multi-channel microelectrode array neural signal recording. (**C**) Histogram of spontaneous firing frequency of CeA in Control group (bin=1 s). (**D**) Histogram of CeA spontaneous discharge frequency in Model group (bin=1 s). (**E**) Histogram of spontaneous discharge frequency of CeA in AP group (bin=1 s). (**F**) Histogram of CeA spontaneous discharge frequency in the Shame AP group (bin=1 s). The horizontal coordinate is the recording time of 180 s, and the vertical coordinate is the number of neurons discharged per second, ie, the discharge frequency. (**G**) Comparison of spontaneous discharge frequency of CeA neurons in 4 groups of mice. Compared with the Control group, *P < 0.05; with the Model group, #P < 0.05; with the AP group, ΔP < 0.05. Data are presented as the mean ± SD, n = 6 mice/group.

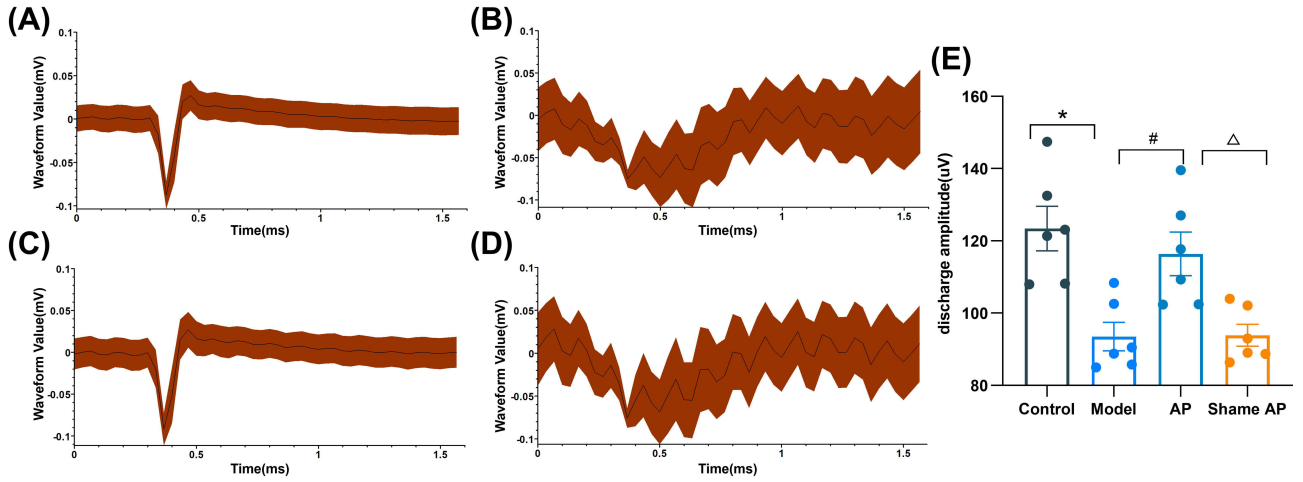

Figure 4 Effects of acupuncture on CeA neuron discharge amplitude: (**A**) CeA neuron discharge waveforms in the Control group; (**B**) CeA neuron discharge waveforms in the Model group; (**C**) CeA neuron discharge waveforms in the AP group; (**D**) CeA neuron discharge waveforms in Shame AP group; the red curves are the action potential waveforms, and the black line in between them is the mean waveform. (**E**) Spontaneous discharge amplitude of CeA neurons in 4 groups of mice. Compared with the Control group, *P<0.05; with the Model group, #P<0.05; with the AP group, ΔP<0.05. Data are presented as the mean ± SD, n = 6 mice/group.

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Acupuncture Can Regulate CeA Peak to Peak Interval (ISI)

The majority of ISI in the Control group mice was concentrated within 0.01 seconds, with dense ISI scatter points and a significantly decreasing distribution from 0 seconds on the vertical axis. The majority of ISI in the Model group mice was concentrated within 0.02 seconds, with sparse ISI scatter and the most miniature decreasing trend from 0 seconds on the vertical axis; most of the ISI in the AP group mice were concentrated within 0.01 seconds, and the ISI scatter points were too dense with a slight decreasing trend from 0 seconds on the vertical axis; most of the ISI in the Shame AP group mice was concentrated within 0.02 seconds, and the ISI scatter points were significantly stratified (Figure 5A–D).

The analysis of the firing amplitude of neurons in each group of mice showed that compared with Control, the ISI of spontaneous firing sequence of CeA neurons in Model group mice was significantly increased ($P < 0.05$), indicating that the firing time interval of CIPD mice was longer. Compared with the Model group, the ISI of CeA discharge sequence in AP group mice was significantly shortened ($P < 0.05$), indicating that acupuncture can shorten the discharge time interval of CIPD mice. Compared with the AP group, the ISI value of CeA discharge sequence in the Shame AP group was higher than that in the AP group, but there was no statistical difference ($P > 0.05$), indicating that the effect of sham acupuncture on shortening the discharge time interval was weaker than that of acupuncture, but it also had some effect (Figure 5E).

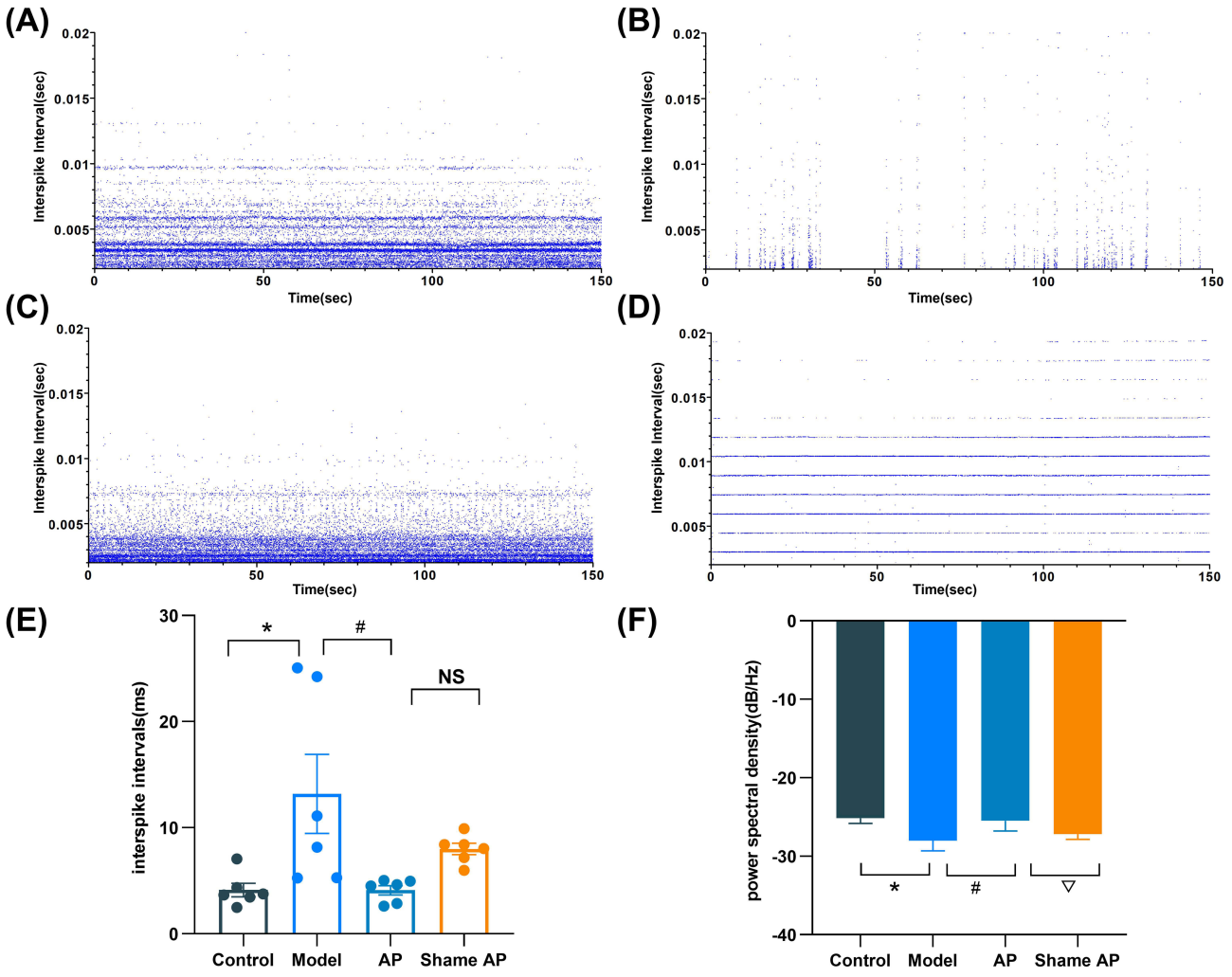

Figure 5 Effect of needling on the inter-peak period and power spectral density of CeA peaks: (**A**) ISI scatter plot of CeA spontaneous discharge in the Control group. (**B**) ISI scatter plot of CeA spontaneous discharge in Model group. (**C**) ISI scatter plot of CeA spontaneous discharge in AP group. (**D**) Shame AP group CeA spontaneous discharge ISI scatter plot. The horizontal coordinates indicate the time-discrete points, and the vertical coordinates indicate the pulse intervals at the corresponding time points. This can reflect the distribution pattern of the peak potential interval sequence over time, thus reflecting the change of neuronal action potential pattern over time. (**E**) ISI values of CeA brain regions. (**F**) CeA self-emitted electrical power spectral density. Note: Compared with the Control group, *P<0.05; compared with the Model group, #P<0.05; compared with the AP group, ΔP<0.05; NS indicates no significant difference. Data are presented as the mean ± SD, n=6 mice/group.

https://doi.org/10.2147/JIR.S501170

The Effect of Acupuncture on the Power Spectral Density of CeA

Figure 5F shows the inter-group comparison of PSD of CeA neurons in each group of mice in the frequency range of 0–100 hz. Compared with Control, the PSD of spontaneous discharge of CeA neurons in Model group mice was significantly reduced ($P < 0.05$), indicating a decrease in discharge power in CIPD mice. Compared with the Model group, the PSD of spontaneous discharge of CeA neurons in AP group mice was significantly increased ($P < 0.05$), indicating that acupuncture can improve the discharge power of CIPD mice. Compared with the AP group, the PSD of spontaneous discharge of CeA neurons in the Shame AP group mice was significantly reduced ($P < 0.05$), indicating that sham acupuncture had no significant effect on the discharge power of CIPD mice.

Discussion

Researches have shown that chronic inflammatory pain and depression have a high degree of comorbidity, such as knee osteoarthritis,[24] rheumatoid arthritis,[25] chronic pelvic inflammatory disease,[26] etc. Traditional Chinese Medicine has had discussions on the relationship between pain and depression since ancient times, such as ”seven emotions causing pain” and ”illness causing depression”, both of which show a close relationship between chronic pain and negative emotions. The ”Difficult Classic” records that ”the disharmony of meridians and qi leads to various pains”, the ”Spirit Pivot: Ben Shen” states that ”those who are worried have blocked qi and cannot function”, and the ”Jin Kui Yao Lue: Diagnosis and Treatment of Various Pain Diseases” mentions that ”pain leads to mental disorders”, all of which point out that ”stagnation of meridians and qi, and pain disturbing the brain and spirit” are the key pathological mechanisms of this disease. Based on this, the research team has condensed the basic treatment methods of ”unblocking collaterals and relieving pain, regulating qi and regulating spirit” through clinical experience and literature review,[27,28] and summarized the acupuncture method of ”relieving pain and regulating spirit” with ”Zusanli”, ”Sanyinjiao”, ”Hegu” and ”Taichong” as the group acupoints. The clinical treatment of inflammatory pain with depression has achieved good results,[29,30] with prominent clinical characteristics and advantages, and has multidimensional comprehensive effects in relieving inflammatory pain with depression.[17] ”Hegu” and Taichong” are collectively known as the four guan acupoints, which complement each other and have the effects of calming the mind, promoting blood circulation, and relieving pain. They are widely used in the treatment of chronic inflammatory pain and depression;[31,32] ”Zusanli” and ”Sanyinjiao” are important acupoints for the treatment of chronic inflammatory pain and depression. The two acupoints are matched to harmonize qi, blood, and clear meridians. Therefore, this study selected ”Zusanli”, Sanyinjiao, ”Hegu”, and ”Taichong” for acupuncture intervention.

Acupuncture has demonstrated multifaceted potential mechanisms in improving pain sensitivity and depression-like behavior in CIPD, which fully demonstrates the good efficacy and unique advantages of acupuncture intervention in CIPD. Studies have shown that acupuncture can regulate the levels of 5-hydroxytryptamine, glutamate, γ-aminobutyric acid, dopamine, and other neurotransmitters in the central nervous system,[33] modulate the expression of neurotrophic factors, improve the synaptic plasticity of neurons, promote the reconstruction of synaptic function, and improve the pain and depression of CIPD mice.[34] Chronic pain is a long-term stress during which stress can lead to a central inflammatory cascade response that affects the development of CIPD.[35] Acupuncture can reduce the inflammatory response by inhibiting microglia and astrocyte cell activation,[36,37] inhibiting the release of the pro-inflammatory factors IL-1β, IL-6, and TNF-α, and increasing the anti-inflammatory factor TGF-β.[38] In addition, acupuncture can exert analgesic and antidepressant effects by modulating the BDNF/TrkB/CREB and ERK signaling pathways and inhibiting the neural circuitry from glutamatergic neurons in the sub-limbic cortex to the basolateral amygdala.[39–41] Combined with the above advances, the current research on acupuncture intervention in CIPD mainly focuses on the level of molecular mechanisms, and the research on the mechanism of acupuncture intervention in CIPD from the neurophysiological point of view is still unclear. Therefore, the present study utilized the M-NEMEA technique to investigate the effects of acupuncture on the alteration and damage of neural information encoding in the CeA brain region of CIPD mice and to provide a basis for studying the neurophysiological mechanism of acupuncture effects.

This study used CFA injection into the left hind sole to replicate the CIPD model. On the first day after modeling, mice showed a significant decrease in the latent period of left hind foot thermal contraction, indicating the successful preparation of the chronic inflammatory pain model. On the 14th day after modeling, the results of forced swimming and

open field experiments showed a significant decrease in exploratory behavior and an increase in despair, indicating the successful preparation of the CIDP model.[17] After acupuncture intervention, the pain and depression behaviors of mice were improved compared to before, indicating that acupuncture not only alleviates pain in the pain perception dimension of chronic inflammatory pain comprehensive treatment but also has unique advantages in adjusting the pain emotion dimension.[42] After intervention in the sham acupuncture group, there was no significant improvement in pain and depressive behavior in mice. It was found that acupuncture could alleviate mechanical nociception and thermal nociception hypersensitivity and improve depressive-like behavior in chronic inflammatory pain with depression mice.[43,44] There was no significant alleviation of pain and depressive-like behavior in mice after the intervention of the sham acupuncture group, which is consistent with the results of the present study.[45]

AMY is an important component of the limbic system and plays a crucial role in various mental illnesses, such as anxiety and depression. Additionally, AMY serves as the regulatory center for the emotional dimension of pain in the brain and regulates the descending facilitation pathway of pain.[7] CeA is the output nucleus of the AMY signal, forming extensive fiber connections with nuclei such as the lateral hypothalamus, striatum, and parathalamic nucleus. It plays an important role in the integration of pain and negative emotional responses such as depression and anxiety.[46] Research has found that damaging CeA can prevent the generation of conditioned aversive emotions and improve pain-induced depressive and anxiety-like behaviors.[47] The results of this experiment showed that the morphology of CeA neurons in CIPD mice underwent significant changes, manifested as a decrease in the number of neurons, disordered arrangement, and a large number of degeneration and damage to neurons, indicating abnormal changes in CeA in CIPD mice.

The encoding characteristics of neuronal information can be reflected explicitly in the temporal and spatial features of the activity of related neuronal clusters.[48] Among them, discharge frequency, waveform amplitude, ISI, and power spectral density are intuitive features of waveform spatiotemporal patterns and action potentials' most basic electrophysiological characteristics.[49] The spatiotemporal patterns of CeA neural encoding can be analyzed by extracting these feature quantities. Among them, the encoding method that uses discharge frequency as the characteristic of neuronal discharge is called frequency encoding, which is a classic way of encoding neuronal discharge information.[50] The waveform amplitude can be obtained by signal separation, screening, and discrimination to obtain the spatiotemporal sequence of neuronal group action potentials, thereby reflecting the intensity of neuronal discharge.[51] The results of this experiment showed that there were abnormal changes in the spatiotemporal pattern of neuronal discharge in CIDP mice, manifested by a decrease in the amplitude of the discharge wave and a reduction in the number of discharging neurons, indicating a decrease in the intensity of CeA discharge and inhibition of discharge activity in CIDP mice. It was found that the action potential firing pattern of glutamatergic neurons in the CeA region of depressed mice was significantly altered, as evidenced by an increase in action potential spacing and a decrease in firing frequency.[52] The spontaneous firing frequency of neurons in the ventral lateral orbital cortex was significantly reduced on postoperative day 13 in pain-associated depressed mice.[53] The above studies are in general agreement with the results of the present study. After acupuncture treatment, the number of discharge neurons in CIDP mice increased, and the amplitude of the discharge wave increased. At the same time, there was no significant change in the sham acupuncture group. Acupuncture has a significant regulatory effect on the spatiotemporal pattern changes of abnormal CeA neurons. Acupuncture can antagonize and reverse the abnormal discharge of CeA neurons caused by CIPD and has an adjusting effect on the plasticity changes of CeA.

An ISl scatter plot is a graph with time intervals as the horizontal axis and discharge pulse intervals at corresponding time points as the vertical axis. It can reflect the distribution of peak potential interval sequences over time, thus reflecting the variation of neuronal discharge patterns.[54] Power spectral density is the power carried by each unit frequency wave obtained by multiplying the spectral density of action potential signals by an appropriate coefficient, representing neuronal electrical activity's coordination and information integration time.[51] This study showed that the ISl sequence of CeA neurons in CIDP mice significantly increased, and the PSD significantly decreased, indicating that the discharge time interval of CIDP mice was longer and the neuronal discharge power was reduced. It was found that the ISI of GABAergic neurons in the CeA area of mice with post-traumatic stress disorder was significantly increased, suggesting that the stress stimulus led to abnormal firing patterns of GABAergic neurons in the CeA area, similar to the results of the present study.[55] After acupuncture, the interval between ISI sequences was significantly shortened. PSD was significantly increased, indicating that acupuncture has improved the abnormal time coding of neurons in the CeA brain area of CIDP mice to some extent.

 https://doi.org/10.2147/JIR.S501170

CIPD mice showed abnormal neuronal cell morphology in the CeA brain region. Neuronal spontaneous firing activity was inhibited, and the ability to encode neural information was impaired, leading to the development of nociceptive hypersensitivity and depressive-like behavior. Acupuncture attenuates neuronal damage in the CeA brain region of CIPD model mice, improves CeA frequency coding and time coding, enhances the neural information coding ability of CeA neurons in CIPD model mice, and improves chronic inflammatory pain and depression-like behavior. In addition, in the current clinical practice of acupuncture, patients with chronic inflammatory pain are often accompanied by depression-like symptoms to varying degrees, and the selection of "Zusanli", "Sanyinjiao", "Hegu" and "Taichong" for acupuncture treatment has a better "analgesic and antidepressant effects". The results of this experiment provide a better experimental mechanism and supporting evidence for the clinical intervention of acupuncture in CIPD, as well as sufficient basis and ideas for the clinical application of the "analgesic and psychotropic" acupuncture method. However, this experiment only explored the functional changes of CeA neurons in CIPD model mice from the perspective of electrophysiology. It did not use neural regulation techniques such as optogenetics and chemogenetics to study CeA and its related projection circuits. Therefore, studying the central mechanism of acupuncture intervention in CIPD from the perspective of CeA neural circuits will be the focus of our next research direction.

Ethics Approval

Experimental Animal Ethics Committee of Gansu University of Traditional Chinese Medicine (SY2023-785).

Consent for Publication

All authors have given final approval of the version and agreed with the publication of this study here.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the Provincial Key Talent Project of Gansu Province in 2023 (No. 2023 [20]); Graduate Innovation and Entrepreneurship Fund Project of Gansu University of Traditional Chinese Medicine in 2025 (No. 2025CXCY-001).

Disclosure

The authors declare that they have no conflicts of interest for this work.

References

1. Brain K, Burrows TL, Rollo ME, et al. A systematic review and meta-analysis of nutrition interventions for chronic noncancer pain. *J Hum Nutr Diet*. 2019;32(2):198–225.
2. Humo M, Lu H, Yalcin I. The molecular neurobiology of chronic pain-induced depression. *Cell Tissue Res*. 2019;377(1):21–43.
3. Zhu X, Tang HD, Dong WY, et al. Distinct thalamocortical circuits underlie allodynia induced by tissue injury and by depression-like states. *Nat Neurosci*. 2021;24(4):542–553.
4. Nerurkar L, Siebert S, McInnes IB, Cavanagh J. Rheumatoid arthritis and depression: an inflammatory perspective. *Lancet Psychiatry*. 2019;6(2):164–173. doi:10.1016/S2215-0366(18)30255-4
5. Wei DF, Wang WY, Wang XW, et al. Study on the preventive and therapeutic effects of rutin sodium in comorbidities of chronic pain and depression. *J Xuzhou Med Univ*. 2023;43(11):789–794.
6. Sun N, Zhang N, Lin LL, et al. Meta analysis on acupuncture improving emotional disorder of patients with chronic pain. *China Acupuncture Moxibustion*. 2020;40(06):657–663.
7. Zhao WN, Hu SW, Zhai XJ, et al. The midbrain dopamine reward system and pain regulation. *Chin J Pain Med*. 2021;27(01):20–30.
8. Douglass AM, Kucukdereli H, Ponserre M, et al. Central amygdala circuits modulate food consumption through a positive-valence mechanism. *Nat Neurosci*. 2017;20(10):1384–1394. doi:10.1038/nn.4623
9. Xu ML, Li FY, Ma HR, et al. Peak potential clustering algorithm for large-scale neural recordings (invited). *Comput Eng*. 2024;50(06):1–34.

10. Shao S, Zheng Y, Fu Z, et al. Ventral hippocampal CA1 modulates pain behaviors in mice with peripheral inflammation. *Cell Rep*. 2023;42 (1):112017. doi:10.1016/j.celrep.2023.112017
11. Cai X, L QIU, Wang C, et al. Hippocampal inhibitory synapsis deficits induced by α5-containing GABAA receptors mediate chronic neuropathic pain-related cognitive impairment. *mol Neurobiol*. 2022;59(10):6049–6061. doi:10.1007/s12035-022-02955-8
12. Pan JW. Construction and application of multi-channel in vivo recording system for rats. *East China Normal Univ*. 2008;2008:1.
13. Wang JY, Luo F, Han JS, et al. In vivo multi-channel synchronous recording technology for central neuronal discharges. *Adv Physiol*. 2003;34 (4):356–358.
14. Wei JH, Luo YJ. Principle and technology of event related potential. *Beijing: Science Press*. 2010;2010:171.
15. Ministry of Science and Technology. Notice on issuing guiding opinions on treating experimental animals well. *Livestock Vet Sci Techn Info*. 2007;2007(04):35–36.
16. Wang YY. Study on the analgesic effect of acupuncture on CFA mice based on the endocytic pathway of spinal cord CXCL1-CXCR2. *Tianjin Univ Trad Chin Med*. 2021;2021:1.
17. Yang P, Su SY, Wang T. The effect of electroacupuncture on the expression of Cyt-C and Caspase-9 in hippocampal tissue of chronic pain and depression comorbid rats. *Chin J Trad Chin Med Info*. 2024;31(03):85–91.
18. Liu GH, Shi DL, Wang ZH, et al. Mechanism study of "dredging the liver and regulating the spirit" acupuncture regulating iron death of hippocampal neurons to improve the co-morbidity of chronic inflammatory pain and depression. *Acupuncture Res*. 2025;1–13. https://kns.cnki.net/kcms/detail/11.2274.R.20241122.1849.004.html.
19. Yao L, Yang FM, Liu YZ, et al. Exploring the clinical point selection pattern of acupuncture for inflammatory pain based on data mining technology. *China TCM Emerg*. 2019;28(05):782–785.
20. China acupuncture and moxibustion Society, Standardization Working Committee of China acupuncture and moxibustion Society. Names and localization of common acupoints for experimental animals part 3: mice T/CAAM 0003-2020. *Beijing: China Standards Press*. 2020;2020:1.
21. Liu XY, Lu H, Qin QQ, et al. Design method of pseudo acupuncture in acupuncture clinical trials. *Chinese J Basic Chin Med*. 2020;26 (10):1531–1534.
22. Interlandi C, Leonardi F, Spadola F, et al. Evaluation of the paw withdrawal latency for the comparison between tramadol and butorphanol administered locally, in the plantar surface of rat, preliminary study. *PLoS One*. 2021;16(7):e0254497. doi:10.1371/journal.pone.0254497
23. George P, B. j K. Franklin. The mouse brain in stereotaxic coordinates. 4th ed. *Acad Press*. 2012;2012:1.
24. Nerurkar L, Siebert S, McInnes IB, et al. Rheumatoid arthritis and depression: an inflammatory perspective. *Lancet Psychiatry*. 2019;6(2):164–173.
25. Harth M, Nielson WR. Pain and affective distress in arthritis: relationship to immunity and inflammation. *Expert Rev Clin Immunol*. 2019;15 (5):541–552. doi:10.1080/1744666X.2019.1573675
26. Karshikoff B, Martucci KT, Mackey S. Relationship between blood cytokine levels, psychological comorbidity, and widespreadness of pain in chronic pelvic pain. *Front Psychiatry*. 2021;12:651083. doi:10.3389/fpsyt.2021.651083
27. Yu WY, Ma LX, Tian Y, et al. Integrating complex networks and data mining to explore the characteristics of acupuncture and moxibustion prescriptions for inflammatory pain. *World Sci Tech Modern Trad Chin Med*. 2022;24(11):4402–4410.
28. Yao L, Yang FM, Liu YZ, et al. Analysis of clinical acupoint selection rules for acupuncture treatment of inflammatory pain based on data mining technology. *Chin Med Emerg*. 2019;28(05):782–785.
29. Wei XL, Tian J, Jia SH, et al. A new perspective of acupuncture and moxibustion to improve chronic pain: "acupuncture and moxibustion to cure the mind" regulates negative emotions and reward/motivation loop. *World J Acupuncture Moxibustion*. 2023;33(1):28–33. doi:10.1016/j.wjam.2022.10.001
30. Wang X, Wu P, Luo Y, et al. Effect of moxibustion on rheumatoid arthritis and related negative emotions. *China Acupuncture Moxibustion*. 2022;42 (11):221–12251232.
31. Li L, Li SR, Guo CY, et al. Treatment of chronic pelvic inflammatory pain by Kaisiguan combined with uterine eight array acupuncture: a randomized controlled trial. *Shanghai J Acupuncture Moxibustion*. 2020;39(2):196–199.
32. Gao Y, Tong QY, Ma W, et al. Treatment of refractory depression with acupuncture of regulating qi and relieving depression: a randomized controlled trial. *China Acupuncture Moxibustion*. 2023;43(4):417–421.
33. Zeng XL, Yang XA, Li SS, et al. Current status of research on the mechanism of action of acupuncture for chronic pain-depression co-morbidity. *Shanghai J Acupuncture Moxibustion*. 2024;43(11):1284–1290.
34. Li S, Huang JP, Luo D, et al. Effects of electroacupuncture on hippocampal AcH3/BDNF in rats with SNI-induced pain-depression model. *J Zhongshan Univ*. 2023;44(01):44–50.
35. Lu GN, Lu YJ, Wei LP, et al. The role of inflammation in the co-morbid process of chronic pain and depression. *Chin J Pain Med*. 2014;20 (12):896–898+903.
36. Li Y, Zhang H, Yang J, et al. P2Y12 receptor as a new target for electroacupuncture relieving comorbidity of visceral pain and depression of inflammatory bowel disease. *Chin Med*. 2021;16(1):139. doi:10.1186/s13020-021-00553-9
37. H ZHANGX, C FENGC, J PEIL, et al. Electroacupuncture attenuates neuropathic pain and comorbid negative behavior: the involvement of the dopamine system in the amygdala. *Front Neurosci*. 2021;15:657507. doi:10.3389/fnins.2021.657507
38. Liu Y. *Research on the Action Mechanism of Electroacupuncture on Pain and Depression in Rats With Neuropathic Pain*. Southwest Medical University; 2021.
39. Cong W, Peng Y, Meng B, et al. The effect of electroacupuncture on regulating pain and depression-like behaviors induced by chronic neuropathic pain[J]. *Ann Palliat Med*. 2021;10(1):104–113. doi:10.21037/apm-20-1900
40. Wu ZM, Wang JL, Xu LL, et al. Effects of electroacupuncture on two-dimensional regulation of sensation and emotion and p-ERK expression in anterior cingulate cortex-primary somatosensory cortex in CFA rats. *World Chin Med*. 2019;14(06):1354–1362.
41. Xie Y, Shen Z, Zhu X, et al. Infralimbic-basolateral amygdala circuit associated with depression-like not anxiety-like behaviors induced by chronic neuropathic pain and the antidepressant effects of electroacupuncture. *Brain Res Bull*. 2024;218:111092. doi:10.1016/j.brainresbull.2024.111092
42. Fang JQ, Shao XM. A new idea of acupuncture analgesia--the feasibility of acupuncture and moxibustion participating in multi-dimensional pain regulation. *Acupuncture Res*. 2017;42(01):85–89.
43. Liao HY, Lin YW. Electroacupuncture attenuates chronic inflammatory pain and depression comorbidity through transient receptor potential V1 in the brain. *Am J Chin Med*. 2021;49(6):1417–1435. doi:10.1142/S0192415X2150066X

44. Yang P, Chen H, Wang T, et al. Electroacupuncture promotes synaptic plasticity in rats with chronic inflammatory pain-related depression by upregulating BDNF/TrkB/CREB signaling pathway. *Brain Behav*. 2023;13(12):e3310. doi:10.1002/brb3.3310
45. Huang HY, Liao HY, Lin YW. Effects and mechanisms of electroacupuncture on chronic inflammatory pain and depression comorbidity in mice. *Evid Based Complement Alternat Med*. 2020;2020:4951591. doi:10.1155/2020/4951591
46. Chang XL, Zhang HY, Zhang LL, et al. Progress in the study of neural circuits underlying chronic visceral pain and the induction of negative emotions. *Chin J Pain Med*. 2024;30(01):57–62.
47. Brandão ML, Coimbra NC. Understanding the role of dopamine in conditioned and unconditioned fear. *Rev Neurosci*. 2019;30(3):325–337. doi:10.1515/revneuro-2018-0023
48. Meng WW, Shao Q, Shao WW, et al. Research progress on the relationship between neural synchronous activity and memory consolidation during non rapid eye movement sleep. *Chin J Physiol*. 2024;76(01):119–127.
49. Wang J. A new method for analyzing multi-channel spike potential signals and its application in the study of hippocampal neuron firing characteristics. *Hangzhou*. 2011;2011:1.
50. Zhu TT, Ma ZB, Yan XK, et al. Study on the intervention mechanism of acupuncture on abnormal neural coding of neurons in area 17 of the visual cortex of monocular deprived rats. *J Acupuncture Tuina Sci*. 2017;15(04):257–262. doi:10.1007/s11726-017-1010-2
51. Zhu TT, Ma ZB, Yan XK, et al. Intervention study of acupuncture on abnormal spatiotemporal patterns of neurons in the 17th area of visual cortex of monocular deprivation amblyopic rats. *China Acupuncture Moxibustion*. 2017;37(01):61–65.
52. Liu DB, Chen ZW, Wang Y, et al. Plasticity changes in central amygdala and prelimbic cortical neural networks in chronic unpredictable mild stress-induced depressed mice. *J Southern Med Univ*. 2024;44(11):2082–2091. doi:10.12122/j.issn.1673-4254.2024.11.04
53. Wu YW, Fu DQ, Gu QF, et al. Study on the mechanism of exogenous cannabinoid HU210 to alleviate anxiety-depression-like behavior induced by chronic neuropathic pain. *Chin J Pain Med*. 2019;25(12):890–897.
54. Zhao ZT. Study on the effect of "soothing the liver and regulating the spirit" acupuncture method on hippocampal neural coding and functional reconstruction in PTSD sleep disorders rats. *Chengdu Univ Trad Chin Med*. 2016;2016:1.
55. Liu DB, Shi Y, Chen HT, et al. Post-traumatic stress disorder induces plasticity changes in neuronal networks in the amygdala subregion of mice. *J Neuroanatomy*. 2022;38(06):641–649.