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Research Article

# Study on the Effect of Acupuncture Point Embedding on the Behavior of Rats with Allergic Rhinitis and its Mechanism of Action

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## Abstract

**Objective:** To observe the interventional effect of Yingxiang acupoint embedding on the behavior of rats with allergic rhinitis and the effect on the levels of serum inflammatory factors IgE, sIgE, IL-4, and IL-5.

**Methods:** The rat model of allergic rhinitis was prepared by selecting ovalbumin, and after the model was successfully constructed, the rats were randomly divided into the model group and the acupoint embedding group, each group consisted of 6 rats, and another 6 normal rats were set up as the control group. In the model group, OVA was used to maintain sensitization, and in the acupuncture point embedding group, bilateral Yingxiang acupoints were buried for 3 weeks. The behavioral performance of the rats in each group was observed and scored before and after the intervention, and the following tests were performed on the serum and nasal mucosa of the rats after the intervention: ELISA for total IgE, sIgE, IL-4, and IL-5; HE staining to observe the histomorphologic changes in the nasal mucosa of rats.

**Results:** The total behavioral score of the rats after the modeling was >5 points, and the nasal mucosa showed obvious pathological changes under light microscopy, indicating that the modeling was successful. The behavioral scores of the rats in the acupoint embedding group decreased compared with that of the model group after the burial intervention (P<0.05). Compared with the model group, levels of the inflammatory factors IgE, sIgE, IL-4, and IL-5 in the serum of rats in the acupuncture group were decreased (P<0.05). HE staining of the nasal mucosa showed inflammatory cell infiltration was seen in the mucosal epithelium and lamina propria of the rats in the model group, dominated by lymphocytes with rounded and deeply stained nuclei and neutrophils with rod-like lobular nuclei. The mucosal epithelial cells were denatured and necrotic, with local epithelial detachment and loss, and the structure of local glands was blurred. The number of cup cells within the mucosal epithelium increased. The pathomorphological changes in the mucosal epithelium of rats in the acupuncture burial group were significantly reduced compared with those in the model group.

**Conclusion:** Acupuncture point embedding can significantly improve the symptoms of nasal sensitivity in AR rats, and the mechanism of action may be related to the inhibition of Th2 cell secretion and synthesis of related inflammatory factors by burrowing.

**Keywords:** Acupuncture point embedding; Allergic rhinitis; Behavioral changes; Inflammatory cytokines

## Introduction

Allergic rhinitis is a common and highly prevalent non-infectious chronic disease caused by IgE-mediated exposure to allergens. These immune responses include mucosal inflammation driven by type 2 cells [1]. The disease is clinically characterized by two or more of the symptoms of itchy nose, sneezing, runny nose, and nasal congestion [2,3].

The pathogenesis of AR is related to environmental exposure, genetics, climate change, and lifestyle. House dust mites, molds, and animal fur are the main sources of sensitization [4-6]. In recent years, with the accelerated pace of life, changes in dietary habits, and the ecological environment, AR has become a global health problem. Some studies have shown that about 500 million people around the world suffer from it, and the prevalence of AR in the central cities of China is 8.7%-24.1% [7]. It shows an increasing trend year by year. Prolonged or recurrent episodes of AR not only prevent people from concentrating on normal studies and work but also affect patients' sleep quality and even mental symptoms such as anxiety and irritability. In addition, AR may interact with other allergic diseases, such as asthma and atopic dermatitis, resulting in a high economic burden on the healthcare system [8-9].

It is currently believed that the pathogenesis of AR is mainly related to the immune imbalance between Th1/Th2 and Th17/Treg cells [10]. Among these, the Th1/Th2 imbalance is considered to be the basis for the occurrence of AR [11]. The treatment of AR in modern medicine includes patient education, allergen avoidance, drug therapy, immunotherapy, and surgery. Commonly used drugs for AR include glucocorticoids, antihistamines, leukotriene receptor antagonists, decongestants, etc. Although these first-line drugs can improve the symptoms of patients with AR in the short term, they are susceptible to drug resistance to a certain extent or bring about localized or even systemic adverse reactions to patients [12].

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Allergic rhinitis is a dominant disease in Chinese medicine treatment, and acupuncture treatment for AR is gradually gaining attention. Acupuncture point embedding belongs to a special kind of acupuncture therapy, and numerous existing clinical studies and animal experiments have shown that acupoint embedding can alleviate the nasal symptoms of AR and obtain good long-term therapeutic effects by inhibiting the neurogenic inflammation of the nasal mucosa and regulating the immune balance between Th1/Th2 cells [13-15]. In this study, we constructed a rat model of AR through OVA, observed the effects of acupoint acupuncture on the behavioral manifestations and related inflammatory cytokines of AR rats, and explored the mechanism of acupoint acupuncture intervention in AR to provide a certain basic experimental basis for the future clinical selection of acupoint acupuncture in the treatment of AR.

## Materials and Methods

### Materials

#### Experimental Animals

Eighteen SPF-grade SD rats, 6-8 weeks old, all males, with an average weight of (200±20) g, were provided by Spivey (Beijing) Biotechnology Co., Ltd, holding Laboratory Animal Production License No. SCXK (Beijing) 2019-0010. All animals were housed in the Animal Center of the Experimental Building of the School of Pharmacy, Chengdu University of Traditional Chinese Medicine, with Laboratory Animal Use License No. SYXK (Chuan) 2020-124. The rats were fed in separate cages, 5 per cage, on a standardized diet, with free access to water. The laboratory was well-ventilated, illuminated by fluorescent lamps, with 12 hours of alternating light and darkness, and maintained an indoor temperature of 24±2°C, a relative humidity of 50-60%, and a noise level of ≤56dB. The experiments were conducted in accordance with the principles of animal ethics, and the present study was approved by the Ethics Committee for Laboratory Animals of Chengdu University of Traditional Chinese Medicine (Ethics Review No: 2024DL-013).

#### Essential Reagents and Instruments

Alum adjuvant (aluminum hydroxide powder): Shanghai McLean Biochemical Technology Co., Ltd, A800852; Ovalbumin (OVA): Sigma, USA, A5503; Rat IgE, IL-4, IL-5, OVA-sIgE kit (Item No. ZC-37001, ZC-36402, ZC-36403, ZC-36993, Shanghai Thrive Color Biotechnology Co.). Enzyme labeling instrument (SpectraMAX Plus384, Meigu Molecular Instrument Co., Ltd.); digital section scanner (Pannoramic 250, 3DHISTECH (Hungary)). Disposable Acupuncture Point Embedding Needle (Specification: No.8 Green Head Embedding Needle, Shandong Boda Medical Supplies Co.)

#### Main Reagent Preparation

**Intraperitoneal Sensitizing Solution:** take 0.3mg ovalbumin and 30mg aluminum hydroxide powder and add it to 1mL saline to form a suspension; Nasal drip stimulating solution: take 1000ug ovalbumin and add saline solution to 20ul to prepare 5% concentration of ovalbumin stimulating solution; Sensitizing maintenance solution: take 100ug ovalbumin and add saline solution to 10ul to prepare 1% concentration of sensitizing maintenance solution. All the solutions were prepared ready to use.

### Methods

#### AR Rat Model Establishment

Referring to the modeling method in the literature [16,17], AR modeling was divided into three stages: (1) basic sensitization: from day 1 to day 13, rats in the modeling group were given 1ml of intraperitoneal sensitizing solution for intraperitoneal injection, and rats in the control group were injected with an equal amount of 0.9% saline intraperitoneally once every other day, for a total of seven times. Nasal drip stimulation: from day 14 to 20, rats in the modeling group were given 100ul of ovalbumin stimulation solution at a concentration of 5%, 50ul/nostril/pupil, and rats in the control group were given an equal amount of 0.9% saline for nasal drip once a day for 7 consecutive days.

**Nasal Drip Maintenance:** from the 21st day to the end of the experiment, rats in the model group were subjected to nasal drip with 1% concentration of ovalbumin solution, 50ul/nostril/each, and rats in the control group were subjected to nasal drip with the same amount of 0.9% saline, once every other day. (Note: AR is an allergic disease, the body is sensitized after modeling, but the allergic reaction is triggered only after exposure to allergens, so the model still needs to be maintained after successful modeling).

#### Model Building Success Criteria

**Behavioral Changes:** Rats were observed scratching their noses and sneezing within 30 minutes after the end of the last nasal drip stimulation. The total score was calculated using the superposition method, with a total score of ≥5 indicating successful modeling [18]. The scoring method is shown in table 1.

| Score | Sneezes (Number) | Scratching Nose (Times) |
|---|---|---|
| 1 point | 3-9 | 2-3 |
| 2 points | 10-14 | 4-5 |
| 3 points | ≥15 | >5 |

**Table 1:** Nasal Symptoms Scale.

Under the light microscope, the following lesions appeared in the nasal mucosa of rats, indicating that the modeling was successful: obvious destruction of the structure of the nasal mucosa, discontinuous and uneven arrangement of the cilia, infiltration of numerous inflammatory cells, obvious expansion and proliferation of the glands and blood vessels around the submucous layer of the mucous membrane, many epithelial cells were detached and necrotic, and the mucous membrane epithelial cup cells proliferated.

#### Intervention Methods

The rats with successful modeling were randomly divided into the model group and the acupoint burrowing group, with 6 rats in each group. The intervention was started on the 2nd day of successful modeling. The rats in the control group were fed normally without intervention; the rats in the model group were given OVA nasal drops to maintain the sensitized state; and the rats in the submerged thread group were given submerged threads at Yingxiang acupoints bilaterally. The specific procedure of thread embedding was as follows.

**Localization of Yingxiang Acupoint:** Referring to the atlas of rat acupoints [19], combined with the comparative anatomical method for localization, when the rats were looking straight ahead and the eyes were downward, it was taken from the posterior end of the

bilateral nostrils and the line connecting the inner canthus of both eyes close to the 1/3 of the area next to both noses.

**Embedding Operation:** Isoflurane anesthesia for rats, when the rat activity is slow, the positioning of the acupuncture point and its surrounding skin disinfection, with disposable sterile tweezers will be in advance with 75% of the length of the medical alcohol soaked protein thread of about 5mm into the embedded needle tube; the left thumb and forefinger pinch up the skin on both sides of the acupoints, the right hand of the thumbs, index fingers, three fingers of the needle, the needle point beveled upward, aligned with the acupoints, fast enter the needle, slowly push the needle, push the needle core to the bottom, push the protein thread into the acupoint, back out of the needle to the subcutaneous when the needle is out of the needle quickly, alcohol cotton ball pressure for about 3 min. After burying the thread daily disinfection of the operation site, for 1 week, to prevent infection. Thread burial cycle: 3 weeks.

### Observation Indexes

#### Behavioral Scores of Rats

Nasal stimulation was performed before modeling and 3 weeks after burying the wires, and nasal allergy symptoms of rats were observed and scored by the symptom superposition scoring method, and the scoring criteria are shown in table 1 above.

### ELISA for Total IgE, OVA-sIgE, IL-4, IL-5 Levels in Rat Serum

After the behavioral observation, the rats were anesthetized with the help of an isoflurane anesthesia machine, placed on the operation table, the abdominal skin and muscles were cut open with scissors, the intra-abdominal organs were pushed to the side with cotton swabs and gauze to fully expose the abdominal aorta, and the abdominal artery was pricked with the tip of the disposable sterile blood-collecting needle, and a disposable vacuum negative-pressure blood-collecting vessel (anticoagulant tube) was connected with the other end to collect about 5ml of blood, and the blood was stored in the room at room temperature for 0.5-1h. After centrifugation at room temperature for 0.5-1h, the blood was centrifuged at 3500r for 15 min, and the supernatant was taken by pipette gun, divided into 1.5ml centrifuge tubes, and stored at -80°C for testing. The levels of rat serum IgE, sIgE, IL-4, and IL-5 were detected according to the instructions of the kit.

### Histopathological and Morphological Changes in Rat Nasal Mucosa Observed by Hematoxylin and Eosin (HE) Staining

After blood collection, the heads of the rats were dissected in a sagittal position. The nasal cavity was exposed, carefully separated, and the mucosal tissue of the nasal cavity was taken out. It was then put into a 10% formaldehyde solution for fixation and embedded in paraffin for storage. Afterwards, it was stained with HE. Under the light microscope, the integrity of the mucosal epithelium covering all parts of the nasal cavity was observed, as well as whether the mucosal epithelial cells had degeneration and necrosis. Additionally, the presence of congestion, edema, and inflammatory cell infiltration in the submucosa was also examined.

### Statistical Analysis

SPSS 22.0 and GraphPad Prism 10 software were used for statistical analysis and graphing. Measurement data were expressed as "mean ± standard deviation." One-way ANOVA was used for comparison between groups, and P<0.05 was considered statistically different.

## Experimental Results

### Comparison of Nasal Symptom Scores of Rats

Before modeling, there was no statistically significant difference in the behavioral scores of the three groups of rats (P>0.05). After modeling, rats in all groups except the control group had nasal allergy symptoms, and the total score of the model group was >5, suggesting that the modeling was successful; scored again within 30 min after the end of the intervention, the total behavioral scores of the rats in the acupoint burrowing group with AR symptoms were significantly lower than those of the model group and were <5 points (P<0.05); the differences in the symptom scores of the rats in the acupoint burrowing group were not statistically significant when compared with those of the control group (P>0.05). See table 2 and figure 1.

| Group | Pre-Modeling | Post-Modeling | Post-Intervention |
|---|---|---|---|
| Control group | 1.00±0.01 | 1.00±0.63 | 0.50±0.55 |
| Model group | 1.00±0.58 | 7.14±1.07* | 6.29±2.29* |
| Acupoint embedding group | 0.80±0.45 | 8.00±1.22* | 1.4±0.55# |
| P | 0.801 | 0.001 | 0.001 |

**Table 2:** Comparison of nasal symptom scores of rats in each group (`x±s, n=6).

**Note:** Compared with control group in the same period: *P<0.05, compared with model group in the same period: #P<0.05

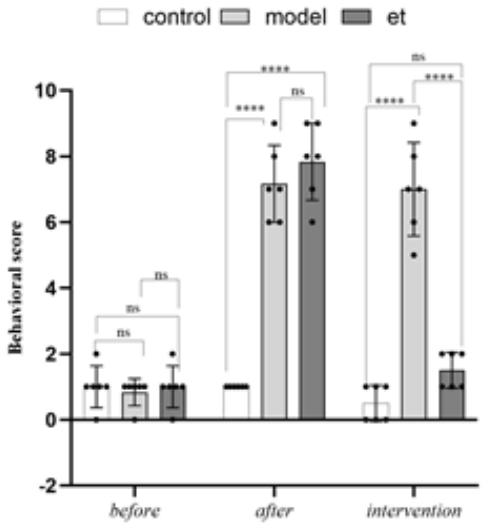


**Figure 1:** Nasal symptom scores of three groups of rats at different stages of modeling.

### Comparison of Serum IgE, sIgE, IL-4, and IL-5 Levels in Rats of Each Group

Compared with the control group, the serum levels of IgE, sIgE, IL-4 and IL-5 in the rats of the model group were significantly higher (P<0.01); compared with the model group, the serum levels of IgE, sIgE, IL-4, and IL-5 in the rats of the acupoint embedding group were lower (P<0.05), with statistical differences. See table 3 and figure 2.

| Group | IgE (μg/mL) | sIgE (ng/mL) | IL-4 (pg/mL) | IL-5 (pg/mL) |
|---|---|---|---|---|
| Control group | 5.934±1.550 | 5.595±1.069 | 8.817±0.982 | 10.694±2.380 |
| Model group | 8.939±0.831** | 9.793±1.989** | 11.184±1.321** | 15.730±2.385** |
| Acupoint embedding group | 7.091±1.766# | 7.258±1.488# | 9.712±0.826# | 11.928±2.581# |
| P | 0.009 | 0.001 | 0.005 | 0.008 |

**Table 3:** Comparison of serum IgE, sIgE, IL-4, and IL-5 levels in rats (`x±s, n=6).

**Note:** Compared with the control group: *P<0.05, **P<0.01; compared with the model group, #P<0.05, ##P<0.01.

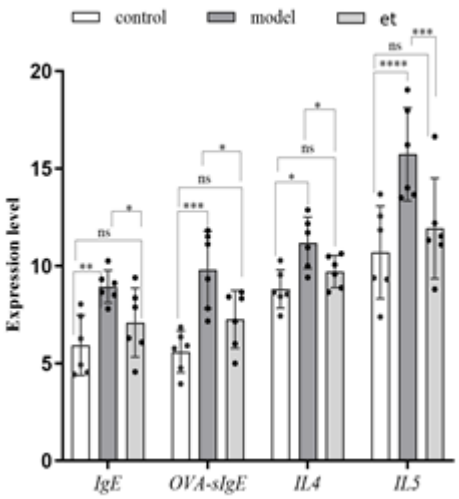


**Figure 2:** Expression of serum inflammatory factor levels in three groups of rats.

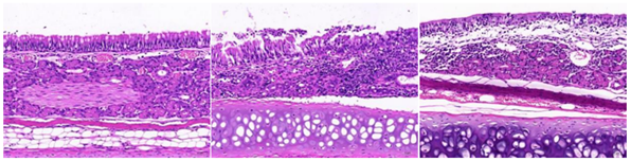


**Figure 3:** Observations on the pathological morphology of the nasal mucosa of rats in each group (HE, ×200).

## Effect of Acupuncture Point Embedding on the Pathologic Morphology of Nasal Mucosa in Rats

In the control group, the epithelium of the nasal mucosa was complete and continuous, the cells were arranged neatly and closely, and a small amount of fibrous tissue and connective tissue were distributed in the lamina propria, and there was no edema and degeneration of the mesenchyme; in the model group, inflammatory cells infiltrated in the epithelium of the nasal mucosa and lamina propria of the rats, and the lymphocytes with rounded and deeply stained nuclei and the neutrophils with rod-shaped lobular nuclei were predominant; the epithelium of the mucous membrane cells was degenerated, necrotic, with cytoplasmic lysis, cytosolic fragmentation, and the epithelium was locally detached and missing. The mucosal epithelial cells were degenerated, necrotic, cytoplasmic lysis, nuclear fragmentation, local epithelial detachment, absence, and blurring of local glandular structure; the mucosal epithelium was thickened, the mucosal epithelial cells were proliferated, and the number of intraepithelial cup cells increased. A small amount of inflammatory cell infiltration was observed in the nasal mucosal epithelium of the rats in the buried wire group, with mild hyperplasia of the glandular tissue, and the mucosal epithelial structure was relatively intact. See figure 3.

## Discussion

According to the clinical manifestations of allergic rhinitis, it is categorized as “Bi Qiu” in Chinese medicine. The treatment of this disease is mainly based on Chinese herbs and acupuncture, of which acupuncture has been recommended as an alternative therapy in the United States [20]. Acupoint embedding belongs to a special acupuncture therapy, which is the extension and development of acupuncture therapy. It is an intervention method mainly developed with the support of acupuncture theory and reference to biology and physics. The needle produces a stimulating effect on the acupoints, stimulating the meridians and qi and blood, and at the same time with the absorbable PGLA thread or protein thread or surgical suture long-term action on the acupoints to produce biochemical effects, which in turn regulates the function of the body and plays a therapeutic role [21-22].

Currently, studies on acupoint catgut embedding for the treatment of AR mainly focus on regulating cytokines, affecting neurotransmitters, and inhibiting the immune molecule immunoglobulin E [23-25]. With the continuous improvement of acupoint embedding theory, the related research literature on embedding therapy for AR has been increasing year by year, and it has achieved satisfactory therapeutic effect in the clinic as an effective external treatment method of Chinese medicine intervention in the treatment of AR. Many clinical studies have shown [26-29], that the use of acupoint catgut embedding alone or in combination can gradually eliminate allergic symptoms by adjusting the systemic immune system and increasing the body’s resistance to achieve long-term relief of the clinical symptoms of AR and even prevent recurrence of the therapeutic effect.

In this study, catgut embedding was performed at Yingxiang point to observe its therapeutic effect and mechanism in treating AR rats. Yingxiang point belongs to the large intestine meridian of hand Yangming and is the intersection point of the meridian and the stomach meridian of foot Yangming. In Chinese medicine theory, the large intestine and the lung are mutually exclusive, both belonging to the metal element in the Five Elements theory, while the stomach belongs to the earth element. Acupuncture at Yingxiang can not only nourish the earth to generate metal, nourish the lung and consolidate the nose for treating the root cause, but also promote nasal ventilation, relieve itching, and stop nasal discharge for treating the symptoms. Thus, Yingxiang point is an important point for treating AR [30]. In modern medical research, Yingxiang point is located above the external nasal branch of the pre-sial nerve and the dorsal nasal branch of the facial artery and vein, and needling Yingxiang can stimulate the nerves of

the nose to achieve the effect of promoting local blood circulation, reducing the sensitivity of the nasal mucous membrane, and improving the ventilation of the nasal cavity.

AR, as an allergic disease, has a complex pathogenesis with numerous cytokines and inflammatory mediators involved in its pathogenesis [31]. IgE is an inflammatory mediator, and allergens can induce the body to produce IgE antibodies, which bind to mast cells [32]. When the sensitized body comes into contact with the same allergen again, the allergen binds to the IgE antibody on the surface of the cell, causing an antigen-antibody reaction. The eosinophils degranulate, releasing large amounts of biologically active mediators, causing local allergic inflammation. Therefore, IgE is an important antibody that induces Type I allergic reactions. The level of serum sIgE antibody objectively reflects the sensitization status of AR, and the higher the level of sIgE antibody, the stronger its correlation with AR. Studies have demonstrated that serum interleukin levels are highly correlated with allergic rhinitis [33], and interleukin assumes a crucial role in transmitting information, activating and regulating immune cells, mediating cell activation, proliferation, and differentiation, as well as inflammation. IL-4 and IL-5 are inflammatory factors secreted by Th2 cells. IL-4 as a specific inducer of IgE, can directly stimulate B cells to produce IgE and IgG4. IL-5 is present in the nasal mucosal environment and is an important cytokine regulating the activity of inflammatory cells, recruiting circulating eosinophils, and also specifically regulating eosinophil proliferation and differentiation, further promoting mast cell degranulation and enhancement of IgE-mediated immune response.

Acupoint embedding can promote local blood circulation, enhance immunity, activate the immune system, and can intervene in serum inflammatory factors and transforming factors, etc., to achieve the purpose of treating allergic rhinitis. The results of this experimental study showed that the behavioral symptom scores of the rats were significantly higher after modeling, and the total score was >5, while the pathological morphology of the nasal mucosa showed changes such as inflammatory cell infiltration, suggesting that the model was successfully established. After the intervention with the buried thread, the behavioral score and the levels of IgE, sIgE, IL-4, and IL-5 in the serum of the buried thread group were lower than those of the model group, and the inflammatory infiltration in the nasal mucosa of rats was significantly improved, indicating that this method can improve the nasal sensitivity symptoms of AR rats and reduce the inflammatory response in the serum. This experiment provides some evidence-based medical evidence for the clinical selection of acupoint catgut embedding for the treatment of AR and has good guiding value for the clinical treatment of AR.

## Conclusion

This study explored the effects of acupoint embedding on nasal symptoms, serum inflammatory factors, and nasal mucosa morphology in Allergic Rhinitis (AR) rats. Three weeks post-embedding, there was a significant reduction in nose scratching and sneezing symptoms. Additionally, nasal mucosal epithelium repair was enhanced, and inflammatory cell infiltration was reduced. The underlying mechanism may involve the suppression of pro-inflammatory mediator secretion by Th2 cells.

## Ethics Approval and Consent to Participate

Not applicable

## Consent for Publication

Not applicable

## Availability of Data and Materials

Not applicable

## Competing Interests

The authors declare that they have no competing interests.

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## Author's Contribution

Tang was accountable for the design of the experimental scheme, the execution of animal experiments and statistical analysis. Wang was responsible for assisting in the development of animal experiments and data collection. Zhang provided financial support for the research and the publication of the article. All authors have read and approved the manuscript.

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