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Association of Subclinical Thyroid Dysfunction with Cognitive Impairment in Rats: The Role of Autophagy

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Background: We investigated the effect of subclinical hyperthyroidism and subclinical hypothyroidism on cognitive function in rats and the role of autophagy in this process.

Methods: Forty Wistar rats were randomized into normal control (NC), hyperthyroidism (Hyper), hypothyroidism (Hypo), subclinical hyperthyroidism (sHyper), and subclinical hypothyroidism (sHypo) groups. Cognitive function (spatial learning and memory) was tested by the Morris water maze test. Hippocampal histopathology was analyzed by H&E staining, and expression levels of caspase-3 in hippocampal CA1 neurons were measured. In addition, immunoblot analysis was performed to detect hippocampal autophagy-related proteins.

Results: Escape latency from day 1 to day 4 was significantly longer in the Hypo, Hyper, and sHyper groups than in the NC group ($P < 0.01$). In addition, the number of rats crossing the virtual platform was significantly lower in the Hypo, Hyper, and sHyper groups than in the NC group ($P < 0.01$). Compared with the NC group, all four groups had significantly lower residence time in the target quadrant ($P < 0.05$). Beclin-1 and LC3-II protein expression in hippocampal tissues was significantly higher in the Hyper and sHyper groups than in the NC group ($P < 0.01$). Beclin-1 and LC3-II protein expression in hippocampal tissues did not significantly differ between the sHypo group and NC group ($P > 0.05$).

Conclusions: Subclinical thyroid dysfunction in rats might lead to cognitive impairment. Subclinical hyperthyroidism might be associated with excessive activation of autophagy and hippocampal neuron damage and necrosis. (J Nippon Med Sch 2023; 90: 372–380)

Key words: subclinical hyperthyroidism, subclinical hypothyroidism, cognitive impairment, LC3-II, Beclin-1

Introduction

Thyroid hormone is closely associated with expression of genes and proteins that are important in learning, memory, changes in synaptic plasticity in the brain, and maintenance of normal cognitive function[1]. Thyroid hormone has an important role in nerve growth in brain regions (such as the hippocampus) that are closely related to memory[2]. Thyroid dysfunction, including subclinical hyperthyroidism and subclinical hypothyroidism, can lead to cognitive and emotional disorders[3]. Biondi et al.[4] reported that subclinical hypothyroidism was associated with cognitive impairment in middle-aged adults. Other studies showed that thyroid dysfunction was associated with cognitive impairment, and more subtle variations of

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thyroid hormone levels within the euthyroid reference range were associated with cognitive function. High-normal FT4 levels were associated with lower cognitive performance and increased risk for dementia[5]. Long-term postoperative TSH suppression therapy can lead to drug-induced subclinical hyperthyroidism. In a prospective cohort study of 110 patients receiving TSH suppression therapy after surgery for differentiated thyroid carcinoma, subclinical hyperthyroidism caused by oral administration of L-T4 impaired attention and short-term memory[6].

Thyroid hormone not only promotes growth and metabolism but also regulates intracellular autophagy[7]. Autophagy removes misfolded proteins and damaged subcellular organelles and is important in synaptic growth and neuronal plasticity needed for learning and memory[8,9]. Changes in autophagy activity are associated with cognitive impairment in neurodegenerative diseases[8–12]. We wondered (i) if subclinical hyperthyroidism and subclinical hypothyroidism cause different forms of cognitive function impairment and (ii) if autophagy is associated with thyroid dysfunction-induced impairment of cognitive function. In this study, we established rat models of various states of thyroid function-namely, hyperthyroidism, subclinical hyperthyroidism, hypothyroidism, and subclinical hypothyroidism-to assess (i) cognitive impairment in subclinical hyperthyroidism and subclinical hypothyroidism in rats and (ii) the level of autophagy in hippocampal tissues. The results show a possible mechanism by which subclinical hyperthyroidism and subclinical hypothyroidism impair cognitive function.

Materials and Methods

Experimental Animals and Groups

The study included clean-grade 8-week-old female Wistar rats weighing between 170 and 230 g supplied by the Laboratory Animal Center of Inner Mongolia Medical University. The rats were housed under standard conditions with a 12/12-h light/dark light regimen at 22-23°C and 45-50% relative humidity, with standard feed and drinking water available ad libitum. Ethical approval was obtained from the biomedical ethics committee of Inner Mongolia Medical University (No. YKD2014063).

The rats were randomly divided into five groups (n = 8 rats per group). ① The normal control (NC) group was provided standard feed and distilled water. ② The hyperthyroidism (Hyper) group was fed adaptively for 1 week, referring to a published experimental method[13]. The rats were injected subcutaneously with 20 μg of L-thyroxine (L-T4) per 100 g body weight per day for 15 days to establish a hyperthyroidism model. Then, the rats were injected with L-T4 for an additional 45 days. After that, the rats were subjected to the water maze test and specimens were collected. ③ The hypothyroidism (Hypo) group was fed adaptively for 1 week, referring to a published experimental method[14]. A longitudinal incision was made in the neck (length 2.0-2.5 cm), bluntly separating the pretracheal sternohyoid muscle along the midline, revealing the elliptical thyroid attached to both sides of the tracheal ring, protecting the posterior recurrent laryngeal nerve, completely removing the bilateral thyroid and the isthmus, and suturing the subcutaneous tissue and skin, layer by layer, to establish the hypothyroidism model by total thyroidectomy. After the operation, the rats were provided standard feed and high-calcium water (0.1% calcium gluconate). After 60 days of feeding, the rats were subjected to the water maze test and specimens were collected. ④ The subclinical hyperthyroidism (sHyper) group: To obtain the optimal TSH concentration, we did not inject the rats directly with L-T4 only. Instead, the rats were fed adaptively for 1 week, and total thyroidectomy was performed as described above. The rats were fed according to the above method. Subclinical hyperthyroidism was established by daily subcutaneous injection of 1.6 μg of L-T4 per 100 g body weight per day from the first day after the operation for 15 days. Then, the rats were injected daily with L-T4 for an additional 45 days. After that, the rats were subjected to the water maze test and specimens were collected. ⑤ The subclinical hypothyroidism (sHypo) group was fed adaptively for 1 week, and total thyroidectomy was performed as described above. The rats were fed according to the above method. Subclinical hypothyroidism was established by daily subcutaneous injection of 1.2 μg of L-T4 per 100 g body weight per day from the first day after operation for 15 days. Then, the rats were injected daily with L-T4 for an additional 45 days. After that, the rats were subjected to the water maze test and specimens were collected.

Behavioral Tests

Cognitive function (spatial learning and memory) was tested by the Morris water maze test[15], which was performed after 60 days of feeding in the NC and Hypo groups, and after daily L-T4 injection for 60 days in the Hyper, sHyper, and sHypo groups. The animals underwent adaptive training for 3 days before the formal experiment. In brief, rats in each group were allowed to swim adaptively in a pool without an underwater plat-

form for 60 s at a fixed time every day. ① In the orientation navigation experiment, which continued for 4 days, the rats were placed in the water at four quadrants (northeast: I, southeast: II, southwest: III, and northwest: IV) every day. The rats were faced toward the pool wall when placed in the water. Escape latency was defined as the time taken by the rats to find the hidden platform (an underwater platform located in quadrant III). If the animals did not find the hidden platform within 120 s, escape latency was recorded as 120 s. The main purpose of this experiment is to test the spatial learning ability of rats. ② The spatial exploration experiment was performed as follows. On the 5th day, the underwater platform was withdrawn, and the rats in each group were placed in the water at quadrant I while facing the pool wall. The number of times the animals crossed the virtual platform, residence time in the target quadrant, and activity distance in the target quadrant were recorded for 120 s. The main purpose of this experiment was to test spatial memory.

Detection of Serum T3, T4, and TSH Levels

After the water maze test, all rats were deprived of food and water for 12 h, and approximately 5 mL of blood was collected from the thoracic cavity (left ventricle). Blood samples were rapidly centrifuged for 15 min at 3,000 rpm. The supernatant was stored in eppendorf (EP) tubes, and serum T3, T4, and TSH levels were measured using a Cobas E601 automatic electrochemiluminescence immunoassay system (Roche). Thyroid hormone reference values in Wistar rats were established in a previous study[16]. Thyroid function was evaluated according to the standard values.

Hippocampal Histopathology and Immunohistochemistry

After cardiac blood collection, the rats were sacrificed by administering a lethal dose of anesthesia. The animals were decapitated, and the skin of the head was cut open. Then, the skull was stripped with bone biting forceps, the hard and soft meninges and cranial nerve root filaments were cut, and the brain was dissected. The hippocampal tissue was quickly extracted on ice and fixed with a 4% paraformaldehyde solution for pathological examination. As instructed by the manufacturers, caspase-3 expression in the CA1 region of the hippocampus was immunohistochemically examined using the streptavidin-biotin-peroxidase complex method. The primary antibodies used were against caspase-3 (Proteintech Group, Inc., USA) protein. The 8-μm thick sections were blocked with endogenous peroxidase blocking agent (Jingmei Biotech, Co., Ltd., CHN) for 10 min at room temperature and later incubated with the primary antibodies for 30 min at room temperature. After washing in distilled water, the sections were incubated with biotinylated secondary antibodies (Jingmei Biotech, Co., Ltd., CHN) and streptavidin-peroxidase (Jingmei Biotech, Co., Ltd., CHN) for 10 min at room temperature. PBS rather than primary antibodies was used as negative control. Cells with cytoplasm stained brown were considered positive.

Western Blot Analysis of Hippocampal Tissue

After cardiac blood collection, the rats were sacrificed by administering a lethal dose of anesthesia. The animals were decapitated, and hippocampal tissue samples were obtained as described above, frozen in liquid nitrogen, and stored at −80°C until Western blot analysis. Total protein was extracted with RIPA buffer, and protein concentration was measured by the Bradford method (MDL biotech Co., Ltd., CHN). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto PVDF membranes, which were blocked with 5% skimmed milk for 2 h. The membranes were washed with TBS twice (5 min each) and incubated with anti-Beclin-1 (MDL biotech Co., Ltd., CHN) or anti-LC3B (MDL biotech Co., Ltd., CHN) overnight at 4°C. Then, the membranes were washed three times with TBST and incubated with secondary antibodies for 2 h at room temperature, followed by another three washes with TBST. Protein bands were visualized through the electrochemiluminescence (ECL) method by using a chemiluminescence imaging system (Bio-rad Co., Ltd., USA). The gray value of each band was detected with Odyssey software, and semi-quantitative analysis was carried out[17].

Statistical Analysis

SPSS 28.0 statistics software was used for statistical analysis, and data are described as mean±SD. One-way analysis of variance (ANOVA) was used to compare escape latency, target quadrant residence time, and number of platform crossings of rats before and after modeling. One-way ANOVA was used to compare intergroup differences in protein expression, and was followed by post hoc testing. When variances were equal, comparison between groups was performed by one-way ANOVA, and the least significant difference t-test was used for multiple comparisons. When variances were not uniform, one-way ANOVA (Welch correction) and the Dunnett T3 test were used. $P < 0.05$ was considered statistically significant.

Table 1 Serum T3, T4, and TSH levels in rats, by group

Group	T3 (ng/mL)	T4 (ng/mL)	TSH (μIU/mL)
NC group	0.83±0.06	6.00±0.77	1.10±0.13
sHyper group	0.88±0.04△	13.85±1.50	0.74±0.09#
Hyper group	1.85±0.06*	22.9±1.80*	0.25±0.02*
Hypo group	0.58±0.06*	1.56±0.11*	20.29±2.5*
sHypo group	0.85±0.04△	10.2±1.6	1.36±0.04#

Note: *P<0.01 *vs.* NC group; # P<0.05 *vs.* NC group; △P>0.05 *vs.* NC group
Normal reference values: 0.73-0.98 ng/mL for T3, 4.2-8.4 ng/mL for T4, and 0.76-1.29 μIU/mL for TSH

Results

Serum T3, T4, and TSH Levels

Compared with the NC group, serum T3 and T4 levels were significantly lower and serum TSH levels were significantly higher in the Hypo group (P < 0.01), indicating hypothyroidism. Compared with the NC group, serum T3 and T4 levels were significantly higher and serum TSH levels were significantly lower in the Hyper group (P < 0.01), indicating hyperthyroidism. Compared with the NC group, serum T3 levels were not significantly different (P > 0.05), but serum TSH levels were slightly higher in the sHypo group (P < 0.05), indicating subclinical hypothyroidism. Compared with the NC group, serum T3 levels were not significantly different (P > 0.05), but TSH levels were slightly lower in the sHyper group (P < 0.05), indicating subclinical hyperthyroidism (see **Table 1**). Daily injection of L-T4 in the sHyper and sHypo groups can increase serum T4 levels; thus, assessment of thyroid function was based on serum T3 and TSH levels.

Behavioral Performance of Rats

Orientation navigation experiment

Compared with the NC group, escape latency from day 1 to day 4 was significantly greater in the Hypo, Hyper, and sHyper groups (P < 0.01), while no significant difference in escape latency was observed between the NC and sHypo groups (P > 0.05). Compared with the sHyper group, escape latency was greater from day 1 to day 4 in the Hypo and Hyper groups (P < 0.05) (**Fig. 1A and B**).

Spatial exploration experiment

Compared with the NC group, the number of rats crossing the virtual platform was significantly lower in the Hypo, Hyper, and sHyper groups (P < 0.01) but not in the sHypo group (P > 0.05) (**Fig. 1C**). Compared with the NC group, the target quadrant residence time was significantly lower in all four groups (P < 0.05) (**Fig. 1D**). Compared with the NC group, the target quadrant activity distance was significantly lower in the Hypo and Hyper groups (P < 0.05) but not in the sHypo and sHyper groups (P > 0.05) (**Fig. 1E**).

Histopathological Changes in Hippocampal Tissues

In the CA1 region of the hippocampus in the NC group, pyramidal cells were intact, evenly distributed, and closely arranged, and intracellular structures were distinct. In the Hypo group, pyramidal cells in the CA1 area of the hippocampus were loose in structure and disordered; cells were swollen and round, and halos around the cytoplasm were present. Cell spacing was large, and few cells showed punctate liquefaction necrosis. In the Hyper group, pyramidal cells in the CA1 area of the hippocampus were loose in structure, disordered in arrangement, and reduced in number; cytoplasm was lightly stained, nuclei were deeply stained for pyknosis, and nucleoli were absent. Some cells showed punctate liquefaction necrosis. In the sHyper group, pyramidal cells in the CA1 area of the hippocampus were loose in structure, sparse, and disordered in arrangement; cell spacing was large, and individual cells showed punctate liquefaction necrosis. In the sHypo group, pyramidal cells in the hippocampal CA1 area were evenly distributed, neatly arranged, and close to normal; some cells were swollen and round (**Fig. 2**).

Immunohistochemistry of Caspase-3

Caspase-3 is activated in apoptotic and necrotic cell death and has been used as a marker of apoptotic and necrotic cell death. The NC group expressed the lowest levels of caspase-3, whereas all experimental groups showed greater expression of caspase-3. Expression of caspase-3 was slightly higher in the sHyper and sHypo groups. Compared with sHyper and sHypo groups, caspase-3 expression was higher in the Hypo and Hyper groups (**Fig. 2**).

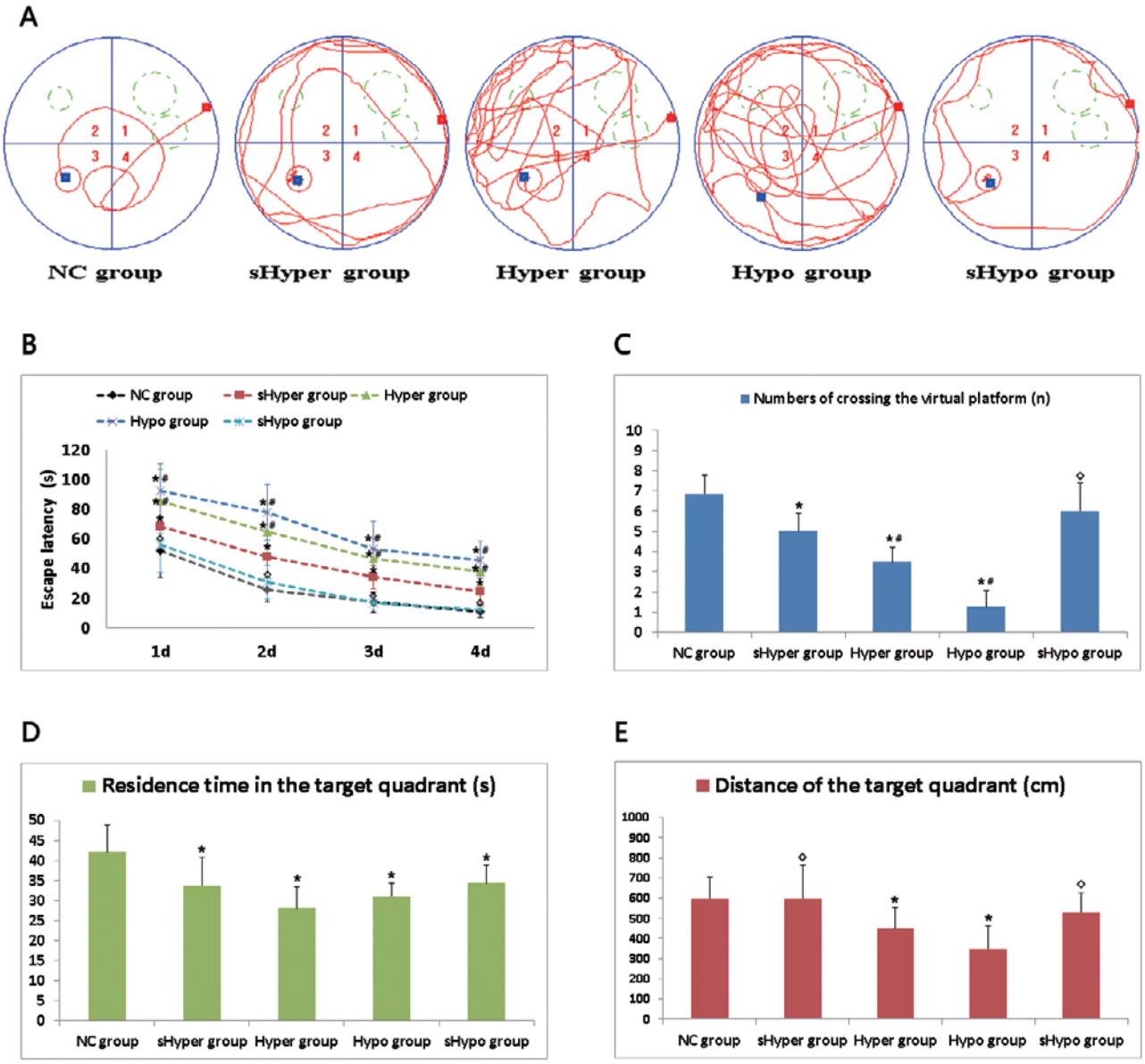

Fig. 1 Spatial learning and memory, as determined using the Morris water maze test

A: Orientation navigation experiment: typical escape route on day 4. B: Orientation navigation experiment: escape latency from day 1 to day 4 (*P < 0.01 vs. NC group; #P < 0.05 vs. sHyper group; ◇P > 0.05 vs. NC group). C: Spatial exploration experiment: numbers of rats crossing the virtual platform (*P < 0.01 vs. NC group; #P < 0.05 vs. sHyper group; ◇P > 0.05 vs. NC group). D: Spatial exploration experiment: residence time in target quadrant (*P < 0.05 vs. NC group). E: Spatial exploration experiment: activity residence time in target quadrant (*P < 0.05 vs. NC group; ◇P > 0.05 vs. NC group).

Expression Levels of Autophagy-related Proteins in Hippocampal Tissues of Rats

Compared with the NC group, Beclin-1 and LC3-II expression in hippocampal tissues was significantly higher in the Hyper and sHyper groups (P < 0.01) (**Fig. 3A**). Moreover, Beclin-1 and LC3-II protein levels were significantly higher in the Hyper group than in the sHyper group (P < 0.05) (**Fig. 3B and C**). Furthermore, the LC3-II/LC3-I ratio was higher in the Hyper and sHyper groups than in the NC group, but the difference was not significant (P > 0.05).

In the Hypo group, Beclin-1 and LC3-II expression in hippocampal tissues was significantly lower than in the NC group (P < 0.01) (**Fig. 3A**). The LC3-II/LC3-I ratio was also significantly lower in the Hypo group than in the NC group (P < 0.05). However, the sHypo group and NC group did not significantly differ in Beclin-1 expression, LC3-II expression, or LC3-II/LC3-I ratio in hippocampal tissues (P > 0.05) (**Fig. 3B-D**).

Discussion

Changes in thyroid function can lead to cognitive impair-

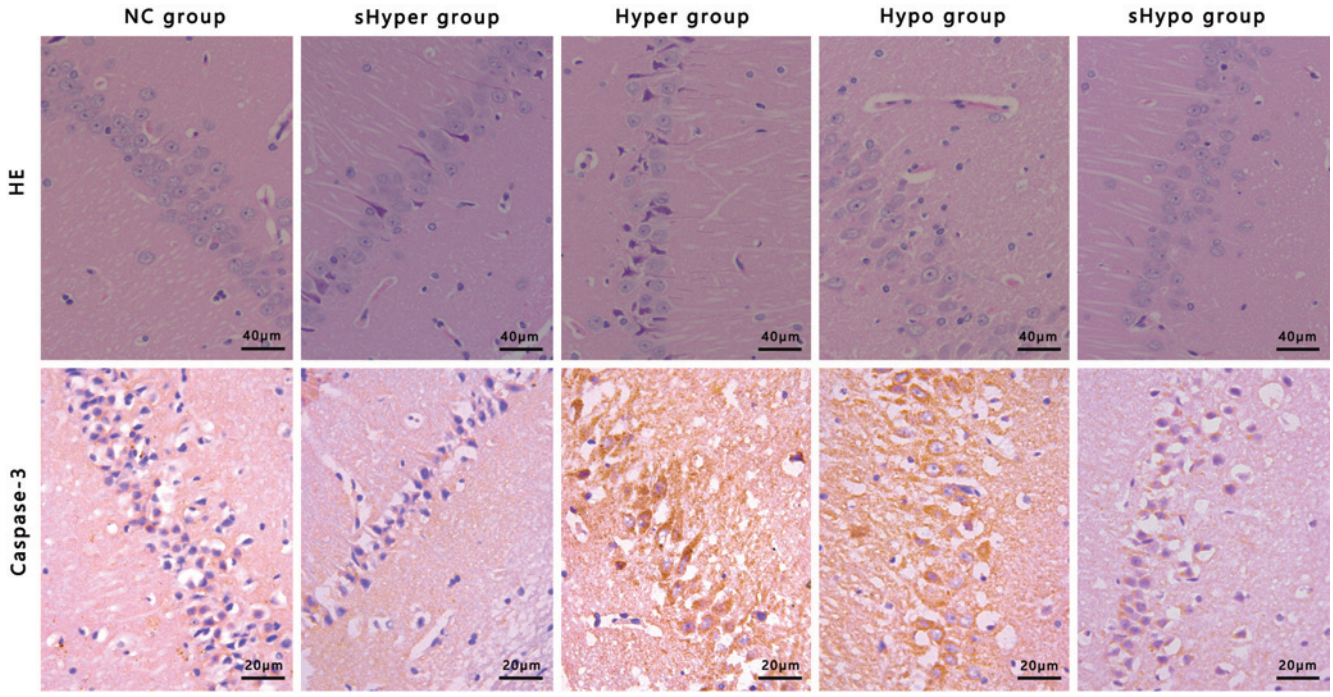

Fig. 2 H&E staining and caspase-3 expression in hippocampal CA1 region
Representative images from the hippocampal CA1 region of rats in the NC, sHyper, Hyper, Hypo, and sHypo groups. HE figures show H&E staining (×200); Caspase-3 figures show immunohistochemical staining of caspase-3 (×400). Brown particles indicate protein expression.

ment. Patients with hypothyroidism or hyperthyroidism may develop cognitive and emotional impairment, such as reduced memory, attention, perception, visuospatial function, and executive function, as well as emotional disorders and symptoms[18]. Our experiments confirmed that the escape latency was longer in rats with subclinical and clinical thyroid dysfunction groups than in control group, while the number of virtual platform crossings, target quadrant residence time, and target quadrant active distance were lower.

These results suggest that subclinical hypothyroidism and subclinical hyperthyroidism are associated with cognitive impairment. In this study, after total thyroidectomy, subclinical hypothyroidism and subclinical hyperthyroidism were induced by L-T4 treatment. The results show that escape latency in the orientation navigation test was significantly longer in the sHyper group. In the spatial exploration test, the number of rats crossing the virtual platform and target quadrant residence time were significantly lower in the sHyper group, but in the sHypo group, for which only target quadrant residence time was significantly lower. Subclinical hyperthyroidism and subclinical hypothyroidism led to cognitive impairment associated with decreased spatial learning and memory. Spatial learning ability and spatial memory ability were significantly better in rats with subclinical hypothyroidism than in those with subclinical hyperthyroidism. These findings suggest that staying within the normal range of thyroid hormone levels can have important effects on learning and memory abilities.

These data are similar to those collected from humans, although contrasting findings have been reported. Kim et al.[19] reported a significant association between low serum TSH levels (<0.5 μIU/mL) and cognitive impairment in 495 community residents in Korea. In a UK population-based cohort of 1,047 older individuals without overt thyroid disease at baseline, Hogervorst et al.[5] found that high TSH levels at baseline (suggesting subclinical hypothyroidism) and high-normal serum FT4 levels were associated with cognitive impairment. High-normal FT4 levels were associated with greater cognitive decline over time and are indicative of dementia[5]. In that study, half the participants were unaware they had thyroid disease. Similarly, de Jong reported that high-normal total and free thyroxine (but not TSH) levels were associated with increased risk for dementia and neuropathology over time[20]. In the Oxford Project To Investigate Memory and Ageing (OPTIMA), TSH levels were lower in patients with Alzheimer disease than in controls, but total thyroxine levels were not different between cases and controls[21].

Pasqualetti et al.[22] performed a meta-analysis of 13 observational studies and found a relationship between

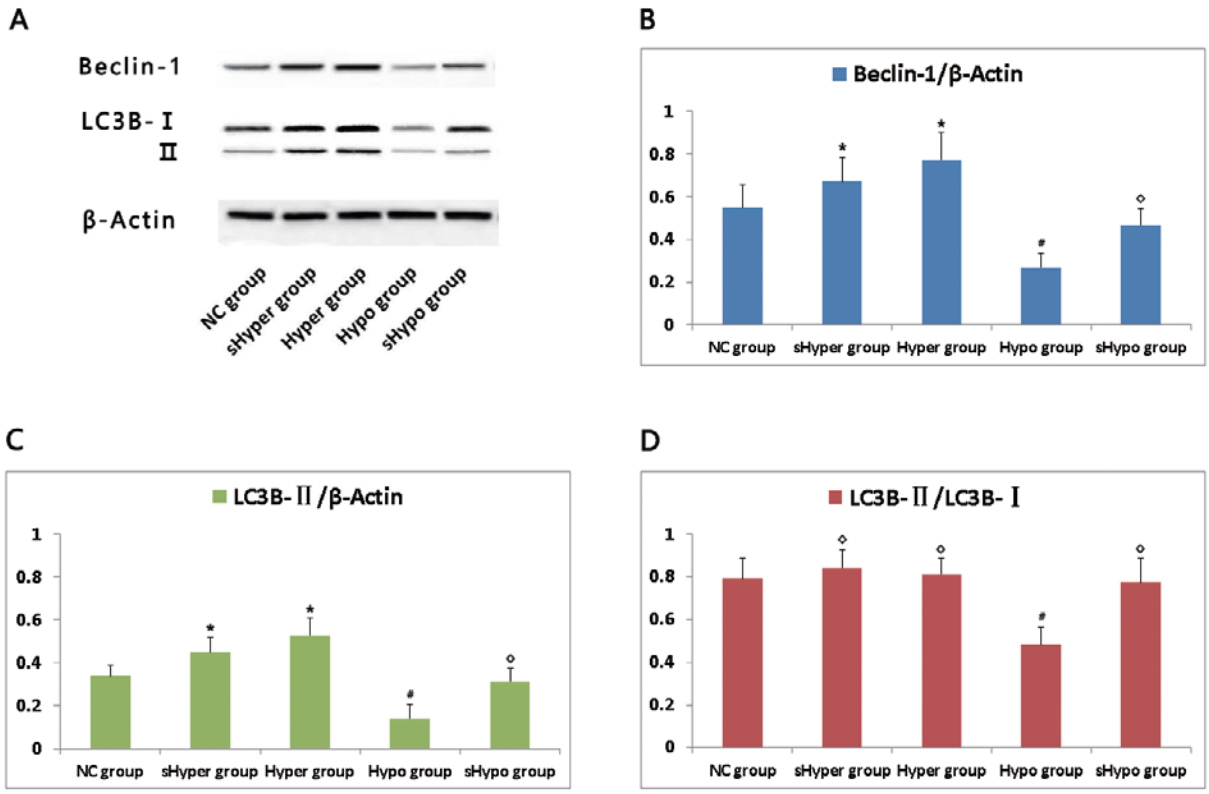

Fig. 3 Expression levels of autophagy-related proteins in hippocampal tissues

A: Representative Western blots and relative density of Beclin-1, LC3B-I, and LC3B-II proteins (β-actin: 42 kDa; Beclin-1: 52 kDa; LC3B-II: 14.6 kDa). B: Expression levels of Beclin-1 protein in hippocampal tissue (*P < 0.01 vs. NC group; #P < 0.01 vs. NC group; ◇P > 0.05 vs. NC group). C: Expression levels of LC3B-II protein in hippocampal tissue (*P < 0.01 vs. NC group; #P < 0.01 vs. NC group; ◇P > 0.05 vs. NC group). D: LC3B-II/LC3B-I ratio (#P < 0.05 vs. NC group; ◇P > 0.05 vs. NC group).

subclinical hypothyroidism and cognitive impairment in persons younger than 75 years. In contrast, a later meta-analysis (2016) of 11 prospective studies reported that subclinical hyperthyroidism, but not subclinical hypothyroidism, was associated with elevated risk for dementia and a faster rate of decline on the MMSE, a dementia screening test. Differences in outcome may be related to the different cut-off scores used to define subclinical thyroid disorders and to differences in the assays used, subjects included (age, with or without thyroid disorders or other morbidities), and cognitive tests used[23].

The basis of cognitive function, including learning and memory, is neuronal synaptic growth and plasticity, and neuronal autophagy is crucial for synaptic development; upregulation and downregulation of autophagy affect the size of synapses[24]. Autophagy is an important process of degradation and digestion of intracellular material components, which are important in intracellular homeostasis, material recycling, and cell morphological integrity. Studies have found that autophagy eliminates misfolded proteins and damaged subcellular organelles and has important roles in neurodegenerative diseases[25]. In common neurodegenerative lesions, a mild increase in autophagy activity likely improves cognitive function in patients. Mild autophagy is thought to help protect cells from harmful conditions, while severe or rapid autophagy induces autophagy-mediated programmed cell death[26,27]. This suggests that autophagy has complex effects on the process of neuronal damage and could result in survival or death-indicating a dual and opposing effect.

Thyroid hormone promotes expression of autophagy-related genes such as MAP1LC3B, ULK1, SQSTM1, and BECN1[28,29]. Tseng et al.[30] showed that thyroid hormone T3 enhances lysosomal activity and promotes formation of autophagosomes. Beclin-1 is an important positive regulator of autophagy and promotes autophagy after binding with type III phosphatidylinositol 3-kinase (PI3K)[31]. During autophagy, LC3 assumes a soluble form (LC3-I) after a series of enzymatic actions, and LC3-I is further transformed into a membrane-bound form (LC3-II) after multiple enzymatic actions. LC3-II is present in the inner and outer membranes of autophagosomes, and LC3-II

levels are directly proportional to the number of autophagic vacuoles. Therefore, LC3-II level and the LC3-II/LC3-I ratio are often used as markers of mammalian autophagosomal membranes[32]. In the present study, Beclin-1 and LC3-II expression levels were significantly higher in hippocampal tissue from rats with subclinical hyperthyroidism and hyperthyroidism. This suggests that subclinical hyperthyroidism and hyperthyroidism both promote autophagy. However, compared with the control group, the LC3-II/LC3-I ratio was not significantly different in the subclinical hyperthyroidism and hyperthyroidism groups, perhaps because thyroid hormone promotes autophagy and enhances protein expression of both LC3-I and LC3-II. Thus, there might be no change in the LC3-II/LC3-I ratio, but data confirm that thyroid hormone activates and promotes autophagy in hippocampal tissue.

In addition, Beclin-1 and LC3-II expression levels in hippocampal tissue were significantly lower in the hypothyroidism group than in the control group, and the LC3-II/LC3-I ratio was significantly lower in the hypothyroidism group. However, there was no significant difference in Beclin-1 expression, LC3-II expression, or LC3-II/LC3-I ratio between the subclinical hypothyroidism group and control group. These findings suggest that clinical hypothyroidism inhibits autophagy but that subclinical hypothyroidism has no effect on autophagy.

Caspase-3 is a well-established and sensitive marker of apoptotic and necrotic cell death[33]. D'Amelio et al.[34] reported that many factors could lead to neuronal death by activating caspase-3. In our study, we assessed caspase-3 levels in the CA1 region of the hippocampus and found increased expression of caspase-3 in all experimental groups. In particular, the Hypo and Hyper groups showed strong positive expression. Furthermore, in rats with hypothyroidism, pyramidal cells in the CA1 area of the hippocampus appeared to be loose in structure and disordered in arrangement. Cells were swollen and round, and halos were found around the cytoplasm. Cell spacing was large, and few cells showed punctate liquefaction necrosis. Cognitive impairment in rats with hypothyroidism was associated with hippocampal neuronal swelling, cellular dysfunction, and spotty necrosis, which might be related to inhibition of autophagy. In rats with hyperthyroidism, pyramidal cells in the CA1 area of the hippocampus were loose in structure, disordered in arrangement, and reduced in number. The cytoplasm was lightly stained, nuclei were deeply stained for pyknosis, and nucleoli had disappeared. Some cells showed punctate liquefaction necrosis. In rats with subclinical hyperthyroidism, pyramidal cells in the CA1 area of the hippocampus were loose in structure, sparse, and disordered in arrangement. Cell spacing was large, and individual cells showed punctate liquefaction necrosis. Cognitive impairment in rats with hyperthyroidism was associated with a reduced number of neurons and hippocampal neuronal necrosis, which might be related to excessive activation of autophagy. Son et al.[35] reported that inhibition of autophagy affects intercellular communication and contributes to neurodegeneration. Conversely, promotion of autophagy may represent a cell death mechanism via excessive removal of key survival factors or cellular organelles[35]. Our experimental evidence supports these conclusions.

In conclusion, thyroid dysfunction in rats led to cognitive impairment (reduced learning and memory abilities), and damage to hippocampal cells was associated with autophagy. Subclinical hyperthyroidism might be related to excessive activation of autophagy and hippocampal neuron damage and necrosis.

Conflict of Interest: The authors declare no conflicts of interest.

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