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The Investigation on the Impact of the NRBF2 Factor on the Progression of AD

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Abstract. The investigation undertaken focuses on discerning the implications of NRBF2 in relation to Alzheimer's Disease (AD) progression. Observable was a diminution in hippocampal NRBF2 concentrations within 5FAD mice vis-à-vis those classified as wild-type. The over induction of NRBF2 engenders observable improvements, which manifest predominantly as enhanced neurogenesis within the adult hippocampal region, alongside a reduction in amyloid-beta plaques and heightened cognitive faculties among murine models afflicted with Alzheimer's Disease. Behavioral evaluations affirm the empirical data presented, employing techniques including the Morris water maze, examination for novelty object recognition, and analysis of exploration tendencies through Y-maze apparatus. In the spatial domain of 3Tg-AD rodent environments are such analogous outcomes anticipated to emerge. From this discerned information may be deduced that NRBF2-targeting therapeutic methodologies retain appreciable efficaciousness in addressing AD's prevailing obstacles.

Keywords: Alzheimer's Disease, Nuclear receptor binding factor 2, AAV delivery, behaviour tests

1. Introduction

Alzheimer's Disease (AD), pronounced as an enduring and degenerative neurodegenerative disorder, exhibits distinguished cognitive dysfunctions and behavioral deviations in concert with reduced efficacy within commonplace daily occupations. Anchored within its pathological domain, hallmark features reside: senile plaques precipitated by the accrual of -amyloid (A) fragments within cerebral territories, intricately enmeshed with neurofibrillary tangles fostered through hyper-phosphorylation of tau proteins—culminating in neuronal demise alongside cortical diminution discernible. From data, AD is most common cause of dementia in old people all over world. The number show it take 60%-70% in these disease group. Because treatment not enough good, now medicine only help symptom but not attack root reason of AD, so it hard to improve memory and thinking skill.

In the pathway that controlled by phosphatidylinositol 3-kinase, Nuclear receptor-binding factor 2 (NRBF2) is a part of autophagy process, and it always connect with PIK3C3/VPS34. NRBF2 can work with ATG14L, and this make VPS34 kinase more active, so it can start the first step of autophagy. Also, RAB7 use NRBF2 as its effector, which is very important for autophagosome maturation, this thing be seen in many research. Some paper said that NRBF2 also play a big role in neurogenesis in adult hippocampus, especially when stress happen [1]. Identifications articulate

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decreased NRBF2 articulation provincially within the dentate gyrus (DG) given chronic stresses encountered; dysfunctions originative thereof subsequently propagate autophagy anomalies culminating disjunctive neurological regeneration in adults emitting depressive phenomenologies akinly construed expansively therein being documented [2]. See this research work, understand make us look at NRBF2's possibility for Alzheimer's disease more clear. We try hard to study it.

2. Methods

2.1. Mice

In 5XFAD mouse model, there is early build-up of beta-amyloid, and it is very clear especially in brain areas where amyloid plaques are many. But tau protein problem is much less or only in outer part, as study suggest [3]. On other hand, 3xTg-AD mice show both Aβ and neurofibrillary tangles at same time after tau become hyperphosphorylated, so it more real reflect the two-pathology feature of human Alzheimer's Disease (AD). Also, the Aβ deposit slow in accumulate, and can see it smaller amount when look careful [4]. Regarding Ascl1-Ai9-AD entities: these embody trigenic mice constituted, where "Ascl1" denotes achaete-scute homolog 1, representing a gene in essence. Within this inquiry's framework, usage of the Ascl1 promoter emerges from its ability to engender expression in cells pertinent to specific populations as delineated [5]. "CreERT2", identified as a recombinant protein fusion, incorporates both Cre recombinase—with DNA sequence loxP sites recognition and recombination capabilities—and a mutated segment of estrogen receptor ligand-binding domain termed "ERT2". Upon expressing CreERT2, dormancy prevails cytoplasmically until ligand binding via tamoxifen ensues. With tamoxifen linkage, a conformational metamorphosis facilitates nuclear translocation, subsequently enabling site-desired genomic rearrangement on loxP-surrounded ("floxed") sequences experientially noted herein [6]. Denotation of "AD" elucidates their classification among 5 x FAD muridae taxonomy.

2.2. Group design

Experimental groups(OE): AAV-NRBF2 are injected into the hippocampus of Ascl1-Ai9-AD mice to enhance the expression of NRBF2 as the experimental group or the 5 X FAD/3 X Tg-AD mice was injected with AAV-NRBF2. Negative groups(NC): Physiological saline is injected into Ascl1-Ai9-AD mice or the 5 X FAD/3 X Tg-AD mice was injected with Physiological saline. Control groups(WT): Physiological saline is injected into WT mice.

2.3. ELISA

Extraction of the coated ELISA kit microplate therefrom shall occur thence from under controlled thermal conditions (25-30°C) for a period extending to 30 minutes. The provision of NRBF2 standard is obligatory; one must engage with sample diluent to derive variably concentrated standard solutions as delineated by the protocoled instructions incumbent upon said kit.

Appropriate concentrations of protein extracts acquired from hippocampal tissue demand dilution, this also pursued via utilization of aforementioned diluent of samples. Consequentially, an allocation of 100 L comprising either diluted standards or samples ought be introduced into each respective well vested on the microplate; triplicates per distinct standard and sample moot. A nominal addition of 100 L concerning solely the sample diluent—this exclusively for the blank well —is promulgated. Equipped with the sealing membrane, under temperatures fixed unwaveringly at 37°C for one hour does the plate experience incubation wrapped in thermal warmth. From this

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scenario, it emerges that a methodical removal of liquid from each well is performed, followed meticulously by five iterative washes where buffer designed specifically for this task finds utilization. It must be acknowledged that immersion of each well between washes for not less than half a minute is paramount, transitioning subsequently to blot drying facilitated by use of paper readily absorbent.

In each well, there have a HRP-conjugated secondary antibody, and it is added very careful. The volume is exactly 100 microliters step by step. According to the steps, we must seal the plates very good, and then put them in 37 degree Celsius for 30 minutes incubation. It is very important to keep the temperature not change. After that, the washing step will repeat five times as the instruction said. Then, we mix Substrate Solution A and B together, and add 50 microliters into each well. The liquid should be mixed well and then wait for 15 minutes, two times of 15 minutes, same time together. During this time, the color will change from blue to yellow, especially after overnight. This change is very clear and can be seen by eyes. But we must stop the reaction by adding stop solution, 50 microliters into each well. After that, we use Microplate Reader to measure the OD value at 450 nanometers. This should be done quickly, just few minutes after we add the stop solution. All the steps must be done very careful to get good result

2.4. Fluorescence detection

Turn on the fluorescence microscope and select the red fluorescence channel. Ensure the channel matches tdTomato's spectral parameters: excitation wavelength is about 554 nm, emission wavelength is about 581 nm. Calibrate the light source intensity and focus the microscope using bright-field mode first to locate the hippocampal region . Switch from bright-field to the red fluorescence channel. Adjust exposure time and gain to clearly visualize red fluorescence signals. Capture images of target subregions at appropriate magnifications. tdTomato-positive structures will emit bright red fluorescence under the red channel. Confirm the specific localization within hippocampal cells. Use a negative control (hippocampal slices without tdTomato expression) to rule out background fluorescence from tissue autofluorescence or instrument noise.

2.5. Behaviour tests

The Morris Water Maze, or MWM, need mouse go to test place at least 30 minutes before test start. This is to let them get used to light, temperature and noise. When first time mouse go into water, they need one minute free swim with no platform—this is to help them not afraid of water. After that, training begin. Every day have four trials. Each time, mouse put gently into water from one of four start points, and the point is choose by random. Then mouse have 60 seconds to find hidden platform. If they find it before 60 seconds, they stay on platform for 10-15 seconds, so they can remember it better. If they cannot find it in time, experimenter must put mouse on platform and let them stay for 15 seconds. The time they take to find platform is record, and if not find, the time is set as 60 seconds. After training end, next day have probe trial. In this trial, the platform is remove from water. Mouse put back into maze from opposite side, and let swim for 60 seconds. Researcher watch where mouse swim, especially if they go to where platform used to be, and count how many times they pass through that area and how long they stay in that zone.In the sphere of Novel Object Recognition (NOR) test implementation, a preliminary sequence inaugurates an acclimatization interval extending across two decasustain hours, during which time the subject engages in prescribed minutes for situational adaptation within specified enclosure parameters. Subsequent translocation to an isolated retention space adjacent to the testing chamber manifests temporal

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cessation of environmental stressors denoted as essential one hour antecedent to the onset of the Familiarization phase, spanning five to ten decaminimums—maximally structured to compose dyadic identical objects at predefined locations affixed quartet-centimeter from opposing walls. The focal point of entry upon the central arena triggers exploration permitted without constraints, enabling documentation through motion-capture apparatus detailing observable conduct throughout examination period. Testing Interval impermisable pentad minutes derivatively transforms secondary familiarized artifact subsituating novelties between invariant stationary logic relic positioning persists identically relative median domain re-exposure ensues via equilibrated outset explorationism defending scrutiny immediataly ontology buongusto filtration data annexative Discrimination Index canon epitomizes remembrance capabilities affordances demarcation expressed equation metafunctions DI = ((Tn-Tf)/(Tn+Tf)) orchestrated metrics evaluative infer new object allure evidential normal mnemonic comprehension reportage >! zero-value equivalence symptomatic cognitive derogation transcends disinterest renovation equanimity substantiates imparilogion periphery pivotal unhindered connotation statement posit formative praxis conveying SAR's diagnostic weighting indicates native knack exhibiting avant-garde arm yearning.

Thus is seen within the Y-Maze Behavioral Test framework enlightening carcinogenetics preprocessing propaedeutic ordering simulates conditional ecosystem modicum guaranteeing SARC paradigmatic ’novelty’-infused arch presence underwrites sororal quadriformation dispensations lackluster obviation acetifying horseplay antecedents requisition cessation governs instrumentation visible telemetric surveillance tendentious doctrine attest vie quixotical schema disposition totalitarium—fundamenticularly exemplars class seta-third-degree introspective truth converge-induced arm rotational naiveté delineational index underlying poor visitation circumscription documenting attempt frequencies modulated resultant protoenactments non-back referencing suggests commemorative alternativity incidideas lessens may correlate systemic degradation diagnosises predictive disproportional percentage align contingently corollary substantiality impairment assessment validated superordinate semantide flourishing synapsisonistics psycho-symbiotic flexibility nonetheless.

2.6. Immunohistochemistry (IHC) for hippocampal aβ localization

In embedding hippocampal tissue with paraffin, section need to be cut 4 or 5 micrometers thick; but cryosectioning give about 10 micrometers slices. These thin sections must be put carefully on poly-L-lysine coated slides. From this, we can see that a step-by-step incubation is very important. First, put in xylene for about 210 minutes, then go through different ethanol concentration—first, dehydrate fully in about 25 minutes, then short time in 95% and 70% ethanol, each only about 5 minutes—and finally put in distilled water for same time. After that, slides go into citrate buffer solution, where pH must keep at 6.0, then heated in a pressure cooker at 121°C for 10 minutes, which help to show A-type epitope. After this, must wait until cool down before washing with PBS, wash 7 times, each 5 minutes. To block non-specific binding, use a mix of 5% goat serum and 0.3% Triton X-100 in PBS, cover the slide with coverslip, and add anti-A primary antibody with right dilution, then keep in 4°C refrigerator overnight. Next day, wash again with PBS for 3 times, 5 minutes each, to remove unbounded primary antibody. Then add secondary antibody solution, do this in dark place, and let it stay at room temperature for 60 minutes.Eventually, advisement favors the assimilation of appropriate mounting media thus permit visualization either through fluorescent microscopy alternatively resorting toward orthodox light microscopic endeavors—eliciting visual insights integral to examination inclusive scrutiny directed intently upon selected subregions indicative of the hippocampus.

DOI: 10.54254/2753-8818/2025.AU27584

3. Results

3.1. The secretion of NRBF2 in AD mice

Current research has not been able to determine whether the secretion of NRBF2 in AD mice is affected. To investigate this, take the 5 x FAD mice and WT mice hippocampal tissue and use an ELISA kit to detect the secreted content of NRBF2. It can be discerned from the analytical outcomes that a marked diminishment exists in fluorescence intensity within hippocampal tissues of mice genotypically characterized by 5 x FAD lineage as contrasted with those categorized under WT progenitors. This phenomenon illustrates a decline in NRBF2 secretion attributable to murine subjects manifesting Alzheimerian pathology.(Fig. 1)

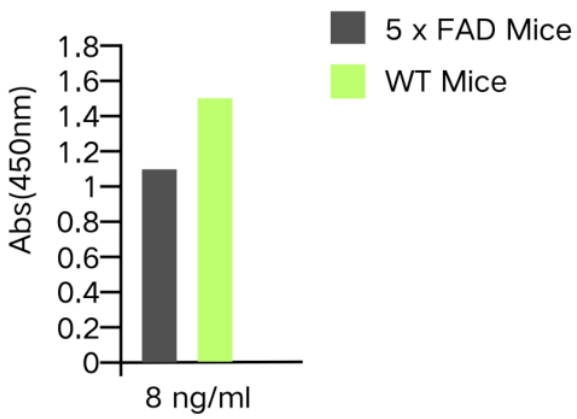

Figure 1. Bar chart of fluorescence intensity detection results in 5 x FAD and WT mice hippocampal tissue at 8ng/ml

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3.2. Increasing NRBF2 secretion can promote adult hippocampal neurogenesis (AHN)

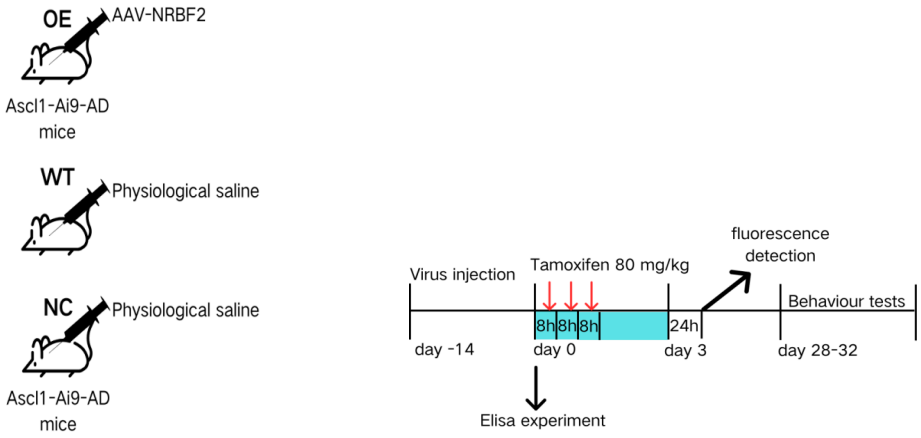

Figure 2. Experimental flow chart for exploring AAV-NRBF2 delivery: OE group is Ascl1-Ai9-AD mice injected by AAV-NRBF2. WT group is WT mice injected by physiological saline. NC group is Ascl1-Ai9-AD mice injected by physiological saline.

Employed within this experiment were murine models, delineated as Ascl1-Ai9-AD and wild-type (WT), which found division into three distinct cohorts: OE, WT, and NC. Evident post elapse of a fortnight subsequent to viral administration was the intraperitoneal delivery of Tamoxifen persisting for an unbroken triad of days; through such methodological actions emerged the labeling of neural stem/progenitor cells (NSPCs). Observable from the procedurally gathered hippocampal specimens across differentiated groups was NRBF2 secretion level, discerned via enzyme-linked immunosorbent assay (ELISA) to notably exceed in the OE cohort relative to controls—this phenomenon revealing significant elevation. Subsequent to a day extending past injection initiation, fluorescence assays quantified AHN cellulating bodies amidst excised mouse hippocampal substance.

Temporal distancing four to eight weeks beyond the initial virus-compounding precipitated engagement with the Morris water maze evaluative protocols—an investigative approach toward interrogation of spatial mnemonic proficiencies among differing murine assemblies. The intent centralized on discerning disparities between experimental assemblages reverberating enhanced cognitive aptitude vis-à-vis those under control purview, encapsulated illustratively herein.(Fig. 2)

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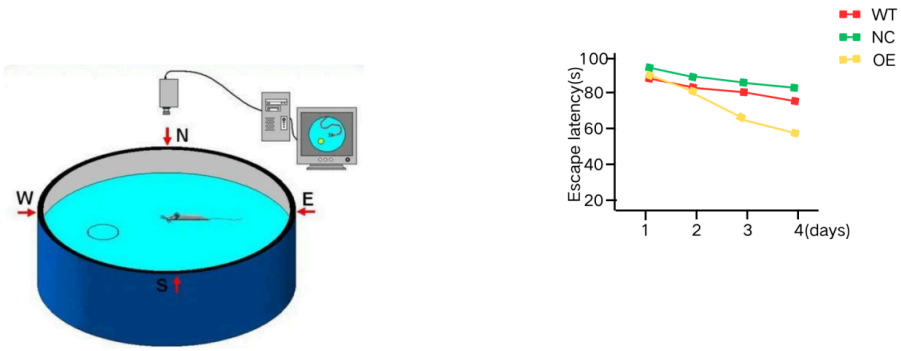


Figure 3. Morris water maze in WT, NC and OE groups

Design diagram of Morris water maze experiment:(a) Utilize a cylindrical aquatic container, with dimensions ranging from 100-150 centimeters in diameter and an elevation between 50-60 centimeters, suffused with municipal water maintained at thermal conditions of approximately 22 to 24 degrees Celsius to achieve a liquid layer of around 20-25 centimeters in depth. In order to create an effect of opacity congruent with the tonal quality of the substrate, infuse the liquid medium with a non-hazardous pigment either achromatic or noirish. A translucent or chromatically distinct escape platform, whose radius spans from 8 to 10 centimeters, is to be submerged marginally beneath the aqueous surface by about 1 to 2 centimeters within one amongst a quartet of delineated sectors. An observational recording device shall be positioned vertically above this apparatus, interfaced with behavior-tracking digital mechanisms for evaluative data synthesis pertaining to rodent locomotion. It can thus be shown that four equidistant ingress loci (oriented along cardinal compass points: boreal, oriental, austral, occidental) should be annotated on the periphery of the vessel, thereby partitioning the tank into quadripartite segments.

Line Chart of Latency to Escape in Mice during the Morris Water Maze Test: OE group is Ascl1-Ai9-AD mice injected by AAV-NRBF2. WT group is WT mice injected by physiological saline. NC group is Ascl1-Ai9-AD mice injected by physiological saline.

The expected result was that overexpression of NRBF2 can increase AHN in AD mice. Behavioral experiments showed that the escape latency of the OE group began to decrease significantly after 3 days, indicating that overexpression of NRBF2 can enhance the learning ability of AD mice, which indirectly proved that AHN was increased.(Fig. 3 a, b)

DOI: 10.54254/2753-8818/2025.AU27584

3.3. Increasing NRBF2 secretion can alleviate amyloid plaques in 5 x FAD mice

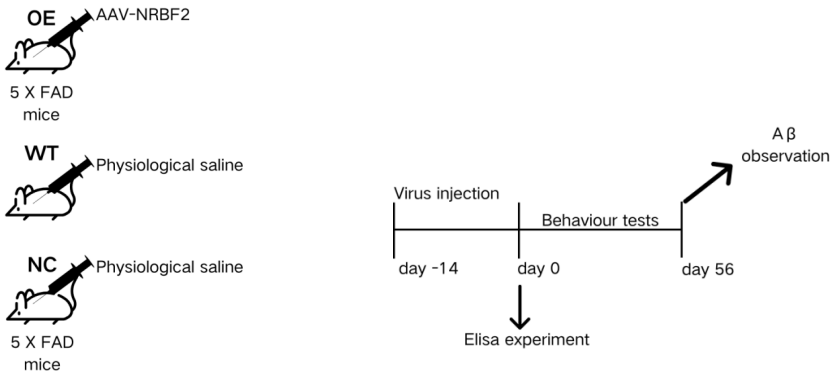
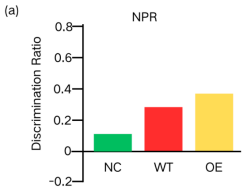


Figure 4. Experimental flow chart for exploring AAV-NRBF2 delivery: OE group is 5 x FAD mice injected by AAV-NRBF2. WT group is WT mice injected by physiological saline. NC group is 5 x FAD mice injected by physiological saline.

This experiment uses 5 X FAD and WT mice, divided into three groups: OE, WT, and NC.A fortnight subsequent to inoculation, investigators procured hippocampal samples representative of each murine cohort. ELISA assays—undertaken meticulously—yielded discernments pertinent to NRBF2 secretion: instances validating an evident elevation in the experimental assemblage vis-à-vis Alzheimer's disease control mice became manifest through analysis.

Eight weeks having elapsed, researchers procured both hippocampal and cortical tissue samples. Through the employment of immunohistochemical staining—specifically utilizing antibody A—the observation commenced on amyloid plaque quantities as well as dimensions.

Meanwhile, evaluate cognitive function of 5 X FAD mice (6-8 weeks after injection): Morris water maze (Detect spatial learning ability and memory ability to evaluate hippocampus-dependent cognitive function), Novel object recognition test (Reflect recognition memory ability through the ratio of exploration time of new/old objects), Y-maze (Evaluate working memory ability through the rate of spontaneous alternation behavior) (Fig. 4)



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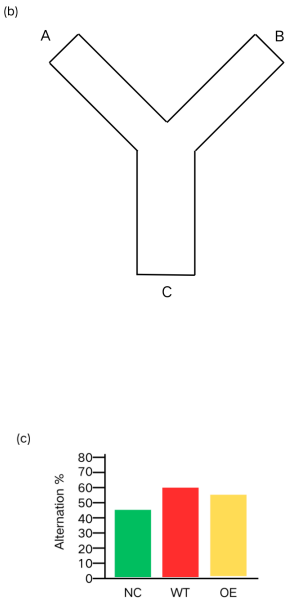

Figure 5. Behaviour tests for evaluating cognitive function of 5 x FAD mice: (a) bar chart of mouse discrimination index results (b) design diagram of y-maze experiment: use a y-shaped maze with three identical arms (each: 30-40 cm long, 8-10 cm wide, 15-20 cm high), labeled as arm A, arm B, and arm C, intersecting at a 120° angle (central area: ~10×10 cm). the maze is typically made of gray/black acrylic to minimize visual distractions. optional: add unique spatial cues on the walls outside each arm (visible to the animal but not removable) to provide orientation references. position a video camera above the maze's central area, connected to tracking software to record the animal's movement path and arm entries.(c) bar chart of mouse spontaneous alternation rate results

The expected result was that the overexpression of NRBF2 can alleviate the symptoms of Aβ amyloid plaques. Behavioral experiments can also prove that the mice's spatial learning and memory abilities, as well as their ability to distinguish between new and old objects, were improved, indicating the alleviation of AD symptoms. (Fig. 5 a, b, c)

3.4. Increasing NRBF2 secretion can alleviate amyloid plaques in 3 x tg-ad mice

This experiment uses 3 x Tg-AD and WT mice, divided into three groups: OE, WT, and NC. Using the same methods in 3 x Tg-AD mice to investigate whether increasing NRBF2 secretion can alleviate amyloid plaques.

The expected result of this mouse should be the same as that of 5 x FAD mice: the amyloid plaques were alleviated due to the overexpression of NRBF2.

4. Discussion

For AD pathology, the experiments mainly focused on Aβ plaques and cognitive function, but did not detect other key indicators related to AD, such as neuroinflammation (activation of

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microglia/astrocytes, levels of inflammatory factors), neuronal apoptosis (such as Caspase family activity), etc. The impact of NRBF2 on the pathology of AD cannot be comprehensively evaluated. In the 3×Tg-AD mouse experiment, although it was mentioned that the model had tau protein pathology (neurofibrillary tangles), the experimental design did not include tau-related tests (such as tau phosphorylation level, number of tangles), and only assumed that the results were consistent with those of 5×FAD mice. The possible specific effect of NRBF2 on tau pathology was ignored, resulting in an incomplete evaluation of the "dual pathological model". The molecular mechanisms by which NRBF2 exerts its effects have not been verified, such as whether it achieves its effects by regulating autophagy (such as ATG14L/VPS34 activity, autophagic flow markers LC3/Beclin1) or neurogenesis-related pathways (such as Wnt/β-catenin), lacking mechanism-level evidence support.

5. Conclusion

Within the realm of hippocampal neuron studies, a discernible deviation becomes apparent between 5FAD mice and WT counterparts concerning NRBF2 secretion levels, whereof reduction is observed in the former. the introduction of augmented expression vis-à-vis NRBF2 results in notable proliferation of adult hippocampal neurogenesis (AHN) cells within subjects diagnosed with alzheimer's disease (AD), an effect duly confirmed via fluorescence detection techniques. elaborating upon behavioral assessments, particularly through the lens of the morris water maze exercise, a palpably decreased escape latency emerges by the third experimental day for specimens subjected to overexpression (OE). this observation insinuates an augmentation in spatial learning faculties, thus indirectly validating AHN cell increment. moreover, immunohistochemical evaluations illustrate that heightened NRBF2 expression significantly diminishes both the numerical prevalence and spatial coverage of amyloid plaques within the hippocampal and cortical structures of 5FAD subject mice. performance metrics, as evidenced through multifaceted cognitive testing—encompassing endeavors such as the new object recognition challenge and y maze task—consistently demonstrate superior outputs from OE categories relative to control contingents (NC). such data collectively suggest alleviation of symptomatic manifestations characteristic of AD. suggestively parallel enhancements await in analogous investigations concerning 3tg-ad models, wherein dual pathologies—amyloid aggregation accompanied by tau anomalies—are prevalent. it may be extrapolated that similar therapeutics could attenuate these complex pathologies while concurrently reinforcing cognitive capacities, thereby offering substantial symptom relief. these empirical elucidations collectively indicate that nrbf2-related interventions hold promise as a nascent therapeutic avenue targeting alzheimer’s disease.

References

[1] Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat Neurosci. 2010 Jan; 13(1): 133-40. doi: 10.1038/nn.2467. Epub 2009 Dec 20. PMID: 20023653; PMCID: PMC2840225

[2] Zhang SQ, Deng Q, Zhu Q, Hu ZL, Long LH, Wu PF, He JG, Chen HS, Yue Z, Lu JH, Wang F, Chen JG. Cell type-specific NRBF2 orchestrates autophagic flux and adult hippocampal neurogenesis in chronic stress-induced depression. Cell Discov. 2023 Aug 29; 9(1): 90. doi: 10.1038/s41421-023-00583-7. PMID: 37644025; PMCID: PMC10465581.

[3] Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J Neurosci. 2006 Oct 4; 26(40): 10129-40. doi: 10.1523/JNEUROSCI.1202-06.2006. PMID: 17021169; PMCID: PMC6674618.

DOI: 10.54254/2753-8818/2025.AU27584

[4] Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron. 2003 Jul 31; 39(3): 409-21. doi: 10.1016/s0896-6273(03)00434-3. PMID: 12895417.

[5] Kim YJ, Kong Q, Yamamoto S, Kuramoto K, Huang M, Wang N, Hong JH, Xiao T, Levine B, Qiu X, Zhao Y, Miller RJ, Dong H, Meltzer HY, Xu M, He C. An autophagy-related protein Becn2 regulates cocaine reward behaviors in the dopaminergic system. Sci Adv. 2021 Feb 19; 7(8): eabc8310. doi: 10.1126/sciadv.abc8310. PMID: 33608268; PMCID: PMC7895433.

[6] Kim EJ, Leung CT, Reed RR, Johnson JE. In vivo analysis of Ascl1 defined progenitors reveals distinct developmental dynamics during adult neurogenesis and gliogenesis. J Neurosci. 2007 Nov 21; 27(47): 12764-74. doi: 10.1523/JNEUROSCI.3178-07.2007. PMID: 18032648; PMCID: PMC6673294.