Effects of Complementary Acupuncture Points on the Expression of 5-HT and Its Receptors in the Acupoint Area and Hippocampal Tissue of Rats with Insomnia

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Abstract [**Objectives**] To investigate the effects of acupuncture at the complementary acupuncture points Neiguan and Waiguan on the expression levels of 5-HT, 5-HIAA, 5-HT_{1A}R, and 5-HT_4R within the acupoint area and hippocampal tissue of insomnic rats. [**Methods**] 72 Wistar rats were randomly divided into the following groups, each comprising 12 animals: the complementary acupuncture point groups (through-acupuncture group, anti-acupuncture group), the single acupoint groups (Neiguan acupoint group, Waiguan acupoint group), the model group, and the blank group. The insomnia rat model was established through intraperitoneal injection of chlorobenzene alanine. Each acupuncture group underwent electroacupuncture treatment following the attainment of the deqi sensation, administered once daily for 20 min per session over a period of consecutive 6 d. Electroacupuncture was administered using dilatational waves, with a stimulation frequency alternating between 2 and 100 Hz, and a current intensity of 1 mA. The contents of 5-HT and 5-HIAA were detected by ELISA; 5-HT_{1A}R levels were assessed using immunohistochemistry, and 5-HT_4R mRNA expression was evaluated through fluorescent in situ hybridization. [**Results**] Compared to the blank group, the expressions of 5-HT, 5-HIAA, 5-HT_{1A}R, and 5-HT_4R mRNA in both the acupoint area and hippocampal tissue of rats in the model group were significantly reduced ($P < 0.05$). These indicators were significantly elevated in all acupuncture groups relative to the model group ($P < 0.05$). Furthermore, the complementary acupuncture point groups (through-acupuncture and anti-acupuncture groups) demonstrated a more pronounced increase compared to the single acupoint groups ($P < 0.05$). No statistically significant differences were observed between the through-acupuncture and anti-acupuncture groups, nor between the single Neiguan and Waiguan acupoint groups ($P < 0.05$). [**Conclusions**] Acupuncture at the Neiguan and Waiguan acupoints effectively ameliorates insomnia in PCPA-treated rats. This therapeutic effect may be associated with increased expression levels of 5-HT, 5-HIAA, 5-HT_{1A}R, and 5-HT_4R mRNA in both the acupoint area and the central hippocampal tissue.

Key words Complementary acupuncture point, Insomnia, 5-HT, 5-HIAA, 5-HT_{1A}R, 5-HT_4R

1 Introduction

With the intensification of social competition and the continual increase in psychological stress among individuals, insomnia has emerged as a significant health concern that warrants serious attention. Insomnia is defined as a disorder characterized by frequent difficulties in initiating or maintaining sleep despite the presence of conducive conditions for sleep. Acupuncture demonstrates a favorable therapeutic effect on insomnia. Nevertheless, numerous acupoint combination protocols are currently employed in clinical practice, resulting in variable therapeutic outcomes. Consequently, the selection of an appropriate acupoint combination is a critical factor in enhancing clinical efficacy. According to the theory of traditional Chinese medicine, the primary pathogenesis of insomnia is attributed to an imbalance between Yin and Yang. Consequently, the regulation of Yin and Yang constitutes a crucial therapeutic principle for the treatment of insomnia. The complementary acupuncture points are believed to produce a synergistic effect by facilitating the mutual assistance of Yin and Yang[1]. Empirical studies have demonstrated that acupuncture, through the stimulation of the complementary acupuncture points Neiguan and Waiguan, effectively alleviates symptoms of insomnia[2-4]. Nevertheless, the precise mechanisms underlying its therapeutic effects remain to be elucidated. Modern medical research indicates that the pathogenesis of insomnia is closely associated with the hyperactivity of the sleep-wake system[5]. Monoamine neurotransmitters are integral to the regulation of the sleep-wake cycle[6]. Notably, 5-hydroxytryptamine (5-HT), a key monoamine neurotransmitter involved in this regulatory process, plays a critical role in both the initiation and maintenance of sleep[7]. This study, grounded in the theory of complementary acupuncture points, examined the expression levels of 5-HT, 5-HIAA, 5-HT_{1A}R, and 5-HT_4R mRNA in both the acupoint area and the central hippocampal tissue of rats with insomnia. The objective was to investigate the potential mechanisms underlying the effects of acupuncture at Neiguan and Waiguan acupoints in the treatment of insomnia, as informed by the theory of complementary acupuncture points.

2 Materials and methods

2.1 Materials

2.1.1 Experimental animals. Seventy-two male Wistar rats, weighing between 180 and 200 g, were procured from Beijing Vital River Laboratory Animal Technology Co., Ltd. (animal license No.: SCXK [Jing] 2021-0006). The rats were housed in the La-

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boratory Animal Center of Chengde Medical University under controlled conditions, including a room temperature of (23 ± 1.0) ℃ and relative humidity of 50%. They were acclimated for one week prior to the commencement of the study. During the experiment, the disposal of rats was conducted in accordance with the *National Standards for the Use of Laboratory Animals* and the *Regulations of the People's Republic of China on the Administration of Laboratory Animals*. This procedure received approval from the Animal Ethics Committee of Chengde Medical University (approval No.: CDMULAC-202100907-013).

2.1.2 Reagents. The primary reagents utilized in this study comprised anhydrous ethanol, xylene, and n-butanol (Sinopharm Chemical Reagent Co., Ltd.); 4% paraformaldehyde (DEPC water, Biosharp); 2 × SSC buffer powder for in situ hybridization and a 5-HT_4R mRNA in situ hybridization detection kit (Wuhan Boster Biological Technology Co., Ltd.); ICAM-1/CD54 antibody (G-5, Santa Cruz Biotechnology); rabbit anti-substance P receptor antibody; general SP kit (Beijing Zhongshan Golden Bridge Biological Technology Co., Ltd.); substance P antibody (Affbiotech); anti-5-HT_{1A} receptor antibody (Abcam); ELISA kits for 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) (mlbio); DEPC (Biosharp); BSA, protease K, DAPI, and anti-fluorescence quenching mounting medium (Servicebio); and TB Green SYBR fluorescent dye (Takara, Japan).

2.1.3 Instruments. The instruments utilized in this study included disposable sterile acupuncture needles (0.25 mm × 25 mm; Suzhou Medical Supplies Factory Co., Ltd.), a mixing instrument (Dalong Xingchuang Experimental Instrument Co., Ltd.), a dehydrator (DIPATH, Italy), an embedding machine (Wuhan Junjie Electronics Co., Ltd.), a tissue spreading machine (Jinhua Kedi Instrument & Equipment Co., Ltd.), a histochemical pen (Wuhan Servicebio Technology Co., Ltd.), a microscope (Nikon Instruments Co., Ltd.), a freezing table (Wuhan Junjie Electronics Co., Ltd.), a microplate reader (Thermo Fisher Scientific), and an electric thermostatic blast drying oven (Shanghai Jinghong Laboratory Equipment Co., Ltd.).

2.2 Methods

2.2.1 Model preparation and grouping. In accordance with the method described in reference[8], a rat model of insomnia was established using p-chlorophenylalanine (PCPA). PCPA was formulated into a 45 mg/mL suspension with normal saline (pH 7.0 – 8.0) and administered via intraperitoneal injection at a dosage of 1 mL/100 g. Except for the control group, rats in all other groups received intraperitoneal injections of the PCPA suspension once every morning at 8:00 for consecutive 2 d. The behavioral changes in the experimental rats were monitored between 27 and 31 h following the administration of the second injection. The successful establishment of the model was indicated by the presence of the following behaviors in the rats: a marked increase in daytime activity, disruption of circadian rhythms, heightened sensitivity to external stimuli (*e.g.*, light and sound), increased aggression, elevated excitability, along with increased food consumption and excretion, and a change in fecal color to grayish-white[9]. Rats in the control group received intraperitoneal injections of an equivalent dose of weakly alkaline normal saline for consecutive 2 d. Following the modeling procedure, 72 rats were randomly assigned to six groups ($n = 12$ per group): the single Neiguan acupoint group, the single Waiguan acupoint group, the complementary point through-acupuncture group, the complementary point anti-acupuncture group, the model group, and the blank group. Distinct regions of the limbs, head, and neck of the rats were marked with bitter acid to facilitate differentiation.

2.2.2 Intervention methods. The intervention commenced on the morning of the 3rd day. The positioning of the Neiguan and Waiguan acupoints was determined according to the method described in *Experimental Acupuncture*[10]. The Neiguan acupoint is located at the distal one-sixth of the inner forearm, situated between the radius and ulna. The Waiguan acupoint is positioned at the distal one-sixth of the outer forearm, also between the radius and ulna. Rats were immobilized using a flat-plate mouse fixator.

(i) Acupuncture group. The acupuncture groups were categorized as follows: the single Neiguan acupoint group, the single Waiguan acupoint group, the complementary point through-acupuncture group (Neiguan and Waiguan through-acupuncture, consistent with acupoints commonly employed in the clinical treatment of insomnia), and the complementary point anti-acupuncture group (Neiguan and Waiguan anti-acupuncture, characterized by a simplified procedure). Acupuncture was administered using a disposable sterile needle measuring 0.25 mm × 25 mm. Single acupoint and anti-acupuncture procedures involve needling the individual Neiguan and Waiguan acupoints, as well as performing anti-acupuncture at these points, with needles inserted perpendicularly to a depth of approximately 1.0 to 1.5 mm. The through-acupuncture technique entails inserting the needle from Neiguan through to Waiguan, advancing the needle approximately 2 to 3.5 mm while ensuring that the needle tip does not penetrate the skin on the opposite side. Following the attainment of the deqi sensation, the SDZ-V electroacupuncture device was connected. In the anti-acupuncture group, the positive electrode was attached to the Neiguan acupoint, while the negative electrode was connected to the Waiguan acupoint. For both the single acupoint group and the through-acupuncture group, the positive electrode was connected to the needle handle, and the negative electrode was attached to the rat's tail. Electroacupuncture was administered using dilatational waves, with a stimulation frequency alternating between 2 and 100 Hz, and a current intensity of 1 mA. The intensity was adjusted to induce slight tremors in the needle handle without causing vigorous struggling in the rats. Each treatment session lasted 20 min, conducted once daily for consecutive 6 d.

(ii) Model group and blank group. The rats in the model group and the blank group were immobilized in the same manner as those in the acupuncture group but did not receive acupuncture treatment. This procedure was conducted for 20 min per session, once daily, over a period of 6 d.

2.2.3 Indicator detection. The contents of 5-HT and its metabolite 5-HIAA in the supernatant of the central acupoint area and hippocampal tissue of rats were measured using ELISA. Immunohistochemistry was employed to determine the distribution of 5-HT_{1A}R in the acupoint area and hippocampal tissue, while fluorescence in situ hybridization was utilized to assess the expression levels of 5-HT_4R mRNA.

2.2.4 Statistical methods. The experimental data were analyzed statistically using SPSS 26.0. Data that conformed to a normal distribution were presented as mean ± standard deviation ($\bar{x} \pm s$). Following assessment with the statistical software, the data satisfied the assumptions of normality and homogeneity of variance. Consequently, one-way analysis of variance (ANOVA) was employed to compare differences between groups. A P-value of less than 0.05 was considered indicative of statistical significance.

3 Results and analysis

3.1 Content of 5-HT and 5-HIAA in the acupoint area and hippocampal tissue of rats Compared to the blank group, the expression levels of 5-HT and 5-HIAA in both the acupoint area and hippocampal tissue of rats in the model group were significantly decreased ($P<0.05$). In contrast, the acupuncture group exhibited significantly increased levels of 5-HT and 5-HIAA compared to the model group ($P<0.05$), with no statistically significant differences observed between the acupuncture and blank groups. Among the various acupuncture treatments, acupuncture was effective in elevating 5-HT and 5-HIAA levels; notably, the through-acupuncture and anti-acupuncture groups demonstrated superior effects relative to the single Neiguan and Waiguan acupoint groups ($P<0.05$). No significant differences were found between the through-acupuncture and anti-acupuncture groups, nor between the single Neiguan and Waiguan acupoint groups (Table 1).

Table 1 Comparison of the contents of 5-HT and 5-HIAA in the acupoint area and hippocampal tissue of rats ($n=12$, $\bar{x} \pm s$, ng/mL)

Group	Hippocampal tissue		Acupoint area	
	5-HT	5-HIAA	5-HT	5-HIAA
Blank	0.85 ± 0.03	35.72 ± 4.74	60.43 ± 4.4	30.45 ± 2.70
Model	$0.71 \pm 0.02^{*}$	$22.14 \pm 3.30^{*}$	$46.96 \pm 8.23^{*}$	$23.00 \pm 3.30^{*}$
Single Neiguan acupoint	$0.77 \pm 0.06^{*\#}$	$28.42 \pm 1.85^{*\#}$	$56.26 \pm 6.95^{*\#}$	$27.82 \pm 2.34^{*\#}$
Single Waiguan acupoint	$0.76 \pm 0.04^{*\#}$	$27.17 \pm 1.69^{*\#}$	$55.90 \pm 4.82^{*\#}$	$27.77 \pm 2.00^{*\#}$
Through-acupuncture	$0.82 \pm 0.03^{*\#\triangle※}$	$32.93 \pm 2.21^{*\#\triangle※}$	$65.32 \pm 7.28^{\#\triangle※}$	$31.91 \pm 2.69^{\#\triangle※}$
Anti-acupuncture	$0.84 \pm 0.03^{*\#\triangle※}$	$34.59 \pm 3.081^{*\#\triangle※}$	$74.16 \pm 7.27^{\#\triangle※}$	$30.85 \pm 1.77^{\#\triangle※}$

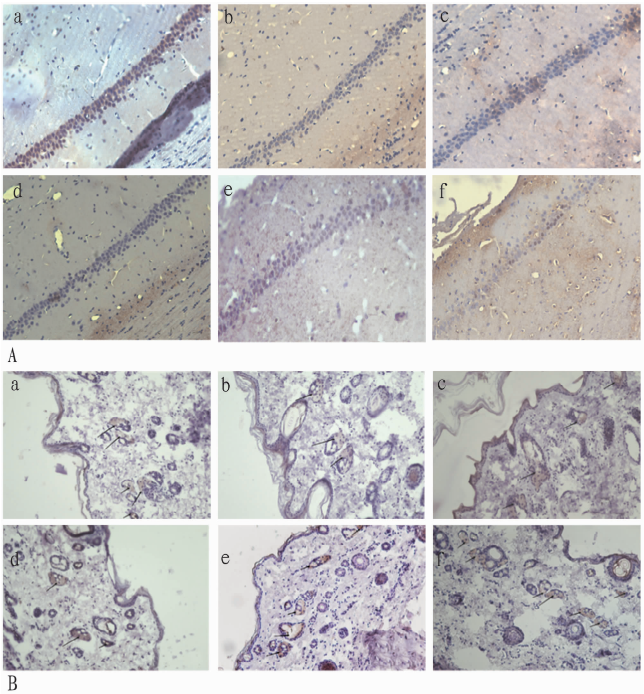
NOTE Compared to the blank group, $^{*}P<0.05$; compared to the model group, $^{\#}P<0.05$; compared to the single Neiguan acupoint group, $^{\triangle}P<0.05$; and compared to the single Waiguan acupoint group, $^{※}P<0.05$. The same below.

3.2 Expression of 5-HT_{1A}R in the acupoint area and hippocampal tissue The expression of 5-HT_{1A}R in the acupoint area and hippocampal tissue of rats from each experimental group was assessed using immunohistochemistry. Microscopic examination demonstrated positive 5-HT_{1A}R expression localized predominantly in the cytoplasm and dendrites, with no staining observed in the nuclei. The positive immunoreactivity was indicated by a pale brownish-yellow coloration and was primarily concentrated in the granule cells of the dentate gyrus and pyramidal cells (Fig. 1). Analysis of the average optical density revealed that the expression level of 5-HT_{1A}R in the acupoint area and hippocampal tissue of the model group was significantly reduced compared to the blank group ($P<0.05$). In contrast, the 5-HT_{1A}R content in the acupoint area and hippocampal tissue of all acupuncture groups was significantly elevated relative to the model group ($P<0.05$). Furthermore, the expression levels of 5-HT_{1A}R in the acupoint area and hippocampal tissue of the two complementary point groups increased more markedly than those in the two single acupoint groups ($P<0.05$). No statistically significant differences were observed between the through-acupuncture and anti-acupuncture groups, nor between the single Neiguan and Waiguan acupoint groups ($P>0.05$, Table 2).

Table 2 Comparison of the average optical density differences of 5-HT_{1A}R and 5-HT_4R mRNA in the acupoint area and hippocampal tissue of rats

Group	5-HT_{1A}R		5-HT_4R mRNA	
	Hippocampal tissue	Acupoint area	Hippocampal tissue	Acupoint area
Blank	0.082 ± 0.013	0.091 ± 0.011	0.00453 ± 0.00089	0.00747 ± 0.00162
Model	$0.026 \pm 0.004^{*}$	$0.056 \pm 0.007^{*}$	$0.00177 \pm 0.00124^{*}$	$0.00560 \pm 0.00089^{*}$
Single Neiguan acupoint	$0.043 \pm 0.005^{*\#}$	$0.073 \pm 0.010^{*\#}$	$0.00273 \pm 0.00053^{*\#}$	$0.00709 \pm 0.00134^{*\#}$
Single Waiguan acupoint	$0.041 \pm 0.005^{*\#}$	$0.075 \pm 0.011^{*\#}$	$0.00268 \pm 0.00096^{*\#}$	$0.00659 \pm 0.00149^{\#}$
Through-acupuncture	$0.058 \pm 0.007^{\#\triangle※}$	$0.084 \pm 0.008^{\#\triangle※}$	$0.00391 \pm 0.00061^{\#\triangle※}$	$0.00851 \pm 0.00138^{\#\triangle※}$
Anti-acupuncture	$0.060 \pm 0.008^{\#\triangle※}$	$0.085 \pm 0.009^{\#\triangle※}$	$0.00412 \pm 0.00083^{\#\triangle※}$	$0.00873 \pm 0.00190^{*\#\triangle※}$

3.3 Expression of 5-HT_4R in the hippocampal tissue of rats The expression of 5-HT_4R mRNA in the hippocampal tissue of rats from each experimental group was assessed using fluorescence in situ hybridization. The results demonstrated that, compared to the



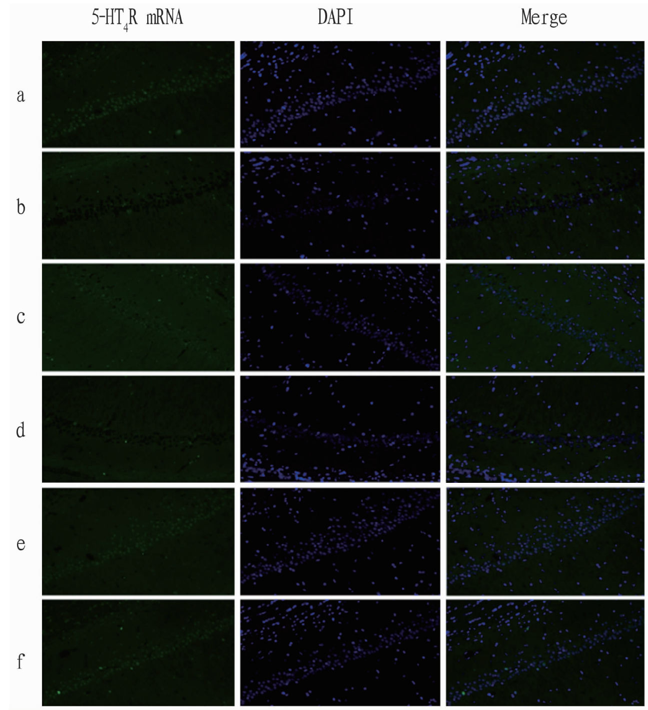
NOTE a. Blank group; b. Model group; c. Single Neiguan acupoint group; d. Single Waiguan acupoint group; e. Through-acupuncture group; f. Anti-acupuncture group.

Fig. 1 Positive expression of 5-HT_{1A}R in the central hippocampal tissue (A) and acupoint area (B) of rats (×200)

blank control group, the 5-HT_4R mRNA expression level in the hippocampal tissue of rats in the model group was significantly reduced ($P<0.01$). In contrast, all acupuncture treatment groups exhibited a significant increase in 5-HT_4R mRNA expression relative to the model group ($P<0.05$). Notably, the complementary acupuncture point groups (through-acupuncture and anti-acupuncture groups) showed a more pronounced increase in 5-HT_4R mRNA expression than the groups treated at single acupoints (Neiguan and Waiguan groups), with this difference reaching statistical significance ($P<0.05$). However, no significant difference was observed between the two complementary acupuncture point groups ($P>0.05$, Table 2 and Fig. 2).

4 Discussion

In traditional Chinese medicine, insomnia is classified under categories such as "inability to sleep", "difficulty closing the eyes", and "inability to lie down". Its pathogenesis is attributed to an imbalance between Yin and Yang; therefore, the primary treatment principle involves regulating this balance. The theory of complementary acupuncture points emphasizes achieving therapeutic outcomes by harmonizing Yin and Yang through the strategic selection of acupoints. Previous clinical trials conducted by the research group have demonstrated that employing complementary acupuncture points in the treatment of insomnia, such as through-acupuncture, offers advantages including precise acupoint selection and favorable therapeutic effects[11]. The Neiguan and Waiguan acupoints, representing Yin and Yang respectively, are interconnected as exterior-interior meridian points. Anatomically, they are positioned opposite each other, reflecting the intrinsic relationship between these two acupoints. Both points are located at the intersection of the eight extraordinary meridians. Specifically, the Neiguan acupoint is associated with the Yinwei meridian, while the Waiguan acupoint corresponds to the Yangwei meridian. The Yinwei meridian links the Yin meridians and functions to coordinate them throughout the body, whereas the Yangwei meridian connects the Yang meridians and facilitates their integration. Together, these meridians connect the Yin and Yang meridians of the human body, thereby regulating the balance between Yin and Yang and promoting their mutual interaction. When the acupuncture technique involves penetrating two acupoints with a single needle, both points simultaneously attain the deqi sensation. This approach facilitates "the guidance of Yang from Yin", thereby promoting smooth interaction between Yin and Yang within the body at these acupoints. Consequently, it achieves the objectives of harmonizing Ying and Wei, regulating qi and blood, and restoring the balance between Yin and Yang.



NOTE a. Blank group; b. Model group; c. Single Neiguan acupoint group; d. Single Waiguan acupoint group; e. Through-acupuncture group; f. Anti-acupuncture group. The green fluorescence signal represents the labeling of 5-HT_4R mRNA, while the blue fluorescence signal corresponds to nuclei stained with DAPI.

Fig. 2 Expression of 5-HT_4R mRNA in the hippocampal tissue of rats (fluorescence in situ hybridization)

Modern medical research indicates that disruption in the balance between factors involved in maintaining wakefulness and sleep, as well as their receptors, may play a significant role in the development of insomnia. The coordinated signal transmission and functional interaction between neurons and target cells constitute the physiological foundation necessary for the normal regulation of the sleep-wake cycle[12]. Recent research has demonstrated that the regulation of sleep-wake rhythms in organisms is associated with various monoamine neurotransmitters, including 5-HT and its metabolite 5-HIAA[13]. 5-HT, synthesized through the biological transformation of tryptophan, functions primarily as an inhibitory neurotransmitter within the central nervous system. It plays a crucial role in the initiation and maintenance of sleep and is extensively involved in multiple physiological processes, such as circadian rhythm regulation, emotional modulation, cognitive performance, and the processing of aversive stimuli[14]. The insomnia rat model prepared by PCPA markedly inhibits the biosynthesis of 5-HT in both the central and peripheral systems of the animals. Previous studies have demonstrated that serum 5-HT concentrations in rats subjected to the PCPA model were significantly decreased[15]. This reduction in neurotransmitter levels was closely correlated with several behavioral abnormalities, including prolonged sleep latency, heightened sensitivity to external stimuli, and increased frequency of aggressive behaviors. Consequently, modulating the 5-HT system function through pharmacological or other interventions to enhance receptor binding has emerged as a critical research focus for improving sleep quality.

5-HIAA is the final metabolite of 5-HT, produced through the sequential catalytic actions of monoamine oxidase A (MAO-A) and aldehyde dehydrogenase (ALDH). Variations in 5-HIAA levels within the peripheral and central nervous systems are commonly employed as critical biomarkers to assess the activity of 5-HT neurons, as well as the synthesis and release dynamics of 5-HT. In studies involving rat models of depression comorbid with sleep disorders[16], significant reductions in the concentrations of 5-HT and 5-HIAA were observed in both the hippocampal region and serum, indicating a potential impairment in the function of the 5-HT nervous system. The 5-HT$_{1A}$R, a significant subtype of the 5-HT receptor implicated in sleep regulation, is among the most widely distributed and abundantly expressed receptor types within the brain[17]. This receptor is predominantly localized in limbic system structures, including the raphe nucleus, prefrontal cortex, and hippocampal tissue, and is closely associated with pathophysiological conditions such as anxiety, depression, insomnia, and disordered eating. Consequently, it has emerged as a critical target in the development of psychotropic medications[18]. As the first fully sequenced 5-HT receptor subtype and the most abundant in the brain, 5-HT$_{1A}$R plays a central role in the regulation of sleep and mood[19].

The 5-HT$_4$R is a type of self-inhibitory receptor derived from chromaffin cells that plays a crucial role in regulating the release of 5-HT[20]. This receptor subtype is expressed at relatively high levels in the hippocampal tissue and contributes to the regulation of sleep rhythms through its interaction with hippocampal functions. The synthesis of 5-HT$_4$R is governed by its genetic template, 5-HT$_4$R mRNA, which serves as the direct carrier of genetic information for the production of the receptor protein. Consequently, the expression level of 5-HT$_4$R mRNA directly influences the amount of functional 5-HT$_4$R produced. An upregulation of 5-HT$_4$R mRNA expression leads to increased synthesis of 5-HT$_4$R, thereby enhancing the neuron's capacity to release 5-HT.

In this study, following the establishment of the PCPA-induced insomnia rat model, a significant decrease in the levels of 5-HT and 5-HIAA was observed in the hippocampal tissue. From a biochemical metabolism perspective, the synthesis of 5-HIAA is directly dependent on the availability of its precursor, 5-HT. Consequently, a reduction in 5-HT neurotransmitter activity and concentration leads to a corresponding decrease in 5-HIAA production. Subsequent to acupuncture intervention, the concentrations of 5-HT and 5-HIAA in the hippocampal tissue of rats across all acupuncture groups increased to varying extents. These findings suggest that acupuncture may enhance the function of the 5-HT system under insomnia conditions and promote the synthesis and metabolism of 5-HT.

This study investigated two distinct acupuncture techniques: through-acupuncture and anti-acupuncture. The through-acupuncture method employed the same needling approach commonly used in the clinical treatment of insomnia, specifically involving the through-acupuncture of Neiguan and Waiguan acupoints. In contrast, the anti-acupuncture method involved needling one acupoint on each side of Neiguan and Waiguan. Observations in rats indicated no significant difference in needle efficacy between the two acupuncture methods. The findings suggest that both acupuncture approaches can enhance sleep quality in insomniac rats. Further analysis demonstrated that acupuncture at Neiguan and Waiguan acupoints significantly increased the expression levels of 5-HT and 5-HIAA in the acupoint area and hippocampal tissue of insomniac rats, as well as elevated the levels of 5-HT$_{1A}$R and 5-HT$_4$R mRNA. Additionally, acupuncture applied to complementary acupuncture points produced superior effects compared to stimulation of a single acupoint, with these differences reaching statistical significance.

In conclusion, the amelioration of insomnia through acupuncture at the Neiguan and Waiguan acupoints may be associated with elevated expression levels of 5-HT, 5-HIAA, 5-HT$_{1A}$R, and 5-HT$_4$R mRNA in both the acupoint area and hippocampal tissue of insomniac rats.

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