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Original Article

A Study on the Mechanism of Herb-Partitioned Moxibustion at the Navel Based on the Gut-Brain Axis Theory in the Prevention and Treatment of Insomnia via the BDNF/TrkB Pathway

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ABSTRACT

Aim/Background: To examine the impact of navel moxibustion, a form of herb-partitioned moxibustion applied at the belly button, on Brain-Derived Neurotrophic Factor (BDNF), Tyrosine Kinase receptor B (TrkB) and 5-Hydroxytryptamine (5-HT) levels in rats with PCPA-induced insomnia. Additionally, to investigate the mechanism by which navel moxibustion improves insomnia. **Materials and Methods:** 27 Specific Pathogen Free (SPF)-grade male three-month-old SD rats were randomly divided by weight into the normal group, model group and navel moxibustion group. Except for the normal group, the latter two groups were administered Para-Chlorophenylalanine (PCPA) via gastric intubation to establish the insomnia rat model. After successful modeling, the navel moxibustion group received treatment, while the normal and model groups received a sham treatment consisting of binding with non-woven fabric at the Shenque (CV 8) point, with treatments administered once every three days for a total of five treatments. After the completion of the treatment, the animals were humanely euthanized in order to obtain hippocampal tissue. The collected samples were then subjected to HE staining, real-time quantitative PCR and Western blot analysis for a comparative assessment of hypothalamic BDNF, TrkB and 5-HT gene and protein expression levels before and after the application of isolated moxibustion therapy. **Results:** HE staining revealed significant pathological symptoms in the hypothalamic tissue of the model group, while the navel moxibustion group showed darker nuclear staining, increased density and more orderly arrangement, resembling the normal group. Compared to the normal group, the model group exhibited reduced gene expression of BDNF, TrkB and 5-HT in the hypothalamic tissue ($p<0.05$), with lower protein levels of BDNF ($p<0.05$), TrkB ($p<0.05$) and 5-HT. After navel moxibustion treatment, the levels of BDNF, TrkB and 5-HT genes were significantly elevated compared to the model group ($p<0.05$), as were the protein expression levels of BDNF ($p<0.05$), TrkB and 5-HT. **Conclusion:** The navel moxibustion method can improve cellular damage in the hypothalamic tissue of PCPA-induced insomnia rats, elevating the gene and protein levels of BDNF, TrkB and 5-HT, thereby confirming the role of navel moxibustion in neurotransmission and effectively alleviating insomnia.

Keywords: 5-hydroxytryptamine (5-HT), Brain-Derived Neurotrophic Factor (BDNF), Insomnia, Tyrosine Kinase receptor B (TrkB).

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INTRODUCTION

Insomnia, also known as sleep disorder, is a condition characterized by reduced sleep due to factors such as challenges in initiating sleep and interruptions in the continuity of sleep.[1] Epidemiological studies have found that the prevalence of insomnia symptoms worldwide is as high as 35%,[2] with the rate in women approximately 1.5 times that of men.[3] The causes are related to high social pressure, excessive use of electronic devices and disrupted sleep rhythms. Currently, the addiction potential and side effects of insomnia medications are significant and their clinical efficacy varies, making insomnia one of the major risk factors affecting human physical and mental health. Scientific research has confirmed that the communication between the brain and gastrointestinal tract occurs via three primary pathways, namely the nervous system, endocrine system and immune system, playing an important role in improving

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insomnia.[4] As a widely present neurotransmitter in the brain-gut axis, 5-HT is one of the earliest factors believed to regulate sleep. The body has a higher level of 5-HT during normal sleep, while insomnia patients exhibit lower levels of 5-HT.[5] Phosphorylation of the cAMP Response Element-Binding protein (CREB), a transcription factor, is observed in various regions of the brain and gastrointestinal tissues. It can induce the phosphorylation of CREB at the serine 133 site (Ser133) of the hippocampal target gene Nerve Growth Factor (NGF), recruit CREB-binding protein to activate the BDNF promoter region, regulate TrkB transcription,[6] promote secretory lineage differentiation, lead to the proliferation of chromaffin cells and enhance the secretion of 5-HT.[7] The aforementioned may be one of the mechanisms for improving insomnia.[8] It has been previously discovered that herb-partitioned moxibustion on the navel (hereinafter referred to as "navel moxibustion") has clinical efficacy in improving insomnia.[9]

Navel moxibustion, an ancient therapeutic method rooted in Traditional Chinese Medicine, involves the application of moxibustion directly to the navel area, which is considered a vital point connecting the brain and the gastrointestinal tract. This method employs a combination of herbal medicine and heat from burning mugwort (*Artemisia argyi*) to stimulate the navel region, thereby modulating the gut-brain axis and potentially influencing sleep patterns.[1] The heat and herbal components are believed to penetrate the skin and affect the underlying tissues, promoting local circulation and enhancing the body's natural healing processes.[10] Navel moxibustion is valued for its potential to target the complex interplay between the digestive, nervous and endocrine systems, offering a holistic approach to addressing insomnia.[11] Recent studies have begun to explore the molecular mechanisms underlying the effects of navel moxibustion, suggesting that it may increase BDNF/TrkB signaling and 5-HT levels, which are crucial for sleep regulation.[12] By integrating these traditional practices with modern scientific insights, we aim to further elucidate the therapeutic potential of navel moxibustion in the management of insomnia.

Therefore, we hereby report our recent research findings as follows.

MATERIALS AND METHODS

Experimental Animals

SPF-grade male 11-week-old SD rats, weighing (400±20) g, purchased from Beijing Huafukang Biotechnology Co., Ltd., License No. SYXK(Jing)2019-0008. Rearing conditions: temperature 20-26°C, humidity 40-70%, with 1 week of acclimatization. The Animal Ethics Committee granted approval for the animal experiments (Approval No.: LL-202308130001).

Experimental Materials

Hematoxylin staining solution (ZLI-9610, Zhongshan Gold Bridge, Beijing, China), hematoxylin blue solution (G1040, Servicebio, Wuhan, China), eosin staining solution (G1100, Solarbio, Beijing, China), ultrapure RNA extraction kit (CW0581M, CWBIO); HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (R223-01, Vazyme, Nanjing, China); ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme, Nanjing, China); RIPA cell lysis buffer (C1053, Beijing Puli Technology Co., Ltd., Beijing, China), BCA protein quantification kit (E-BC-K318-M, Elabscience, Wuhan, China), Mouse Anti-β-Actin (HC201, TransGen Biotech, 1/2000, Beijing, China), secondary antibody: HRP conjugated Goat Anti-Mouse IgG (H+L) (GB23301, Servicebio, 1/2000, Wuhan, China), Primary antibodies: Rabbit Anti 5-HT (Affinity AF5453, 1:1000, Beijing, China), Rabbit Anti BDNF (Affinity df6061, 1:1000, Beijing, China), Rabbit Anti TrkB (Affinity AF6461, 1:1000, Beijing, China), secondary antibody: HRP conjugated Goat Anti-Rabbit IgG (H+L) (GB23303, Servicebio, 1/2000, Wuhan, China).

Experimental Equipment

Tissue dehydrator (KD-TS3S1, Jinhua Kedi Instrument Co., Ltd., Zhejiang Province), tissue embedding machine (HistoCore Arcadia, Leica, Wetzlar, Germany), microtome (HistoCore BIOCUT, Leica), slide warmer (Leica HI1210, Leica, Wetzlar, Germany), fully automated microplate reader (SuPerMax 3100, Shanghai Shamp, Shanghai, China), vertical protein electrophoresis apparatus (UPS600, Yamei, Shanghai, China), fully automated chemiluminescence imaging analysis system (Tanon-5200, Shanghai Tianen Technology Co., Ltd., Shanghai, China), fluorescent PCR instrument (CFX Connect™ Real-Time, Bio-Rad Life Science Products (Shanghai) Co., Ltd., Shanghai, China), ultra-sensitive chemiluminescence imaging system (Chemi Doc™ XRS+, Bio-Rad Life Science Products (Shanghai) Co., Ltd., Shanghai, China).

Methods

Animal Grouping and Model Creation

A cohort of 27 male SD rats was carefully chosen and their weights were recorded. Subsequently, the rats were randomly allocated into three distinct groups, each comprising 9 individuals: a control group, an experimental model group and a navel moxibustion intervention group. All groups except the normal group underwent modeling. The modeling experiment used a modified method of intraperitoneal injection of PCPA to create the insomnia rat model. The insomnia model using PCPA is a commonly used pharmacological technique to study the sedative and hypnotic effects. The modeling time is short and the characteristics of insomnia are obvious, which is especially suitable for the evaluation of the treatment effect of insomnia.[13] Dissolve $NaHCO_3$ tablets in 30-40°C warm water to prepare

a 5% $NaHCO_3$ solution, then add 0.9% NaCl and test the pH with precise pH paper until it reaches between pH 7 and 8, add PCPA, stir, then add 10% gum arabic and sonicate for 15 min to prepare a weakly alkaline physiological saline and administer an intraperitoneal injection at a dosage of 0.50 $g \cdot kg^{-1}$ for 2 consecutive days.

Criteria for Successful Modeling

Sodium pentobarbital was given at a dosage of 35 mg per kilogram, dissolved in physiological saline at 50 mg/mL for intraperitoneal injection. The rats' sleep latency and duration were measured, while their behavioral responses were monitored. If significant differences were observed compared to the normal group, this indicated successful modeling.

Experimental Methods

In the navel moxibustion group, the rat's Shenque point (positioned at the intersection where the upper three-fourths and lower one-fourth of the line extending from the upper border of the sternum to the external genitalia meet) and surrounding fur were shaved. The rat was fixed in a supine position and the Shenque point was routinely disinfected with 75% ethanol. A circle was made from flour mixed with warm water (approximately 0.5 cm inner diameter) and placed on the Shenque point. The prepared herbal powder (approximately 3 g) was evenly filled into the Shenque point to be level with the flour ring, A small moxa cone (approximately 0.3 cm in diameter and height) was placed on the herbal powder for moxibustion. Continuous moxibustion was performed for 5 cones over approximately 20 min, taking care to prevent burns. After moxibustion, the herbal powder was left in place and covered with medical non-woven fabric to seal the Shenque area, which was removed 24 hr later. Treatment was performed once every 3 days for a total of 5 times, followed by evaluation and testing (Figure 1). The herbal composition included Xiangfu, Qingbanxia, Wuweizi and Rougui, which were mixed in equal proportions, finely powdered and sealed for future use. The rats in both the control group and experimental group were immobilized and subjected to non-woven fabric treatments applied at the Shenque acupoint.

Specimen Extraction

After administering treatment to the rats in each of the three groups, an intraperitoneal injection of 10% chloral hydrate (50 mg/kg) was given. Once the rats entered a deep anesthetic state, the skull of the rat was secured with forceps and then the whole brain was removed. A surgical blade was used to bluntly separate the cortex from the hippocampus after incising. The hippocampus was transferred into a 1.5 mL EP cryotube for HE staining, PCR and Western blot detection.

Table 1: **Primer sequence list.**

Primer name	Primer sequences (5′-3′)	Product length(bp)	Annealing temperature (°C)
GAPDH F	GACAACTTTGGCATCGTGGA	133	58
GAPDH R	ATGCAGGGATGATGTTCTGG		
BDNF F	CTCTGTCCCTGAAGCCCACT	376	57.3
BDNF R	CTTGCCCTTGATGTCCGT		
TrkB F	GGCTCTGGGGCTTATGCTT	469	58.9
TrkB R	TCGGGGCTGGATTTCGT		
5-HT F	GTCACCTGCGACCTGTTTATC	400	58.3
5-HT R	TGCCTGCTCCCTTCTTTTC		

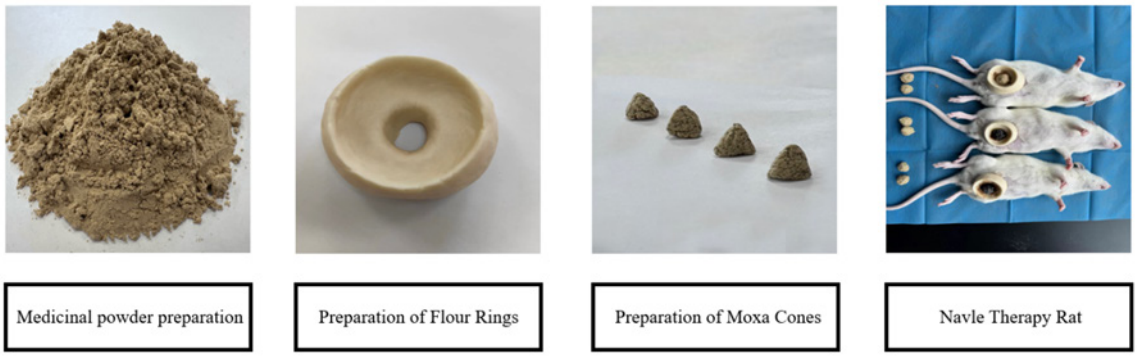

Figure 1: Navel moxibustion method for PCPA insomnia model rats.

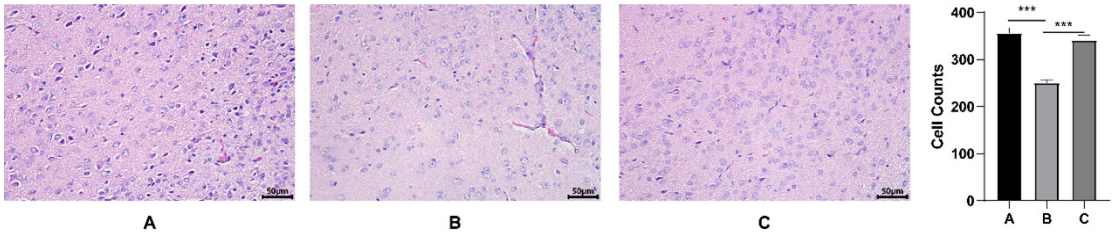

Figure 2: HE staining results of hypothalamus tissue in PCPA insomnia rats (×400); A: Normal group; B: Model group; C: Navel Moxibustion group.

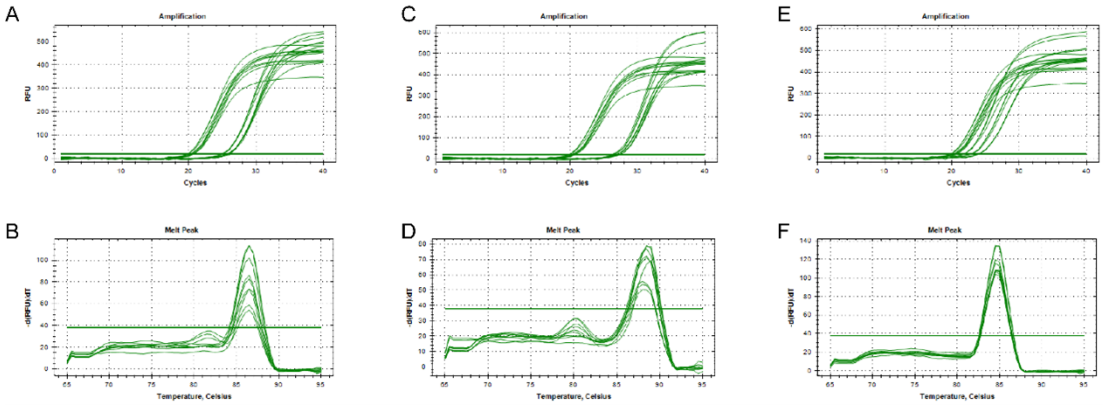

Figure 3: Amplification and dissolution curves of BDNF, TrkB and 5-HT gene samples in hypothalamus tissue of PCPA insomnia rats; A: 5-HT gene amplification curve; B: 5-HT gene dissolution curve; C: BDNF gene amplification curve; D: BDNF gene dissolution curve; E: TrkB gene amplification curve; F: TrkB gene dissolution curve.

Indicator Detection

HE Staining

SD rat hypothalamic tissue sections were prepared, baked, deparaffinized and rehydrated. The sections underwent staining using hematoxylin (ZLI-9610, Zhongshan Jinqiao) for a duration of 3 to 5 min. Subsequently, they were rinsed with water, differentiated using alcohol containing 1% hydrochloric acid and finally blued utilizing a bluing solution. After staining with eosin (G1100, Solarbio) for 3-5 min, the segments underwent dehydration and were subsequently mounted. The samples were observed under a microscope (BX43, Olympus).

PCR Detection

Fluorescent quantitative PCR (SYBR Green method) was applied, using R-GAPDH as the internal reference gene to detect the expression levels of BDNF, TrkB and 5-HT genes in the rat hypothalamic tissue. The reaction proceeded in the following manner: initial pre-denaturation at a temperature of 95°C for duration of 10 min, followed by denaturation at the same temperature for a period of 10 sec. Subsequently, annealing took place at a temperature of 58°C for an interval of 30 sec and extension occurred at a temperature of 72°C lasting for another duration of 30 sec. This entire process was repeated for a total number of 40 cycles. GAPDH was employed as the endogenous control and the $2^{-\Delta\Delta Ct}$ method was utilized to determine the relative expression levels of the genes. The primer sequences can be found in Table 1.

Western Blot Detection

Measurement of protein expression levels of BDNF, TrkB and 5-HT in the hypothalamic tissue of rats with insomnia induced by PCPA. Specific procedures include extracting proteins, determining protein concentrations, loading and conducting electrophoresis on samples, transferring membranes, blocking them, incubating with primary antibodies, incubating with secondary antibodies and detecting chemiluminescence.

Statistical Analysis

All experiments were conducted in triplicate. The results are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS 19.0 software, employing one-way ANOVA to compare multiple groups. A significance level of α=0.05 was chosen for determining statistical significance. GraphPad Prism 9.0 software was utilized for data visualization.

RESULTS

Pathological Effects of Navel Moxibustion on Hypothalamic Tissue in PCPA-Induced Insomnia Rats

As depicted in Figure 2, the hypothalamic nuclei of rats with insomnia induced by PCPA exhibited a blue staining pattern, while the cytoplasm displayed a red staining. Compared to the

control group, the experimental group showed reduced intensity of nuclear staining in hypothalamic tissue, decreased density and a more disordered arrangement. In the navel moxibustion group, the hypothalamic nuclei showed deeper staining, increased density and a more orderly arrangement, resembling that of the normal group. These results indicate that the model group rats developed significant pathological changes in hypothalamic tissue and the insomnia symptoms were significantly improved after navel moxibustion treatment.

Effects of Navel Moxibustion on the Expression Levels of BDNF/TrkB Pathway-Related Genes in the Hypothalamic Tissue of PCPA-Induced Insomnia Rats

As depicted in Figures 3 and 4, the expression levels of BDNF, TrkB and 5-HT genes in the hypothalamic tissue of the PCPA-induced insomnia model group were found to be significantly decreased compared to those in the normal group ($p<0.05$). Conversely, when comparing with the model group, it was observed that the herbal-partitioned moxibustion group exhibited a notable increase in the expression levels of BDNF, TrkB and 5-HT genes ($p<0.05$).

Effects of Navel Moxibustion on the Expression Levels of BDNF, TrkB and 5-HT Related Proteins in the Hypothalamic Tissue of PCPA-Induced Insomnia Rats

As depicted in Figures 5 and 6, the PCPA-induced insomnia model group exhibited a significant decrease in the expression of BDNF ($p<0.05$), TrkB ($p<0.05$) and 5-HT proteins within the hypothalamic tissue when compared to the normal group. Compared to the control group, there was a significant decrease in BDNF expression ($p<0.05$), TrkB and 5-HT genes in the herbal-partitioned moxibustion group was upregulated. The observed upregulation after treatment was consistent with expectations.

DISCUSSION

Based on the theoretical foundation that "the Shenque point (navel) is the key point for the transformation of yin and yang qi and connects the internal organs," in our previous research, we confirmed that herb-partitioned moxibustion at the navel has the effect of improving insomnia. Our experimental results confirm that herb-partitioned moxibustion at the navel can effectively improve hypothalamic tissue damage caused by the PCPA-induced insomnia rat model and increase the expression of BDNF, TrkB and 5-HT proteins. This suggests that herbal-partitioned moxibustion at the navel is an effective external therapy for improving insomnia and its mechanism may

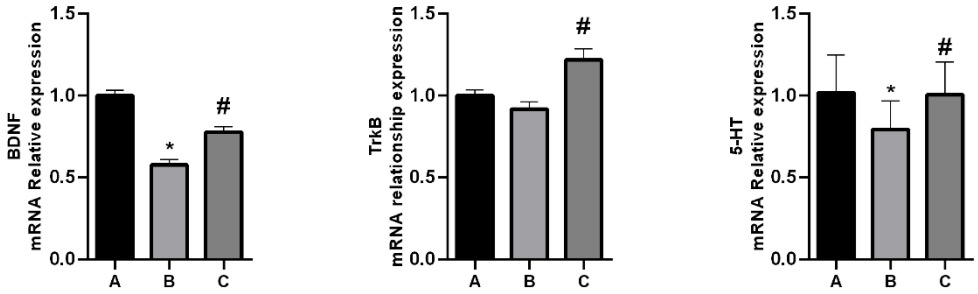

Figure 4: Relative expression levels of BDNF, TrkB and 5-HT genes in the hypothalamus of PCPA insomnia rats; (A: normal group B: model group C: navel moxibustion group *Compared with the normal group, $p<0.05$; #Compared with the model group, $p<0.05$).

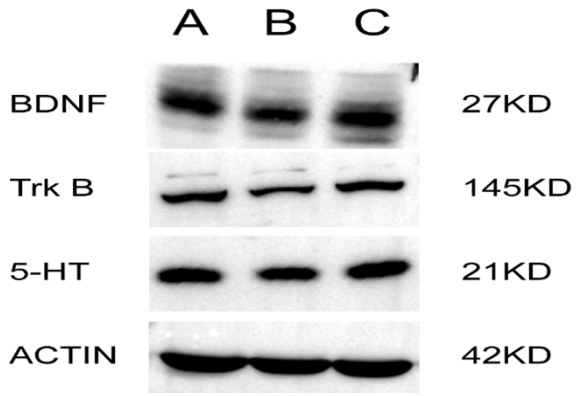

Figure 5: Electrophoresis of BDNF, TrkB and 5-HT proteins in hypothalamus of PCPA insomnia rats.

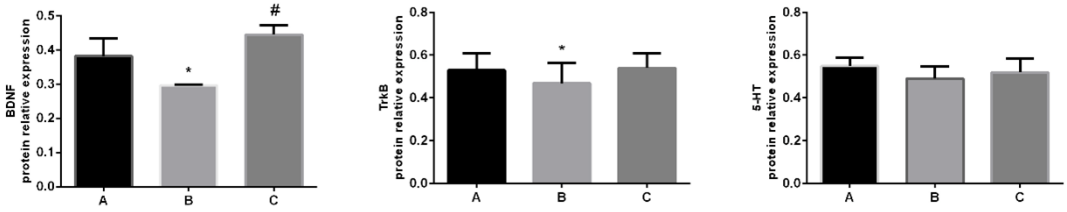

Figure 6: Relative expression of BDNF, TrkB and 5-HT proteins in the hypothalamus of PCPA insomnia rats; (A: normal group B: model group C: navel moxibustion group *Compared with the normal group, $p<0.05$; #Compared with the model group, $p<0.05$).

be related to involvement in the BDNF/TrkB signaling pathway and increased expression of 5-HT.

The development of insomnia is strongly linked to the interplay between monoamine neurotransmitters and their receptors, including 5-HT, Gamma-Aminobutyric Acid (GABA) and its corresponding receptors. Additionally, it involves cytokine inflammatory responses as well as hormones associated with the Hypothalamus-Pituitary-Adrenal (HPA) axis.[14-16] 5-HT functions as a vital neurotransmitter and intracellular messenger, widely present in both the brain and intestinal tissues. It plays a crucial role in regulating diverse physiological functions, such as pain perception, sleep patterns and body temperature regulation.[17] BDNF, a growth factor with multiple functions, is involved in the development and functioning of the brain. It acts through the TrkB receptor to support the survival and differentiation of neurons, as well as facilitate synaptic plasticity. These processes are closely linked to insomnia and behaviors associated with anxiety.[17] According to relevant research, BDNF/TrkB has been found to have a stimulating effect on the production of 5-HT in both brain and intestinal tissues. This includes its impact on tryptophan hydroxylase, which is an enzyme that plays a crucial role in the synthesis of 5-HT.[18-20] A deficiency in BDNF signaling can interfere with 5-HT in the basolateral amygdala.[16] In Traditional Chinese Medicine, insomnia is referred to as "bu mei" (sleeplessness), generally attributed to "disharmony between Ying and Wei and Yang failing to enter yin.[21]" In <Lingshu•Intestines and Stomach>, it is recorded: "The small intestine attaches to the spine from behind... its flow to the large intestine is externally connected above the navel." The "large intestine" refers to the colon; the large and small intestines are interconnected and both attach to the navel. The deep layer of the navel corresponds to the large intestine, so<Lingshu•Intestines and Stomach>also states: "The large intestine is associated with the navel area." In terms of anatomical position, the Shenque point connects with both the large and small intestines. Research has shown that drugs administered via the navel can directly act on the intestines, exhibiting high sensitivity and rapid absorption, with bioavailability approximately six times higher than other administration routes.[22] In<Li Yue Pian Wen>, it is also stated: "Entering through the navel is no different from entering through the mouth." Apart from its anatomical structure, the navel is often concave, making it easy to store and seal medicines after application. This makes the Shenque point the most ideal site for medication administration in the body. In addition, the ignition of the moxa cone plays an important role in herbal-partitioned moxibustion at the navel. Mugwort (*Artemisia argyi*) has the function of warming meridians and unblocking channels. Pharmacological studies have shown that the volatile components in moxa smoke come from the volatile oils of Qiai (*Artemisia argyi*), oxidation products and incomplete combustion products, which can significantly increase monoamine neurotransmitters such as 5-HT and NE in mice.[23] Therefore, studying the use of herbal-partitioned moxibustion at the navel to improve insomnia is valuable.

The traditional Chinese medicines used in herbal-partitioned moxibustion at the navel for treating insomnia are spleen-strengthening and mind-calming herbs, such as *Cyperus rotundus* (Xiangfu), *Pinellia ternata* (Qing Banxia), *Schisandra chinensis* (Wuweizi) and *Cinnamomum cassia* (Rougui), among others. Xiangfu enters the liver, spleen and triple burner meridians and has therapeutic effects such as soothing the liver to relieve depression and regulating qi to alleviate chest discomfort. Banxia is warm and dry in nature, bitter in taste, descending rebellious qi and harmonize the stomach, pungent to disperse stagnation and travels through the meridians associated with the spleen, stomach and lungs. It is an essential herb for drying dampness, resolving phlegm and warming cold phlegm. It is particularly effective in treating damp phlegm in the viscera, excels at descending rebellious qi to stop vomiting and eliminating distention and dispersing nodules. The <Zhu Zhi Mi Yao> states: "It dries stomach dampness, resolves phlegm, benefits spleen and stomach qi, reduces swelling and disperses stagnation and removes phlegm and saliva from the chest." Modern research indicates that Banxia has the potential to induce sedation and promote a sense of calmness through its ability to regulate the imbalance of GLU/GABA neurotransmitters and modulate 5-HT levels.[24] *Schisandra chinensis* (Wuweizi) has functions of astringing to prevent leakage, benefiting qi and generating fluids, tonifying the kidneys and mind pacification. Research indicates that Wuweizi can improve sleep by modulating the TLR/NF-κB signaling pathway.[25] *Cinnamomum cassia* (Rougui) possesses properties that promote warmth in the digestive system, alleviate

cold sensations, regulate energy flow and provide pain relief. Clinically, it is often combined with *Coptis chinensis* (Huanglian) to harmonize the heart and kidneys, facilitating the downward movement of heart fire and the upward movement of kidney water, attaining equilibrium between fire and water, thus enhancing sleeplessness. The entire formula is based on the principle of regulating qi and strengthening the spleen, which aligns with the theory from the <Huangdi Neijing> that "if the stomach is disharmonious, one cannot rest peacefully," corresponding with the mechanism of improving insomnia via the "brain-gut axis."

Therefore, based on the "brain-gut axis" theory, we infer that herbal-partitioned moxibustion at the navel can improve insomnia by regulating the protein expression of BDNF/TrkB and adjusting the body's 5-HT levels. Our study innovatively proposes that, starting from the brain-gut axis theory, regulating 5-HT through the BDNF/TrkB signaling pathway can improve substances related to insomnia. Moreover, herbal-partitioned moxibustion at the navel has the characteristics of good efficacy, fewer side effects and high compliance, making it worthy of clinical promotion and application. However, in this study, our number of PCPA-induced insomnia rats was small and the detection of downstream related proteins was not comprehensive enough; thus, we will continue our in-depth research in the next phase.

CONCLUSION

This study highlights the efficacy of herb-partitioned navel moxibustion in alleviating insomnia through modulation of the gut-brain axis. The treatment improved hypothalamic tissue damage in PCPA-induced insomnia rats and significantly increased BDNF, TrkB and 5-HT gene and protein expression. These findings suggest that navel moxibustion enhances neurotransmitter regulation and synaptic plasticity, contributing to improved sleep quality. By integrating traditional Chinese medicine with modern scientific insights, this non-invasive therapy demonstrates potential as a viable, low-risk alternative for insomnia management. Future studies are recommended to explore additional downstream mechanisms for comprehensive validation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BDNF: Brain-derived neurotrophic factor; **TrkB:** Tyrosine kinase receptor B; **5-HT:** 5-hydroxytryptamine (serotonin); **PCPA:** Para-chlorophenylalanine; **SPF:** Specific pathogen-free; **SD rats:** Sprague-Dawley rats; **HE staining:** Hematoxylin and eosin staining; **PCR:** Polymerase chain reaction; **RIPA:** Radioimmunoprecipitation assay; **BCA:** Bicinchoninic acid; **HRP:** Horseradish peroxidase; **GAPDH:** Glyceraldehyde 3-phosphate dehydrogenase; **CREB:** cAMP response element-binding protein; **NGF:** Nerve growth factor; **HPA axis:** Hypothalamus-pituitary-adrenal axis; **GLU/GABA:** Glutamate/Gamma-aminobutyric acid; **TLR:** Toll-like receptor; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **NE:** Norepinephrine; **EP:** Eppendorf (cryotube).

REFERENCES

1. Cohen ZL, Eigenberger PM, Sharkey KM, Conroy ML, Wilkins KM. Insomnia and other sleep disorders in older adults. Psychiatr Clin North Am. 2022;45(4):717-34. doi: 10.1016/j.psc.2022.07.002, PMID 36396275.
2. Morin CM, Drake CL, Harvey AG, Krystal AD, Manber R, Riemann D, *et al.* Insomnia disorder. Nat Rev Dis Primers. 2015;1:15026. doi: 10.1038/nrdp.2015.26, PMID 27189779.
3. Suh S, Cho N, Zhang J. Sex differences in insomnia: from epidemiology and etiology to intervention. Curr Psychiatry Rep. 2018;20(9):69. doi: 10.1007/s11920-018-0940-9, PMID 30094679.
4. Kasarello K, Cudnoch-Jedrzejewska A, Czarzasta K. Communication of gut microbiota and brain via immune and neuroendocrine signaling. Front Microbiol. 2023;14:1118529. doi: 10.3389/fmicb.2023.1118529, PMID 36760508.
5. Landolt HP, Wehrle R. Antagonism of serotonergic 5-HT2A/2C receptors: mutual improvement of sleep, cognition and mood? Eur J Neurosci. 2009;29(9):1795-809. doi: 10.1111/j.1460-9568.2009.06718.x, PMID 19473234.
6. Castro J, Harrington AM, Garcia-Caraballo S, Maddern J, Grundy L, Zhang J, *et al.* Alpha-conotoxin Vc1.1 inhibits human dorsal root ganglion neuroexcitability and mouse colonic nociception via GABA(B) receptors. Gut. 2017;66(6):1083-94. doi: 10.1136/gutjnl-2015-310971, PMID 26887818.
7. Ford AC, Mahadeva S, Carbone MF, Lacy BE, Talley NJ. Functional dyspepsia. Lancet. 2020;396(10263):1689-702. doi: 10.1016/S0140-6736(20)30469-4, PMID 33049222.
8. Daftary SS, Calderon G, Rios M. Essential role of brain-derived neurotrophic factor in the regulation of serotonin transmission in the basolateral amygdala. Neuroscience. 2012;224:125-34. doi: 10.1016/j.neuroscience.2012.08.025, PMID 22917617.
9. Ren Y, Zhong L. Influence of moxibustion at Shenque (CV 8) on senile symptoms and melatonin. World J Acupunct Moxibustion. 2013;23(2):6-10. doi: 10.1016/S1003-5257(13)60036-7.
10. Huang C, Liang J, Han L, Liu J, Yu M, Zhao B. Moxibustion in early Chinese medicine and its relation to the origin of meridians: A study on the unearthed literatures. Evid Based Complement Alternat Med. 2017; 2017:8242136. doi: 10.1155/2017/8242136, PMID 28298936.
11. Shi J, Zhang X, Chen J, Shen R, Cui H, Wu H. Acupuncture and moxibustion therapy for cognitive impairment: the microbiome-gut-brain axis and its role. Front Neurosci. 2023;17:1275860. doi: 10.3389/fnins.2023.1275860, PMID 38274501.
12. Deng H, Shen X. The mechanism of moxibustion: ancient theory and modern research. Evid Based Complement Alternat Med. 2013; 2013:379291. doi: 10.1155/2013/379291, PMID 24159344.
13. Bao Y, Zhou H, Fu Y, Wang C, Huang Q. Zhumian Granules improves PCPA-induced insomnia by regulating the expression level of neurotransmitters and reducing neuronal apoptosis. J Ethnopharmacol. 2024;327:118048. doi: 10.1016/j.jep.2024.118048, PMID 38484955.
14. Rosenberg R, Citrome L, Drake CL. Advances in the treatment of chronic insomnia: A narrative review of new nonpharmacologic and pharmacologic therapies. Neuropsychiatr Dis Treat. 2021;17:2549-66. doi: 10.2147/NDT.S297504, PMID 34393484.
15. Modica MN, Lacivita E, Intagliata S, Salerno L, Romeo G, Pittalà V, *et al.* Structure-activity relationships and therapeutic potentials of 5-HT(7) receptor ligands: an update. J Med Chem. 2018;61(19):8475-503. doi: 10.1021/acs.jmedchem.7b01898, PMID 29767995.
16. Roman V, Hagewoud R, Luiten PG, Meerlo P. Differential effects of chronic partial sleep deprivation and stress on serotonin-1A and muscarinic acetylcholine receptor sensitivity. J Sleep Res. 2006;15(4):386-94. doi: 10.1111/j.1365-2869.2006.00555.x, PMID 17118095.
17. Berumen LC, Rodríguez A, Miledi R, García-Alcocer G. Serotonin receptors in hippocampus. ScientificWorldJournal. 2012; 2012:823493. doi: 10.1100/2012/823493, PMID 22629209.
18. Madhav TR, Pei Q, Zetterström TS. Serotonergic cells of the rat raphe nuclei express mRNA of tyrosine kinase B (trkB), the high-affinity receptor for brain derived neurotrophic factor (BDNF). Brain Res Mol Brain Res. 2001;93(1):56-63. doi: 10.1016/s0169-328x(01)00183-8, PMID 11532338.
19. Siuciak JA, Clark MS, Rind HB, Whittemore SR, Russo AF. BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain. J Neurosci Res. 1998;52(2):149-58. doi: 10.1002/(SICI)1097-4547(19980415)52: 2<149::AID-JNR3>3.0.CO;2-A, PMID 9579405.
20. Kronenberg G, Mosienko V, Gertz K, Alenina N, Hellweg R, Klempin F. Increased brain-derived neurotrophic factor (BDNF) protein concentrations in mice lacking brain serotonin. Eur Arch Psychiatry Clin Neurosci. 2016;266(3):281-4. doi: 10.1007/s00406-015-0611-3, PMID 26100147.

21. Li Y, Fan Y, Mao J, Zhang N, Abulimiti A, Zhong K. The law of oral traditional Chinese medicine for insomnia based on Chinese medical code. Med Data Min. 2019;2(4):160-8. doi: 10.53388/mdm2019123004.
22. Chien YW. Navel absorption: transdermal bioavailability of testosterone. J Pharm Sci. 1984;73(2):283-5. doi: 10.1002/jps.2600730241, PMID 6707906.
23. Xu H, Zhao B, Cui Y, Lim MY, Liu P, Han L, *et al.* Effects of moxa smoke on monoamine neurotransmitters in SAMP8 mice. Evid Based Complement Alternat Med. 2013; 2013:178067. doi: 10.1155/2013/178067, PMID 23762105.
24. Wang M, Li N, Jing S, Wang C, Sun J, Li H, *et al.* Schisandrin B exerts hypnotic effects in PCPA-treated rats by increasing hypothalamic 5-HT and gamma-aminobutyric acid levels. Exp Ther Med. 2020;20(6):142. doi: 10.3892/etm.2020.9271, PMID 33093880.
25. Urry Z, Xystrakis E, Richards DF, McDonald J, Sattar Z, Cousins DJ, *et al.* Ligation of TLR9 induced on human IL-10-secreting Tregs by 1alpha,25-dihydroxyvitamin D3 abrogates regulatory function. J Clin Invest. 2009;119(2):387-98. doi: 10.1172/JCI32 354, PMID 19139565.

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