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Therapeutic potential of HSP70 in correcting cognitive deficits and its effect on beta-amyloid formation in Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by the progressive accumulation of beta-amyloid and impaired cognitive function. Existing treatments are not effective enough, it's necessary to search for new therapeutic strategies targeting key pathogenetic mechanisms.

The aim. To investigate the therapeutic potential of intracellular and extracellular forms of heat shock protein HSP70 for correcting cognitive deficits and reducing amyloid load in AD.

Materials and methods. The study was performed on APPswe/PS1dE9/Blg transgenic mice, modeling AD, and lines created on their basis expressing intracellular (Tg_h) or extracellular (Tg_h_mod) forms of human HSP70. Behavioral tests were used to assess cognitive functions: Open Field, Novel Object Recognition, Y-maze, Barnes Maze. Amyloid load was assessed by histological method.

Results. The extracellular form of HSP70 (Tg_h_mod) significantly reduced amyloid load by 37% (p = 0.0033) and demonstrated marked cognitive improvement — by 40–45% in the Y-maze and Barnes Maze tests, whereas the intracellular form (Tg_h) reduced amyloidosis by 23.6% (p = 0.0273) but did not show significant memory recovery. The results indicate that the neuroprotective effect of extracellular HSP70 is likely mediated not only by chaperone activity but also by additional mechanisms critical for synaptic function.

Conclusion. A comparative study of the effectiveness of intracellular and extracellular forms of HSP70 in correcting both molecular and behavioral disorders in an AD model was conducted for the first time. It was found that the modified form of HSP70 has therapeutic potential. HSP70, especially its extracellular form, is a promising target for the development of AD therapy, providing a comprehensive effect on amyloid pathology and cognitive functions.

Keywords: Alzheimer's disease; HSP70; amyloid plaques; cognitive functions; neuroprotection

Abbreviations: AD — Alzheimer's disease; Aβ — beta-amyloid; APP/PS1 — APPswe/PS1dE9/Blg; Tg_h_mod — C57Bl/6-Tg_h(HSPA1A)-/+mod; Tg_h — C57Bl/6-Tg_h(HSPA1A)-/+; HSPA1A — human protein HSP70; WT — wild-type mice; IP — preference index; ID — discrimination index.

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Терапевтический потенциал HSP70 в коррекции когнитивного дефицита и его влияние на образование бета-амилоида при болезни Альцгеймера

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Болезнь Альцгеймера (БА) характеризуется прогрессирующим накоплением бета-амилоида и нарушением когнитивных функций. Существующие методы лечения недостаточно эффективны, что требует поиска новых терапевтических стратегий, направленных на ключевые патогенетические механизмы.

Цель. Исследовать терапевтический потенциал внутриклеточной и внеклеточной форм белка теплового шока HSP70 для коррекции когнитивного дефицита и снижения амилоидной нагрузки при БА.

Материалы и методы. Исследование выполняли на трансгенных мышах линии APPswe/PS1dE9/Blg, моделирующих БА, и созданных на их основе линиях, экспрессирующих внутриклеточную (Tg_h) или внеклеточную (Tg_h_mod) формы человеческого HSP70. Для оценки когнитивных функций применяли поведенческие тесты: Открытое поле, Распознавание нового объекта, У-лабиринт, Лабиринт Барнса. Амилоидную нагрузку оценивали гистологическим методом.

Результаты. Внеклеточная форма HSP70 (Tg_h_mod) значимо снижала амилоидную нагрузку на 37% (p=0.0033) и демонстрировала выраженное когнитивное улучшение — на 40–45% в тестах У-лабиринт и Лабиринт Барнса, тогда как внутриклеточная форма (Tg_h) уменьшала амилоидоз на 23,6% (p=0,0273), но не показывала значимого восстановления памяти. Полученные результаты указывают на то, что нейропротекторный эффект внеклеточного HSP70, вероятно, опосредован не только шаперонной активностью, но и дополнительными механизмами, критически важными для синаптической функции.

Заключение. Впервые проведено сравнительное исследование эффективности внутриклеточной и внеклеточной форм HSP70 в коррекции как молекулярных, так и поведенческих нарушений в модели БА. Установлено, что модифицированная форма HSP70 обладает терапевтическим потенциалом. HSP70, особенно его внеклеточная форма, является перспективной мишенью для разработки терапии БА, оказывая комплексное воздействие на патологию амилоида и когнитивные функции.

Ключевые слова: болезнь Альцгеймера; HSP70; амилоидные бляшки; когнитивные функции; нейропротекция

Список сокращений: БА — Болезнь Альцгеймера; Аβ — бета-амилоида; APP/PS1 — APPswe/PS1dE9/Blg; Tg_h_mod — C57Bl/6-Tg_h(HSPA1A)-/+mod; Tg_h — C57Bl/6-Tg_h(HSPA1A)-/+; HSPA1A — человеческий белок HSP70; WT — беспородные мыши; ИП — индекс предпочтения; ИД — индекс дискриминации.

INTRODUCTION

Alzheimer's disease (AD) remains the most common cause of dementia worldwide, posing a serious medical and social problem. Its pathogenesis is based on the accumulation of extracellular senile plaques in the brain, consisting of beta-amyloid (Aβ), and intraneuronal neurofibrillary tangles of hyperphosphorylated tau protein. These processes trigger a cascade of neurodegenerative changes, including synaptic dysfunction, chronic neuroinflammation, and neuronal death, ultimately leading to progressive cognitive deficits [1].

Despite progress in understanding the molecular basis of AD (amyloid and tau pathology, neuroinflammation), most approved drugs are only symptomatic. There is currently no therapy capable to slow or stop the progression of the disease, making the investigation of approaches targeting key pathogenetic links, such as proteostasis disruption and the accumulation of toxic protein aggregates, highly relevant [2, 3].

In this regard, considerable attention is paid to the search for endogenous neuroprotective factors capable of modulating key disease links. One such promising agent is the 70 kDa heat shock protein — HSP70 [4, 5].

Heat shock proteins (HSPs) are involved in a wide

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range of cellular housekeeping processes, including the folding of newly synthesized polypeptides, refolding of metastable proteins, assembly of protein complexes, degradation of misfolded proteins, and dissociation of protein aggregates. Under normal conditions, HSPs constitute 5–10 % of the total cellular protein content and function as an integrated network to maintain proteostasis [6]. Under extreme conditions, heat shock transcription factors are activated in response to stress to mitigate damage, leading to the transcription of a large number of HSPs. Based on their primary function as molecular chaperones (they are also involved in numerous processes in eukaryotic cells), impaired HSP function is linked to many diseases [7, 8].

HSP90 / HSP70 acts an important role in maintaining the normal physiological state of tau protein, as well as blocking its abnormal phosphorylation and accumulation, and participates in the pathological process associated with tau protein and Aβ [9, 10].

In neurodegenerative pathologies such as AD, Parkinson's disease, and Huntington's disease, HSP70 acts as a primary protective mechanism, correcting protein folding (including α-synuclein) and suppressing apoptosis. However, excessive expression can exacerbate neuroinflammation through TLR4 activation [11, 12]. It is important to note that HSP70 functions not only as an intracellular chaperone but also as an extracellular signaling mediator, interacting with receptors (TLR2/4) and modulating the inflammatory response, which is particularly significant for long-lived neurons, ensuring their resistance to stress and premature death [13].

HSP70 is often released from cells under stress conditions and/or in transformed cells. According to studies, endogenous HSPs exhibit neuroprotective activity in rodent models of Huntington's disease [14].

THE AIM. To determine the effect of intracellular and extracellular HSP70 on amyloid plaque accumulation in the brain and to evaluate its impact on cognitive functions in mice using a battery of behavioral tests.

MATERIALS AND METHODS

Study Design

The study design and animal housing conditions were selected in accordance with the recommendations of the Eurasian Economic Commission Board dated November 14, 2023 No. 33. Sexually mature mice were used in the study. Starting from week 17, behavioral screening was conducted to identify short-term and long-term memory impairments using the following tests: Open Field, Novel Object Recognition, Y-maze, and Barnes Maze. After the tests were completed, histological analysis of amyloid plaque accumulation in the brain was performed.

Study Conditions and Duration

Experimental and control animals were housed in the pathogen-free vivarium of the Belgorod National Research University under artificially regulated light-dark cycles (12 / 12 hours) at a temperature of +22–26 °C; they had free access to food and water. The study was conducted from January to September 2025.

Animals

The following lines were used in the study: APPswe/PS1dE9/Blg is overexpressing human mutant *APP* and *PSEN1* genes cDNA; C57Bl/6-Tg_h(HSPA1A)-/+mod — expressing a modified human HSP70 protein (HSPA1A) in milk; C57Bl/6-Tg_h(HSPA1A)-/+ is expressing a modified human HSP70 protein (HSPA1A) in cells. Crosses between the APPswe/PS1dE9/Blg line and the C57Bl/6-Tg_h(HSPA1A)-/+mod (Tg_h_mod) and C57Bl/6-Tg_h(HSPA1A)-/+ (Tg_h) lines were used in the experiment (n=11). Outbred mice derived from these crosses (WT, *n* = 11) were as controls.

Ethics Approval

Animal experiments were conducted in accordance with the "Rules of Laboratory Practice in the Russian Federation" dated April 01, 2016 No. 199n. The study was approved by the Commission for the Control of Laboratory Animal Husbandry and Use of Belgorod National Research University (Expert Opinion No. 01-01i/24 dated 09.01.2024).

Open Field Test

The animal was placed in an "Open Field" apparatus (NPK Otkrytaya Nauka, Russia), and its movements were recorded. The apparatus is a square chamber with a base of 50×50 cm, made of opaque acrylic glass. Animal behavior was assessed based on one parameter characterizing mouse behavior — locomotor activity. The EthoVision software (Noldus Information Technology, Netherlands) allows for

automatic acquisition of selected parameters: distance traveled, activity, and average speed of all movements in cm/sec. Each animal was tested for 5 minutes under 40 lux (dim lighting) [15].

Novel Object Recognition Test

A simple behavioral test based on the innate exploratory behavior of rodents. The test is divided into three phases: habituation, training / adaptation, and testing. On the first day of the test, the animal was placed in an empty 50×50 cm arena to explore it for 5 minutes under 40 lux. The second day of the test (adaptation) involves placing the animal in the same arena with two identical objects. On the third day (testing) the animal was placed in the arena with one of the familiar objects from the previous phase and one new object [16]. The following parameters were recorded: number of approaches to the new and old object and time spent near them; preference index (IP), calculated by formula 1; and discrimination index (ID), calculated by formula 2.

$$PI = \left(\frac{T_n}{T_n} + T_o\right) \times 100, \quad (1)$$

$$DI = \frac{T_n - T_o}{T_n + T_o}, \quad (2)$$

where PI — preference index; DI — discrimination index; T_n — time spent exploring the new object; T_o — time spent exploring the old object.

Barnes Maze Test

This test is used to investigate spatial learning and memory in animals. The apparatus (NPK Otkrytaya Nauka, Russia) consists of a circular platform 122 cm in diameter, containing 40 holes 5 cm in diameter, one of which is an exit (shelter). Distal visual cues are represented by 4 black and white images with different figures and patterns, located in different cardinal arms — North, South, West, East. Video recording is performed for 5 minutes. Measurements include total distance traveled by the animal, speed of movement, and time to find the exit within the allotted period.

Training days (Days 1–4): The animal gets acquainted with its surroundings for 3 minutes to locate the "shelter". Each mouse has 4 trials per day with a 15-minute interval.

Test day (day 5): The "shelter" area is covered by a flap. The animal remains on the platform for 5 minutes, during which time spent in the exit, number of approaches, and time spent in this zone are recorded [17].

Y-Maze

Working memory was assessed using the Y-Maze Test (NPK Otkrytaya Nauka, Russia) with arm dimensions of 32.5×8.5×15 cm (L×W×H). The test was conducted under dim lighting (40 lux). Mice were allowed to explore two arms of the maze for 5 minutes, while the third arm was blocked. After a 30-minute break between trials, a second trial was conducted, during which animals were allowed to explore all three arms for 5 minutes. Entry into the arm was recorded when more than half of the mouse's body crossed the boundary between two others. The number of entries and time spent in each one were recorded. Analysis was performed in two scenarios: the entire 5-minute test duration, or the first 2 minutes of "active exploration" [18].

Histology

Animals were subjected to terminal anesthesia; their brains were dissected, and fixed in Carnoy's solution (6 parts 96% ethanol, 3 parts chloroform, 1 part glacial acetic acid) for 12 hours. The tissue was dehydrated by sequential passage through ethanol solutions of increasing concentration: 75 % — 1 hour, 96 % (I) — 5 minutes, 96 % (II) — 45 minutes, 100 % (I) — 5 minutes, 100 % (II) — 45 minutes. Then, it was incubated for 30 minutes in a mixture of 100% ethanol–chloroform (1:1), 1 hour in chloroform (I), left overnight in chloroform (II), after which the tissues were infiltrated with paraffin (3 changes of 1 hour each) at 60°C. Paraffin sections 8 µm thick were mounted on polylysine-coated slides.

Sections were deparaffinized for 20 minutes in xylene and rehydrated by sequential incubation in ethanol: 10 minutes in 100 %, 5 minutes in 95 %, 5 minutes in 50 %, then washed three times in deionized water for 5 minutes each. Sections were stained with Congo red solution (0.5 % Congo red in 50 % ethanol) for 5 minutes and differentiated in a 0.2 % potassium hydroxide solution in 80 % ethanol for 1 minute, washed three times in deionized water for 5 minutes, and mounted using Glasseal mounting medium (Labiko LLC, Russia) [19].

Microscopy of samples was performed using a Nikon Eclipse Ti microscope equipped with a motorized stage. Panoramic visualization of mouse brain sections in TRITC fluorescence mode was performed using a 10x objective with NIS Elements AR software (version 4.6), with frame stitching, 10 % overlap, and automatic

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post-processing. The resulting images were loaded into QuPath software (version 0.5.1) for detection and analysis of aggregates. Object detection was based on the threshold brightness of amyloid fluorescence spots relative to the background brightness of intact brain tissue. Morphometric data were expressed as %area of plaques/mm² of cerebral cortex. After automatic object detection, manual verification was performed to exclude false identifications. To confirm reproducibility, two independent researchers also performed manual counts [20].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism Software 8.0 ("GraphPad Software Inc.", USA). Data are presented as M ± SD. Depending on the type of distribution and equality of variances, the significance of the results was assessed using parametric (ANOVA, Tukey's test) or non-parametric (Mann-Whitney U test) criteria. An unpaired Student's t-test was used to identify differences in intergroup comparisons. Differences were considered significant at $p \leq 0.05$.

RESULTS

Effect of HSP70 on Locomotor Activity

The Open Field test was used to assess general locomotor activity. As shown in Figure 1, no statistically significant differences were observed between the control group and the experimental groups, indicating no impairment of locomotor function in the animals, which allows us to compare the results of further tests without any adjustments [21].

Using Open Field test, we can also analyze the anxiety states of animals by looking at the time spent in the center versus the periphery, as these two indicators are mutually interchangeable. We assessment the time spent in the periphery and the number of transitions to this zone. There were no statistically significant differences in the number of transitions between the two sectors, but there were differences between the control group and the APP/PS1 group ($F(3, 35) = 3.860$; $p = 0.0391$).

Effect of HSP70 on Short-Term Memory Formation

The Novel Object Recognition test was performed to assess long-term memory. The perirhinal cortex is responsible for object recognition and spatial memory; impairments in its structure or function manifest as a lack of interest in new objects [22]. Thus, we observe that the number of approaches to the "new" toy increases when it is replaced 24 hours after the initial familiarization with the toys, in almost all mouse lines except for the positive control and the double transgenic Tg_h animals. The percentage of interest in the new object was statistically significantly lower between the WT group and the APP/PS1 and Tg_h groups ($F(7, 70) = 1.782$; $p = 0.0108$ and $p = 0.0319$). A significant difference can be observed in the IP, which indicates the degree of preference for the unfamiliar object. In the negative control and Tg_h_mod groups, it is above 50%; in the APP/PS1 and Tg_h groups, it differs significantly by 23% and 22% respectively ($F(3, 34) = 4.526$; $p = 0.0204$ and $p = 0.0209$) from the control group. A similar pattern is observed for the ID ($F(3, 42) = 3.874$; $p = 0.0368$ and $p = 0.0435$), indicating a decline in long-term memory functions and physiological changes in the perirhinal cortex.

To further confirm hippocampal impairments, the Y-Maze Test was conducted to analyze pathologies in short-term memory formation.

Figure 3 presents data on the preference for the new arm for exploration. Exploratory activity is driven by the innate curiosity of rodents, who strive to explore unvisited places. A mouse with intact working memory and, consequently, intact prefrontal cortex functions will remember previously visited arms and will tend to enter the less visited arm. Spatial memory, determined by the hippocampus, is also involved in this test by opening a new arm half an hour after exploring two others. As seen from the percentage of time spent by animals exploring the new arm, the negative control shows a typical pattern for healthy mice, with more time spent exploring the new arm (35 %) compared to the old one (29 %). The positive control also shows a typical pattern for AD mice, where the total time exploring the new arm does not exceed 20%. The experimental Tg_h_mod line shows a similar pattern to the negative control, while the Tg_h line, although showing a pronounced difference in visiting the new and old arms, exhibits a more blurred pattern of exploration of both the "old" and "new" arms, differing only in the time spent in the "landing" arm versus the "new" arm. The percentage of entries into the new arm decreases by 14 % ($p = 0.0398$) in the positive control group compared to the negative control, and by 28 % ($p = 0.0099$) in the Tg_h group. The Tg_h_mod group's

indicators increased statistically significantly by 40 % (p = 0.0156) compared to the Tg_h group.

Effect of HSP70 on Long-Term Memory Formation

The Barnes Maze test measures spatial learning and memory. The test is based on rodents' aversion to open spaces, which motivates the subject to seek shelter. The first 4 days are training days: to establish a correct trajectory for mice in finding the "shelter" and reduce the percentage of errors in hiding in false shelters, mice use visual cues to develop this trajectory. The latency to find the platform indicates the learning speed of the mice. As shown in Figure 4, progress in learning is observed in the positive control only on the third day; thereafter, almost identical parameters are observed, indicating a lack of progress in remembering the platform's location. The same pattern was observed in the Tg_h group — as seen from the graph, the learning process in both groups occurs almost identically. The negative control shows a typical pattern for healthy mice, with a tendency to learn each subsequent day. The tendency for a decrease in latency to find the platform in the Tg_h_mod group is similar to the negative control, but there are no statistically significant differences on the third and fourth days. The speed of the negative control increases daily, and the distance decreases, which fully correlates with the tendency for reduced time to find the platform. The positive control shows the opposite trend with increased speed and distance in the test and increased time to find the platform, indicating a lack of learning in this mouse line. In the Tg_h group, a decrease in total distance and speed in the test should be noted, resulting in almost no change in latency to find the shelter, but based on the overall data, it can be concluded that the learning process in this animal line is weakly expressed. The Tg_h_mod line shows a jump-like change in speed and total distance on the second day and almost unchanged values of these parameters on days 3 and 4 of training, while we see a decrease in latency to find the platform. Considering all the data, high learning ability in the test can be stated.

On the fifth day of the test, we also investigated learning and spatial memory. The latency in both experimental groups shows an intermediate result and does not statistically differ from the control. The number of entries into the shelter zone shows a statistical difference between the positive and negative controls (p=0.0409). The total time spent in the "shelter" zone in the positive control group was statistically significantly different from the Tg_h_mod group (p = 0.0184) and the negative control group (p = 0.0020). Compared to the WT group, this parameter is practically unchanged in the Tg_h group and increases by 45% in the Tg_h_mod group.

Effect of HSP70 on Amyloid Plaque Formation

To confirm the theory of slowing AD development, in addition to studying short-term and long-term memory, a histological analysis of amyloid plaques in the cortex and hippocampus of mouse brains was performed at 5 months of age. Amyloid aggregates are one of the most prominent markers of the disease, and their quantity and area indicate the degree of disease progression. Both investigated forms of HSP70 protein significantly reduced amyloid load compared to the control APP/PS1 group in the cerebral cortex (Fig. 5A). The extracellular form of HSP70 showed a more pronounced effect: the difference in mean ranks with the control was 10.00 (p = 0.0033), whereas for the intracellular form, this value was 8.00 (p = 0.0273). Critically, a direct comparison between the two experimental groups revealed no statistically significant differences (mean rank difference -2.00, p > 0.9999). This indicates that although both forms are effective in reducing plaque numbers, their anti-amyloidogenesis action likely relies on similar or overlapping molecular mechanisms.

Analysis of amyloid deposit distribution by size revealed a selective and region-specific action of HSP70. In the cerebral cortex, a significant reduction under the influence of both protein forms was observed exclusively in the pool of small plaques with a diameter less than 100 µm (p = 0.0104 for the extracellular and p = 0.0015 for the intracellular form). The difference between the experimental groups was not significant (p = 0.7061). The number of medium (100–500 µm) and large (>500 µm) plaques in the cortex did not statistically differ from the control or from each other. In the hippocampus, the picture was different: extracellular HSP70 (Tg_h_mod) demonstrated the most pronounced effect, significantly reducing the number of both small plaques (<100 µm, p = 0.0013) and large conglomerates (>500 µm, p = 0.028). The intracellular form (Tg_h) in the hippocampus showed a tendency towards a reduction in small deposits (p = 0.0884). These data suggest that the neuroprotective mechanism,

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especially for extracellular HSP70, may be related not only to a general reduction in amyloid load but also to the selective inhibition of early aggregation stages (small plaques) in the hippocampus, a structure crucial for memory, which aligns with the better cognitive performance observed in this experimental group.

Both variants of HSP70 (extracellular and intracellular) significantly reduce amyloid load compared to the APP/Ps1 control group.

DISCUSSION

The conducted study demonstrates the comprehensive neuroprotective effect of heat shock protein HSP70 in a transgenic AD model. The obtained data not only confirm the key role of HSP70 in maintaining proteostasis but also reveal a fundamentally important difference in the functional consequences of expressing its intracellular and extracellular forms. This difference places HSP70 among promising agents with a multimodal mechanism of action, which is critically important for the development of therapies for complex neurodegenerative diseases.

The most intriguing result of the study is the dissociation between the effect on the neuropathological marker (amyloid plaques) and the cognitive phenotype. Both investigated forms of HSP70 showed a statistically significant and comparable reduction in total amyloid load in the cerebral cortex. This result is in full agreement with the canonical chaperone function of HSP70, which involves preventing Aβ peptide aggregation and stimulating its clearance, as also described in the literature [23]. However, a detailed analysis of plaque distribution by size revealed important nuances: effective reduction was observed predominantly in the pool of small plaques (<100 μm), which may indicate that HSP70 inhibits early stages of Aβ aggregation or enhances the clearance of small, potentially more toxic oligomers.

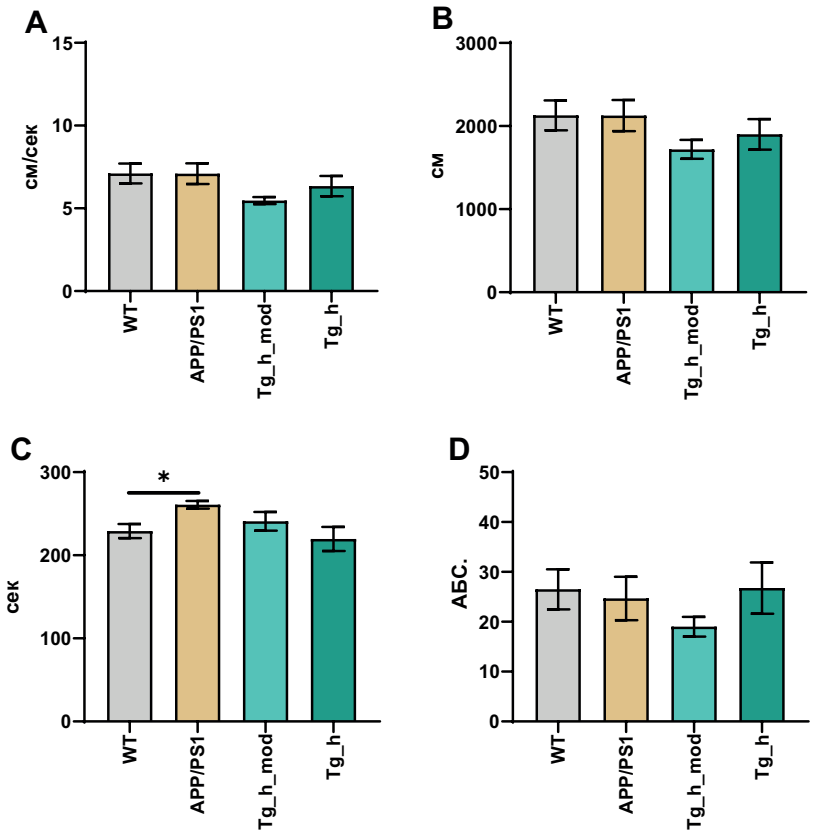

Figure 1 — Open Field Test results.
Note: A, speed of movement; B, distance traveled in 5 minutes; C, time spent in the periphery; D, number of transitions between sectors.
* — $p < 0.05$ (Tukey's test).

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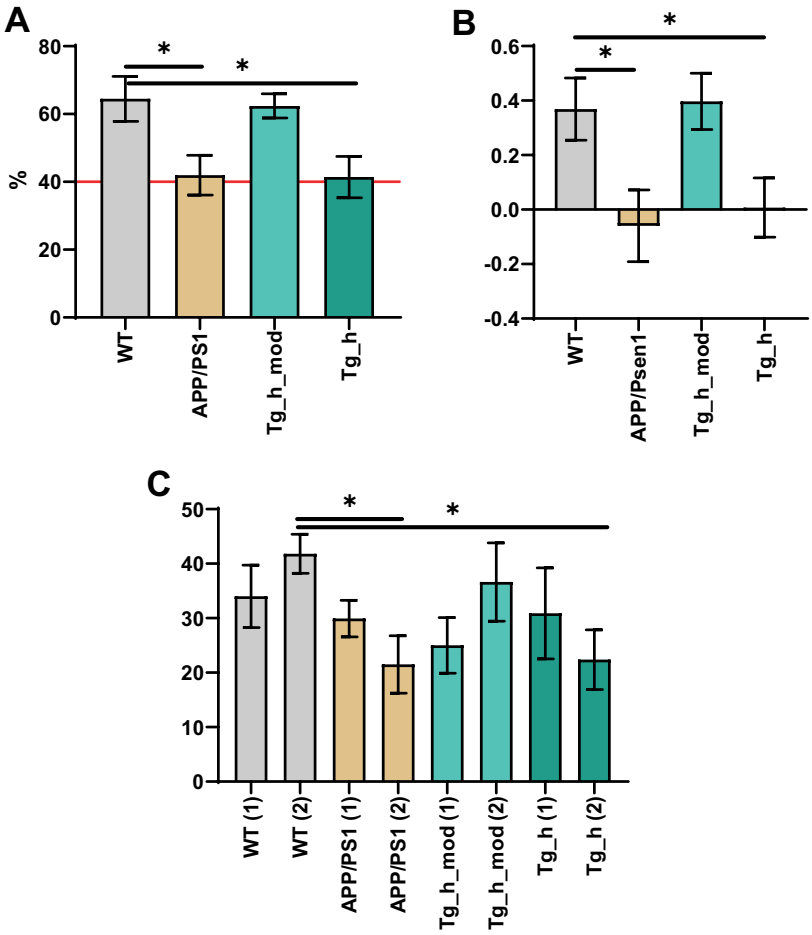

Figure 2 — Novel Object Recognition Test Activity results.
Note: A, preference index; B, discrimination index; C, number of approaches to the new toy (1-training day, 2-test day).
* — $p < 0.05$ (Tukey's test).

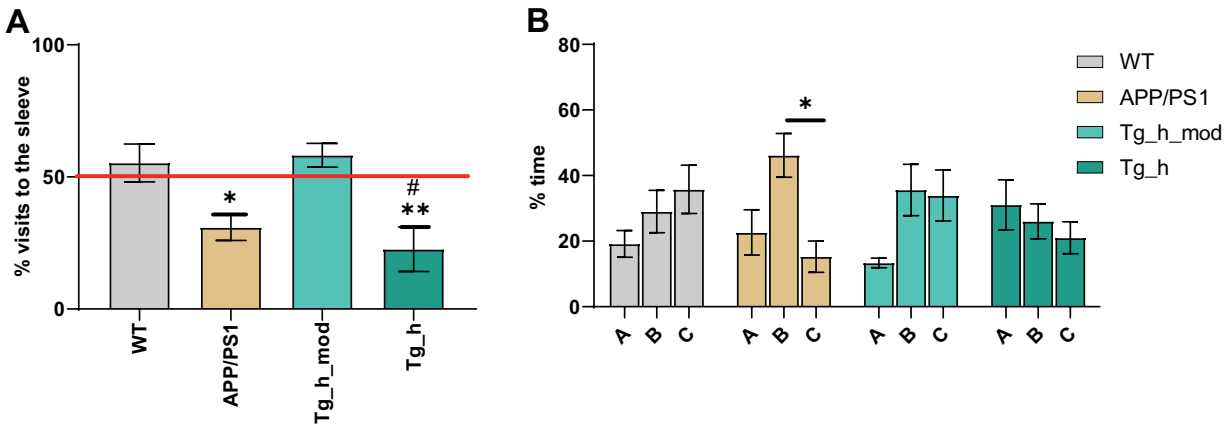

Figure 4 — Barnes Maze Test results.
Note: A, number of entries into the shelter zone on day 5; B, time spent in the shelter zone on day 5; C, latency to find the shelter zone from days 1 to 4 of training. * — $p < 0.05$; ** — $p < 0.01$ (Kruskal-Wallis test).

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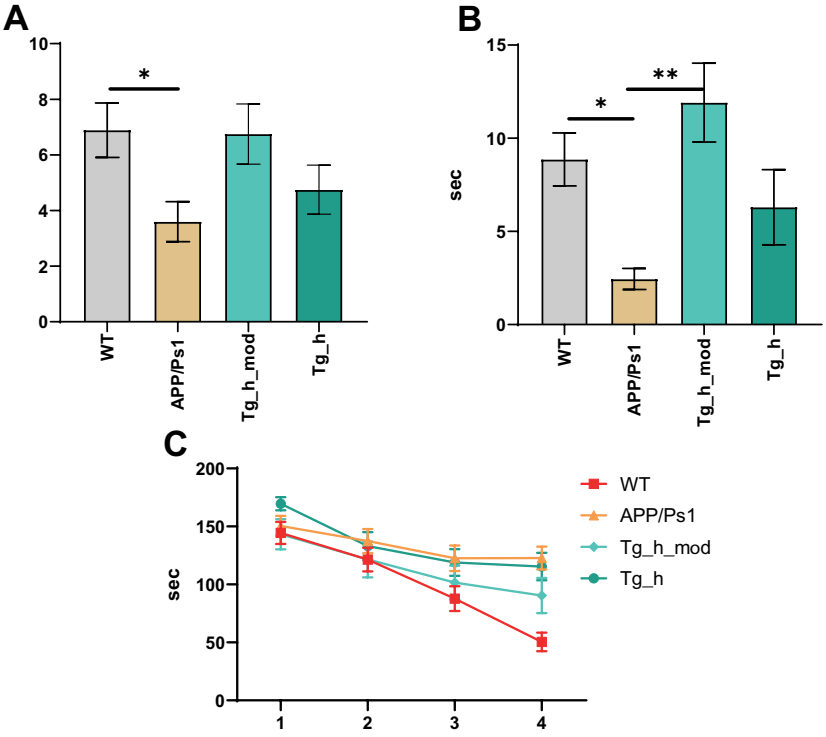

Figure 3 — Y-Maze Test "New" Arm Preference results.
Note: A, number of entries into the "new" arm; B, distribution of time spent in arms (C-new arm). * — $p < 0.05$; ** — $p < 0.01$ (Kruskal-Wallis test, comparison with WT); # — $p < 0.05$ (Kruskal-Wallis test, comparison with Tg_h_mod).

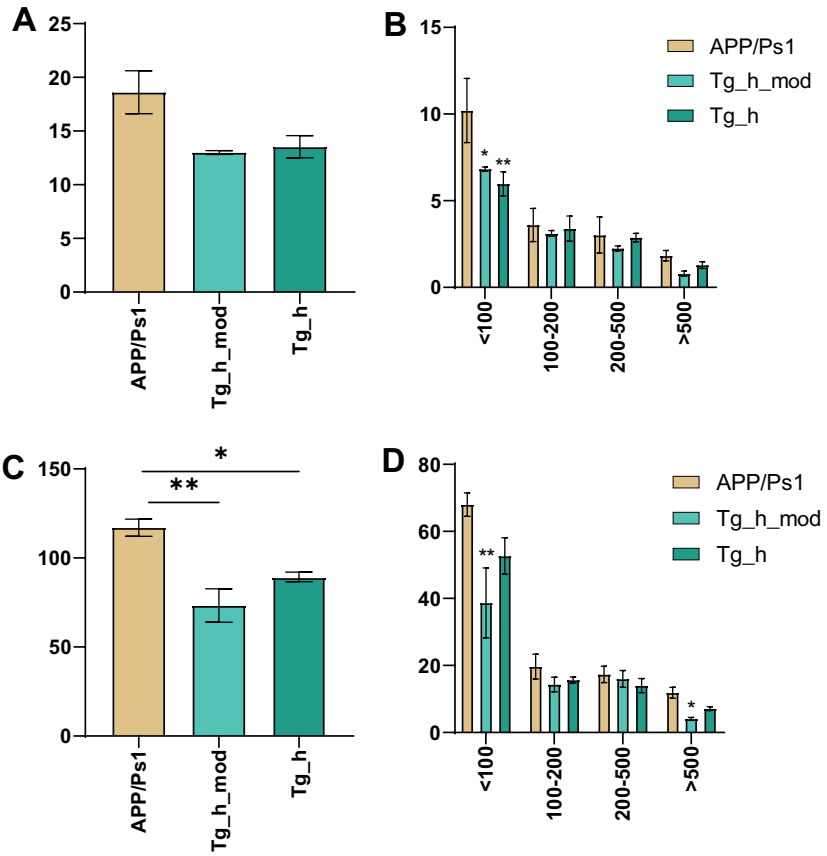

Figure 5 – Histology results.
Note: The figure shows the total number of amyloid plaques in the hippocampus (A) and cerebral cortex (C); distribution of amyloid deposits by size in the hippocampus (B) and cerebral cortex (D). * — $p < 0.05$; ** — $p < 0.01$ (Kruskal-Wallis test).

Despite similar anti-amyloid efficacy, the extracellular form of HSP70 (Tg_h_mod) demonstrated incomparably more pronounced positive effects on cognitive functions in all behavioral tests, reaching levels close to the wild-type (WT) group. In contrast, Tg_h mice expressing intracellular HSP70, although having a lower amyloid load, showed only minor or partial improvements in memory and learning. This observation is of key importance — merely reducing the number of amyloid plaques is insufficient to restore synaptic transmission and neuronal network functions. Extracellular HSP70 likely mediates additional protective mechanisms critically important for cognitive function. Intracellular HSP70 may directly impede the formation of Aβ oligomers, while the secreted form, as shown in other studies, can modulate neuroinflammation by interacting with microglia, stimulating phagocytosis and amyloid clearance [24].

The data from the Barnes Maze test are of particular interest. The fact that the Tg_h_mod line showed a learning dynamic comparable to the wild-type (WT) group suggests that HSP70 supports the functional reserves of neuronal networks responsible for navigation and spatial memory formation. It is known that HSP70 induction can alleviate synaptic defects and improve NMDA receptor-dependent signaling in the hippocampus, which is a key mechanism for spatial learning [25].

Within the scope of the conducted study, the obtained statistical data convincingly confirm the main conclusions. In behavioral tests, the extracellular form of HSP70 (Tg_h_mod) showed a statistically significant advantage in restoring cognitive functions. In the Novel Object Recognition test, the IP in this group was significantly higher than in the APP/PS1 and Tg_h groups — ($F(3, 34) = 4.526$; $p = 0.0204$ compared to APP/PS1). In the Y-maze, the Tg_h_mod group not only differed significantly from the positive control ($p = 0.0398$) but also demonstrated a 40 % better result in the percentage of entries into the new arm compared to the Tg_h group ($p = 0.0156$, Kruskal-Wallis test). In the Barnes Maze test, the time spent in the shelter zone in Tg_h_mod was 45% longer than in WT and statistically significantly differed from APP/PS1 ($p = 0.0184$). Meanwhile, the intracellular form (Tg_h) showed no significant improvements in most tests compared to APP/PS1, except for a tendency towards improvement. Histological data revealed a different picture: both forms of HSP70 significantly and approximately equally reduced the total amyloid load in the cortex compared to the APP/PS1 control ($p = 0.0033$ for extracellular and $p = 0.0273$ for intracellular form), with no significant difference in their efficacy when compared directly ($p > 0.9999$). However, analysis by plaque size showed that in the hippocampus, only extracellular HSP70 significantly reduced the number of both small (<100 µm, $p = 0.0013$) and large (>500 µm, $p = 0.028$) plaques.

Notably, in all cognitive tests, the Tg_h_mod line demonstrated a more pronounced positive effect compared to the Tg_h line [26]. This may be related to specific expression patterns or post-translational modifications of the protein in this model, which enhance its stability, chaperone activity, or secretion capacity. This observation has important practical implications, suggesting that the therapeutic efficacy of HSP70 can be optimized through targeted modification, which is a promising arm for drug development [27, 28].

Study Limitations

The conclusions are drawn based on a specific transgenic mouse line that primarily models the amyloid pathway of pathogenesis. Extrapolating the results to sporadic forms of human AD requires further verification. Additionally, the study involved a transgenic animal line expressing a modified human HSP70 protein in milk, which is a limiting factor for projecting this effect onto an exogenous protein.

CONCLUSION

As evidenced by the behavioral tests, crossing a mouse line with a neurodegenerative disease with mice producing heat shock protein does not lead to changes in locomotor function or the psychological state of the animals. However, tests such as Novel Object Recognition, Y-maze, and Barnes Maze show positive changes in learning processes, short-term, and long-term memory. The expression of extracellular HSP70 protein shows a greater effect on the restoration of both short-term and long-term memory, as well as an improvement in spatial memory functions. The expression of intracellular HSP70 protein influences these processes to a lesser extent. Although histological data indicate that both proteins affect the slowing of amyloid plaque formation to approximately the same degree.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Elena V. Kuzubova, Alexandra I. Radchenko — supervision, investigation; Yulia V. Stepenko, Amina O. Rumyantseva, Nikita S. Zhunusov — data analysis, writing — original draft; Marina A. Rzhevskaya, Alina A. Apostol — investigation; Elena B. Artyushkov, Anastasia Yu. Adonina — data analysis, writing — review & editing; Oleg S. Gudyrev — visualization; Liliya V. Korokina — conceptualization, supervision. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

REFERENCES

1. Pokrovsky VM, Deikin AV, Zhang T, Verlov NA, Konevega AL, Korokin MV. The influence of exogenous recombinant HSP 70 on the alteration of membrane stiffness in hippocampal neurons following the modeling of neonatal hypoxic-ischemic injury in mice. Research Results in Pharmacology. 2024;10(4):87–97. DOI: 10.18413/rrpharmacology.10.547
2. Kim JY, Barua S, Huang MY, Park J, Yenari MA, Lee JE. Heat Shock Protein 70 (HSP70) Induction: Chaperonotherapy for Neuroprotection after Brain Injury. Cells. 2020;9(9):2020. DOI: 10.3390/cells9092020
3. Richter K, Haslbeck M, Buchner J. The heat shock response: life on the verge of death. Mol Cell. 2010;40(2):253–66. DOI: 10.1016/j.molcel.2010.10.006
4. Rane MJ, Pan Y, Singh S, Powell DW, Wu R, Cummins T, Chen Q, McLeish KR, Klein JB. Heat shock protein 27 controls apoptosis by regulating Akt activation. J Biol Chem. 2003;278(30):27828–35. DOI: 10.1074/jbc.M303417200
5. Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B. The Hsp70 chaperone network. Nat Rev Mol Cell Biol. 2019;20(11):665–80. DOI: 10.1038/s41580-019-0133-3
6. Hu C, Yang J, Qi Z, Wu H, Wang B, Zou F, Mei H, Liu J, Wang W, Liu Q. Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. MedComm (2020). 2022;3(3):e161. DOI: 10.1002/mco2.161
7. Avdeeva NV. Novel mGluR4 agonist Rapitalam ameliorates motor dysfunction in mice with tau-associated neurodegeneration. Research Results in Pharmacology. 2020;6(2):9–17. DOI: 10.3897/rrpharmacology.6.52098
8. Bakthisaran R, Tangirala R, Rao ChM. Small heat shock proteins: Role in cellular functions and pathology. Biochim Biophys Acta. 2015;1854(4):291–319. DOI: 10.1016/j.bbapap.2014.12.019
9. Fernández-Fernández MR, Gragera M, Ochoa-Ibarrola L, Quintana-Gallardo L, Valpuesta JM. Hsp70 – a master regulator in protein degradation. FEBS Lett. 2017;591(17):2648–60. DOI: 10.1002/1873-3468.12751
10. Mahat DB, Salamanca HH, Duarte FM, Danko CG, Lis JT. Mammalian Heat Shock Response and Mechanisms Underlying Its Genome-wide Transcriptional Regulation. Mol Cell. 2016;62(1):63–78. DOI: 10.1016/j.molcel.2016.02.025
11. Weiss C, Jebara F, Nisemblat S, Azem A. Dynamic Complexes in the Chaperonin-Mediated Protein Folding Cycle. Front Mol Biosci. 2016;3:80. DOI: 10.3389/fmolb.2016.00080
12. Bobkova N, Guzhova I, Margulis B, Nesterova I, Medvinskaya N, Samokhin A, Alexandrova I, Garbuz D, Nudler E, Evgen'ev M. Dynamics of endogenous Hsp70 synthesis in the brain of olfactory bulbectomized mice. Cell Stress Chaperones. 2013;18(1):109–18. DOI: 10.1007/s12192-012-0359-x
13. Panza F, Lozupone M, Logroscino G, Imbimbo BP. A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nat Rev Neurol. 2019;15(2):73–88. DOI: 10.1038/s41582-018-0116-6
14. Almohmadi NH, Al-Kuraishy HM, Albuhadily AK, Al-Gareeb AI, Abdelaziz AM, Alexiou A, Papadakis M, El-Saber Batiha G. Alzheimer disease: Amyloid peptide controversies and challenges of anti-Aβ immunotherapy. J Pharmacol Exp Ther. 2025;392(8):103639. DOI: 10.1016/j.jpet.2025.103639
15. Polikarpova AV, Egorova TV, Bardina MV. Genetically modified animal models of hereditary diseases for testing of gene-directed therapy. Research Results in Pharmacology. 2022;8(2):11–26. DOI: 10.3897/rrpharmacology.8.82618
16. Lysikova EA, Kuzubova EV, Radchenko AI, Patrakhanov EA, Chaprov KD, Korokin MV, Deykin AV, Gudyrev OS, Pokrovskii MV. [APPswe/PS1dE9/Blg Transgenic Mouse Line for Modeling Cerebral Amyloid Angiopathy Associated with Alzheimer's Disease]. Mol Biol (Mosk). 2023;57(1):85–94. Russian. DOI: 10.31857/S0026898423010081
17. Kuzubova E, Radchenko A, Pokrovskii M, Shcheblykina O, Chaprov K, Nesterov A, Avtina T, Pokrovskii V, Korokin M. Sex-Dependent Phenotypic and Histomorphometric Biomarkers in the APPswe/PS1dE9/Blg Mouse Model of Alzheimer's Disease. Brain Sciences. 2025; 15(11):1237. DOI: 10.3390/brainsci15111237
18. Nikitina IL, Gaisina GG, Klen EE, Tkachenko LA. Assessment of the 3-substituted thietane-1,1-

dioxide derivative antidepressant effect using rat model of depression induced by reserpine. Research Results in Pharmacology. 2025;11(1):13–26. DOI: 10.18413/rrpharmacology.11.542

19. Shmigerova VS, Stepenko YV, Kurbatova AA, Zhunusov NS, Lyapkalov NS, Sviridova MS, Avtina TV, Nesterov AV, Popov AA, Nesterova NI, Goltsova MI, Pokrovskaya TG. Investigation of the pharmacological activity of the tetrapeptide HAEE, zinc, and human serum albumin in a transgenic mouse model with tau protein overexpression (P301S). Research Results in Pharmacology. 2025;11(1):49–57. DOI: 10.18413/rrpharmacology.11.493
20. de Munter JPJM, Tsoy A, Sitdikova K, Wolters EC, Chaprov K, Yenkoyan KB, Torosyan H, Askarova S, Anthony DC, Strekalova T. Therapeutic Effects of Neuro-Cells on Amyloid Pathology, BDNF Levels, and Insulin Signalling in APPswe/PSd1E9 Mice. Cells. 2025;14(16):1293. DOI: 10.3390/cells14161293
21. Evans CG, Wisén S, Gestwicki JE. Heat shock proteins 70 and 90 inhibit early stages of amyloid beta-(1-42) aggregation in vitro. J Biol Chem. 2006;281(44):33182–91. DOI: 10.1074/jbc.M606192200
22. Hoshino T, Murao N, Namba T, Takehara M, Adachi H, Katsuno M, Sobue G, Matsushima T, Suzuki T, Mizushima T. Suppression of Alzheimer's disease-related phenotypes by expression of heat shock protein 70 in mice. J Neurosci. 2011;31(14):5225–34. DOI: 10.1523/JNEUROSCI.5478-10.2011
23. Kakimura J, Kitamura Y, Takata K, Umeki M, Suzuki S, Shibagaki K, Taniguchi T, Nomura Y, Gebicke-Haerter PJ, Smith MA, Perry G, Shimohama S. Microglial activation and amyloid-beta clearance induced by exogenous heat-shock proteins. FASEB J. 2002;16(6):601–3. DOI: 10.1096/fj.01-0530fje
24. Hachani K, Ghanem M, Pockley AG, Wollenberg B, Bashiri Dezfouli A, Multhoff G. Heat shock protein 70 (Hsp70) as a target for advancing immunotherapy in solid tumors. Cytokine Growth Factor Rev. 2025;86:83–95. DOI: 10.1016/j.cytogfr.2025.09.002
25. Bobkova NV, Garbuz DG, Nesterova I, Medvinskaya N, Samokhin A, Alexandrova I, Yashin V, Karpov V, Kukharsky MS, Ninkina NN, Smirnov AA, Nudler E, Evgen'ev M. Therapeutic effect of exogenous hsp70 in mouse models of Alzheimer's disease. J Alzheimers Dis. 2014;38(2):425–435. DOI: 10.3233/JAD-130779
26. Lu RC, Tan MS, Wang H, Xie AM, Yu JT, Tan L. Heat shock protein 70 in Alzheimer's disease. Biomed Res Int. 2014;2014:435203. DOI: 10.1155/2014/435203
27. Valle-Medina A, Calzada-Mendoza CC, Ocharan-Hernández ME, Jiménez-Zamarripa CA, Juárez-Cedillo T. Heat shock protein 70 in Alzheimer's disease and other dementias: A possible alternative therapeutic. J Alzheimers Dis Rep. 2025;9:25424823241307021. DOI: 10.1177/25424823241307021
28. Turturici G, Sconzo G, Geraci F. Hsp70 and its molecular role in nervous system diseases. Biochem Res Int. 2011;2011:618127. DOI: 10.1155/2011/618127

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