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Hippocampal NLRP1 inflammasome mediates anxiety-like behavior in mice with hypothyroidism

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Hypothyroidism is associated with anxiety and depression. However, the mechanisms underlying these neuropsychiatric symptoms remain largely unknown. This study aimed to investigate the role of the NLRP1 inflammasome in anxiety-like behavior in mice with hypothyroidism. Male C57BL/6j mice were divided into three groups: euthyroid controls, a hypothyroid model group induced by propylthiouracil, and a hypothyroid group treated with levothyroxine (L-T4). Anxiety-like behavior was assessed using both the open field test and the elevated plus maze. Protein levels of NLRP1 inflammasome components and associated cytokines in the hippocampus were examined by Western blot analysis. Mice with hypothyroidism exhibited anxiety-like behavior, as evidenced by decreased activity in the central area of the open field and reduced time spent in the open arms of the elevated plus maze. These behavioral changes were accompanied by an increased expression of NLRP1 inflammasome components (NLRP1, ASC, and Caspase-1) and associated cytokines (IL-1β, IL-18, and IL-6) in the hippocampus. L-T4 treatment reversed both the behavioral deficits and inflammatory changes. Our findings highlight the crucial role of NLRP1 inflammasome activation in the hippocampus in mediating anxiety-like behavior in hypothyroid mice, shedding light on the mechanisms underlying hypothyroidism-related psychiatric comorbidities and identifying potential therapeutic targets.

Keywords Anxiety, Hippocampus, Hypothyroidism, Levothyroxine, NLRP1 inflammasome

Hypothyroidism occurs when there is an inadequate production or release of thyroid hormones, leading to a systemic low metabolic syndrome[1]. The prevalence of hypothyroidism varies by region, age, gender, and diagnostic criteria, affecting approximately 4.6–13.95% of the global population. Subclinical hypothyroidism is more prevalent than overt hypothyroidism[2–4]. The intricate relationship between thyroid hormones and the central nervous system is well established, with hypothyroidism often leading to alterations in mood, cognitive function, and emotional regulation[5–7]. The development of these symptoms is multifaceted and include alterations in neurotransmitter levels, decreased metabolic rate, and the physical symptoms affecting patients' self-esteem and psychological well-being[8,9]. Recent studies have implicated inflammation in the pathophysiology of anxiety and depression in hypothyroidism[10,11]. Among the various inflammatory pathways, the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 1 (NLRP1) inflammasome has emerged as a key mediator of neuroinflammation in autoimmune thyroid disease[12,13]. However, the role of NLRP1 inflammasome in hypothyroidism-related anxiety remains underexplored.

Inflammation within the CNS has emerged as a critical factor in the pathogenesis of anxiety and depression[14,15]. The inflammatory response, mediated by inflammasomes, involves the release of cytokines that play a pivotal role in modulating neuronal function and behavior[16]. Inflammasomes are complexes made up of multiple protein complexes comprising a cytosolic pattern-recognition receptor from either the nucleotide oligomerization domain-like receptor family or the HIN domain-containing family, an adapter protein known as apoptosis-associated speck-like protein that includes a caspase-activation and recruitment domain (ASC), and pro-caspase-1[17]. Inflammasomes act as sensors of cellular stress and damage, triggering the activation of caspase-1 and the subsequent cleavage and release of pro-inflammatory cytokines, such as interleukin-1β

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(IL-1β), IL-18, IL-6, and tumor necrosis factor-α (TNF-α)[18]. The NLRP1 inflammasome has been shown to be activated in response to various stressors, including chronic stress, which is a known risk factor for the development of anxiety and depression. The activation of the NLRP1 inflammasome in response to chronic stress results in the release of pro-inflammatory cytokines, which can exacerbate neuroinflammation and contribute to the pathogenesis of anxiety disorders[19,20]. Therefore, understanding the role of the NLRP1 inflammasome in neuroinflammation and its relationship with anxiety disorders in patients with hypothyroidism is crucial for developing novel therapeutic strategies.

The hippocampus is intimately involved in emotional regulation and cognitive function[21]. Notably, the hippocampus is highly sensitive to thyroid hormones, making it particularly vulnerable to alterations in thyroid function[22,23]. In hypothyroidism, structural and functional abnormalities within the hippocampus have been observed, such as reduced hippocampal volume and impaired neuronal function[22,24,25]. These abnormalities are believed to contribute to the development of anxiety and depression disorders. Furthermore, the NLRP1 inflammasome within the hippocampus has been implicated in neuroinflammation and neuronal pathological damage, both linked to anxiety and depression[26–28]. It is conceivable that in hypothyroidism, the cellular inflammation of hippocampal neurons mediated by the NLRP1 inflammasome could trigger or exacerbate anxiety disorders. To test this hypothesis, we conducted an animal study in propylthiouracil-induced hypothyroid mice. The open field test and elevated plus maze were used to assess anxiety-like behavior. Additionally, the protein levels of NLRP1 inflammasome components and their associated cytokines in the hippocampus were analyzed using Western blot. Our results show that hypothyroidism-induced anxiety-like behavior is associated with the activation of NLRP1 inflammasome-driven inflammatory signaling pathway, and L-T4 treatment ameliorated these behaviors and hippocampus inflammation.

Results

Establishment of hypothyroidism model in mice

The experimental procedure is shown in Fig. 1A. Mice were fed with propylthiouracil (PTU) in their chow for 6 weeks, after which serum thyroid hormones were measured to evaluate the success of the model. In the hypothyroid group, serum TSH levels were elevated ($p < 0.01$, Fig. 1B), demonstrating a compensatory response of the pituitary gland to low thyroid hormone levels. Conversely, serum T3 levels were markedly reduced compared to the euthyroid (EU) group ($p < 0.01$, Fig. 1C), and serum T4 levels were also lower ($p < 0.01$, Fig. 1D), indicating decreases in biologically active and precursor hormones, respectively. After levothyroxine (L-T4) replacement therapy, serum T3 and T4 levels in the L-T4 group restored towards normal values (Figs. 1C,D). Notably, TSH levels in these mice were significantly decreased compared to the hypothyroid group ($p < 0.01$, Fig. 1B), indicating an improvement in thyroid function. In addition, mouse weights were monitored throughout the experiment to track their health status. Compared to the EU mice, only during the second week after the experiment commenced, the mice in the L-T4 group exhibited a decrease in body weight when undergoing the induction of hypothyroidism. Subsequently, during L-T4 replacement therapy, no significant changes in body weight were observed ($p < 0.05$, Fig. 1E). These findings collectively demonstrate the effectiveness of L-T4 treatment in ameliorating the biochemical abnormalities associated with hypothyroid mice.

Hypothyroidism induced anxiety-like behavior in open field test

We employed the open field test to evaluate the general locomotor activity and levels of anxiety in mice. Figure 2A presents representative locomotor trajectories for EU, hypothyroid, and L-T4 treated mice. Hypothyroid mice exhibited decreased central zone activity compared to EU mice, while L-T4 treatment restored activity to EU levels (Fig. 2A). Total distance traveled (Fig. 2B) and average speed (Fig. 2C) were similar among all groups. Central zone distance traveled by hypothyroid mice was significantly less than EU mice ($p < 0.01$, Fig. 2D), and L-T4 treatment increased this distance ($p < 0.01$), though still less than EU ($p < 0.05$). Hypothyroid mice spent less time in the central zone ($p < 0.01$, Fig. 2E), which increased with L-T4 treatment ($p < 0.01$). Peripheral zone activity was similar among groups (Figs. 2F,G). The percentage of time spent in the central zone was lower for hypothyroid mice ($p < 0.01$, Fig. 2H) and increased with L-T4 treatment ($p < 0.05$). Similarly, the ratio of central zone distance to total distance was reduced in hypothyroid mice and increased with L-T4 treatment ($p < 0.01$, Fig. 2I). Hypothyroid mice made fewer central zone entries ($p < 0.01$, Fig. 2J), which increased with L-T4 treatment ($p < 0.01$). These results suggest hypothyroid mice exhibit anxiety-like behavior without locomotor impairments, and L-T4 treatment reduces anxiety, although some indicators do not fully recover to EU levels.

Hypothyroidism induced anxiety-like behavior in elevated plus maze test

Next, the Elevated Plus Maze (EPM) test was employed to assess anxiety-like behaviors in mice. Figure 3A shows trajectories of mice from EU, hypothyroid, and L-T4 groups in the EPM. Hypothyroid mice exhibited decreased open arm activity compared to EU mice, which was restored to EU levels by L-T4 treatment. Hypothyroid mice spent significantly less time in the central zone than EU mice ($p < 0.01$, Fig. 3B). L-T4 treatment increased this time significantly compared to hypothyroid mice ($p < 0.01$). Hypothyroid mice spent significantly less time in the open arms than EU mice ($p < 0.01$, Fig. 3C). L-T4 treatment increased this time but remained lower than EU levels ($p < 0.05$). Conversely, hypothyroid mice spent more time in the closed arms than both EU and L-T4 mice ($p < 0.01$, Fig. 3D). Hypothyroid mice had significantly fewer open arm entries than EU mice ($p < 0.01$, Fig. 3E), which increased with L-T4 treatment. Similarly, hypothyroid mice had fewer closed arm entries ($p < 0.01$, Fig. 3F), also increased by L-T4 treatment ($p < 0.01$). The ratio of open arm entries to total entries was significantly lower in hypothyroid mice than EU mice ($p < 0.01$, Fig. 3G). L-T4 treatment showed a non-significant trend towards increasing this ratio. The ratio of time spent in the open arms relative to the total recording time was calculated for each group. Hypothyroid mice showed significantly lower ratios of time and distance in the open arms compared to EU mice ($p < 0.01$, Fig. 3H,I), with L-T4 treatment restoring time but

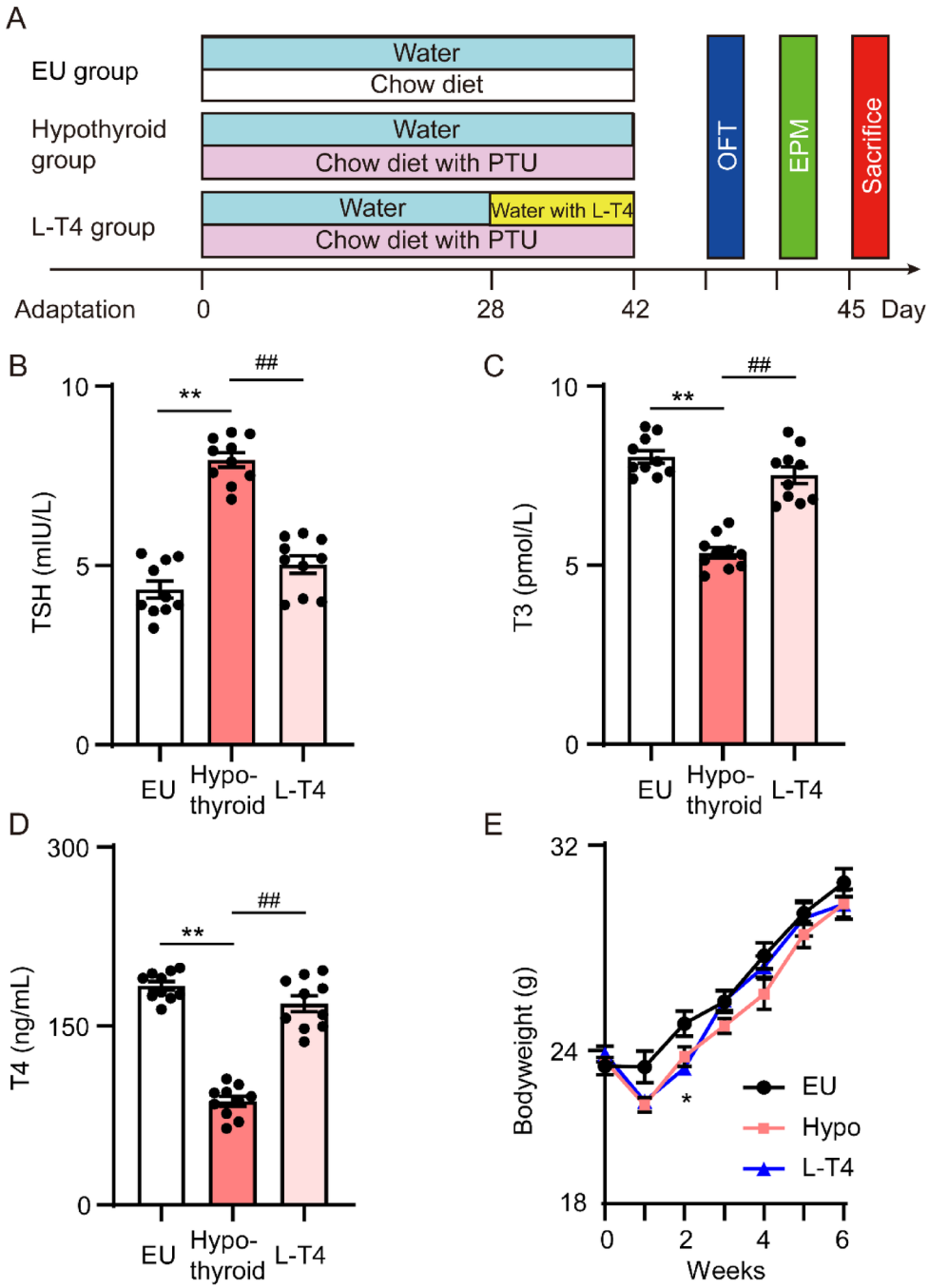

Fig. 1. Serum TSH, T3, T4 levels, and body weight changes in mice. (**A**) Schematic representation of the experimental workflow, including animal model induction and treatment, behavioral testing, and sample collection and analysis. (**B**) Changes in serum TSH levels in mice from different treatment. (**C**) Serum T3 level variations among 3 groups of mice. (**D**) Changes in serum T4 levels in mice from various groups. (**E**) Body weight fluctuations during the experiment in mice from the 3 groups. The data are presented as means ± SEM, with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the EU group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the Hypothyroid group. $n = 10$.

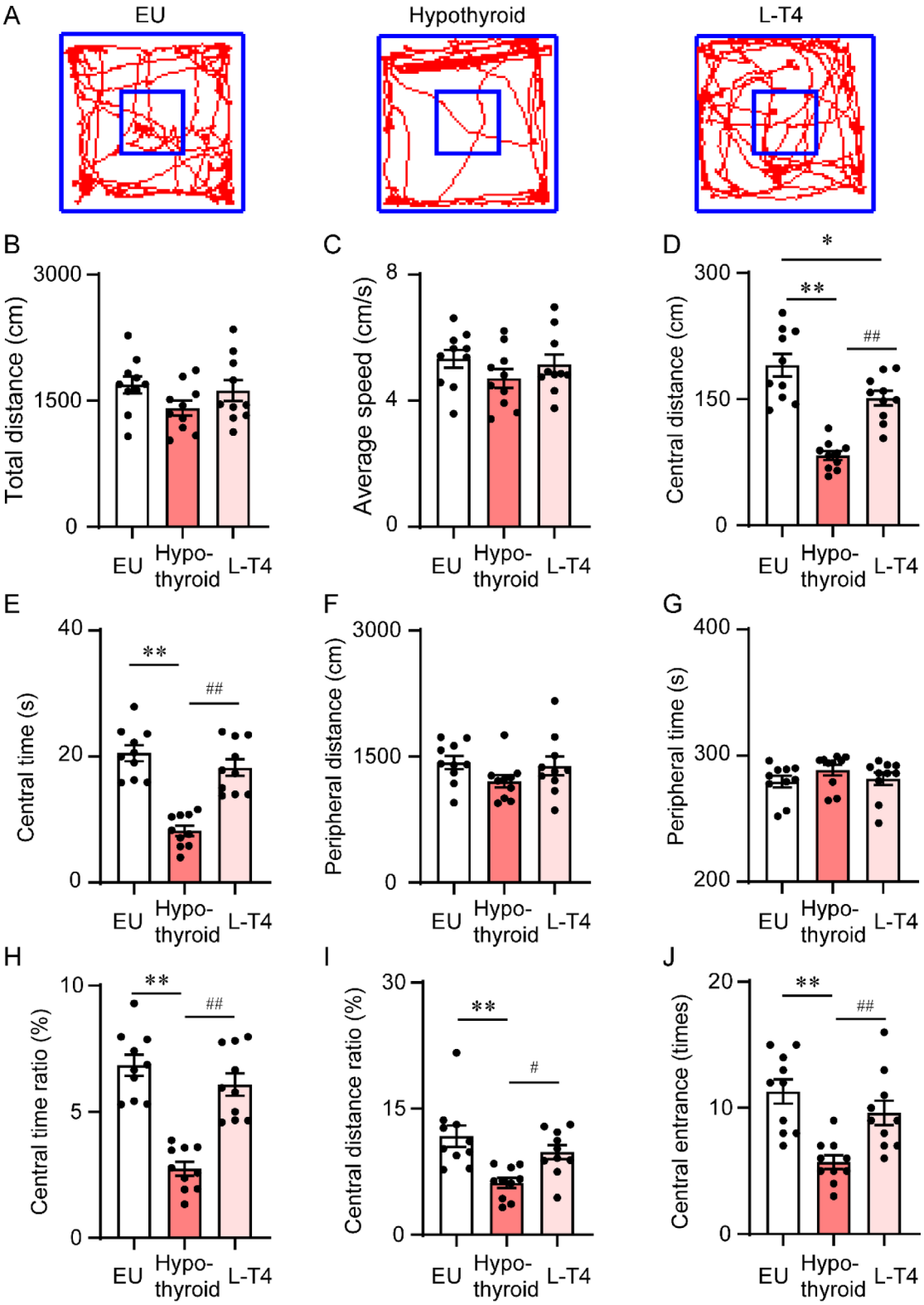

Fig. 2. Behavioral changes in the open field test induced by hypothyroidism in mice. (**A**) Representative trajectory plots of mice from EU, hypothyroid, and L-T4 group in the open field test; (**B**) Total distance traveled within 5 min; (**C**) Average speed of movement; (**D**) Distance traveled in the central zone; (**E**) Time spent in the central zone; (**F**) Distance traveled in the peripheral zone; (**G**) Time spent in the peripheral zone; (**H**) Ratio of total time spent in the central zone; (**I**) Ratio of total distance traveled in the central zone relative to the total distance traveled; (**J**) Number of entries into the central zone. The data are presented as means ± SEM, with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the EU group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the hypothyroid group. $n = 10$.

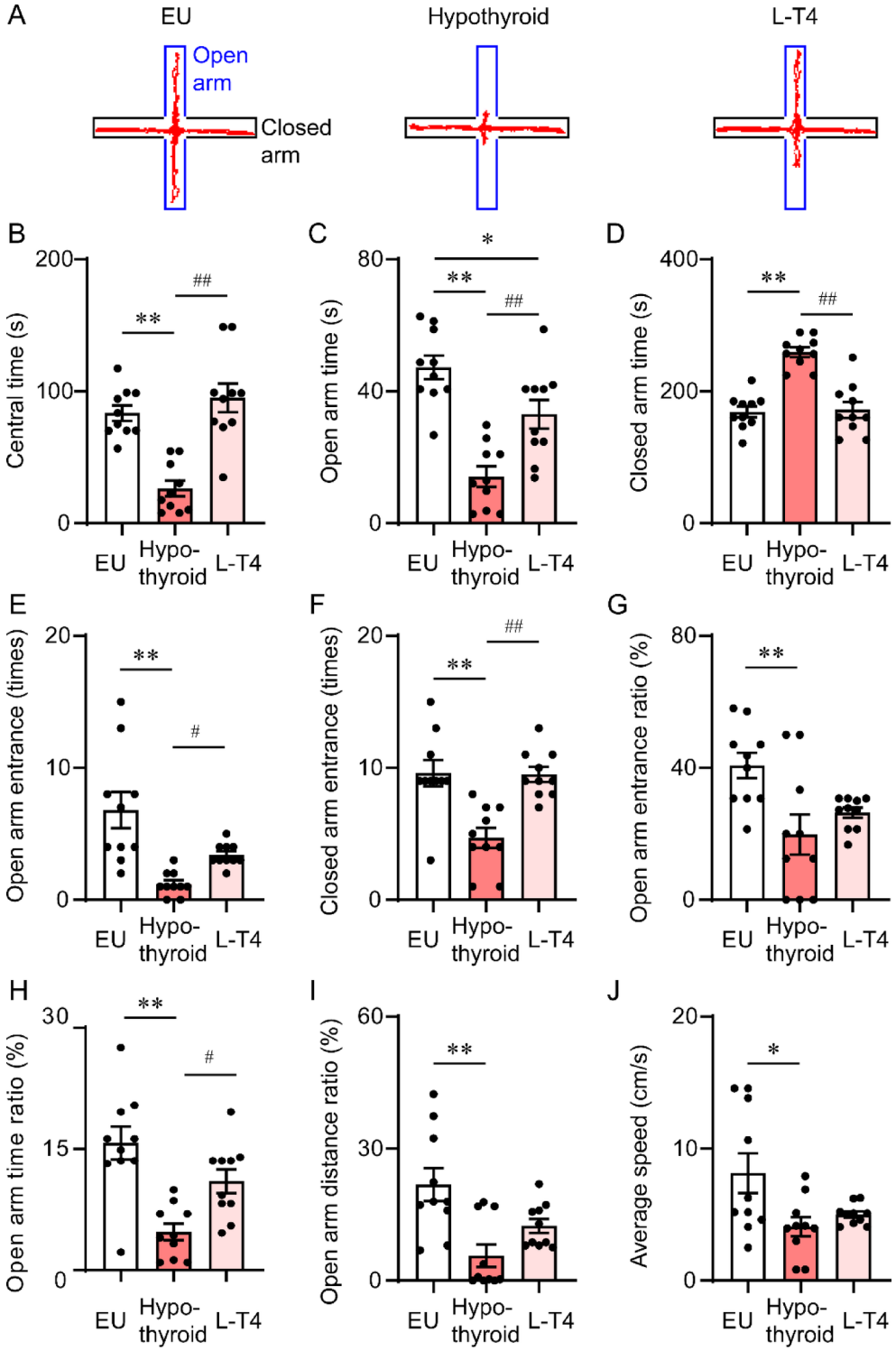

Fig. 3. Behavioral changes in the elevated plus maze induced by hypothyroidism in mice. (**A**) Representative trajectory plots of mice from each group in the elevated plus maze; (**B**) Time spent in the central zone; (**C**) Time spent in the open arms; (**D**) Time spent in the closed arms; (**E**) Number of entries into the open arms; (**F**) Number of entries into the closed arms; (**G**) Ratio of open arm entries relative to the total entries into both open and closed arms; (**H**) Ratio of time spent in the open arms relative to the total recording time; (**I**) Ratio of distance traveled in the open arms relative to the total distance traveled; (**J**) Average speed of movement. The data are presented as means ± SEM, with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the EU group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the hypothyroid group. $n = 10$.

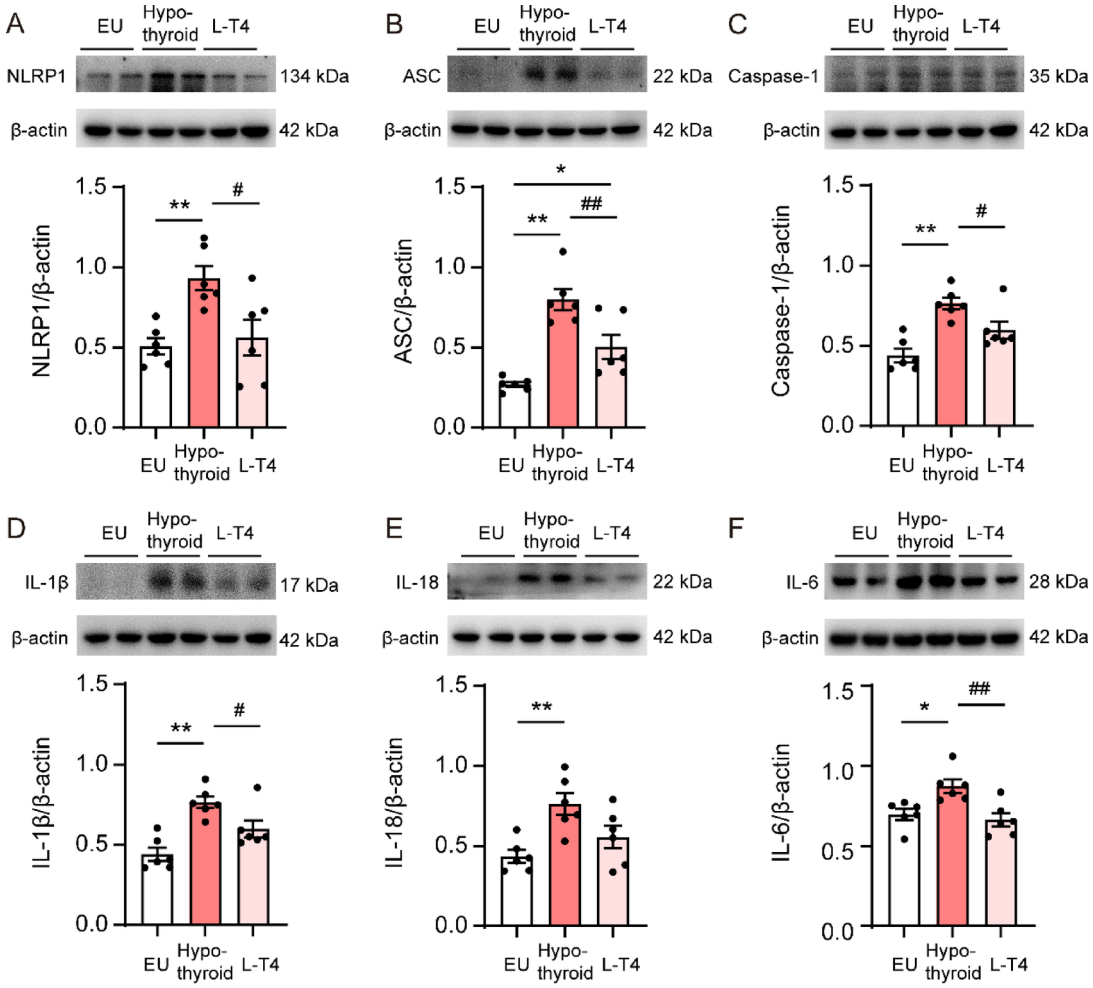

Fig. 4. Activation of the NLRP1 inflammasome signaling pathway and increased inflammatory cytokines expression in the hippocampus of hypothyroid mice. (**A**) Representative immunoblots and quantitative analysis of NLRP1 inflammasome; (**B**) Representative immunoblots and quantitative analysis of ASC; (**C**) Representative immunoblots and quantitative analysis of Caspase-1; (**D**) Representative immunoblots and quantitative analysis of the inflammatory cytokine IL-1β; (**E**) Representative immunoblots and quantitative analysis of the inflammatory cytokine IL-18 protein expression; (**F**) Representative immunoblots and quantitative analysis of the inflammatory cytokine IL-6. The data are presented as means ± SEM, with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the EU group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the hypothyroid group. $n = 6$.

not distance ratios. Locomotor speed was significantly reduced in hypothyroid mice ($p < 0.01$, Fig. 3J). These results indicate that hypothyroidism induces anxiety-like behaviors, which can be partially alleviated by L-T4 replacement therapy.

Hypothyroidism induced anxiety-like behavior is mediated by activation of hippocampal NLRP1 inflammasome

To investigate the mechanism of anxiety disorders in hypothyroid mice, we used western blot analysis to examine NLRP1 inflammasome complex protein levels in the hippocampus. Hypothyroid mice showed increased NLRP1 inflammasome expression compared to EU mice. After L-T4 replacement therapy, NLRP1 inflammasome expression decreased to levels similar to EU mice ($p < 0.01$, Fig. 4A). ASC expression in the hippocampus of hypothyroid mice was significantly higher than in EU mice ($p < 0.01$, Fig. 4B). Although ASC expression decreased with L-T4 treatment, it remained elevated compared to EU mice ($p < 0.05$, Fig. 4B). Caspase-1 expression in hypothyroid mice was also higher than in EU mice ($p < 0.01$, Fig. 4C), but returned to EU levels after L-T4 therapy. These results suggest the formation of an NLRP1 inflammasome complex comprising NLRP1, ASC, and Caspase-1 in hypothyroid mice, potentially leading to inflammatory cytokine expression. Further examination revealed higher levels of IL-1β ($p < 0.01$, Fig. 4D), IL-18 ($p < 0.01$, Fig. 4E), and IL-6 ($p < 0.05$, Fig. 4F) in the hippocampus of hypothyroid mice compared to EU mice. After L-T4 treatment, IL-1β ($p < 0.05$, Fig. 4D) and IL-6 ($p < 0.01$, Fig. 4F) expression decreased compared to hypothyroid mice, but IL-18 expression did not significantly decrease ($p = 0.096$, Fig. 4E). These findings imply that the formation of

the NLRP1 inflammasome complex and the expression of inflammatory cytokines IL-1β, IL-18, and IL-6 in the hippocampus of hypothyroid mice may mediate anxiety disorders.

Discussion

Our study demonstrated that hypothyroidism induces anxiety-like behavior in mice. These behavioral changes were accompanied by increased expression of NLRP1 inflammasome components and associated cytokines in the hippocampus. Importantly, treatment with L-T4 reversed both the behavioral deficits and the inflammatory changes, suggesting a causal link between hippocampal inflammation and anxiety-like behavior in hypothyroidism.

Hypothyroidism is associated with a range of neurological and psychiatric symptoms, including cognitive impairment, anxiety, and depression[29–31]. Patients with hypothyroidism often report feelings of unease, worry, and irritability, which can significantly impact their daily lives. Despite the widespread use of L-T4 replacement therapy to normalize thyroid hormone levels, this treatment often fails to completely reverse all cognitive and emotional disturbances in hypothyroid patients[32,33]. This observation highlights the complexity of the underlying mechanisms involved in the neuropsychiatric manifestations of hypothyroidism. In both the OFT and EPM tests, we observed clear indicators of anxiety-like behavior in the hypothyroid group, such as reduced time spent in the open arms of the EPM and decreased activity in the central zone of the OFT (Figs. 2 and 3). In anxiety disorders, animals often exhibit risk-averse behavior, leading to reduced exploration of both open and potentially threatening closed spaces[34,35]. This cautious approach to exploration is a hallmark of anxiety-like behavior and is consistent with our findings. Despite the hypothyroid mice showing a reduction in both open and closed arm entries in the EPM test (Fig. 3E,F), we did not observe any overt signs of motor deficits or locomotor impairment throughout the experimental period. Moreover, compared to the euthyroid controls, the hypothyroid mice did not exhibit any significant differences in total distance traveled or average speed (Fig. 2B,C). These findings suggest that the reduced exploration in the EPM is more likely attributed to anxiety-like behavior rather than a general impairment in locomotion.

Neuroinflammation has emerged as a critical factor in the pathogenesis of anxiety and depression[15]. The NLRP1 inflammasome, a key component of the innate immune system, has been implicated in the pathogenesis of various neurological disorders, including anxiety and depression[36,37]. The inflammasome is a multiprotein complex that plays a central role in initiating inflammatory responses by activating caspase-1, that cleaves pro-inflammatory cytokines such as IL-1β and IL-18 into their active forms[18]. Previous studies have shown that chronic stress activates NLRP1 inflammasome in the hippocampus of rodents, leading to the release of cytokines like IL-1β, IL-18, IL-6, and TNF-α. These cytokines are known to contribute to neuroinflammation and subsequently trigger anxiety and depressive-like behaviors[20,26]. For instance, IL-1β has been shown to directly affect neuronal activity and synaptic plasticity, leading to alterations in emotional processing and cognitive function. A recent study found that NLRP1 inflammasome activation was inhibited by knockdown of EndophilinA1, leading to reduced neuroinflammation and alleviation of depressive-like symptoms[19]. This finding suggests that targeting specific components of the inflammasome pathway may provide novel therapeutic strategies for treating neuropsychiatric disorders. Similarly, knockdown of Nlrp1a rescued the impaired autophagy and alleviated depressive-like behaviors in mice, suggesting that activation of NLRP1 inflammasome disrupts autophagy, a cellular process critical for maintaining neuronal health and function. Furthermore, rapamycin treatment, which induces autophagy, exhibited neuroprotective effects by inhibiting the expression of NLRP1 inflammasome[38]. These findings highlight the intricate interplay between inflammation and neuropsychiatric symptoms. In hypothyroidism, the altered hormonal milieu may activate the NLRP1 inflammasome, results in increased production of IL-1β, IL-6, and IL-18, which could contribute to the manifestation of anxiety symptoms.

The hippocampus, a crucial brain region implicated in emotion regulation and spatial cognition, is particularly susceptible to the impacts of hypothyroidism[8,39,40]. Structural and functional impairments within the hippocampus, such as decreased volume and compromised synaptic function, have been documented in hypothyroid patients[24,25]. These abnormalities are thought to contribute to the emotional disturbances associated with hypothyroidism. In our current study, we found that hypothyroid mice exhibited significantly higher protein levels of NLRP1, ASC, Caspase-1, IL-1β, IL-18, and IL-6 in the hippocampus compared to the EU group ($p < 0.05$ for all comparisons, Fig. 4). This upregulation indicates a pro-inflammatory state in the brains of hypothyroid mice, aligning with the established link between hypothyroidism and heightened inflammation[10,41]. The elevated inflammatory markers in the hippocampus offer a plausible explanation for the anxiety-like behaviors exhibited by hypothyroid mice. Another crucial factor to consider is the potential impact of hypothyroidism on hippocampal neurogenesis. In hypothyroidism, reduced thyroid hormone levels can impair the proliferation and differentiation of neural progenitor cells in the hippocampus, ultimately leading to decreased neuronal density and compromised neuronal function[23,42–44]. This reduction in neurogenesis may impair the hippocampus's capacity to process and integrate emotional information, potentially resulting in anxiety and other emotional disturbances. Following L-T4 treatment, the protein levels of NLRP1, ASC, Caspase-1, IL-1β, and IL-6 in the hippocampus were markedly reduced compared to the hypothyroid group. These findings suggest that L-T4 replacement therapy can mitigate the pro-inflammatory state in hypothyroidism by downregulating the expression of NLRP1 inflammasome components and their downstream cytokines. By restoring thyroid hormone levels and alleviating inflammation, L-T4 treatment may help normalize hippocampal function and ameliorate anxiety symptoms. Furthermore, the functional connectivity between the hippocampus and other neuroanatomical structures, such as the prefrontal cortex and amygdala, is vital for regulating emotional responses[45,46]. Disruptions in this connectivity, potentially driven by neuroinflammation, could underlie the anxiety observed in hypothyroidism.

Although our study offers valuable insights into the role of the NLRP1 inflammasome in anxiety-like behavior associated with hypothyroidism, several limitations should be considered. First, the use of male mice in our

study may not fully capture the complexity of hypothyroidism in humans, where females are more commonly affected. Future research should strive to include female subjects to ensure the generalizability of findings. Second, our study was limited to examining the NLRP1 inflammasome and did not investigate other potential inflammatory pathways. It is possible that multiple inflammatory mechanisms contribute to the neuropsychiatric manifestations of hypothyroidism.

Conclusion

In summary, our study provides evidence that the NLRP1 inflammasome in the hippocampus plays a crucial role in mediating anxiety-like behavior in mice with hypothyroidism. These results are significant for comprehending the mechanisms behind psychiatric comorbidities associated with hypothyroidism and indicate possible therapeutic targets for their management.

Materials and methods

Experimental animals

Male C57BL/6j mice, aged 8–9 weeks and weighing 22–25 g, are obtained from the Animal Center at Anhui Medical University. The mice are housed in a controlled environment with an ambient temperature of 22 ± 1 °C and a relative humidity of 60 ± 5%. A 12-h light/dark cycle is maintained, with lights on at 07:00. The mice are allowed to consume standard rodent chow and water freely. All procedures involving animals were performed in accordance with the ARRIVE guidelines 2.0[47], and were approved by Anhui Medical University's Animal Care and Use Committee (LLSC: 20220741). All methods reported in this study were conducted in compliance with relevant ethical guidelines and regulatory standards.

Reagents and antibodies

Propylthiouracil (PTU, P3755) was purchased from Sigma–Aldrich (St Louis, MO.). Levothyroxine (L-T4, L819046) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, P.R.C). Primary antibodies of NLRP1 (sc-390133), IL-1β (sc-12742), and IL-6 (sc-57315) were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Primary antibodies of Caspase-1 (22915-1-AP), ASC (67494-1-lg), IL-18 (10663-1-AP) and β-actin (66009-1-lg) were purchased from Proteintech (Wuhan, Hubei, P.R.C). Horseradish peroxidase (HRP)-conjugated secondary antibodies, Goat anti-Rabbit IgG (E-AB-1003) and Goat anti-Mouse IgG (E-AB-1001), were obtained from Elabscience Biotechnology Co.,Ltd. (Wuhan, Hubei, P.R.C.).

Grouping and induction of hypothyroidism in mice

The mice are randomly assigned to three groups: EU group, hypothyroid group, and hypothyroidism with L-T4 treatment (L-T4) group. The mice in EU group were fed with standard chow and water without restriction during the whole experiment. The hypothyroid mice were induced by adding PTU to the rodent chow at a concentration of 1500 mg/kg for 6 weeks. This led to a significant reduction in serum T3 and T4 levels, with a concurrent elevation in TSH. Mice in the L-T4 group were fed chow that included PTU for 6 weeks. And L-T4 was administered in the drinking water at a concentration of 0.1 μg/ml from the fifth week and until the end of the experiment.

Open field test

The open Field Test (OFT) is performed in a quiet, dimly lit room to minimize external stressors. The apparatus consists of a square arena (50 cm × 50 cm) with 45 cm high walls, divided into a central and peripheral zone. The arena is disinfected with 70% ethanol to remove any smell cues before testing. Each mouse is placed in the middle and allowed to explore freely for 5 min. Behavioral parameters such as total distance traveled, average speed, time spent in the center zone, and time spent in the peripheral zone are recorded and measured using SuperMaze software (Shanghai Xinruan Information Technology Co., Ltd, China). Increased anxiety-like behavior is manifested as reduced entries into the center zone and decreased exploratory behavior.

Elevated plus maze

The elevated plus maze (EPM) is conducted in a quiet, dimly lit room to minimize external stressors. Before conducting tests, the maze is wiped with 70% ethanol to clear any scent markers. Each mouse starts on the central platform, facing an enclosed arm, and is given 5 min to explore the maze. Behavioral parameters such as the percentage of time spent in the open arms, the average speed, and the number of entries into the open arms are recorded using SuperMaze software (Shanghai Xinruan Information Technology Co., Ltd, China). An increase in anxiety-like behavior is indicated by reduced time spent and fewer entries into the open arms.

Serum T3, T4, and TSH measurements

Serum levels of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) are measured to assess thyroid function in experimental mice. Blood samples were collected via cardiac puncture under deep anesthesia (pentobarbital, 50 mg/kg, intraperitoneal injection). The collected blood was allowed to clot for 60 min on ice before being centrifuged at 3000 × g for 10 min at 4 °C. Enzyme-specific immunoassay kits were used to determine the concentrations of T3, T4, and TSH, following the manufacturer's instructions (Rui Ying Biotechnology Co., Ltd. Suzhou, P.R.C). The assays were performed in duplicate, and the absorbance is read at 450 nm using a spectrophotometer.

Tissue preparation

Mice were euthanized under deep anesthesia (pentobarbital, 50 mg/kg, intraperitoneal injection). After the blood collecting, the brains were then rapidly removed and placed on ice to minimize degradation of tissue. The hippocampi were carefully dissected using fine surgical instruments under a dissecting microscope. The dissected hippocampi were immediately snap-frozen in liquid nitrogen and stored at − 80 °C until further analysis.

Western blot analysis

The hippocampal tissue (50 mg) was homogenized with 400 µl RIPA lysis buffer and 10 µl of PMSF by a homogenizer. Subsequently, the hippocampal tissue was lysed on ice for an additional 30 min. Following centrifugation at 12,000 rpm for 15 min at 4 °C, the supernatant was collected. The BCA protein assay kit (P0399, Beyotime Biotech Inc, Shanghai, P.R.C) was used to assess the protein concentration. Next, equal amounts of protein were separated by SDS-PAGE electrophoresis and transferred onto a PVDF membrane (88585, Thermo Scientific, Rockford, lL). The membrane was blocked with Tris-buffered saline with 0.1% Tween-20 containing 5% non-fat milk for 60 min at room temperature, followed by incubation with the primary antibody (anti-NLRP1, 1:500; anti-ASC, 1:5000; anti-Casepase-1, 1:2000; anti- IL-1β, 1:500; anti-IL-18, 1:5000; anti-IL-6, 1:500; anti-β-actin, 1:10,000) overnight at 4 °C. After rinsing the PVDF membrane, it was incubated with the HRP-conjugated secondary antibody (Goat anti-Rabbit IgG or Goat anti-Mouse IgG, 1:5000) for 60 min at room temperature. Finally, the membrane was exposed to ECL and images were captured. In the end, an enhanced chemiluminescence detection system (JS-1070, Shanghai Peiqing Science & Technology Co., Ltd, Shanghai, P.R.C) is used to visualize the protein bands.

To quantify the expression of each protein, we utilized Image J Fiji software to perform quantitative measurements of the protein bands. Specifically, we calculated the grayscale values of both the target protein bands and the internal control bands (β-actin). The expression level of each target protein was then determined by normalizing its grayscale value to that of β-actin, yielding a ratio that represents the relative protein expression level. Each target protein was analyzed in six samples in total, ensuring adequate statistical power for our analyses.

Statistical analysis

The data obtained from the study are presented as the mean value accompanied by the standard error of the mean (SEM). To assess whether there are any statistically significant differences between three groups, a one-way analysis of variance (ANOVA) was employed. Following the ANOVA, Bonferroni's post hoc test was utilized to pinpoint the specific groups that differ significantly. A p-value less than 0.05 was established as the threshold for statistical significance.

Data availability

Data available on request due to privacy/ethical restrictions request by contact with the corresponding author.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
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