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BI-D1870 Causes the Rats' Learning and Memory Acquisition Ability Impairment

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Abstract

Aim: To observe the rats' learning and memory acquisition ability disturbance induced by BI-D1870. **Methods:** Male SD rats were randomly divided into control group, solvent control group and BI-D1870 group. The rats in the control group were intraperitoneally injected with saline, while those in the solvent control group were intraperitoneally injected with DMSO + sulfobutyl-β-cyclodextrin solvent, and those in the BI-D1870 group were intraperitoneally injected with BI-D1870. All the rats' appearance and behavior were daily observed, and body weight was recorded on the day 15, 30, 45, 60, 75 and 82 of BI-D1870 injected. Morris water maze was used to screen the rats' learning and memory acquisition ability on the day 22 - 25, 52 - 55, and 82 - 85 of training by BI-D1870 treated. The successful rates of the rats' memory impairment were respectively calculated for three times screening. **Results:** During the whole experiment, there was no obvious difference in appearance and fur color in all rats. The rats' agitation began to appear on the day 10th of BI-D1870 given. The agitation rats' number and rats' body weight gradually increased along with BI-D1870 treated ($P < 0.05$, $P < 0.01$). According to the latency of rats on the day 25, 55 and 85 in Morris water maze training, the rats' successful rate in the learning and memory acquisition ability impairment induced by BI-D1870 was 50.00%, 62.00% and 82.00%, respectively. **Conclusion:** Intraperitoneal injection of BI-D1870 can induce the rats' learning and memory acquisition ability disorder.

Keywords

BI-D1870, Learning and Memory Acquisition Impairment, Morris Water Maze, RSK Inhibitor

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1. Introduction

Learning and memory is the most basic neurological function of the human brain, and abnormal nerve tissue structure is accompanied by learning and memory dysfunction [1]. Many neurodegenerative diseases, such as Alzheimer's Disease (AD) exist neuron loss and neural structure damage [2]. A series of studies have shown that neurotrophic factors directly support learning and memory function and neurogenesis decline and neuron loss are primary factors in causing cognitive impairment in AD [3]. Then, it is necessary to study the learning and memory impairment caused by decreased neurogenesis.

Cyclic-AMP Response Element Binding (CREB) protein is a kind of protein in the biological nucleus of eukaryotic cells and is regarded as a nuclear transcription factor, which can regulate gene transcription to synthesize the neurotrophic factors for neurogenesis [4]. BDNF-ERK-CREB, involved in CREB, is an important signal pathway in regulating neurogenesis [5]. Ribosomal S6 Kinase (RSK), an upstream molecule in the BDNF-ERK-CREB signal pathway, can activate CREB phosphorylation at the 133 site, which can promote the neurotrophic factors for neurogenesis, learning and memory formation [6]. When the activity of RSK is inhibited, CREB phosphorylation at the Ser133 site is also inhibited, then, the expressions of downstream genes and proteins are reduced [7] [8]. The result is that the production of neurotrophic factors is decreased, which may lead to neurogenesis reduction, learning and memory ability impairment [9].

BI-D1870, an inhibitor of RSK, can inhibit RSK activity and CREB phosphorylation [10], which may reduce neurotrophic factors production. Whereas, whether BI-D1870 can result in the rats learning and memory ability impairment, is not reported. The aim of the present study was to explore the rats' learning and memory acquisition ability impairment induced by BI-D1870.

2. Materials and Methods

2.1. Animals

70 healthy male rats (SD, 260 - 280 g) were purchased from Beijing Huafukang Biotechnology Co., Ltd. (Certification No. SCXK (Jing) 2019-0008), and the experimental animal production license was No. 1401180. Before the experiment, all rats were housed in the SPF-grade Experimental Animal Center of Chengde Medical College. The environment was kept at constant temperature (23˚C ± 1˚C) and humidity (55% ± 5%) with a light-dark cycle of 12-hour. Five rats were raised in one cage and ate daily 100 g of food and freely drank water. The experiment was begun after 7 days of feeding in animal center. The experimental protocol abided by the relevant provisions of "Guidelines for Animal Ethics and Welfare" and was approved by the Animal Ethics Committee of Chengde Medical College. The approval number was CDMULAC-20210407004. All animal experiments were followed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of China on Oct. 31, 1988.

2.2. Reagents and Instruments

BI-D1870 (Lot. 501437-28-1) and sulfobutyl-β-cyclodextrin (Lot. 182410-00-0) were purchase from Shanghai Yuanye Bio-Technology Co., Ltd. DMSO (cell-grade, Lot. D8371) was bought from Beijing Solarbio Technology Co., Ltd. Morris water maze was provided by Institute of Materia Medica, Chinese Academy of Medical Science.

2.3. Morris Water Maze and Training of Rats

Morris water maze is the equipment for testing the rats' learning and memory ability. It is a stainless-steel circular pool with the diameter 1.2 m and the height 0.5 m. The pool is artificially divided into four quadrants and the pool walls are marked different type labels as reference and designated four entry points. A 10 cm diameter, 30 cm high circular glass cylindrical platform is as a safe platform for rats to escape. During the Morris water maze training, the laboratory temperature was controlled at 24˚C - 25˚C, the water was added to high 31.5 cm in the pool and the temperature was kept at (23˚C ± 1˚C). When the experiment was conducted, the platform was placed in the middle of any quadrant of the pool and a small amount of edible melanin was added into the maze until the water became black and the platform was not visible. A camera is installed above the pool connected to a computer with capture card, which recorded the swimming behavior of rats in the pool. Before the day of training, all rats were placed in the pool swimming 180 s for adaptive the water environment. When the rats were performed the learning and memory ability assayed using Morris water maze, the rats were conducted two trials by difference intra-point to water to search for the platform at 4 Quadrants (Q4). On the first point of training, the rats were put into the water by facing the pool wall from a distal point away from the platform, and the latency required for finding the platform was record. The rats found the platform within 60 s and stayed the platform for 20 s. If the platform was not found within 60 s, the rats were guided onto the platform and stayed there for 20 s and the latency was recorded as 60 s. On the second point of training, the rats were put into the water by facing the pool wall from proximal point away from the platform and perform the same above procedure. The rats were allowed have physical recovery for 10 s outside the pool between the two trials. The rats were trained once a day in the morning and afternoon for four consecutive days. The average latency was calculated from the four times training for the learning and memory acquisition performance of rats.

2.4. Experimental Design

2.4.1. Experimental Grouping and Administration of Rats

After 7 days of adaptive feeding in the barrier environment, 70 rats were randomly divided into control group and solvent control group, with 10 rats in each group. The remained 50 rats were as BI-D1870 group. The BI-D1870 group rats were daily intraperitoneally injected with 0.35 mg/kg BI-D1870, the solvent control group rats were daily intraperitoneal injected 10% DMSO + 90% sulfo-

butyl-β-Cyclodextrin solvent and the control group rats were daily intraperitoneally injected with equal volume of saline.

2.4.2. Record of the Appearance, Behavior and Weight of Rats

During the injection of BI-D1870, each rat was fed with 20 g diet per day. The changes in appearance and behavior of rats were observed. The body weight of rats in the three groups was recorded on day 15, 30, 45, 60, 75 and 82, and the weight changes of rats were calculated.

2.4.3. Test the Rats' Learning and Memory Acquisition Ability with Morris Water Maze

The rats' learning and memory acquisition abilities were evaluated on day of 22 - 25, 52 - 55 and 82 - 85 of BI-D1870 injected by consecutive Morris water maze training. The latency for each rat in BI-D1870 group to find the hidden platform on the day 4th training, corresponding to day 25, 55 and 85 of BI-D1870 injected, was designated as A, and that for the solvent control group was designated as B. The Screening Ratio (SR) of learning and memory acquisition impairment in BI-D1870 group rats was SR = (A − B)/B × 100%. When the SR of any a rat in the BI-D1870 group was ≥20%, the learning and memory acquisition abilities of this rat was regarded as disorder. Then, the successful rate of the rats' learning and memory acquisition abilities impairment on the day 25, 55 and 85 of BI-D1870 injected was calculated. The administration of the BI-D1870 was continually given during the Morris water maze training period.

2.5. Statistical Analysis

All data were analyzed by SPSS 26.0 Statistical Software, and were expressed as mean ± standard deviation. One-way ANOVA was used to calculate the mean values of multiple samples. The Least Significant Difference (LSD) test was applied to the groups with homogeneous variance, whereas the Games-Howell test was used for the groups with heterogeneous variance. Values at $P < 0.05$ were considered statistically significant.

3. Results

3.1. Influence of BI-D1870 on Appearance and Behavior of Rats

During the whole experiment, there was no obvious difference in the appearance in all rats, with the pure and white, bright and smooth fur. One rat appeared agitation on the day 10th of intraperitoneal injection of BI-D1870. With the prolongation of intraperitoneal injection of BI-D1870, some rats appeared varying degrees agitation and the number of rats with agitation increased to 32.00% on the day 85th of BI-D1870 given.

3.2. Influence of BI-D1870 on Body Weight of Rats

Figure 1 shows the influence of BI-D1870 on the body weight of rats. The food intake of rats in each group was not significantly changed, but, the rats' body weight in BI-D1870 group was higher than that of control group and solvent

control group. The rat' body weight in BI-D1870 group increased by 27.53% ($P < 0.05$) and 94.66% ($P < 0.01$) on day 15 respectively, by 6.55% ($P > 0.05$) and −16.15% ($P > 0.05$) on day 30 respectively, by −8.78% ($P > 0.05$) and −19.64% ($P > 0.05$), respectively on day 45, by −18.93% ($P > 0.05$) and 17.65% ($P > 0.05$) respectively on day 60, by 88.13% ($P < 0.01$) and 50.66% ($P < 0.05$) on day 75 respectively, by 23.13% ($P > 0.05$) and 26.13% ($P < 0.05$) on day 82 respectively, as compared with control group and solvent control group.

3.3. Influence of BI-D1870 on the Rats' Learning and Memory Acquisition Abilities

3.3.1. Successful Rate of the Rats' Learning and Memory Acquisition Abilities Impairment on Day 25 of BI-D1870 Injected

Figure 2 is the result of rats' learning and memory acquisition abilities tested during 22 - 25 days of intraperitoneal injection BI-D1870 treated. The latency of three groups to find the platform was gradually shortened during the 4 days training in the Morris water maze. There was no significant difference in the latency to find the hidden platform between the control group and the solvent control group. Compared with the control group, the latency of the BI-D1870 group to find the platform was increased by 10.63% (22 d, $P > 0.05$), 91.63% (23 d, $P < 0.05$), 148.96% (24 d, $P < 0.01$) and 172.96% (25 d, $P < 0.01$), respectively, during 4 days training in the Morris water maze. Compared with the solvent control group, the latency of rats in the BI-D1870 group to find the platform was increased by 1.57% (22 d, $P > 0.05$), −13.63% (23 d, $P > 0.05$), 84.01% (24 d, $P < 0.05$) and 185.86% (25 d, $P < 0.01$), respectively, during 4 days training in the Morris water maze. According to the rats' swimming performance in each group on day 25 of Morris water maze training, 50.00% of the rats treated with BI-D1870 got the impaired learning and memory acquisition abilities.

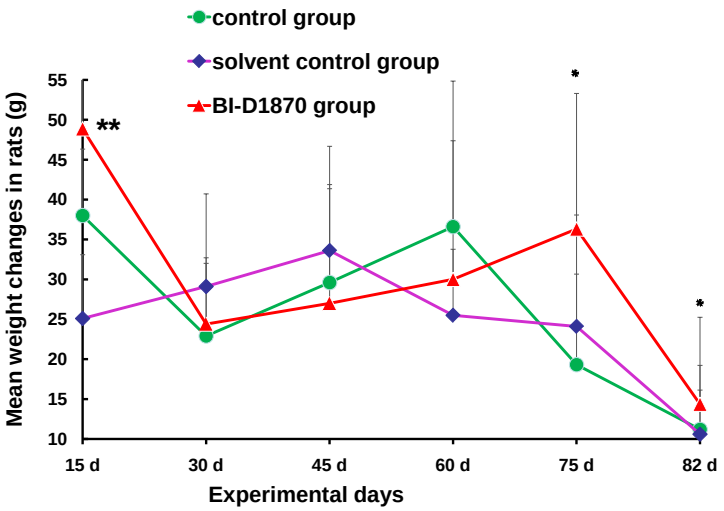

Figure 1. The influence of BI-D1870 on the body weight of rats. Mean weight changes of rats in control group, solvent control group and BI-D1870 group were recorded on day 15, 30, 45, 60, 75 and 82. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* solvent control group.

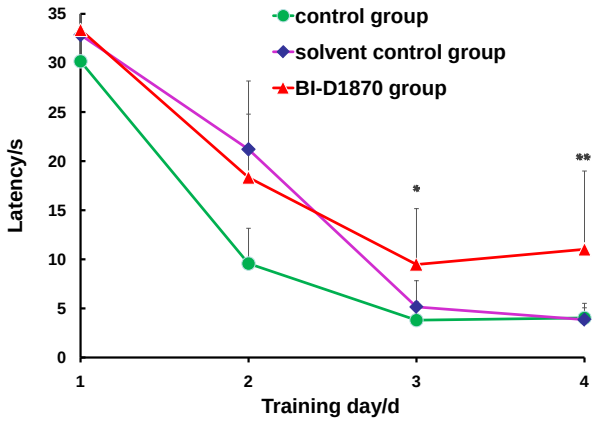

Figure 2. The latency of rats' learning and memory acquisition abilities tested on the day 22 - 25 of intraperitoneal injection of BI-D1870 with Morris water maze. The average value of latency every day was regarded as the learning performance of the rats and the results on day 25 of intraperitoneal injection of BI-D1870 was the successful rate of rats' learning and memory acquisition ability impairment. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* solvent control group.

Figure 3 shows the swimming distance of rats to find the hidden platform in each group on the day 4th, corresponding to the day 25 of intraperitoneal injection of BI-D1870, for measurement of learning and memory acquisition abilities by Morris water maze training. BI-D1870 can result in the longer swimming distance to find the hidden platform than that of control group. The swimming distance in the BI-D1870 group increased 104.48% ($P < 0.01$) and 68.97% ($P < 0.05$), as compared with the control group and solvent control group. Meanwhile, there was no significant difference between the control group and the solvent control group in the swimming distance.

Figure 4 is the rats' swimming trajectory in Morris water maze training from day 22 to 25 of BI-D1870 treated. The swimming trajectory is the edge type → trend type → linear type for control group and solvent control group, and is the edge type → trend type for BI-D1870 group in looking for the hidden platform. The trajectory of BI-D1870 group in Morris water maze swimming was significantly longer than that of control group and solvent control group, which was consistent the swimming distance of the result in **Figure 3**.

3.3.2. Successful Rate of the Rats' Learning and Memory Acquisition Abilities Impairment on Day 55 of BI-D1870 Injected

Figure 5 is the result of the rats' learning and memory acquisition abilities tested during 52 - 55 days of intraperitoneal injection BI-D1870 treated. Along with the increase of training days, the latency of rats to find the hidden platform of three groups was gradually shortened in Morris water maze training. The latency of BI-D1870 group to find the hidden platform increased by 2.76% (52 d, $P > 0.05$), 3.53% (53 d, $P > 0.05$), 6.51% (54 d, $P > 0.05$) and 126.66% (55 d, $P < 0.01$), respectively, as compared with the control group, by 13.48% (52 d, $P > 0.05$), −2.96% (53 d, $P > 0.05$), 8.64% (54 d, $P > 0.05$) and 121.89% (55 d, $P < 0.01$), re-

spectively, as compared with the solvent control group. There was no significant difference between the control group and the solvent control group. According to the rats' swimming performance in each group on day 25 of Morris water maze training, 62.00% of the rats treated with BI-D1870 got the impaired learning and memory acquisition abilities.

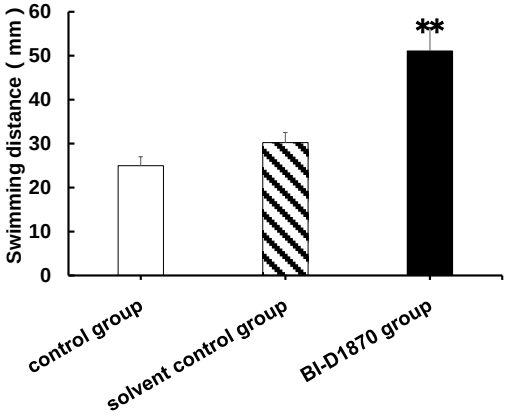

Figure 3. The swimming distance of rats' learning and memory acquisition abilities tested on the day 22 - 25 of intraperitoneal injection of BI-D1870 with Morris water maze. The average value of swimming distance on day 25 was regarded as the learning performance of the rats. $^{**}P < 0.01$ *vs* solvent control group.

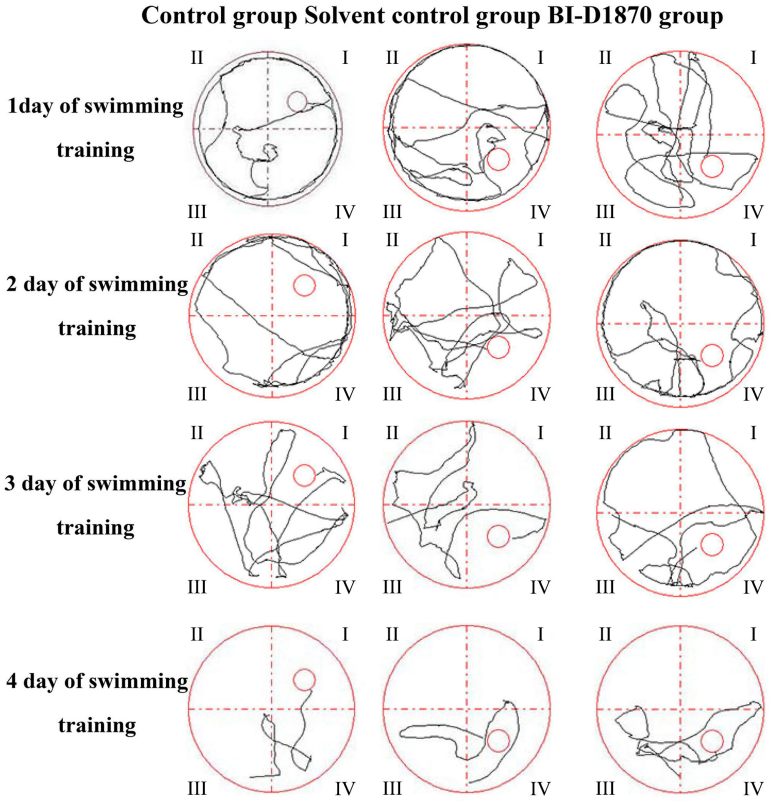

Figure 4. The swimming trajectory of rats' learning and memory acquisition abilities tested on the day 22 - 25 of intraperitoneal injection of BI-D1870 with Morris water maze.

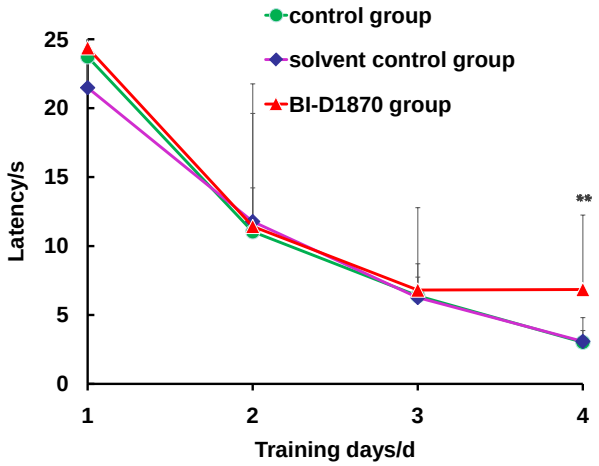

Figure 5. The latency of rats' learning and memory acquisition abilities tested on the day 52 - 55 of intraperitoneal injection of BI-D1870 with Morris water maze. The average value of latency every day was regarded as the learning performance of the rats and the results on day 55 of intraperitoneal injection of BI-D1870 was the successful rate of rats' learning and memory acquisition ability impairment. $^{**}P < 0.01$ *vs* solvent control group.

Figure 6 is the swimming distance of rats in each group to find the hidden platform on the day 4th, corresponding to the day 55 of intraperitoneal injection of BI-D1870, for determination of learning and memory acquisition abilities by Morris water maze training. The swimming distance in BI-D1870 group was prolonged by 41.65% ($P < 0.01$) and 27.58% ($P < 0.05$), respectively, than that of control group and solvent control group. There was no significant difference between the control group and the solvent control group in the swimming distance.

Figure 7 is the swimming trajectory of Morris water maze training in rats from day 52 to 55. The swimming trajectory of control group and the solvent control group is the trend type → linear type and the BI-D1870 group rats freely swam to search for the hidden platform. The trajectory in the BI-D1870 group was significantly longer than that of the control group and the solvent control group on day 55 of intraperitoneal injection of BI-D1870, which was parallel the swimming route of the result in **Figure 6**.

3.3.3. Successful Rate of the Rats' Learning and Memory Acquisition Abilities Impairment on Day 85 of BI-D1870 Injected

Figure 8 is the measured data of the rats' learning and memory acquisition abilities during 82 - 85 days of intraperitoneal injection BI-D1870 treated. Along with the increase of Morris water maze training days, the latency of rats in three groups to find the hidden platform was gradually decreased. There was no notable difference in the latency between the control group and the solvent control group. the latency of the BI-D1870 group for finding the hidden platform increased by −1.17% (82 d, $P > 0.05$), 22.73% (83 d, $P > 0.05$), 52.13% (84 d, $P > 0.05$) and 92.64% (85 d, $P < 0.05$), as compared with the control group, and in-

creased by −24.60% (82 d, $P > 0.05$), 35.25% (83 d, $P > 0.05$), 56.56% (84 d, $P > 0.05$) and 97.02% (85 d, $P < 0.05$), as compared with the solvent control group, for consecutive 4 days Morris water maze training. According to the rats' swimming performance on day 85 of Morris water maze training, 82.00% rats received intraperitoneal injection of BI-D1870 appeared the impaired learning and memory acquisition abilities.

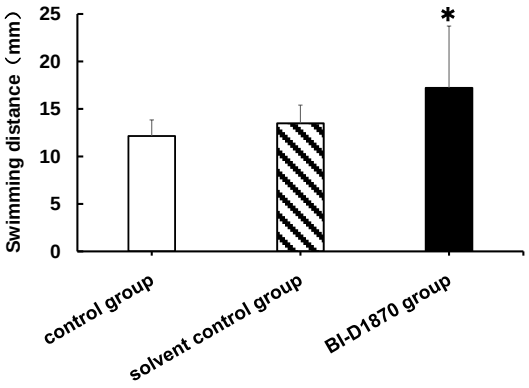

Figure 6. The swimming distance of rats' learning and memory acquisition abilities tested on the day 52 - 55 of intraperitoneal injection of BI-D1870 with Morris water maze. The average value of swimming distance on day 55 was regarded as the learning performance of the rats. $^{*}P < 0.05$ *vs* solvent control group.

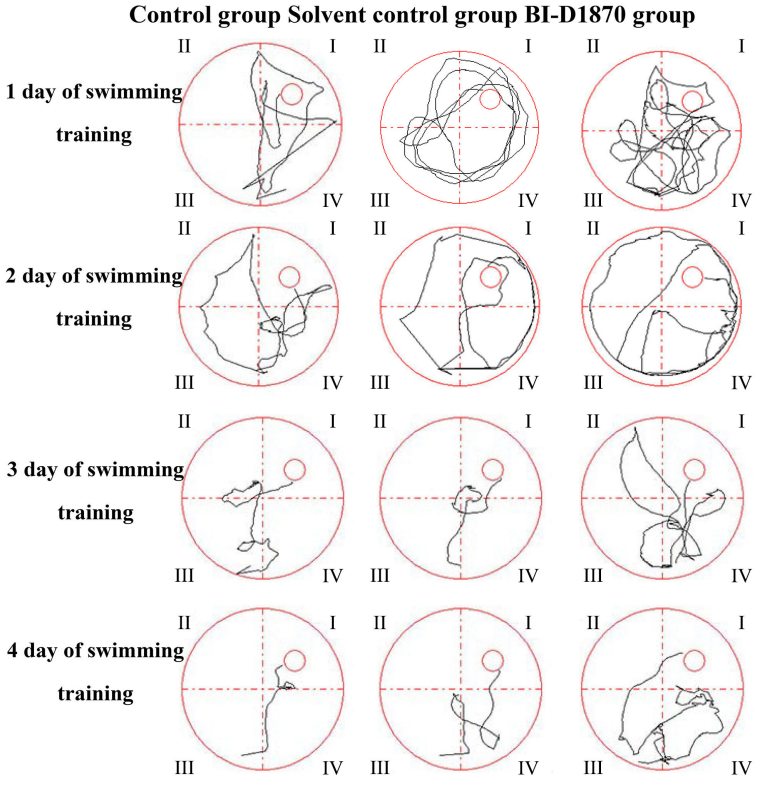

Figure 7. The swimming trajectory of rats' learning and memory acquisition abilities tested on the day 52 - 55 of intraperitoneal injection of BI-D1870 with Morris water maze.

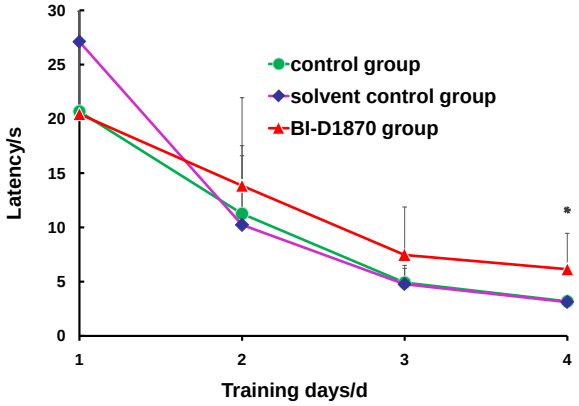

Figure 8. The latency of rats' learning and memory acquisition abilities tested on the day 82 - 85 of intraperitoneal injection of BI-D1870 with Morris water maze. The average value of latency every day was regarded as the learning performance of the rats and the results on day 55 of intraperitoneal injection of BI-D1870 was the successful rate of rats' learning and memory acquisition ability impairment. $^{*}P < 0.05$ compared with solvent control group.

Figure 9 is the swimming distance of each group to find the platform on day 4th, corresponding to the day 85 of intraperitoneal injection of BI-D1870, for assay of learning and memory acquisition abilities by Morris water maze training. There was no significant difference between the control group and the solvent control group in the swimming distance. The swimming distance in the BI-D1870 group was respectively, higher 24.73% ($P < 0.05$) and 22.67% ($P < 0.05$) than that of the control group and solvent control group.

Figure 10 is the swimming trajectory of Morris water maze training in each group from day 82 to 85. The swimming trajectory was trend type → linear type in control group and solvent control group, and was edge type or trend type in BI-D1870 group for searching for the hidden platform. Moreover, the swimming trajectory in the BI-D1870 group on day 85 of Morris water maze training was significantly longer than that of the control group and the solvent control group, which was consistent the swimming distance of the result in **Figure 9**.

4. Discussion

It is well known that the learning and memory are the basic cognitive function of human and may appear disorder in the neurodegenerative diseases, including AD [11]. Studies have shown that the neurotrophic factors can directly affect the learning and memory function [12]. The neurogenesis reduction and neuronal loss also contribute to the cognitive impairment of animals.

The neurogenesis plays the important role in compensating the neuron loss and neuronal damage, and the neurogenesis is also regulated by multiple signal pathways and signal molecular [13]. Of which, RSK is a Ser/Thr protein kinase and can modulate the cell division, survival and differentiation [14] [15]. RSK can independently move into the nucleus and catalyzes CREB to phosphorylate and promote the genes and protein expression for nerve growth factor. These

nerve growth factors can expedite the neural cell proliferation and differentiation, prompt the neurogenesis and advance to ameliorate the cognitive deficits [16] [17]. BI-D1870 is RSK inhibitor and can inhibit the activity of RSK and suppress the transcription activity of CREB [18]. This result can result in the decreased nerve growth factors, and advance to disturb the neuronal repair, neurogenesis and differentiation.

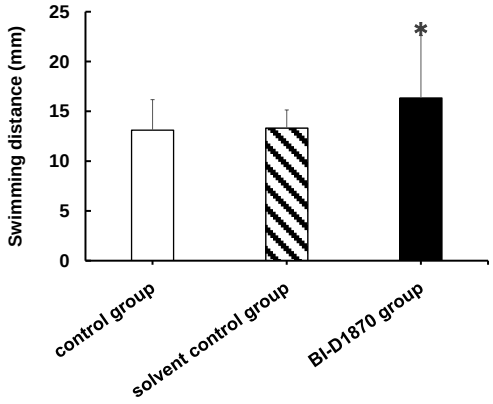

Figure 9. The swimming distance of rats' learning and memory acquisition abilities tested on the day 82 - 85 of intraperitoneal injection of BI-D1870 with Morris water maze. The average value of swimming distance on day 85 was regarded as the learning performance of the rats. $^{*}P < 0.05$ *vs* solvent control group.

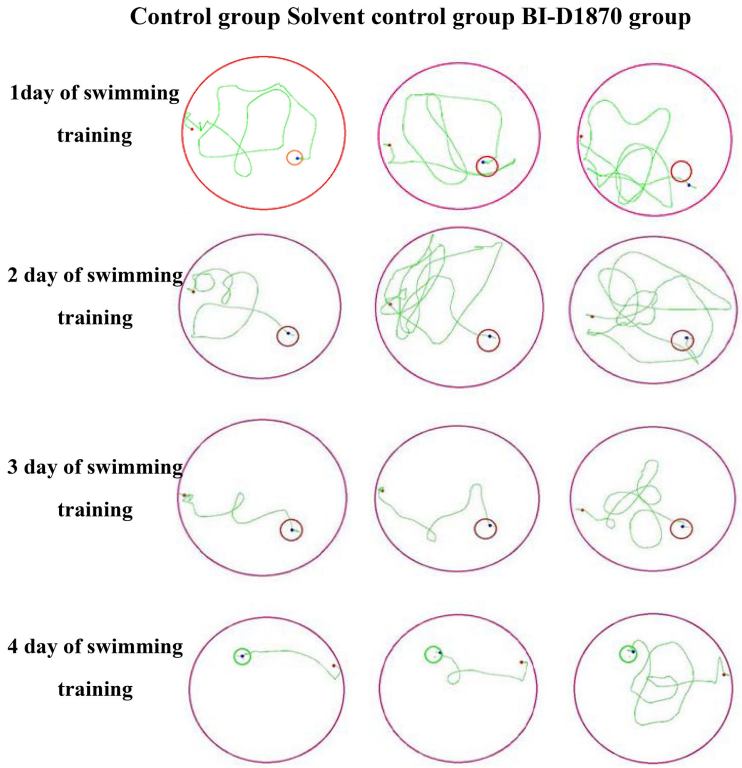

Figure 10. The swimming trajectory of rats' learning and memory acquisition abilities tested on the day 82 - 85 of intraperitoneal injection of BI-D1870 with Morris water maze.

There has been evidence showing that Neuropsychiatric Symptoms (NPSs), such as anxiety, apathy and irritability, are often associated with cortical damage of temporal lobe, middle frontal gyrus and limbic system in AD patients [19]. Aβ sedimentation is an important driving factor for the development of apathy and anxiety in the aged [20]. The frequency and severity of NPS may increase along with the deterioration of cognitive ability [21]. The continuous response of agitation and stress can cause excessive neuronal autophagy and lead to the irreversible deterioration even the death of neurons, which further aggravates AD development [22] [23]. In this experiment, it was found that BI-D1870 could result in the rats' agitation. This effect of BI-D1870 may produce the neurotoxicity, destroy the brain's limbic system and cortical function, which may simulate the anxiety, irritability and other neurological symptoms in rats.

TRKB is the start switch for intracellular and extracellular signal transmission [24] [25]. The extracellular signal molecular BDNF can combine with TRKB and activate CREB to regulate neurogenesis and neuronal function [26]. TRKB and BDNF are mainly expressed in the hypothalamus regions, and the hypothalamus is the key area in regulating animal diet and energy balance [27]. It was reported that the energy consumption of TRKB knockout mice is markedly decreased, while, the daily food intake of BDNF knockout mice is dramatically increased and the brown fat decomposition and energy consumption are notably decreased, which may result in the mice obesity [28]. The present study found that BI-D1870 resulted in the rats' body weight increased, which may be from BI-D1870 inhibiting RSK and CREB, and advance to lower BDNF and TRKB expression, thereby reducing the lipolysis ability and energy consumption, finally the rats' weight was increased.

Morris water maze is the "gold standard" for testing the learning and memory ability of rats [29] [30]. In the present study, the learning and memory acquisition of rats were determined with Morris water maze. The latency refers to the time when the rats successfully find the platform after entering the water. The longer latency spent of rats finding the platform indicates the impaired learning and memory ability of rats. The results of the present experiment showed that BI-D1870 significantly prolonged the latency of rats to find the hidden platform, which indicated that BI-D1870 can destroy the rats' learning and memory.

The swimming trajectory of rats represents different strategies for finding the hidden platform [31]. With prolonging the training time, the trajectory of control group and the solvent control group swam to search for the hidden platform was usually "edge type → random type → trend type → linear type", and that BI-D1870 group swam to look for the platform was usually by "edge type → random type or random type → edge type". This result was consistent with the rats' searching for strategy with the learning and memory disorders.

The present study found that there were 50.00%, 62.00% and 82.00% of rats to appear learning and memory impairment by intraperitoneal injection of 0.35 mg/kg BI-D1870 for 25, 55 and 85 days. The results indicated that BI-D1870 can

disturb the rats' learning and memory acquisition ability and the number of rats with learning and memory acquisition disorder increased to more than 80.00% with BI-D1870 treated over 85 days. In addition, we also found on day 85 of treatment of BI-D1870 caused the inhibition in RSK activity and CREB phosphorylation at the Ser133 site in rats' hippocampus and cerebral cortex by Western blot and immunofluorescence measured (Data in press other journals).

5. Conclusion

In conclusion, intraperitoneal injection of 0.35 mg/kg BI-D1870 for 85 days to rats offers a valuable *in vivo* animal model to better understand learning and memory acquisition impairment. This model provides a fast and simple experimental protocol with a high model successful rate. Later, if BI-D1870 will be found to result in the rats' two characteristic pathological changes of SP and NFTs in the brain like AD, this rats' model may be as an AD model to study the neuronal pathology, including the neurogenesis.

Availability of Date and Materials

The data are to support the findings of this study and are available from the corresponding author upon request. Availability is dependent upon compliance with personal data protection laws and ethics committee laws and regulations.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Mishra, R., Phan, T., Kumar, P., Morrissey, Z., Gupta, M., Hollands, C., Shetti, A., Lopez, K.L., Maienschein-Cline, M., Suh, H., Hen, R. and Lazarov, O. (2022) Augmenting Neurogenesis Rescues Memory Impairments in Alzheimer's Disease by Restoring the Memory-Storing Neurons. *Journal of Experimental Medicine*, **219**, e20220391. https://doi.org/10.1084/jem.20220391

[2] Yan, Y., Yang, H.H., Xie, Y.X., Ding, Y.L., Kong, D.L. and Yu, H.B. (2020) Research Progress on Alzheimer's Disease and Resveratrol. *Neurochemical Research*, **45**,

989-1006. https://doi.org/10.1007/s11064-020-03007-0

[3] Moradi, H.R., Hajali, V., Khaksar, Z., Vafaee, F., Forouzanfar, F. and Negah, S.S. (2021) The Next Step of Neurogenesis in the Context of Alzheimer's Disease. *Molecular Biology Reports*, **48**, 5647-5660. https://doi.org/10.1007/s11033-021-06520-9

[4] Lai, H.-C., Chang, Q.-Y. and Hsieh, C.-L. (2019) Signal Transduction Pathways of Acupuncture for Treating Some Nervous System Diseases. *Evidence-Based Complementary and Alternative Medicine*, **2019**, Article ID: 2909632. https://doi.org/10.1155/2019/2909632

[5] Ding, S.K., Liu, Q.Q. and Shang, Y.Z. (2022) The Effects and Regulatory Mechanism of Flavonoids from Stems and Leaves of Sutellaria Baicalensis Georgi in Promoting Neurogenesis and Improving Memory Impairment Mediated by the BDNF-ERK-CREB Signaling Pathway in Rats. *CNS & Neurological Disorders-Drug Targets*, **21**, 354-366. https://doi.org/10.2174/1871527320666210827112048

[6] Naqvi, S., Martin, K.J. and Arthur, J.S.C. (2014) CREB Phosphorylation at Ser^{133} Regulates Transcription via Distinct Mechanisms Downstream of cAMP and MAPK Signalling. *Biochemical Journal*, **458**, 469-479. https://doi.org/10.1042/BJ20131115

[7] Chae, H.-D., Dutta, R., Tiu, B., Hoff, F.W., Accordi, B., Serafin, V., Youn, M., Huang, M., Sumarsono, N., Davis, K.L., Lacayo, N.J., Pigazzi, M., Horton, T.M., Kornblau, S.M. and Sakamoto, K.M. (2020) RSK Inhibitor BI-D1870 Inhibits Acute Myeloid Leukemia Cell Proliferation by Targeting Mitotic Exit. *Oncotarget*, **11**, 2387-2403. https://doi.org/10.18632/oncotarget.27630

[8] Li, G.N. and Liu, X.Y. (2012) Research Progress of NHE-1 Phosphorylation Kinase RSK and Its Inhibitors. *Chinese Pharmaceutical Journal*, **47**, 241-245.

[9] Xing, J., Ginty, D.D. and Greenberg, M.E. (1996) Coupling of the RAS-MAPK Pathway to Gene Activation by RSK2, A Growth Factor-Regulated CREB Kinase. *Science*, **273**, 959-963. https://doi.org/10.1126/science.273.5277.959

[10] Harada, K., Fukuda, E., Hirohashi, N. and Chiba, K. (2010) Regulation of Intracellular pH by p90Rsk-Dependent Activation of an Na^+/H^+ Exchanger in Starfish Oocytes. *Journal of Biological Chemistry*, **285**, 24044-24054. https://doi.org/10.1074/jbc.M109.072553

[11] Rabbito, A., Dulewicz, M., Kulczyńska-Przybik, A. and Mroczko, B. (2020) Biochemical Markers in Alzheimer's Disease. *International Journal Molecular Sciences*, **21**, Article 1989. https://doi.org/10.3390/ijms21061989

[12] Jiang, Q.S., Liang, Z.L., Wu, M.J., Feng, L., Liu, L.L. and Zhang, J.J. (2011) Reduced Brain-Derived Neurotrophic Factor Expression in Cortex and Hippocampus Involved in the Learning and Memory Deficit in Molarless SAMP8 Mice. *Chinese Medical Journal* (*English*), **124**, 1540-1544. https://pubmed.ncbi.nlm.nih.gov/21740813/

[13] Costa, V., Lugert, S. and Jagasia, R. (2015) Role of Adult Hippocampal Neurogenesis in Cognition in Physiology and Disease: Pharmacological Targets and Biomarkers. *Handbook of Experimental Pharmacology*, **228**, 99-155. https://doi.org/10.1007/978-3-319-16522-6_4

[14] Anjum, R. and Blenis, J. (2008) The RSK Family of Kinases: Emerging Roles in Cellular Signalling. *Nature Reviews Molecular Cell Biology*, **9**, 747-758. https://doi.org/10.1038/nrm2509

[15] Lara, R., Seckl, M.J. and and Pardo, O.E. (2013) The p90 RSK Family Members: Common Functions and Isoform Specificity. *Cancer Research*, **73**, 5301-5308. https://doi.org/10.1158/0008-5472.CAN-12-4448

[16] Kazim, S.F. and Iqbal, K. (2016) Neurotrophic Factor Small-Molecule Mimetics Mediated Neuroregeneration and Synaptic Repair: Emerging Therapeutic Modality

for Alzheimer's Disease. *Molecular Neurodegeneration*, **11**, Article No. 50. https://doi.org/10.1186/s13024-016-0119-y

[17] Nasrolahi, A., Javaherforooshzadeh, F., Jafarzadeh-Gharehziaaddin, M., Mahmoudi, J., Asl, K.D. and Shabani, Z. (2022) Therapeutic Potential of Neurotrophic Factors in Alzheimer's Disease. *Molecular Biology Reports*, **49**, 2345-2357. https://doi.org/10.1007/s11033-021-06968-9

[18] Sapkota, G.P., Cummings, L., Newell, F.S., Armstrong, C., Bain, J., Frodin, M., Grauert, M., Hoffmann, M., Schnapp, G., Steegmaier, M., Cohen, P. and Alessi, D.R. (2007) BI-D1870 is a Specific Inhibitor of the p90 RSK (Ribosomal S6 Kinase) Isoforms *in Vitro* and *in Vivo*. *Biochemical Journal*, **401**, 29-38. https://doi.org/10.1042/BJ20061088

[19] Xiang, Y.Y. and Zhang, Q.M. (2021) Comparative Analysis of Cognitive Function and Mental Behavior Characteristics between Alzheimer's Disease and Parkinson's Disease Dementia. *Neural Injury and Function Reconstruction*, **16**, 258-261. (In-Chinese) https://kns.cnki.net/kcms/detail/detail.aspx?dbcode=CJFD&dbname=CJFDLAST2021&filename=GWKF202105003&uniplatform=NZKPT&v=HVS8C-sPC28hBcDRu0nCoufMbJb9Jg84RCegMWBKGsiG_QJ-3VjFiHDI52n5rOAC

[20] Johansson, M., Stomrud, E., Johansson, P.M., Svenningsson, A., Palmqvist, S., Janelidze, S., Van Westen, D., Mattsson-Carlgren, N. and Hansson, O. (2022) Development of Apathy, Anxiety, and Depression in Cognitively Unimpaired Older Adults: Effects of Alzheimer's Disease Pathology and Cognitive Decline. *Biological Psychiatry*, **92**, 34-43. https://doi.org/10.1016/j.biopsych.2022.01.012

[21] Zhao, Q.F., Tan, L., Wang, H.F., Jiang, T., Tan, M.S., Tan, L., Xu, W., Li, J.Q., Wang, J., Lai, T.J. and Yu, J.T. (2016) The Prevalence of Neuropsychiatric Symptoms in Alzheimer's Disease: Systematic Review and Meta-Analysis. *Journal of Affective Disorders*, **190**, 264-271. https://doi.org/10.1016/j.jad.2015.09.069

[22] Yi, S.Y., Chen, K., Zhang, L.H., Shi, W.B., Zhang, Y.X., Niu, S.B., Jia, M.M., Cong, B. and Li, Y.M. (2019) Endoplasmic Reticulum Stress Is Involved in Stress-Induced Hypothalamic Neuronal Injury in Rats via the PERK-ATF4-CHOP and IRE1-ASK1-JNK Pathways. *Frontiers in Cellular Neuroscience*, **13**, Article 190. https://doi.org/10.3389/fncel.2019.00190

[23] Wolinsky, D., Drake, K. and Bostwick, J. (2018) Diagnosis and Management of Neuropsychiatric Symptoms in Alzheimer's Disease. *Current Psychiatry Reports*, **20**, Article No. 117. https://doi.org/10.1007/s11920-018-0978-8

[24] Numakawa, T., Suzuki, S., Kumamaru, E., Adachi, N., Richards, M. and Kunugi, H. (2010) BDNF Function and Intracellular Signaling in Neurons. *Histology and Histopathology*, **25**, 237-258. https://doi.org/10.14670/HH-25.237

[25] Ribeiro, F.F. and Xapelli, S. (2021) Intervention of Brain-derived Neurotrophic Factor and Other Neurotrophins in Adult Neurogenesis. *Advances in Experimental Medicine and Biology*, **1331**, 95-115. https://doi.org/10.1007/978-3-030-74046-7_8

[26] Ahn, S.M., Kim, Y.R., Shin, Y.I., Ha, K.T., Lee, S.Y., Shin, H.K. and Choi, B.T. (2019) Therapeutic Potential of a Combination of Electroacupuncture and TrkB-Expressing Mesenchymal Stem Cells for Ischemic Stroke. *Molecular Neurobiology*, **56**, 157-173. https://doi.org/10.1007/s12035-018-1067-z

[27] Woo, J., Shin, K.O., Park, S.Y., Jang, K.S. and Kang, S. (2013) Effects of Exercise and Diet Change on Cognition Function and Synaptic Plasticity in High Fat Diet Induced Obese Rats. *Lipids in Health and Disease*, **12**, Article No. 144. https://doi.org/10.1186/1476-511X-12-144

[28] Yang, H.L., An, J.J., Sun, C. and Xu, B.J. (2016) Regulation of Energy Balance via BDNF Expressed in Nonparaventricular Hypothalamic Neurons. *Molecular Endocrinology*, **30**, 494-503. https://doi.org/10.1210/me.2015-1329

[29] Gehring, T.V., Luksys, G., Sandi, C. and Vasilaki, E. (2015) Detailed Classification of Swimming Paths in the Morris Water Maze: Multiple Strategies within One Trial. *Scientific Reports*, **5**, Article No. 14562. https://doi.org/10.1038/srep14562

[30] Othman, M.Z., Hassan, Z. and Che, Has, A.T. (2022) Morris Water Maze: A Versatile and Pertinent Tool for Assessing Spatial Learning and Memory. *Experimental Animals*, **71**, 264-280. https://doi.org/10.1538/expanim.21-0120

[31] Vouros, A., Gehring, T.V., Szydlowska, K., Janusz, A., Tu, Z., Croucher, M., Lukasiuk, K., Konopka, W., Sandi, C. and Vasilaki, E. (2018) A Generalised Framework for Detailed Classification of Swimming Paths Inside the Morris Water Maze. *Scientific Reports*, **8**, Article No. 15089. https://www.nature.com/articles/s41598-018-33456-1