Ind. J. Pharm. Edu. Res., 2026; 60(3):1256-1267.
https://www.ijper.org

Original Article

TSH Suppression Induces Anxiety- and Depression-like Behaviors in Rats, With Hippocampal 5-HT Loss, Notch Activation, and a Neurogenesis-Apoptosis Imbalance

Yikun Zhao[1], Honglin Jiang[2], Huiyuan Shang[1], Zhongyuan Xia[3,*]

[1]Clinical Medical College of China-Japan Friendship Hospital, Beijing University of Chinese Medicine, Beijing, CHINA.
[2]Department of Acupuncture and Mini-invasive Oncology, Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing, CHINA.
[3]Department of Chinese Medicine Surgery, China-Japan Friendship Hospital, Beijing, CHINA.

ABSTRACT

Background: TSH suppression is widely used in thyroid cancer management; however, its neurobehavioral consequences are not well understood. **Objectives:** To evaluate the impact of TSH suppression on emotion-related behaviors, thyroid axis hormones, hippocampal serotonin (5-HT) levels, neurogenesis, Notch signaling, and apoptosis in a rat model. **Materials and Methods:** Rats were assigned to three groups: blank control, TSH replacement, and TSH suppression. TSH suppression was induced using total thyroidectomy combined with graded L-T4. Behavioral assessments were performed using open field and tail suspension tests. We measured serum levels of FT3, FT4, and TSH via ELISA, while hippocampal 5-HT levels were also quantified. Neural stem-cell proliferation and differentiation were assessed with BrdU, Nestin, NeuN, and GFAP immunohistochemistry. Notch signaling and apoptosis markers were analyzed using qPCR and Western blot techniques. **Results:** Compared with control and replacement groups, TSH suppression led to anxiety- and depression-like behaviors, demonstrated by greater immobility, longer rest periods, and delayed entry into the center of the open field. Additionally, we observed elevated FT3 and FT4 levels with reduced TSH, decreased levels of hippocampal 5-HT, and significant upregulation of Notch signaling pathway markers. Neurogenesis was disrupted, characterized by enhanced stem-cell proliferation but decreased neuronal differentiation, coupled with an increase in astrocyte differentiation. Furthermore, the Bax/Bcl-2 ratio increased, indicating a pro-apoptotic environment. **Conclusion:** The findings indicate that TSH suppression is linked to adverse mood-related behaviors, 5-HT depletion, Notch pathway activation, and an imbalance in neurogenesis and apoptosis. These results suggest that excessive suppression of the thyroid hormone axis may carry neuropsychiatric risks that need to be carefully balanced against the oncological benefits during thyroid cancer treatment.

Keywords: 5-HT, Bax/Bcl-2, Neurogenesis, Notch, Open field, Tail suspension, TSH suppression.

Correspondence:
Dr. Zhongyuan Xia
Department of Chinese Medicine Surgery, China-Japan Friendship Hospital, Beijing, CHINA.
Email: zg810056888@163.com

Received: 16-12-2025;
Revised: 27-02-2026;
Accepted: 06-04-2026.

INTRODUCTION

The relationship between thyroid dysfunction and mood disorders has been recognized for decades; patients with hyperthyroidism frequently present with anxiety, irritability and insomnia, whereas hypothyroidism is associated with depressive symptoms and cognitive impairment.[1,2] In current clinical practice, however, neuropsychiatric complaints are also encountered in patients whose thyroid axis is pharmacologically manipulated. In differentiated thyroid cancer, long-term Thyroid-Stimulating Hormone (TSH)-suppressive therapy with supraphysiologic doses of Levothyroxine (L-T4) is routinely prescribed after total thyroidectomy to reduce the risk of recurrence.[3] By chronically driving serum TSH into a subnormal range, this regimen achieves oncologic benefit but may simultaneously disturb central monoaminergic systems. Growing clinical observations suggest that patients on long-term TSH suppression frequently report anxiety, depressive symptoms and impaired quality of life, raising concern that aggressive endocrine therapy may carry underappreciated neuropsychiatric risks.[4]

The pathogenesis of emotional disorders is multifaceted, with the hippocampus playing a crucial role in regulating emotions and cognition.[5] Thyroid hormones can cross the blood-brain barrier and directly influence the activities of hippocampal neurons, thereby modulating neurotransmitter metabolism, synaptic plasticity, and neurogenesis.[2] Existing evidence suggests that abnormal thyroid function can lead to a decrease in the level of 5-Hydroxytryptamine (5-HT), a core neurotransmitter involved

DOI: 10.5530/ijper.20264130

Copyright Information :
Copyright Author (s) 2026 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

in emotional stability and antidepressant mechanisms. This 5-HT deficiency is considered an important pathological basis for anxiety and depression.[6] Notably, changes in 5-HT levels not only directly affect neuronal activities but also participate in the regulation of hippocampal neurogenesis by modulating the proliferation and differentiation of Neural Stem Cells (NSCs).[7] Therefore, TSH suppression may indirectly contribute to the development of emotional disorders through the pathway of "decreased 5-HT → impaired neurogenesis".

The Notch signaling pathway is a critical molecular network that regulates the fate determination of Neural Stem Cells (NSCs) during neurogenesis.[8] Previous studies have suggested that excessive Notch signaling is associated with decreased neuronal differentiation, increased astrocyte production, and the development of depression-like behaviors.[9] It can be hypothesized that the decrease in serotonin (5-HT) caused by Thyroid-Stimulating Hormone (TSH) suppression may abnormally activate the Notch pathway, disrupt the balance between neurogenesis and apoptosis, and ultimately manifest as anxiety/depression-like behaviors.

The present study employed a rat model to systematically investigate the neurobiological mechanisms underlying the association between Thyroid-Stimulating Hormone (TSH) suppression therapy and the risk of emotional disorders. Levothyroxine-induced TSH suppression was used to establish the experimental model, and a comprehensive assessment was conducted to evaluate the effects on emotion-related behaviors, hippocampal serotonin (5-HT) levels, Notch signaling activity, and the neurogenesis/apoptosis process across behavioral, neurochemical, and molecular levels. The findings of this study aim to elucidate the potential mechanisms by which TSH suppression may contribute to the development of emotional disorders, providing experimental evidence to inform the assessment and management of neuropsychiatric risks in clinical patients undergoing long-term TSH suppression therapy for thyroid cancer.

MATERIALS AND METHODS

Experimental Animals

In this experiment, 120 SPF-grade male Wistar rats, sourced from Beijing Vital River Laboratory Animal Technology Co., Ltd., (License No. SCXK (Beijing) 2016-0006), were used. At purchase, the rats were approximately 6 weeks old, weighing 200±10 g, and measuring 12.9±1.4 cm in length. They were housed at the Laboratory Animal Center of Beijing University of Chinese Medicine under controlled conditions: 22±2°C temperature, 50%-60% humidity, and a 12-hr light/dark cycle. Standard pellet feed and water were provided ad libitum. The rats underwent a 7-day acclimation period to mitigate stress from transport and environmental changes. The study adhered to the Regulations for the Administration of Laboratory Animals and received approval from the Laboratory Animal Ethics Committee of Beijing University of Chinese Medicine (Approval No. BUCM-2024060102-2228).

Experimental Drug

Levothyroxine (L-thyroxine, L-T4; Sigma-Aldrich, Merck, product number T2501, 50 µg/tablet) was dissolved in sterile normal saline to achieve the desired concentration and used immediately to maintain drug efficacy.

Grouping and Modeling

A cohort of 120 rats was randomly allocated into two groups: 12 rats served as the blank control group, while the remaining 108 underwent total thyroidectomy to establish a model, following the methodology outlined by Jin S, Sugitani I, *et al.*,[10] Under pentobarbital sodium anesthesia (3%, 0.1 mL/100 g, intraperitoneally), bilateral thyroidectomy was performed microscopically. One week post-operation, the survival rate of the model rats was approximately 56.5%, resulting in 61 successfully modeled survivors. These 61 rats were further divided into two groups using a random number table, with 12 rats per group: the hormone replacement group and the TSH suppression group. Both groups received daily morning drug administration for model establishment. Experiment approach was shown in Figure 1.

1. Blank control group: No thyroidectomy was performed. The animals were reared conventionally without any drug intervention.
2. Hormone replacement group: Rats after total thyroidectomy were subcutaneously injected with L-T4 (1.2 µg/100 g body weight) once a day for 15 consecutive days.
3. TSH suppression group: Rats after total thyroidectomy were subcutaneously injected with L-T4 (1.6 µg/100 g body weight) once a day for 15 consecutive days.

Throughout the modeling and drug administration phases, we meticulously monitored the rats' body weight, activity levels, and overall condition to assess the impact of the modeling and interventions.

Behavioral Testing

Open Field Test (OFT)

The experimental setup consisted of a transparent polycarbonate cube (100 × 100 × 50 cm^3), with the bottom surface divided into 9 equal-area quadrants. A 4K camera (60 Hz) was mounted at the top, and XR-SuperMaze 3.0 software was used for video recording and trajectory analysis. The experiment was conducted in a quiet environment maintained at approximately 40 lux illuminance. Each rat was placed in the center of the apparatus and allowed

to move freely for 6 min. The recorded behavioral measures included: total displacement, average speed, immobility duration, latency to first enter the central area, frequency of visits to the central area, and proportion of time spent in the central area. Immediately after each trial, the apparatus was cleaned with 75% ethanol, ventilated, and dried before the next animal was tested.

Tail Suspension Test (TST)

The experiment took place during the dark cycle (illuminance≤10 lux) at a room temperature of 22±1°C and a relative humidity of 50%±5%. The rat's tail was clamped 1.0±0.2 cm from the tip. Prior to the test, rats acclimated for 60 sec before the formal timing commenced for 360 sec. Immobility time during passive suspension was recorded, defined as the complete stillness of all four limbs, with only respiratory movements observed.

Specimen Preparation

Serum Preparation

Following a 12-hr fast, rats were weighed and anesthetized via an intraperitoneal injection of 3% pentobarbital sodium (0.1 mL/100 g). Once anesthesia was confirmed, the abdominal aorta was exposed, and approximately 5 mL of blood was drawn using a vacuum blood collection tube, completing the procedure within 5 min. The blood samples stood for 30 min before being centrifuged at 3000 r/min for 10 min to separate the serum, which was then stored at -80°C for future analysis.

Brain Tissue Collection

Following blood collection, the rats were sacrificed by decapitation, and the entire brain was extracted within 120 sec. The brains were placed on a dissection table cooled to 4°C. Under these low-temperature conditions, the surface was rinsed with sterile saline to eliminate residual blood. The hippocampal region was meticulously dissected under a binocular microscope, divided into aliquots in pre-frozen 2 mL cryotubes, labeled, and promptly stored at -80°C.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum thyroid function indices (TSH, FT3, FT4) and hippocampal 5-hydroxytryptamine (5-HT) levels were quantified using commercial ELISA kits, following the manufacturers' protocols. Optical density values at 450 nm were measured with a Bio-Rad Model 680 microplate reader, and a standard curve was constructed for quantitative analysis.

Double-Label Immunofluorescence

Hippocampal tissues were fixed in 4% paraformaldehyde, dehydrated with 30% sucrose, and sectioned into 10 μm paraffin slices. Following antigen retrieval and blocking, primary antibodies were applied and incubated overnight at 4°C. The next day, tissues were washed with PBS, then incubated with fluorescently labeled secondary antibodies for 1 hr at room temperature in the dark. Nuclei were stained with DAPI, visualized under a fluorescence microscope, and the number and distribution of positive cells were analyzed using ImageJ software.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA from the hippocampus was extracted via the TRIzol method, with purity and concentration assessed by Nanodrop. cDNA synthesis was conducted through reverse transcription, followed by qPCR using SYBR Green Master Mix in a 20 μL reaction system over 40 cycles. The target genes were pivotal to the Notch signaling pathway, with GAPDH serving as the internal reference. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blot

Hippocampal tissues were homogenized and lysed using RIPA buffer with PMSF, followed by centrifugation at 12,000 g for 10 min to collect the supernatant. Protein concentration was assessed via the BCA method. Proteins (30 μg) were separated by SDS-PAGE, transferred to a membrane, and blocked with 5% non-fat milk for 1 hr. Primary antibodies were applied and incubated overnight at 4°C. After TBST washing, HRP-labeled secondary antibodies were added and incubated for 1 hr at room temperature. Bands were visualized using ECL and captured with a ChemiDoc MP imaging system. Quantitative analysis was conducted with ImageJ, normalizing relative expression levels to β-actin. All the experimental reagents are listed in Table 1.

Statistical Analysis

The data are presented as mean±standard deviation ($\bar{x}$±s). Normality and homogeneity of variance were assessed prior to analysis. One-way Analysis of Variance (ANOVA) was used for comparisons among multiple groups, followed by the Least Significant Difference (LSD) method for pairwise comparisons. Non-parametric tests were employed if the data did not conform to the normal distribution. Statistical analyses were performed using SPSS 26.0, and figures were generated with GraphPad Prism 10. The threshold for statistical significance was set at $p<0.05$. Effect sizes (e.g. η^2 for ANOVA) were calculated to quantify the magnitude of group differences.

RESULTS

Behavioral Performance

In the Tail Suspension Test (TST), immobility time varied significantly among groups (ANOVA, F=7.42, p=0.000398). Post-hoc analysis revealed that rats in the TSH suppression group exhibited significantly longer immobility times compared to the blank group (p=0.0003) and the replacement group (p=0.005). No significant difference was observed between the replacement and blank groups (p=0.3255) (Figure 2).

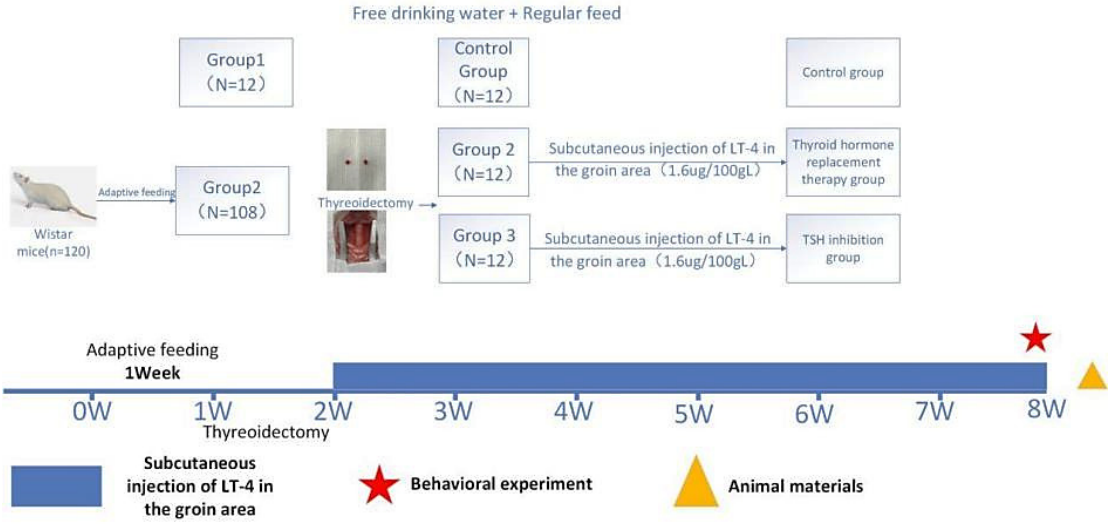

Figure 1: Experiment Approach.

Table 1: Main reagents, suppliers and catalog numbers.

Reagent name	Supplier	Catalog No.
Rat TSH ELISA KIT	Ruixin Biotech	RX302808R
Rat FT3 ELISA KIT	Ruixin Biotech	RXJ302109R
Rat 5-HT ELISA KIT	Ruixin Biotech	RXJ303050R
Rat FT4 ELISA KIT	Ruixin Biotech	RXJ302110R
Anti-Brdu Mouse mAb	Servicebio (Wuhan, China)	GB12051
Anti-GFAP Rabbit pAb	Servicebio (Wuhan, China)	GB11096
Anti-Nestin Mouse mAb	Servicebio (Wuhan, China)	GB12138
Anti-NeuN Rabbit pAb	Servicebio (Wuhan, China)	GB11138
BAX Antibody	MCE	HY-P80027
Bcl-2 Antibody	MCE	HY-P80029
GAPDH Antibody	Affinity	AF7021
Hes1 Antibody	Beyotime Biotechnology	AF2167
HES5 Antibody	Servicebio (Wuhan, China)	A9768
Jagged1 Antibody	MCE	HY-P80195
Notch1 Antibody	Zenbio	R380355

The Open Field Test (OFT) revealed distinct behavioral patterns in the inhibition group compared to controls (Figure 3A). Rats in the inhibition group exhibited a significant reduction in the number of entries into the central arena (p<0.0001, Figure 3B). Additionally, these animals displayed a marked prolongation in the latency to enter the central area (p<0.0001, Figure 3C) and the time to first enter the central zone (p=0.0002, Figure 3D). Furthermore, the total distance traveled by the inhibition group was significantly decreased (p<0.0001, Figure 3E).

Changes in thyroid axis function

ELISA assay results demonstrated that TSH suppression markedly influenced thyroid hormone levels. In the suppression group, FT3 and FT4 levels were significantly elevated compared to the blank group (p<0.0001), whereas no significant difference was observed between the replacement and blank groups. TSH levels significantly decreased in the suppression group (p<0.0001), with no significant difference between the replacement and blank groups (Table 2).

5-HT level in the hippocampus

The ELISA assay revealed a significant reduction in hippocampal 5-HT content in the inhibition group compared to the blank group (1.916±0.37 vs. 3.415±0.42, p<0.0001). Conversely, the substitution group exhibited no statistically significant difference from the blank group (p=0.054) (Figure 4).

Changes in Hippocampal Neurogenesis

Double immunofluorescence staining revealed that, compared to the blank and substitution groups, the inhibition group exhibited significantly elevated levels of BrdU/Nestin (neural stem cells; p=0.0013 and p=.0014, respectively; Figure 5), significantly reduced levels of BrdU/NeuN (newborn neurons; p=0.0006; Figure 6), and significantly increased levels of BrdU/GFAP (newborn astrocytes; p=0.0008 and p=0.0009, respectively; Figure 7).

Activity of the Notch signaling pathway

Quantitative PCR (qPCR) analysis revealed significant upregulation of Notch1, Hes1, Hes5, and Jagged-1 mRNA expression in the inhibition group compared to controls (all p<0.05, Figure 8). Western blotting further corroborated this trend, with Notch1, HES1, HES5, and Jagged-1 protein levels demonstrating a statistically significant increase in the inhibition

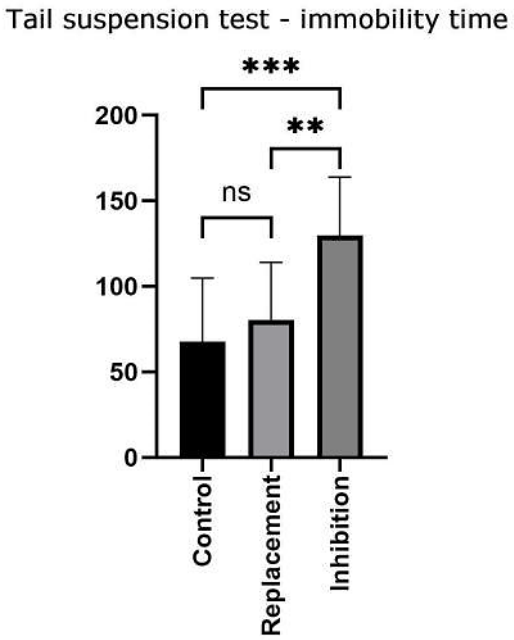

Figure 2: Tail suspension test - immobility time. (ns, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001).

group relative to the blank and substitution groups (Notch1: F=14.69, p=0.0012; HES5: F=23.04, p=0.0002; HES1: F=14.59, p=0.0013; Jagged-1: F=26.60, p=0.0002, Figure 9).

Changes in Apoptosis-Related Molecules

The Bax/Bcl-2 ratio, a key indicator of apoptotic signaling, was significantly elevated at the mRNA level in the inhibition group compared to both the blank and replacement groups (6.510±0.799 vs. 0.667±0.015 and 0.677±0.015, respectively; p<0.01 for both comparisons). In contrast, no statistically significant difference in the Bax/Bcl-2 ratio was observed between the blank and replacement groups (p=0.072) (Table 3). This trend was recapitulated at the protein level, where the Bax/Bcl-2 ratio was markedly increased in the inhibition group relative to the blank and replacement groups (0.867±0.015 vs. 0.667±0.015 and 0.677±0.015, respectively) (Table 4).

DISCUSSION

This study systematically demonstrated that prolonged TSH suppression elicits substantial metabolic and endocrine alterations, as well as anxiety- and depression-like behaviors,

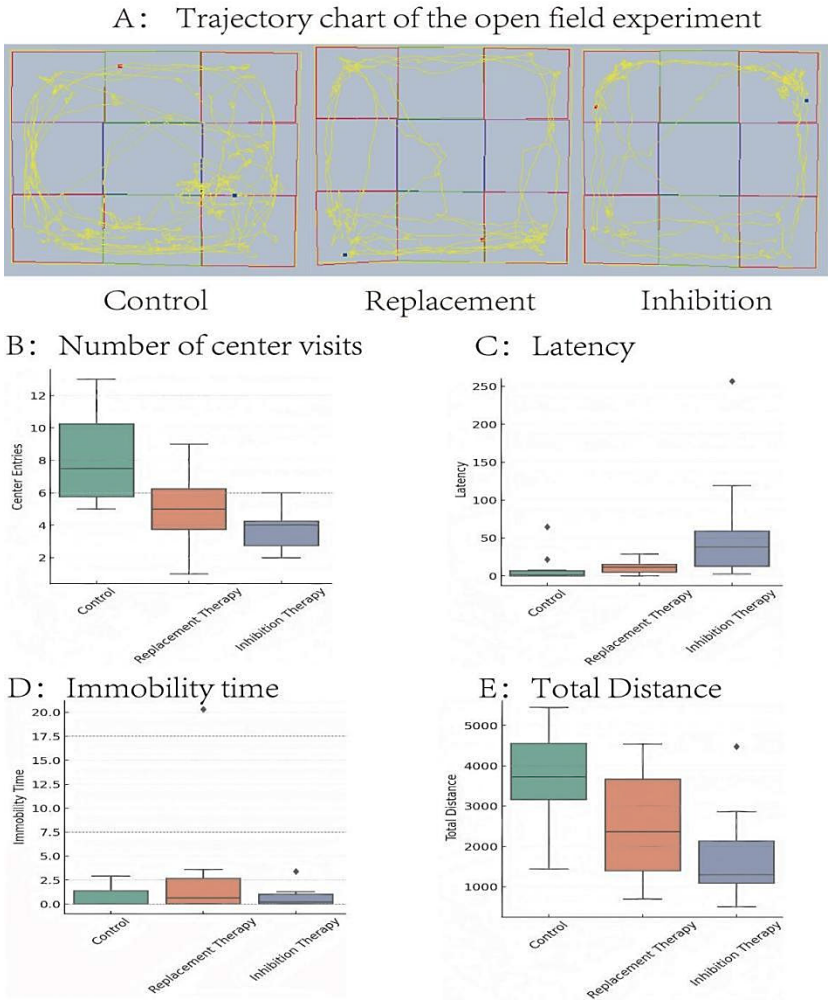

Figure 3: The results of the open field test.

by modulating hippocampal neurotransmitters, neurogenesis, and apoptosis in a rat model of TSH suppression. These findings offer an experimental foundation for understanding the mental health symptoms experienced by clinical thyroid cancer patients following TSH suppression therapy, and suggest potential underlying molecular mechanisms.

The present study demonstrates that rats subjected to Thyroid-Stimulating Hormone (TSH) suppression exhibit distinct behavioral phenotypes characteristic of depression and anxiety. Specifically, these animals displayed prolonged immobility in the tail suspension test and reduced exploration with increased latency in the open field test. This finding aligns with clinical observations in thyroid cancer patients undergoing long-term TSH suppression therapy, who frequently report symptoms such as anxiety, depression, and emotional instability.[11-13] Importantly, the control group receiving hormone replacement therapy maintained euthyroid status and did not exhibit significant emotional disturbances. These results suggest that the emotional abnormalities observed are not simply a consequence of thyroid hormone deficiency, but are directly linked to the persistent low levels of TSH and the artificial disruption of the thyroid axis regulation.

The present study provides further mechanistic insights into the observed behavioral alterations associated with Thyroid-Stimulating Hormone (TSH) suppression. The findings indicate that TSH suppression was accompanied by a significant decrease in serotonin (5-HT) levels within the hippocampus. This is noteworthy, as extensive evidence has demonstrated that thyroid hormones can regulate the metabolism and transport of monoamine neurotransmitters, including 5-HT, in the brain, and the impact on the 5-HT system is particularly crucial.[14] Serotonin is not only the classic neurotransmitter basis for emotional disorders, but its deficiency has also been linked to inhibited neurogenesis and reduced neural plasticity.[15] In the current study, the decrease in 5-HT levels coincided with the observed behavioral abnormalities, suggesting a pivotal upstream role of 5-HT dysregulation in the emotional disturbances caused by TSH suppression. Importantly, clinical studies have reported similar findings, with hyperthyroid patients exhibiting 5-HT metabolic disorders[16] and hypothyroid patients showing decreased 5-HT synthesis and release,[17] both of which are strongly correlated

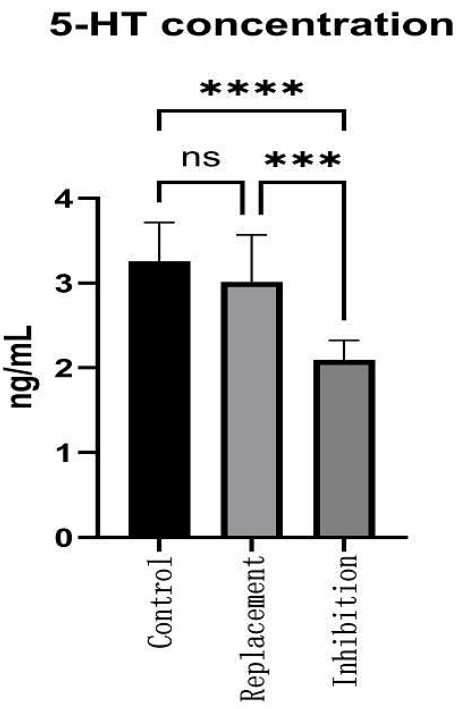

Figure 4: 5-HT concentration. (ns, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001).

Table 2: The contents of FT3, FT4 and TSH in the serum of rats in different groups.

Group	Sample size	Average concentration of FT3	±SD	Minimum	Maximum
Control	12	3.635	±0.140	3.454	3.803
Replacement	12	3.704	±0.142	3.504	3.862
Inhibition	12	5.448	±0.171	5.217	5.673
	Sample size	**Average con-centration of FT4**	**±SD**	**Minimum**	**Maximum**
Control	12	15.122	±0.799	14.000	16.268
Replacement	12	15.104	±0.828	13.442	16.426
Inhibition	12	22.342	±1.205	20.974	24.850
	Sample size	**Average con-centration of TSH**	**±SD**	**Minimum**	**Maximum**
Control	12	7.014	±0.509	6.167	7.561
Replacement	12	6.858	±0.640	5.508	7.595
Inhibition	12	2.702	±0.418	2.112	3.268

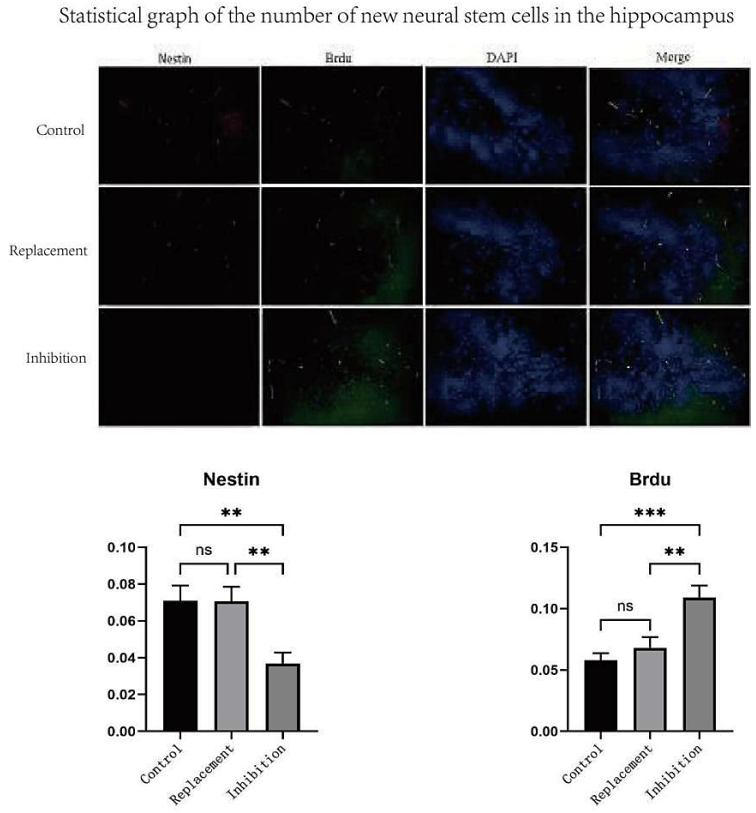

Figure 5: Statistical graph of the number of new neural stem cells in the hippocampus. (ns, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001).

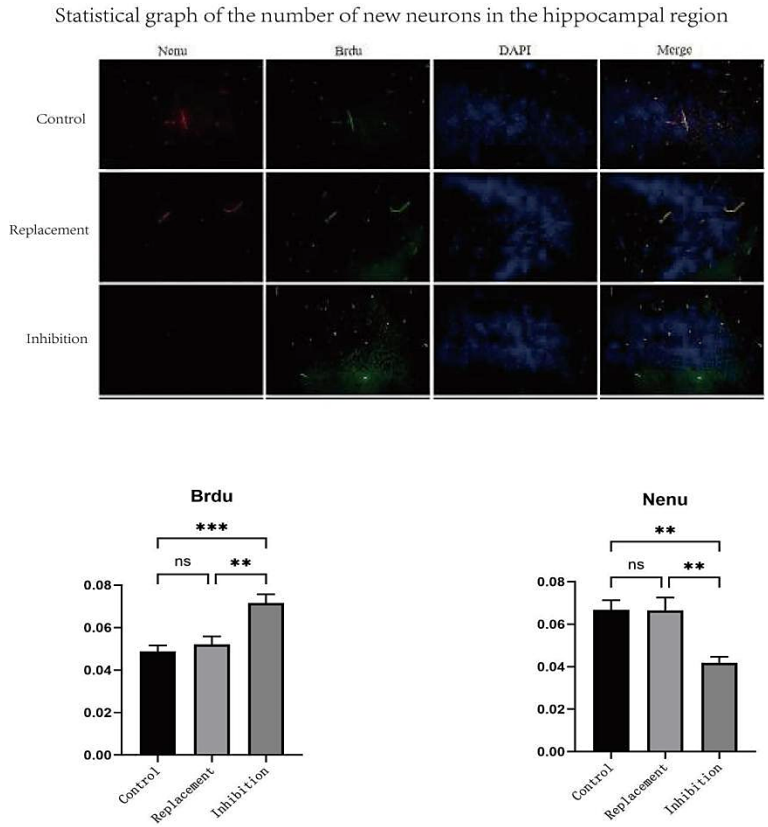

Figure 6: Statistical graph of the number of new neurons in the hippocampal region. (ns, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001).

with depressive and anxious symptoms. Collectively, these results indicate that TSH suppression may lead to a hormonal imbalance by over-activating the thyroid axis, which in turn alters the homeostasis of the 5-HT system, ultimately resulting in emotional abnormalities.

The present study observed that TSH suppression resulted in a significant imbalance in hippocampal neurogenesis, characterized by enhanced proliferation, restricted neuronal differentiation, and increased glial differentiation. Hippocampal neurogenesis is a core pathological mechanism implicated in emotional disorders. Previous animal studies have demonstrated that both chronic stress and depression models are accompanied by reduced hippocampal neuron generation and enhanced glial reactivity.[18] The abnormal neurogenesis pattern reported here is highly consistent with the decline in 5-HT signaling, as 5-HT can directly regulate the fate determination of neural stem cells. Diminished 5-HT not only weakens neuronal differentiation signals but may also lead to functional impairment of neural circuits by triggering a deviation towards the glial lineage.[19]

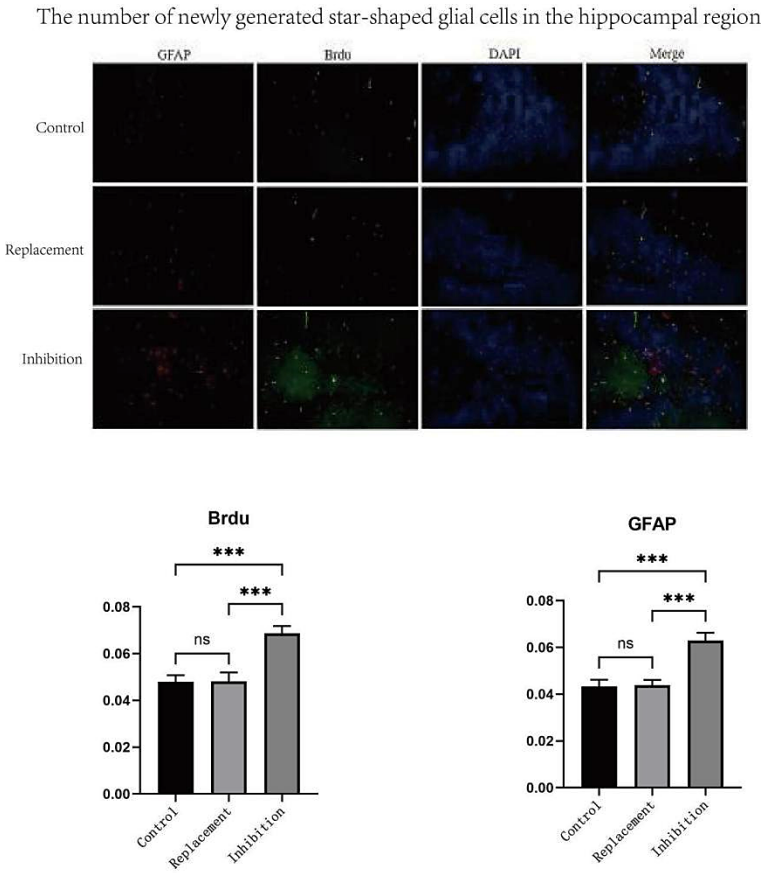

Figure 7: The number of newly generated star-shaped glial cells in the hippocampal region. (ns, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001).

Table 3: Relative expression level of Bax/Bcl-2 mRNA.

Group	Bax RQ	Bcl-2 RQ	Bax/Bcl-2
Control 1	1.000	1.000	1.000
Control 2	2.297	1.548	1.484
Control 3	3.106	2.415	1.286
Replacement 1	3.800	1.732	2.194
Replacement 2	4.120	2.041	2.019
Replacement 3	3.950	1.876	2.106
Inhibition 1	5.200	0.850	6.118
Inhibition 2	5.500	0.920	5.978
Inhibition 3	5.800	0.780	7.436

Table 4: Relative expression level of Bax/Bcl-2 protein.

Group	Sample 1	Sample 2	Sample 3	Mean±SD
Control	0.65	0.68	0.67	0.667±0.015
Replacement	0.66	0.69	0.68	0.677±0.015
Inhibition	0.85	0.88	0.87	0.867±0.015

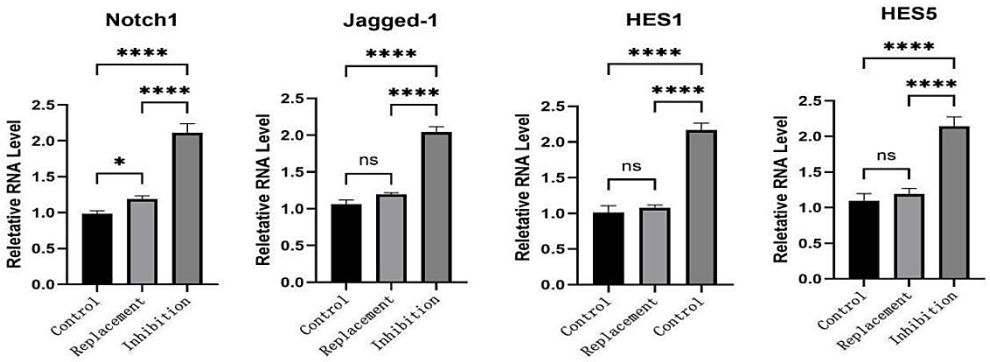

Figure 8: The mRNA expression levels of key target genes of the Notch signaling pathway in the hippocampus of each group of rats. (ns, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001).

The Notch signaling pathway appears to be a critical mediator of this neurogenic imbalance. We observed significant upregulation of Notch1, Hes1, Hes5 and Jagged-1 at both the mRNA and protein levels in the TSH suppression group, indicating robust activation of the Notch cascade. Notch signaling maintains neural stem cells in an undifferentiated state and inhibits neuronal lineage commitment, thereby promoting astroglial differentiation when overactivated.[20] Previous work has reported hyperactivation of Notch signaling in depression models and stress-exposed animals, accompanied by reduced hippocampal neurogenesis.[21] Our findings extend these observations by showing that pharmacological TSH suppression, likely via upstream 5-HT disturbance, is associated with aberrant Notch activation and subsequent disruption of the neurogenesis-apoptosis balance.

From a pharmacological perspective, the Notch pathway may represent a potential target to mitigate TSH-suppression-induced neurobehavioral effects.[22] Experimental studies have shown that pharmacological modulators of Notch signaling-including γ-secretase inhibitors and ligand-blocking strategies-can dampen Notch activity, restore a more favorable neuronal/glial differentiation balance and ameliorate depression-like behaviors in certain rodent models.[23] While the systemic inhibition of Notch raises safety concerns, especially in oncology, these preclinical data suggest that selective, brain-targeted or temporally constrained modulation of Notch signaling could, in principle, help preserve hippocampal plasticity and mood in contexts where endocrine therapy perturbs this pathway.[24] Future work combining TSH suppression with pharmacological manipulation of Notch could clarify whether this signaling axis is a viable therapeutic entry point.[25]

This study identifies increased apoptosis as a significant finding alongside impaired neurogenesis. Rats subjected to TSH suppression displayed a higher Bax/Bcl-2 ratio, indicating a pro-apoptotic state. Previous research links abnormal thyroid function to oxidative stress and mitochondrial damage, accelerating neuronal apoptosis.[26] The combined impact of heightened neuronal apoptosis and impaired neurogenesis compromises hippocampal plasticity and functionality, contributing to emotional disorders.[27] Mechanistically, reduced 5-HT levels not only diminish neuronal survival but may also disrupt Notch signaling and increase apoptosis by impairing BDNF signaling.[28] Consequently, emotional disorders may arise from intertwined pathological processes: hormonal imbalance reduces 5-HT, impairing neurogenesis via Notch signaling and apoptotic pathways, ultimately leading to anxiety and depression in behavioral assessments.

This study elucidates a comprehensive mechanism: TSH suppression disrupts thyroid hormone levels, leading to reduced hippocampal 5-HT, aberrant Notch pathway activation, and restricted neuronal differentiation. This results in increased glial differentiation, heightened apoptosis signals, and reduced neural network plasticity, culminating in depressive and anxiety-like behaviors. Aligning with previous clinical findings, this mechanism offers a robust experimental foundation for understanding mood disorders associated with TSH suppression therapy, highlighting potential neuropsychiatric risks of excessive suppression.

Beyond elucidating mechanisms, our findings also have implications for pharmacological intervention and for pharmaceutical education and research. First, given the central involvement of hippocampal 5-HT depletion, impaired

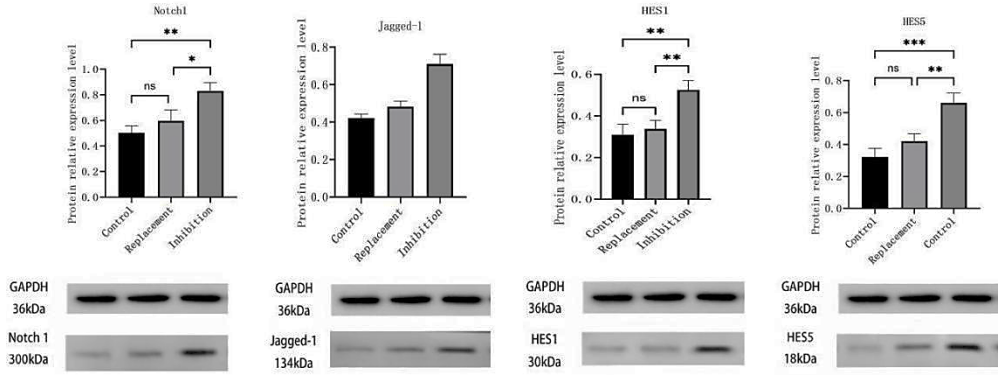

Figure 9: The expression levels of key target proteins of the Notch signaling pathway in the hippocampus of each group of rats. (ns, **p*<0.05, ***p*<0.01, *** *p*<0.001, **** *p*<0.0001).

neurogenesis and aberrant Notch activation, clinically available serotonergic antidepressants and other neuroplasticity-enhancing agents may be considered as adjunctive options in thyroid cancer patients who develop mood symptoms during TSH-suppressive therapy, provided that drug-drug interactions and endocrine status are carefully monitored. Second, the present work underscores the importance of integrating neuroendocrine and neuropsychiatric concepts into pharmacy curricula. Training future pharmacists and clinical pharmacologists to recognize the potential neuropsychiatric sequelae of long-term endocrine manipulations, to counsel patients about mood and cognitive changes and to participate in the rational selection of psychotropic co-medications may help to improve the comprehensive management of patients requiring chronic TSH suppression. Third, our rat model offers a preclinical platform for pharmaceutical research to screen novel compounds that modulate 5-HT signaling, Notch activity or apoptosis-related pathways in the context of endocrine-induced neuropsychiatric dysfunction.

Despite its strengths, this study has several limitations. First, the dosing regimen, route and duration of levothyroxine administration in rats do not perfectly recapitulate the chronic, oral TSH-suppressive therapy used in differentiated thyroid cancer patients. The temporal dynamics and reversibility of mood-related changes may therefore differ between species, and caution is required when extrapolating these results to the clinic. Second, we focused primarily on hippocampal 5-HT, Notch signaling and Bax/Bcl-2-mediated apoptosis, whereas other mechanisms highly relevant to mood regulation-such as neuroinflammation, oxidative stress, the BDNF/TrkB pathway and alterations in the hypothalamic-pituitary-adrenal axis-were not systematically explored. Third, our data are correlational; we did not directly manipulate 5-HT or Notch signaling *in vivo* to establish causality. Future studies that combine pharmacological or genetic modulation of these pathways with longitudinal behavioral testing, as well as clinical cohorts in which the intensity of TSH suppression is quantified alongside standardized neuropsychiatric assessments and neuroimaging, will be essential to bridge the gap between animal models and human practice.

In summary, this study elucidates the mechanisms by which TSH suppression may lead to emotional disorders, examining behavioral science, neurotransmitters, neurogenesis, and molecular signaling pathways. The findings indicate that prolonged TSH suppression poses risks, necessitating a balance between tumor control and mental health. Further clarification of the 5-HT-Notch-neurogenesis pathway and exploration of drug interventions could inform comprehensive management strategies for thyroid cancer patients.

CONCLUSION

TSH suppression is associated with pronounced anxiety- and depression-like behaviors in rats, accompanied by decreased hippocampal 5-HT content, activation of the Notch signaling pathway and an imbalance between neurogenesis and apoptosis. These findings suggest that excessive suppression of the thyroid axis may pose neuropsychiatric risks by impairing hippocampal plasticity and monoaminergic homeostasis. Clinically, in differentiated thyroid cancer patients receiving long-term TSH-suppressive levothyroxine therapy, our data support careful titration to the lowest effective level of TSH suppression, systematic monitoring of mood and cognitive symptoms and consideration of adjunctive pharmacological strategies when emotional disturbances emerge. Balancing tumor recurrence prevention with preservation of mental health should therefore be a key principle in the comprehensive management of patients requiring chronic TSH suppression.

ABBREVIATIONS

5-HT: 5-Hydroxytryptamine (Serotonin); **ANOVA:** Analysis of Variance; **Bax:** Bcl-2-Associated X Protein; **Bax/Bcl-2:** Bax to Bcl-2 Ratio; **BCA:** Bicinchoninic Acid (Assay); **Bcl-2:** B-Cell Lymphoma 2; **BDNF:** Brain-Derived Neurotrophic Factor; **BrdU:** 5-Bromo-2'-Deoxyuridine; **BUCM:** Beijing University of Chinese Medicine; **cDNA:** Complementary DNA; **CRediT:** Contributor

Roles Taxonomy; **DAPI:** 4′,6-Diamidino-2-Phenylindole; **ECL:** Enhanced Chemiluminescence; **ELISA:** Enzyme-Linked Immunosorbent Assay; **FT3:** Free Triiodothyronine; **FT4:** Free Thyroxine; **GFAP:** Glial Fibrillary Acidic Protein; **HES1:** Hairy and Enhancer of Split-1 (HES family bHLH TF 1); **HES5:** Hairy and Enhancer of Split-5 (HES family bHLH TF 5); **HRP:** Horseradish Peroxidase; **L-T4:** Levothyroxine; **LSD:** Least Significant Difference; **mRNA:** Messenger Ribonucleic Acid; **NeuN:** Neuronal Nuclei (RBFOX3); **ns:** Not Significant; **NSC(s):** Neural Stem Cell(s); **OFT:** Open Field Test; **PBS:** Phosphate-Buffered Saline; **PMSF:** Phenylmethylsulfonyl Fluoride; **qPCR:** Quantitative Polymerase Chain Reaction; **qRT-PCR:** Quantitative Reverse Transcription PCR; **RIPA:** Radioimmunoprecipitation Assay Buffer; **SCXK:** Laboratory Animal Production License (PRC); **SD:** Standard Deviation; **SDS-PAGE:** Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis; **SPF:** Specific Pathogen-Free; **SPSS:** IBM SPSS Statistics; **TBST:** Tris-Buffered Saline with Tween-20; **TrkB:** Tropomyosin Receptor Kinase B (NTRK2); **TSH:** Thyroid-Stimulating Hormone; **TST:** Tail Suspension Test.

ETHICAL STATEMENT

The study adhered to the Regulations for the Administration of Laboratory Animals and received approval from the Laboratory Animal Ethics Committee of Beijing University of Chinese Medicine (Approval No. BUCM-2024060102-2228).

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING

This work was supported by the National Natural Science Foundation of China [Grant No.81973855]; the Clinical Research Business Expenses of Central High-level Hospitals at the provincial-ministerial level (Research No.: 2022-NHLHCRF-LX-02-0115).

AUTHOR CONTRIBUTIONS

Yikun Zhao: Writing - original draft, Project administration, Investigation, Conceptualization. Honglin Jiang: Writing - original draft, Project administration, Investigation, Conceptualization. Zhongyuan Xia: Conceptualization, Investigation. Huiyuan Shang: Validation, Investigation. All authors have read and agreed to the published version of the manuscript.

SUMMARY

This study investigated how thyroid-stimulating hormone (TSH) suppression-commonly used in thyroid cancer management-affects emotional behavior and hippocampal function in rats. Using a total thyroidectomy model followed by graded levothyroxine administration, rats were assigned to blank control, hormone-replacement, or TSH-suppression groups. Behavioral tests showed that TSH-suppressed rats displayed prominent anxiety- and depression-like behaviors, including increased immobility and reduced exploration. Biochemically, these rats exhibited elevated FT3/FT4, markedly reduced TSH, and significantly decreased hippocampal serotonin (5-HT). TSH suppression also induced abnormal neurogenesis, characterized by increased neural stem-cell proliferation, reduced neuronal differentiation, and enhanced astrocyte formation. Concurrently, Notch signaling components (Notch1, Hes1, Hes5, Jagged-1) were upregulated, and the Bax/Bcl-2 ratio increased, indicating a pro-apoptotic environment. Together, these findings reveal that excessive TSH suppression disrupts hippocampal neurotransmission, neurogenesis, and apoptosis, ultimately producing mood-related behavioral disturbances. The results highlight potential neuropsychiatric risks of aggressive TSH-suppressive therapy.

REFERENCES

1. Davis PJ, Zhou M, Davis FB, Lansing L, Mousa SA, Lin HY. Mini-review: cell surface receptor for thyroid hormone and nongenomic regulation of ion fluxes in excitable cells. Physiol Behav. 2010;99(2):237-9. doi: 10.1016/j.physbeh.2009.02.015, PMID 19248795.
2. Ritchie M, Yeap BB. Thyroid hormone: influences on mood and cognition in adults. Maturitas. 2015;81(2):266-75. doi: 10.1016/j.maturitas.2015.03.016.
3. Schlumberger M, Leboulleux S. Current practice in patients with differentiated thyroid cancer. Nat Rev Endocrinol. 2021;17(3):176-88. doi: 10.1038/s41574-020-00448-z, PMID 33339988.
4. Jin S, Yang YT, Bao W, Bai Y, Ai JW, Liu Y, *et al.* Naming difficulties after thyroid stimulating hormone suppression therapy in patients with differentiated thyroid carcinoma: a prospective cohort study. Endocrine. 2019;65(2):327-37. doi: 10.1007/s12020-019-01943-8, PMID 31056722.
5. Mistry N, Wass J, Turner MR. When to consider thyroid dysfunction in the neurology clinic. Pract Neurol. 2009;9(3):145-56. doi: 10.1136/jnnp.2008.167163.
6. Bauer M, Whybrow PC. Role of thyroid hormone therapy in depressive disorders. J Endocrinol Invest. 2021;44(11):2341-7. doi: 10.1007/s40618-021-01600-w, PMID 34129186.
7. Vancamp P, Butruille L, Demeneix BA, Remaud S. Thyroid hormone and neural stem cells: repair potential following brain and spinal cord injury. Front Neurosci. 2020;14:875. doi: 10.3389/fnins.2020.00875, PMID 32982671.
8. Ding MR, Qu YJ, Hu B, An HM. Signal pathways in the treatment of Alzheimer's disease with traditional Chinese medicine. Biomed Pharmacother. 2022;152:113208. doi: 10.1016/j.biopha.2022.113208, PMID 35660246.
9. Ran LC, Shang H, Yuan Y, Chen LR, Yang T, Li L, *et al.* [Mechanism of electroacupuncture on hippocampal neuron regeneration in depression rats based on Wnt/beta-catenin and Notch signaling pathway]. Zhen Ci Yan Jiu. 2024;49(12):1282-8. doi: 10.13702/j.1000-0607.20230664, PMID 39681486.
10. Jin S, Sugitani I. Development of a rat model for evaluating thyroid-stimulating hormone suppression after total thyroidectomy. J Nippon Med Sch. 2021;88(4):311-8. doi: 10.1272/jnms.JNMS.2021_88-409, PMID 32863348.
11. Botella-Carretero JI, Galán JM, Caballero C, Sancho J, Escobar-Morreale HF. Quality of life and psychometric functionality in patients with differentiated thyroid carcinoma. Endocr-Relat Cancer. 2003;10(4):601-10. doi: 10.1677/erc.0.0100601, PMID 14713270.
12. Roerink SH, de Ridder M, Prins J, Huijbers A, de Wilt HJ, Marres H, *et al.* High level of distress in long-term survivors of thyroid carcinoma: results of rapid screening using the distress thermometer. Acta Oncol. 2013;52(1):128-37. doi: 10.3109/0284186X.2012.723822, PMID 23101467.

13. Jung MS, Visovatti M. Post-treatment cognitive dysfunction in women treated with thyroidectomy for papillary thyroid carcinoma. Support Care Cancer. 2017;25(3):915-23. doi: 10.1007/s00520-016-3481-y, PMID 27837323.
14. Kotkowska Z, Strzelecki D. Depression and autoimmune hypothyroidism-their relationship and the effects of treating psychiatric and thyroid disorders on changes in clinical and biochemical parameters including BDNF and other cytokines-A systematic review. Pharmaceuticals (Basel). 2022;15(4):391. doi: 10.3390/ph15040391, PMID 35455388.
15. Mele C, De Marchi L, Pitino R, Costantini L, Cavigiolo B, Caputo M, *et al.* The interplay between thyrotropic axis, neurological complications, and rehabilitation outcomes in patients with traumatic brain injury. Best Pract res Cl En. 2025;39(3):102001.
16. Tesfaye E, Getnet M, Anmut Bitew D, Adugna DG, Maru L. Brain functional connectivity in hyperthyroid patients: systematic review. Front Neurosci. 2024;18:1383355. doi: 10.3389/fnins.2024.1383355, PMID 38726033.
17. Osnaya-Brizuela N, Valenzuela-Peraza A, Santamaría-Del Ángel D, García-Martínez Y, Pacheco-Rosado J, Pérez-Sánchez G, et al. Is the acquired hypothyroidism a risk factor for developing psychiatric disorders? Front Psychiatry. 2024;15:1429255. doi: 10.3389/fpsyt.2024.1429255, PMID 39100850.
18. Ahmed OM, Ahmed RG, El-Gareib AW, El-Bakry AM, Abd El-Tawab SM. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: II-the developmental pattern of neurons in relation to oxidative stress and antioxidant defense system. Int J Dev Neurosci. 2012;30(6):517-37. doi: 10.1016/j.ijdevneu.2012.04.005, PMID 22664656.
19. Baghcheghi Y, Beheshti F, Shafei MN, Salmani H, Sadeghnia HR, Soukhtanloo M, *et al.* The effects of vitamin E on brain derived neurotrophic factor, tissues oxidative damage and learning and memory of juvenile hypothyroid rats. Metab Brain Dis. 2018;33(3):713-24. doi: 10.1007/s11011-017-0176-0, PMID 29290043.
20. Xu Y, Yang L, Teng Y, Li J, Li N. Exploring the underlying molecular mechanism of tri(1,3-dichloropropyl) phosphate-induced neurodevelopmental toxicity via thyroid hormone disruption in zebrafish by multi-omics analysis. Aquat Toxicol. 2023;258:106510. doi: 10.1016/j.aquatox.2023.106510, PMID 37003012.
21. Maglione AV, Do Nascimento BP, Ribeiro MO, de Souza TJ, Da Silva RE, Sato MA, *et al.* Triiodothyronine Treatment reverses Depression-Like Behavior in a triple-transgenic animal model of Alzheimer's disease. Metab Brain Dis. 2022;37(8):2735-50. doi: 10.1007/s11011-022-01055-9, PMID 35951206.
22. Zhang Q, Zheng J, Sun H, Zheng J, Ma Y, Ji Q, *et al.* The Notch signaling pathway: A potential target for mental disorders. Mol Neurobiol. 2025;62(11):14717-33. doi: 10.1007/s12035-025-05034-w, PMID 40372672.
23. Tong Y, Zhao G, Shuang R, Wang H, Zeng N. Saikosaponin a activates tet1/dll3/notch1 signalling and promotes hippocampal neurogenesis to improve depression-like behavior in mice. J Ethnopharmacol. 2024;319(3):117289. doi: 10.1016/j.jep.2023.117289, PMID 37844745.
24. Li C, Xie Z, Xing Z, Zhu H, Zhou W, Xie S, *et al.* The Notch signaling pathway regulates differentiation of NG2 cells into oligodendrocytes in demyelinating diseases. Cell Mol Neurobiol. 2022;42(7):1-11. doi: 10.1007/s10571-021-01089-0, PMID 33826017.
25. Yang J, Sun Q, Liu X, Yang Y, Rong R, Yan P, *et al.* Targeting Notch signaling pathways with natural bioactive compounds: a promising approach against cancer. Front Pharmacol. 2024;15:1412669. doi: 10.3389/fphar.2024.1412669, PMID 39092224.
26. Liao J, Zeng G, Fang W, Huang W, Dai X, Ye Q, *et al.* Increased Notch2/NF-κB signaling may mediate the depression susceptibility: evidence from chronic social defeat stress mice and WKY rats. Physiol Behav. 2021;228:113197. doi: 10.1016/j.physbeh.2020.113197, PMID 33017602.
27. Schroer J, Warm D, De Rosa F, Luhmann HJ, Sinning A. Activity-dependent regulation of the BAX/BCL-2 pathway protects cortical neurons from apoptotic death during early development. Cell Mol Life Sci. 2023;80(6):175. doi: 10.1007/s00018-023-04824-6, PMID 37269320.
28. Ru D, Zhang Z, Liu M, Fan X, Wang Y, Yan Y, *et al.* Downregulation of Notch signaling-stimulated genes in neurovascular unit alterations induced by chronic cerebral hypoperfusion. Immun Inflamm Dis. 2024;12(11):e70082. doi: 10.1002/iid3.70082, PMID 39607309.

Cite this article: Zhao Y, Jiang H, Shang H, Xia Z. TSH Suppression Induces Anxiety- and Depression-like Behaviors in Rats, With Hippocampal 5-HT Loss, Notch Activation, and a Neurogenesis-Apoptosis Imbalance. Indian J of Pharmaceutical Education and Research. 2026;60(3):1256-67.