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Original Research Article

Enhancement of memory and reduction of amyloid plaques in alzheimer's disease mouse model using cyclic glycine-proline

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ABSTRACT

Background: Amyloid plaques and neurofibrillary tangles are two of the most important signs that can be used to diagnose Alzheimer's disease (AD), which is a neurodegenerative condition. Animal models, such as APP/PS1 mice, are extremely important for advancing our understanding of the factors that contribute to Alzheimer's disease and evaluating potential treatments.
Aim: The purpose of this study is to investigate the effects of cyclic glycine-proline (cGP), a stable cyclic dipeptide that originates from glutamate, on spatial memory and the accumulation of amyloid plaque in mice with Alzheimer's disease and Parkinson's disease type 1.
Materials and Methods: In order to evaluate the spatial memory of APP/PS1 mice, the Morris Water Maze was utilised after the mice had been administered cGP. To determine the amount of amyloid plaque in the brain, first the staining was done using thioflavin-S, then imaging was performed, and last the results were quantified.
Results: Treatment with cGP resulted in a considerable reduction in the quantity of amyloid plaque and an improvement in spatial memory in mice with APP/PS1 mutations. There was a decline in escape latency times, amyloid plaque numbers, and plaque covering percentages in the cortex and hippocampus.
Conclusion: There is evidence to suggest that cGP may have therapeutic potential; in a rat model of Alzheimer's disease, it was found to improve cognitive performance and reduce amyloid pathology. The therapeutic potential of cGP in reducing AD pathology and cognitive impairment is demonstrated by these results.

1. Introduction

Dementia most commonly occurs in those aged 65 and up due to Alzheimer's disease. The defining trait of Alzheimer's disease is the accumulation of misfolded proteins. Specifically, this includes neurofibrillary tangles, made up of hyperphosphorylated tau within neurons, and amyloid plaques, formed from amyloid-β (Aβ) outside the cells.Neuronal loss and cellular malfunction are both exacerbated by these protein aggregates. The amyloid cascade theory, which is generally agreed upon, implies that Aβ is pivotal in advancement of AD. The amyloid precursor protein (APP) is the main source of Aβ, which can exist in various forms, with the oligomeric form being the most toxic. Brain problems, malfunctioning synapses, harm to neurones, inflammation of the nervous system, and oxidative stress are all associated with this type of Aβ.[1]

Researchers often use transgenic animal models to replicate the symptoms and cognitive impairment related with Alzheimer's disease. One such example is the APP/PS1

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mouse model, which features mutations in both presenilin-1 and APP.[2] To bypass the blood-brain barrier, insulin-like growth factor-1 (IGF-1), a crucial factor for brain development, can be administered intravenously.Increasing IGF-1 levels may give neuroprotection; intranasal IGF-1 has exhibited neuroprotective benefits in cerebral ischaemia models. This is because lower levels of IGF-1 are associated to elevated risk of developing Alzheimer's disease. IGF-1 has been demonstrated to reduce the amyloid-βproduction, and alterations in its signaling have been observed in Alzheimer's disease.[3]

Interleukin-1 (IGF-1) peptide metabolites include cyclic glycine-proline (cGP), a lipophilic and more stable version of glycine-proline-glutamate (GPE). Because it is stable, cGP improves brain uptake and controls IGF-1 homeostasis through modifying the interaction between IGF-1 and binding proteins.[4] But the effect that cGP has on Alzheimer's disease is not yet recognised. The study adopted the APP/PS1 double transgenic mouse model of Alzheimer's disease.The aim of thestudy was to investigate how cGPimpacted the spatial memory and the deposition of amyloid plaques ?

1.1. Alzheimer's disease's symptoms

Progressive neurodegenerative disorders like Alzheimer's cause behavioural abnormalities, cognitive decline, and memory loss. There are usually three phases to the disease's clinical manifestations: early, medium, and late. There are different symptoms associated with each stage, and they get worse over time.[5]

1.2. Early Stage Symptoms

1. *Memory Loss:* Memory loss, especially of new material, is one of the earliest symptoms
2. *Disorientation:* Disorientation in Time and Space
3. *Difficulty Planning or Solving Problems:* Difficulties in Planning, Problem-Solving, and Numerical Tasks
4. *Misplacing Items:* Misplacing Items and Inability to Retrace Steps
5. *Difficulty Completing Familiar Tasks:* Difficulty performing routine tasks at home, work, or during leisure activities
6. *Changes in Personality and Mood:* Increased anxiety, confusion, or depression
7. *Challenges with Vocabulary:* Difficulty Finding the Right Words or Misnaming Objects

1.3. Middle stage symptoms

1. *Increased Memory Loss and Confusion:* Increased Difficulty Recalling Personal History and Recognizing Friends and Family
2. *Problems with Language:* Significant issues with speaking, reading, and writing
3. *Difficulty with Coordinated Movements:* Trouble with motor functions and coordination
4. *Disorientation and Misinterpreting Spatial Relationships:* Getting lost in familiar places
5. Hallucinations and Delusions: Experiencing Hallucinations and Delusions
6. Sleep Disturbances: *Changes in sleep patterns, including i*nsomnia or sleeping too much
7. *Inappropriate Behavior:* Acting out in socially unacceptable ways

1.4. Late stage symptoms

1. *Severe Memory Loss:* Loss of Recognition of Close Family Members and even Self
2. *Complete Dependence:* Need for Full-Time Assistance with Daily Activities Such as Eating, Dressing, and Bathing
3. *Physical Decline:* Decline in Physical Capacities, Including Walking, Sitting, and Eventually Swallowing
4. *Increased Vulnerability to Infections:* Higher susceptibility to illnesses, particularly pneumonia
5. *Inability to Communicate:* Severe Decline in Verbal Skills, Resulting in Minimal or No Speech
6. *Significant Changes in Physical Health:* Weight Loss, Skin Infections, Seizures, Difficulty Swallowing, and Increased Sleep.

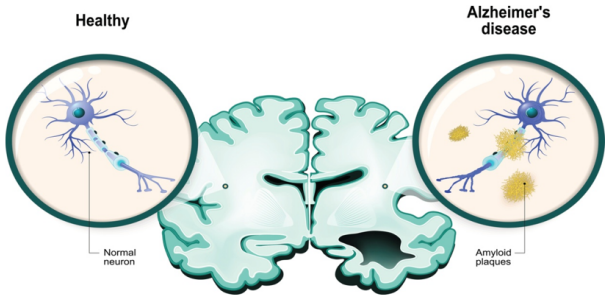


Figure 1: Comparison of healthy neurons and neurons affected by Alzheimer's disease

2. Materials and Methods

2.1. Animal model and housing conditions

At the National Brain Research Centre, we breed transgenic mice with specific genes (APP/PS1, PSEN1dE9)85Dbo/Mmjax), initially sourced from India's IGSS Animal Care Laboratory. In these animals, a mutant form of presenilin-1 (PS1-dE9) and a hybrid of human and mouse amyloid precursor protein (Mo/HuAPP695swe) are overexpressed, regulated by a prion promoter. Using APP and PS1 primers that were unique to these transgenes, their existence was confirmed.[6]All investigations were

conducted using male mice, eitherwild-type (WT) or transgenic (APP/PS1), meaning they did not carry any transgenes. The mice, aged 9–11 months and weighing 20–40 grams, were maintained on a 12-hour photoperiod. All tests were carried out in the light phase, and the mice were given continuous access to water and rodent food for the duration of the study. All procedures received approval by the Institutional Animal Ethics Committee.[7]

Figure 2: Laboratory mice in a cage

2.2. Treatment with Cyclo(-Gly-Pro) (cGP)

The animals were split into two groups—one to serve as a control and another to undergo the experiment—to guarantee equity. Over the course of five days, we touched each animal for five to ten minutes per day. An 80 $\mu g/\mu l$ solution of cGP (Cyclo(-Gly-Pro); Bachem, cat. no. G-1720) was produced in phosphate-buffered saline (PBS; 137 mMNaCl, 2.7 mMKCl, 10.14 mM Na2HPO4, 1.76 mM KH2PO4, pH 7.4). The dosage of cGP was 20 mg/kg of body weight and given intranasally. The 28-day treatment plan was derived on prior research that chronically administered cGPutilisinggraphene quantum dot-conjugated GPE and piracetam dipeptide analogues. Equal volume of PBS was administered to the control group as well as the group treated with cGP. Figure 1 in the Supplementary Materials provides information on the pilot experiments for cGP dose determination.[8]

3. Spatial Memory Assessment

The spatial memory test, referred to as the Morris Water Maze (MWM) test, was employed as a reliable tool in the scientific community. According to the previous research, APP/PS1 transgenic mice have been reported to have a diminished capacity for spatial memory.[9]

3.1. Apparatus

The experiment known as the Morris Water Maze was carried out in a pool that was circular in shape and measured 1.68 meters in diameter and 60 centimetres in height. In order to maintain a temperature of 24 ± 2 degrees Celsius, the water was maintained at a level that was halfway filled with the pool.A ten-centimeter-diameter white plexiglass platform lay one centimetre below the water's surface. Applying non-toxic white paint to the water made[10] it opaque, which allowed the platform to remain invisible. Around forty to sixty centimetres from the pool, there were a number of spatial hints. A black cardboard box, a tall multi-shelf rack, a big toy, and a white rectangle with a black cross on it were among the clues. The pool was illuminated by four lamps with a power output of one hundred watts each, which were placed in the four corners of the chamber. There was no movement of the platform or spatial signals throughout training. While testing, the platform was taken down but the spatial clues were kept where they had been.[11]

3.2. Training

The Morris Water Maze (MWM) challenge, which has been established previously, was modified and used to teach both wild-type and APP/PS1 mice.[12,13] During the training phase, the remaining eight days of pharmaceutical therapy were carried out simultaneously. The animals were put through four repetitions of the test every day and aten-minute break was given between each trial. Beginning one hour after the delivery of the medicine and continuing for no more than three hours, the trials were initiated. In each of the trials, the mice were given a time limit of two minutes to successfully discover the hidden platform.[13] They were escorted to the platform, and if they were unable to remain there for thirty seconds, they were instructed to remain there. Every day, the starting positions were pseudo-randomly selected. The time it took to reach the platform, or escape latency, was determined by averaging the four trials performed daily.[14]

3.3. Testing

An evaluation of memory was carried out by a probing trial twenty-four hours subsequent to the final training session. The experiment allowed the animals two minutes to swim around in the pool at their own pace. For the purpose of performance evaluation, a number of metrics were monitored and recorded. These metrics included escape latency, the rate of platform crossings, and the total duration spent in each platform quadrant.[15] A Logitech webcam was used to record the animals' actions during the testing and training sessions, and ANYMAZE software was used to track their progress. The video material was recorded and then examined by hand using a hard disc.[14]

3.4. Thioflavin-S staining

This experimentation made use of two distinct sets of mice. After 28 days of intranasal cGP treatment, both APP/PS1 and wild-type mice were euthanised4-5 hours following

their last dose. The hemispheres were separated after the brain tissue was washed with ice-cold PBS, which followed brain extraction. Every mouse had one hemisphere frozen in 4% paraformaldehyde for the night before being moved in sequence into PBS solutions with 20% and 30% sucrose, respectively. Cryostat (Leica CM3050 S) brain slices were cut to a thickness of 40 μm and then set in 24-well plates with PBS and 0.01% sodium azide. Six sections were placed onto slides for each experimental group. Thioflavin-S, a fluorescent dye that binds to fibrillar β-sheets and makes them visible for analysis, was used to stain these sections (Sigma Aldrich; cat. no. T1892).[16]

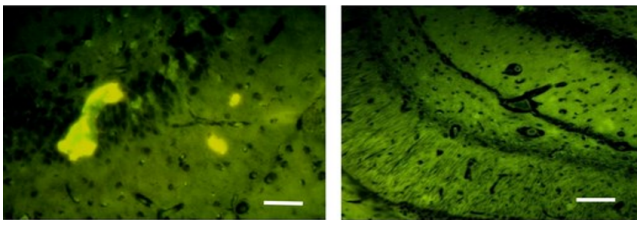


Figure 3: Thioflavin-S staining

Visualization and Quantification of Amyloid PlaquesAmyloid plaques were visualised using the Thioflavin-S staining procedure.[6] Leica DM RXA2 microscope was fitted with a Leica DFC 320 camera that utilised in order to take photographs of the stained sections. Both the excitation and emission wavelengths were determined to be as follows: 390 nm and 428 nm, respectively. For every experimental group, the imaging parameters were standardised in the first portion and maintained uniformly in all subsequent sections.[14,15]

We used ImageJ (NIH) to quantify the plaque burden. The free-hand tool was used to outline the ROIs in the cortex andhippocampus. After converting the RGB images to 8-bit binary representation, the fine tuning of thresholding parameters was adjusted by hands to ensure precise plaque detection. To verify accurate plaque identification, the thresholded images were contrasted with the initial RGB images. Four critical parameters were evaluated using the "Analyse Particles" plugin in Image 3 plaque density (plaques per mm^2), plaque size, percentage of area covered by plaques, and total area analysed. Each animal's total score was determined by averaging data from all five regions of its respective hemisphere. To ensure similar clarity in the representative photographs, brightness and contrast modifications were done uniformly to both groups.[16]

3.5. Data analysis

Figures 3 and 4 represent data obtained from a single set of animals, while Figure 1 and the Supplementary Figures were derived from another set. Two-way analysis of variance (ANOVA) was applied to data from Figure 1 and Supplemental Figures 2 and 3, followed by Fisher's Least Significant Difference (LSD) test for pairwise comparisons. For Figures 2, 3 and 4, an unpaired, two-tailed Student's t-test was used. Statistical significance was considered at $p < 0.05$. Results are presented as the mean ± standard error of the mean (SEM) unless stated otherwise.[17]

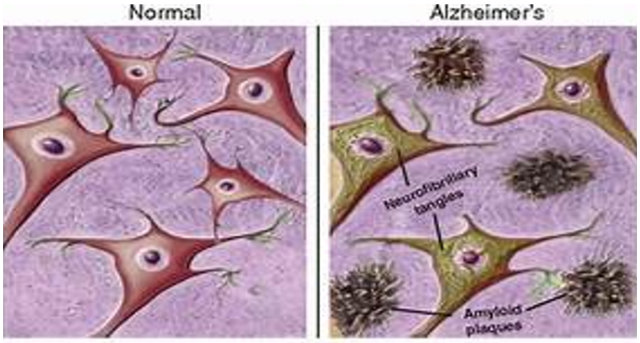


Figure 4: Difference between normal and alzheimer's amyloid plaques

Figure 4 specifically illustrates the presence and levels of amyloid β-protein aggregates and tau protein (Amyloid β-Tau) as observed in the study.

4. Results

4.1. cGP treatment enhances memory in APP/PS1 Mice

APP/PS1 mice have been shown to have difficulty remembering information about spatial relationships, according to studies.[3,12] It can be concluded that the findings of those investigations are consistent with these findings. Using the Morris water maze paradigm, an investigation into the impact that cGP therapy has on spatial memory was carried out. After eight days of training and a probing trial, mice with and without APP/PS1 gene mutations were administered cGP and assessed for spatial learning and memory. When comparing APP/PS1 and wild-type mice during training, a notable difference in escape latency was found. These variations suggested that APP/PS1 mice had trouble picking up the skill. Their performance was significantly improved by the cGP treatment, as seen by the escape latencies on the final day of training. Escape latencies in the APP/PS1 group were 67.2 ± 5.5 seconds, significantly higher than the controls' 18.2 ± 2.8 seconds ($p < 0.01$). Latencies for escape were 32.9 ± 7.2 seconds in the APP/PS1 group that was treated with cGP ($p < 0.01$). Scientists discovered that there was no discernible change in the reaction times of untreated and cGP-treated wild-type mice (18.2 ± 2.8 seconds for WT and 19.9 ± 2.5 seconds for cGP-WT, $p > 0.8$).

On the long-term memory (LTM) test, APP/PS1 animals performed significantly worse than wild-type mice across the board. Among these shortcomings were escape latency, platform quadrant time, and annulus crossing frequency.[18]

these parameters were significantly improved in APP/PS1 animals after receiving cGP, while in wild-type mice (WT versus cGP-WT, all p-values were more than 0.7), the drug had no effect. The sample track graphs obtained during the probe experiment are shown in Figure 1d. While cGP treatment improved the memory skills of APP/PS1 mice, these graphs show that the mice themselves had weak spatial memory. [19]

4.2. Swimming speed and body weight

There was no statistical difference in swimming speeds among the wild-type mice, APP/PS1 animals, and cGP-treated APP/PS1 mice during either the training phase or the probe trial (Supplementary Figure 2). Additionally, no group treated with cGPdemonstrated any statistically significant changes in body weight.

4.3. cGP reduces amyloid plaque load in APP/PS1 Mice

The effects of cyclic glycine-proline (cGP) on amyloid pathology were not investigated until the augmentation in spatial memory was substantiated. This confirmation followed the observed enhancement in cognitive performance. The deposition of amyloid plaques in the cortex and hippocampus of APP/PS1 mice was the primary focus of the investigation. This was conducted to gain a deeper understanding of the phenomena. A considerable reduction in plaque burden was detected in these regions due to the administration of cGP, providing further evidence of the cognitive gains observed.

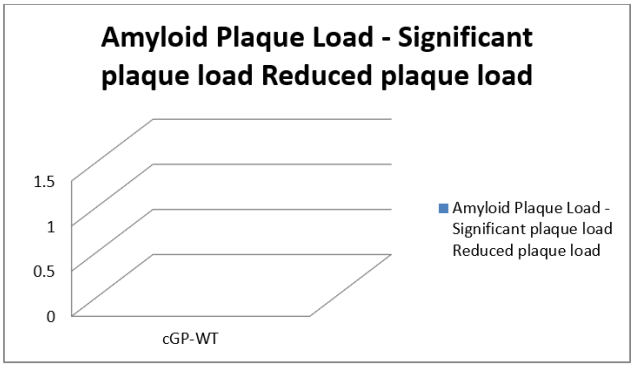
1. A summary of the findings from the study, highlighting the effects of cGP treatment on spatial memory and amyloid plaque deposition in APP/PS1 mice.Graphical representation of the spatial memory enhancement in APP/PS1 mice treated with cGP compared to controls (Table 1) (Graph 1).
2. Illustration of the amyloid β-protein aggregates and tau pathology data, focusing on the cortex and hippocampus of APP/PS1 mice (Figure 5).

4.4. cGP improves memory in APP/PS1 Mice

The main purpose of the study is to examine the effect of cGPintervention on memory performance in APP/PS1 mice compared to that of wild-type (WT) mice.

4.5. Escape latency

An evaluation of escape latencies and long-term memory (LTM) was carried out during the course of a training period that lasted for eight days. Each group consisted of nine individuals.By day 4, notable differences in escape latency between wild-type (WT) mice and APP/PS1



Graph 1: Amyloid plaque load reduced significants

animals had become evident, with these variations being quite pronounced. The differences were confirmed to be statistically significant. APP/PS1 mice treated with cGP demonstrated a significant decrease in escape time compared to their untreated counterparts.This was observed throughout the entirety of the training period as well as the test of long-term memory comprehension. It is essential to pay attention to the fact that this decrease was detected. At all stages of the study research, the escape latencies of wild-type mice and cGP-treated wild-type mice (cGP-WT) remained similar.

4.6. Time spent in quadrants during probe trial

In the probe trial of the long-term memory test, APP/PS1 mice spent notably lesser time in the platform quadrant than wild-type mice, highlighting a significant difference among the groups. However, the cGP treated APP/PS1 mice (cGP-APP/PS1) spent significantly more time in the platform quadrant than the groups without cGP treatment (APP/PS1). Additionally, wild-type mice treated with cGP (cGP-WT) spent the same amount of time in the platform quadrant as untreated wild-type mice (WT), regardless of treatment.

4.7. Number of annulus crossings during probe trial

The APP/PS1 mice exhibited a significantly lower number of annulus crossings when compared to wild-type mice. However, untreated APP/PS1 mice exhibited notable lower number of crossings compared to cGP-APP/PS1 mice, which demonstrated a notably higher number of annulus crossings. Additionally, the crossover rates between wild-type mice and cGP-treated wild-type mice (cGP-WT) did not show any significant difference. This pattern held consistent for the entire duration of the research.

4.8. Representative track plots during probe trial

The track plots generated during the probe session provided further insight into the variations in spatial navigation between the groups. These plots highlighted the distinctive

Table 1: Impact of cGP treatment on spatial memory and amyloid pathology in APP/PS1 Mice

Parameter	Wild-Type (WT)	APP/PS1	cGP-APP/PS1	cGP-WT
Escape Latency (last day of training)	18.2 ± 2.8 s	67.2 ± 5.5 s	32.9 ± 7.2 s	19.9 ± 2.5 s
p-value (vs. WT)	-	< 0.01	< 0.01	> 0.8
Long-Term Memory Test (Escape Latency)	-	Significant impairment	Significant improvement	-
Time spent in platform quadrant	-	Significant impairment	Significant improvement	-
Number of annulus crossings	-	Significant impairment	Significant improvement	-
Swimming Speed	No significant difference	No significant difference	No significant difference	No significant difference
Body Weight	No significant difference	No significant difference	No significant difference	No significant difference
Amyloid Plaque Load	-	Significant plaque load	Reduced plaque load	

features of wild-type (WT) and APP/PS1 mice, with asterisks that shows statistically significant differences having p value< 0.05. In contrast, hashtags marked the significant discrepancies between APP/PS1 mice and cGP-APP/PS1 mice. To investigate the impact of treatment, amyloid plaque levels in brain slices stained with thioflavin-S were compared between APP/PS1 and WT mice. Notably, no amyloid plaques were found in the hippocampus of either WT or cGP-treated WT mice. However, APP/PS1 mice showed substantial amyloid plaque deposition in the hippocampus (Tables 2 and 3)(Graph 2). Following cGP treatment, a significant reduction in plaque burden was observed. cGP-treated APP/PS1 mice displayed a marked decrease in both plaque density and the percentage of the hippocampal area occupied by plaques, as demonstrated . This contrasts with untreated APP/PS1 mice. Quantitative analysis revealed that APP/PS1 mice had an average plaque count of 42.3 ± 6.9 plaques per square millimeter, while cGP-treated APP/PS1 mice exhibited a notably lower plaque count.

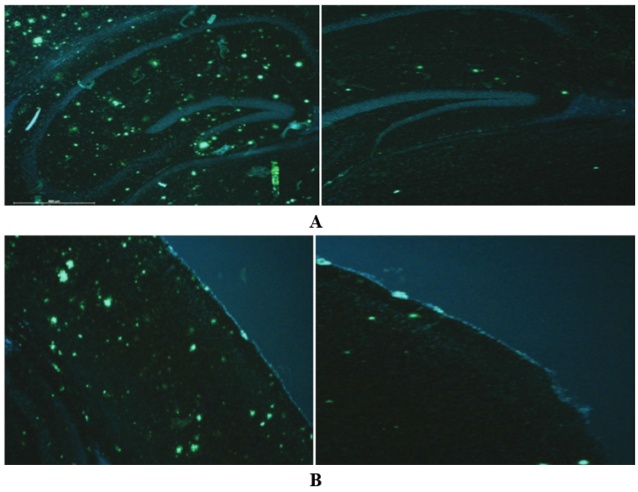


Figure 5: A**:** Microscopic picture APP/PS1 cGP-APP/PS1 B**:** Microscopic Picture APP/PS1 cGP-APP/PS1

The administration of cGP, however, was shown to potentially reduce amyloid plaque accumulation in the hippocampus of mice with Alzheimer's disease and Parkinson's disease type 1. To assess the plaque levels in the brain, hippocampal slices from untreated APP/PS1 mice and cGP-treated APP/PS1 mice (cGP-APP/PS1) were compared.Thioflavin-S staining was used for visualisation in this study. An image that is representative of the total is displayed in panel a, which has a scale bar that measures 500 micrometres. Within the cGP-APP/PS1 group, both the plaque density and the percentage of the hippocampal area that was filled by plaques (shown in panel b2) were found to be significantly reduced. The same group was used for both of these measures, which were taken between them. This study's findings are consistent with those of previous research that has been conducted. It is interesting to notice that the average size of the plaques that were left behind in cGP-APP/PS1 mice was comparable to that of untreated APP/PS1 mice. This is something that should be taken into consideration. Additionally, the total area that was taken into consideration for the quantification of plaque was comparable between the groups. This was the case. In order to demonstrate statistically significant differences among the groups, asterisks are employed. On the other hand, the notation "N.S." is employed to indicate whether or not the results are not significant. When compared to untreated APP/PS1 mice (0.5 ± 0.1), the administration of cGP resulted in a significant reduction in the proportion of the hippocampus region that was covered by plaques in cGP-treated APP/PS1 mice (0.2 ± 0.03). This reduction was accompanied by a p-value that was less than 0.03, as indicated by the findings of the study. The average size of the residual plaques remained comparable between treated animals (94.7 ± 19.9 μm) and untreated mice (92.8 ± 17.6 μm) (Figure 2(b)b3, p > 0.9). This was observed despite the fact that the plaque area was reduced. Furthermore, it

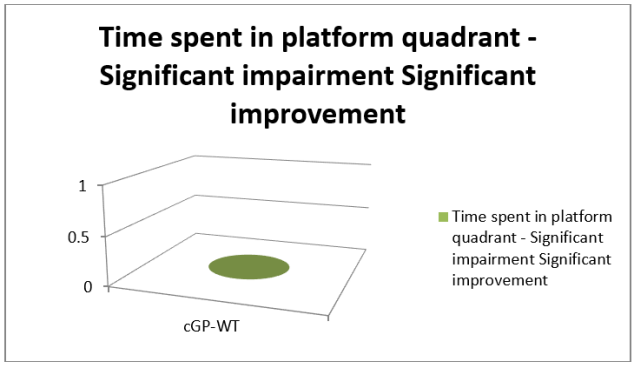
is important to note that the overall area under evaluation remained consistent in both groups. The ratio of APP to PS1 was 1.5 ± 0.1 mm2, and the ratio of cGP to APP was 1.6 ± 0.1 mm2, with a statistical significance of $p > 0.2$. There was no significant difference between the two groups. The results of this study suggest that therapy with cGP significantly reduces the number of amyloid plaques that are seen in the hippocampus of rats with Alzheimer's disease and Parkinson's disease type 1.

The amyloid opathy in the cortex was examined in the same mice in a manner that was equivalent to the previous methodology. In agreement with the findings that were gathered from the hippocampi, it was discovered that neither wild-type nor cGP-WT animals possessed any plaques in their cortex. In APP/PS1 mice that had been treated with cGP, the number of cortical amyloid plaques was significantly reduced in comparison to APP/PS1 animals that had not been treated. This was observed in contrast to mice that had not been treated. To be more specific, the plaque density and the percentage of the cortical area that was occupied by plaques were found to be significantly lower in mice that were treated with cGP in APP/PS1 mice, plaque count/mm2: APP/PS1, 42.7 ± 7.7; cGP-APP/PS1, 15.4 ± 1.2, $p < 0.02$), percentage area: APP/PS1, 0.5 ± 0.1; cGP-APP/PS1, 0.2 ± 0.01, $p < 0.04$). From the data presented it was discovered that the average size of the cortical plaques that survived was comparable between the treated group and the untreated group. The size of the plaque was determined to be in micrometres and was as follows: It was observed that the APP/PS1 ratio was 136.2 ± 11.1 and the cGP-APP/PS1 ratio was 114.1 ± 17.1; the p-value was greater than 0.3. In addition, it is important to mention that the total area that was taken into consideration remained the same across all groups (b4, APP/PS1: 2.5 ± 0.1 mm2; cGP-APP/PS1: 2.4 ± 0.1 mm2; $p > 0.3$). These data, which are in agreement with those obtained in the hippocampus, suggest that cGP therapy is effective in reducing amyloid pathology in both the hippocampus and the cortex of rats with Alzheimer's disease and Parkinson's disease type two.

Table 2: Hippocampal amyloid plaque findings

Parameter	APP/PS1	cGP-APP/PS1	p-value
Plaque Density (plaques/mm^2)	-	Significant reduction	< 0.05
Percentage Area Covered by Plaques	0.5 ± 0.1	0.2 ± 0.03	< 0.03
Average Size of Plaques (μm)	92.8 ± 17.6	94.7 ± 19.9	> 0.9 (N.S.)
Total Area Analyzed (mm^2)	1.5 ± 0.1	1.6 ± 0.1	> 0.2 (N.S.)

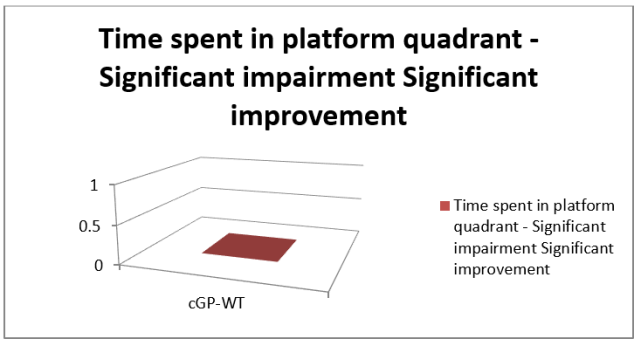
To evaluate the effectiveness of cyclic glycine-proline (cGP) in reducing the quantity of amyloid plaques in



Graph 2: Time in platform quardrant improvement

Table 3: Cortex findings

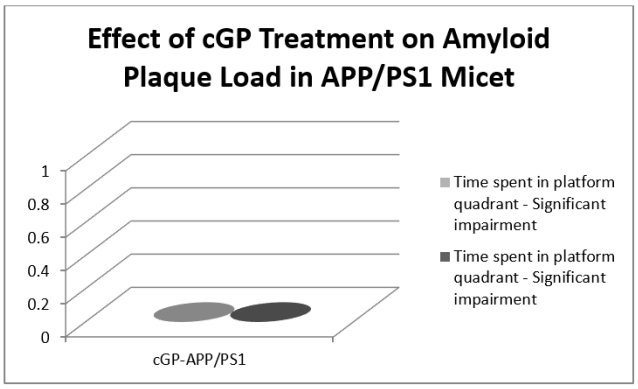
Parameter	APP/PS1	cGP-APP/PS1	p-value
Plaque Density (plaques/mm^2)	42.7 ± 7.7	15.4 ± 1.2	< 0.02
Percentage Area Covered by Plaques	0.5 ± 0.1	0.2 ± 0.01	< 0.04
Average Size of Plaques (μm)	136.2 ± 11.1	114.1 ± 17.1	> 0.3 (N.S.)
Total Area Analyzed (mm^2)	2.5 ± 0.1	2.4 ± 0.1	> 0.3 (N.S.)



Graph 3: Significant impairment improvement

Table 4: Effect of cGP treatment on amyloid plaque load in APP/PS1 Mice

Parameter	APP/PS1 Mice (n = 4)	cGP-APP/PS1 Mice (n = 4)	Statistical Significance
Plaque Density	Higher	Significantly Reduced	p < 0.05
Percentage Area Covered by Plaques	Higher	Significantly Reduced	p < 0.05
Average Plaque Size	Comparable	Comparable	N.S. (Not Significant)
Total Cortical Area Assessed	Similar	Similar	N/A



Graph 4: Effect of cGP treatment on amlyloid plaque load APP/PS1 MICE

the cortex of APP/PS1 mice and cGP-treated APP/PS1 mice (cGP-APP/PS1), an experiment was conducted. The purpose of the experiment was to understand the therapeutic potential of cGP. Thioflavin-S staining was applied to cortical slices obtained from both groups to assess plaque deposition.

Results showed that both plaque density and the percentage of cortical surface area covered by plaques were significantly reduced in cGP-APP/PS1 mice (n = 4 per group) compared to untreated APP/PS1 mice. However, the average size of the plaques remaining in cGP-APP/PS1 animals was comparable to the plaques observed in untreated APP/PS1 mice. No significant differences were found in the total cortical area examined for plaque burden between the groups.

Upon statistical analysis, the following key observations were made:

1. Significant decreases ($p < 0.05$) in both the plaque density and the percentage of cortical area covered by plaques were noted in cGP-treated groups.
2. significant differences (N S) were observed in the size of individual plaques
3. Quantitative summary of amyloid plaque density and percentage of cortical area covered by plaques in APP/PS1 and cGP-APP/PS1 mice (Table 3).
4. Statistical analysis comparing the size of plaques and overall cortical area burden in APP/PS1 and cGP-APP/PS1 groups.Graphical representation of plaque density and cortical surface area covered by plaques (Table 3)(Graph 3).Bar graph illustrating plaque size comparisons and statistical significance (Table 4) (Graph 4).

5. Discussion

Individuals aged 65 and older are at a higher risk of developing Alzheimer's disease (AD), the most common condition leading to dementia. To study the progression of AD, researchers often utilize transgenic animal models that exhibit abnormalities in long-term potentiation and depression.[9]This is a common approach in Alzheimer's research. In particular, genetically modified mice that overexpress mutant human amyloid precursor protein (APP) tend to accumulate amyloid plaques and show impairments in learning and memory function .These amyloid plaques, characteristic of Alzheimer's, are a hallmark of these genetically modified mice.Moreover, double transgenic mice, which carry mutations in both amyloid precursor protein (APP) and presenilin 1 (PS1), display rapid accumulation of amyloid-beta (Aβ) in the brain.[18] However, despite these mice showing cognitive decline and amyloid plaque formation,[3] they do not develop tau-related pathologies commonly observed in human Alzheimer's disease. This limits their ability to fully mimic the human condition. Despite this limitation, these transgenic models are crucial for studying the fundamental mechanisms of Alzheimer's disease and for investigating the potential therapeutic interventions. The aim of this study was to assess the effects of a cyclic dipeptide on amyloid plaque deposition in the hippocampus and cortex of these mice, as well as its influence on spatial memory. Specifically, the study sought to investigate how amyloid plaques accumulate in these regions and the impact this has on the cognitive abilities of the mice.[20]

Insulin-like growth factor a key factor in brain development and has been shown to have neuroprotective properties.[16] When IGF-1 is broken down, it produces two fragments: glycine-proline-glutamate (GPE) and des-IGF-I.[21] These fragments are generated as part of the breakdown process. Previous studies have investigated the effects of GPE conjugated with nanomaterials in animal models of Alzheimer's disease.Cyclic glycine-proline (cGP), a metabolite of IGF-1 likely derived from GPE,[8] has the advantage of being able to penetrate the central nervous system more effectively and is resistant to enzyme degradation. Due to its increased lipophilicity, cGP is able to efficiently cross the blood-brain barrier and permeate the central nervous system. These properties make cGP a more effective candidate for brain targeting compared to its parent peptide. Both cGP and its analog, NNZ 2591, have demonstrated neuroprotective effects in models of hypoxic-ischemic injury.[NNZ 2591 has also been demonstrated to improve sensory-motor function and protect against motor deficits in models of Parkinson's disease , as well as scopolamine-induced memory impairment,[17] likely by modulating acetylcholine neurotransmission. However, the specific role of cGP in Alzheimer's disease has not been explored until now.

In this study, the APP/PS1 mouse model of Alzheimer's disease was used to investigate the effects of cGP on spatial memory and amyloid plaque accumulation. The purpose of this research was to evaluate cGP's potential as a

therapeutic agent. The findings suggest that cGP effectively reduces amyloid-related pathologies and improves memory deficits in the examined animals. Despite these promising results, the precise mechanisms by which cGP impacts memory and reduces amyloid plaque load remain unclear and require further investigation.It is hypothesized that cGP is responsible for regulating the homeostasis of IGF-1 by modifying the interaction that it has with factors that bind to it. Because of this, the interaction is altered, which leads to an increase in IGF-1 activity when levels are low and a decrease in activity when levels are too high.[2,3] IGF-1 has been shown to have protective effects against Aβ toxicity and mutant APP, that it has the ability to reduce amyloid load,[17] and that it improves memory while simultaneously lowering Aβ levels in mice that have previously been treated with APP/PS2.[15] Despite the fact that the complete role of IGF-1 in Alzheimer's disease is not yet fully understood, research has shown that it has these effects. Given that cGP is able to influence the activity of IGF-1, it is conceivable that the beneficial effects of cGP on memory and amyloid plaque burden that were described in this experiment are related with IGF-1 signalling molecules. This is because cGP has the ability to modulate the activity of IGF-1.

In this particular investigation, the presence of cGP in the brain after intranasal delivery was not measured. On the other hand, prior studies have proven that NNZ 2591 can be found in cerebral fluid following subcutaneous injection and in the gastrointestinal system following intraperitoneal treatment. With this in mind, it is plausible to hypothesise that cGP that is supplied intranasally has the potential to reach the brain. Further research is needed to confirm the presence of cGP in the brain and to explore the molecular pathways through which it exerts its effects in transgenic mouse models of Alzheimer's disease.

6. Conclusion

A short cyclic peptide derived from IGF-1, known as cGP, has demonstrated promising potential as a therapeutic agent for Alzheimer's disease. In studies using APP/PS1 transgenic mice—widely accepted models for this condition—cGP significantly improved memory function while reducing amyloid plaque accumulation in critical brain regions, including the cortex and hippocampus. These findings suggest that cGP may target one of the hallmark features of Alzheimer's disease, the buildup of amyloid plaques, thereby mitigating cognitive decline. Additionally, its ability to enhance spatial memory indicates its potential to alleviate the severity of dementia-related symptoms. As such, cGP represents a compelling candidate for future research into therapeutic strategies aimed at managing and slowing the progression of Alzheimer's disease.

7. Ethical Statement

All procedures outlined in this study received approval from the Institutional Animal Ethics Committee. The transgenic mice were handled responsibly, adhering to ethical guidelines for the care and use of laboratory animals. The mice were housed in controlled environments with a 12-hour light/dark cycle and were provided with standard rodent food and water.

8. Conflict of Interest

None.

9. Source of Funding

None.

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