Distinct Effects of The Hippocampal Transplantation of Neural And Mesenchymal Stem Cell In a Transgenic Model of Alzheimer's Disease

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Short Report

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Abstract

Alzheimer's disease (AD) is a highly disabling condition, with no cure currently available that accounts for 60-70% of all dementia cases worldwide. Therefore, the study of possible therapeutic strategies for AD is required. For that, animal models which resemble the main aspects of AD has been largely employed. Similar to AD patients, the double transgenic APP^{swe}/PS1^{dE9} (APP/PS1) mice presents amyloid- β (A β) plaques in the cortex and hippocampus, hyperlocomotion, cognitive deficits, and exacerbated inflammatory response. Recent studies showed that these neuropathological features were reversed by the transplantation of stem cells. However, the comparison of the effects induced by neural (NSC) or mesenchymal (MSC) stem cells was never investigated in an AD animal model before. In view of that, the present study aimed to evaluate whether NSC or MSC transplantation into the hippocampus of APP/PS1 mice reverse AD-related alterations, namely locomotor activity (open field test), short- and long-term memory (object recognition test), A β plaques formation (6-E10 immune staining) and microglia activation (Iba-1 immune staining) in the hippocampus. NSC and MSC engraftment reduced the number of hippocampal A β plaques in the hippocampus of APP/PS1 mice, and NSC reverted the peripheral hyperlocomotion activity displayed by APP/PS1 mice. Surprisingly, NSC increased microglia activation in the hippocampus of APP/PS1 mice and no impairment in short or long-term memory was observed in APP/PS1 mice. Altogether, this study reinforces the possible beneficial effects of NSC or MSC transplantation in the AD treatment.

Introduction

Alzheimer's disease (AD) is the main form of dementia, accounting for more than 60% of the cases (1). Currently, AD has no cure and only palliative treatments are available, which implies in high economic burden due to the use of health care resources, treatment costs and indirect costs resulting from the incapacitation of the patients (2). AD is mainly characterized by gradual memory loss, with cognitive dysfunctions, as well as personality and behavioral changes. Neuropathological features, such as amyloid- β (A β) plaques and neurofibrillary tangles, as well as synaptic and neuronal loss, are found mainly in the cortex and hippocampus of AD patients and animal models, which also present exacerbated neuroinflammatory responses (3). All these processes result in behavioral impairment which have been associated with the memory deficits and cognitive decline observed during the progression of the disease (1).

Although several advances have been made towards understanding AD, no efficient treatment for this disease has been found. In this context, it is important to develop new therapeutic approaches to prevent or control the progression of neurodegeneration and neuroinflammation caused by the accumulation and deposition of A β peptide. Stem cells have a range of applications in therapies for various neurological diseases for which there is still no effective treatment, such as AD. This fact has stimulated new researches, not only to investigate the potential of these cells in the treatment of different neuropathological processes, but also to understand the mechanisms of protection and tissue repair, as well as disease modification effects (4).

Promising studies using transplantation of mesenchymal (MSC) and neural (NSC) stem cells in animal models of neurological diseases, including AD, have shown significant functional recovery, and motivated the emergence of new prospects for clinical applications (5; 6; 7; 8; 9). The most important effect of transplanted MSC is the immunomodulatory function through crosstalk with the immune cells or paracrine actions that has been shown to reduce the A β deposition in AD experimental models (10; 11). Advantageously, the protective mechanisms attributed to MSC include the secretion of substances involved in anti-inflammatory, proliferative and anti-apoptotic responses, as well as to rapidly migration and interaction with microglia, neurons, and astrocytes (12; 13; 14). While MSC preferably have paracrine effects inducing actions on resident cells, NSC are more likely to proliferate, self-renew and differentiate into astrocytes, oligodendrocytes and neurons (15;16), and are able to restore the working memory and short-term memory in AD (5; 6). Since these two cell populations act differently when transplanted into the brain, it is interesting to compare them in relation to their therapeutic effects in AD.

In order to reproduce the neuropathology present in patients with AD, double mutant transgenic mice carrying genes associated with AD (APP^{swe} and PS1), shortly called as APP/PS1, have been produced (17). APP^{swe} mutation favors its processing by β - and γ -secretases leading to A β formation and aggregation (18; 19). PS1 is a core protein in the γ -secretase complex and the deletion of exon 9 in PS1 gene has also been associated with increased A β deposition (3). Histochemical studies in this *in vivo* model showed deposition of A β starting at 6 months (symptomatic middle stage) with a progressive increase of neuroinflammatory responses, plaques formation and consequently inducing behavioral alterations (17; 20). The consistent deposition of A β in early APP/PS1 mice confirms its utility for behavioral and histological studies, making it a valuable tool for new therapeutic approaches (17). In view of that, the present study aimed to compare the effects of MSC and NSC hippocampal transplantation in A β plaque formation, microglial expression, and in behavioral responses in a murine model (APP/PS1) of AD.

Materials And Methods

2.1. Animals and maintenance

All animals were provided by CEDEME (Center for the Development of Animal Models in Biology and Medicine at the Universidade Federal de São Paulo). They were housed in polypropylene home cages (41 x 34 x 16.5cm) in a pathogen-free facility, under controlled temperature (22–23°C) and lighting (12h light, 12h dark; lights on at 6:45 a.m.) conditions. Chow for rodents and water were available *ad libitum*. The Ethics Committee of the Federal University of São Paulo approved all experiments under the protocol #9268250618.

Male C57/Bl6-eGFP + mice (n = 8), with 6–8 weeks and approximately 25g, were used as MSC donors. Embryos of C57/Bl6 mice (13.5 days post conception) constitutively expressing green fluorescent protein (GFP) (21) were used as donors of NSC for transplant.

Adult male APP^{swe}/PS1^{dE9} transgenic mice (APP/PS1) and their wild-type (WT) littermates negative for the mutations, with 6–7 months old, were used as hosts for NSC and MSC transplantation.

2.3. MSC extraction and cell culture

To obtain MSC, 8 male transgenic animals expressing Green Fluorescent Protein (GFP), with 6 to 8 weeks of age, were euthanized by anesthetic overdose (thiopental 100 mg/kg, i.p.) after lidocaine administration (10 mg/kg, i.p.). Both femurs and tibias were removed, and muscles and cartilage were dissected. Inside in the vertical laminar flux the largest epiphysis of both bones was cut and the MSC were removed by flushing with DMEM and centrifuged (1700 Xg for 5 minutes). MSC was extracted from the supernatant and cultivated in DMEM Low Glucose and 10% FCS (fetal bovine serum). Thereafter, cells were expanded and used between passages 7–10, when they reached 70–90% confluence.

2.4. NSC extraction and cell culture

Pregnant females aged 13.5 days were euthanized due to an overdose of anesthetics (Thiopental 150mg / kg) after application of lidocaine 10 mg / kg (i.p.). Fetuses were removed from the uterus and, with the help of a fluorescence microscope (Nikon) at excitation b-2e/c FITC, fetuses expressing green fluorescent protein GFP were selected. The telencephalon region was extracted with the help of a stereomicroscope (Nikon). Tissues were placed in an Eppendorf tube and incubated in a 0.05% sterile trypsin solution for 5 minutes at 37°C, followed by inactivation with FBS (Gibco). The tubes were centrifuged at 1500 Xg for 7 minutes and the tissue mechanically dissociated with the aid of micropipettes (P1000 and P200) in DMEM-F12 + DNase. After a new centrifugation, the pellet was resuspended in 0.5 mL of DMEM. Cells were grown at a density of approximately 100,000 cells/mL in 25 cm² plastic bottles, previously treated with poly 2-hydroxyethyl methacrylate (Sigma), a polymer to prevent cell adhesion. The standard culture medium used for neurosphere growth was composed of DMEM-F12 50:50 (Invitrogen), supplemented with 1% N2 (Invitrogen), 1% L-glutamine (200 mM; Invitrogen), penicillin (100 IU / mL), streptomycin (100 ug / mL) and amphotericin B (0.25 ug / mL), in addition to the factors EGF (20 ng / mL; Sigma) and FGF2 (10 ng / mL; P&D). The cells were kept in an oven at 37°C and 5% CO₂ for approximately 5 days (21).

2.5. Stem cell transplantation

APP/PS1 mice received intrahippocampal infusion of NSC or MSC. Animals under deep anesthesia (ketamine 115 mg/kg + xylazine 10 mg/kg) were placed in stereotaxic. Skull surface was exposed after scalp incision and cleansed to expose the bregma. Two orifices were opened and gengival needles were used for stem cell infusion according to coordinates: AP = -2,06 mm, ML = ± 1,3 mm, DV = -2,7 mm. 400.000 cells of NSC or MSC, previously dissociated, were diluted in a saline solution and 4µL was slowly infused. Orifices were then closed with bone wax and scalp was closed.

After the procedure, all animals received ibuprofen (30 mg/kg, orally administered) right after surgery and 12h intervals for 3 days and were monitored. Transplanted mice were distributed in experimental groups

according to their genotypes (positive or negative for transgenicity), and treatment of stem cell they received, as follows:

- WT (control): APP/PS1-negative mice (n = 15)
- APP/PS1: APP/PS1-positive mice (n = 15)
- APP/PS1 + MSC: APP/PS1-positive mice with MSC transplantation (n = 15)
- APP/PS1 + NSC: APP/PS1-positive mice with NSC transplantation (n = 15)

2.6. Behavioral tests

The animals were subjected to Open Field and Novel Object Recognition behavioral tests four weeks after stem cell transplantation.

2.6.1. Open Field (OF)

The Open Field test arena consists of a cylindric apparatus with 40cm-high and 2cm-thick walls as previously described (22). The arena floor is divided into 19 sections of approximately equal dimensions: 12 peripheric sections (near the wall), 6 intermediate sections and 1 central section, in three concentric circles of different radiuses (20, 14 and 8 cm, respectively).

Animals were placed in the arena for 10 min and allowed to explore it freely. All tests were video-recorded. Locomotion frequency was defined as the number of sections explored by the animal during the test; each locomotion unit corresponds to the animal's four paws placed inside one of the sectors.

2.6.2. Novel object recognition (NOR)

The same arena used in the OF test was used for NOR started 24h after OF test. The test consists of two phases: training and test. In both phases, each animal was allowed to explore freely for 5 minutes. The training phase consists of one trial, where two equal objects (A and A') were fixed on opposite sides of the arena with adhesive tape. The test phase consists of one trial, 1h after training, where one object was replaced by a new one (B) (23) Object exploration was defined as smelling and touching objects with snout or front paws. Sitting or walking around the object were not considered object exploration.

The discrimination index (DI) was used to analyze exploration time between new and familiar objects. DI is calculated as the difference between familiar object exploration (FT) and new object exploration (NT) divided by the total time spent exploring both objects [$DI = (NT - FT) / (NT + FT)$]. Positive DI values indicate newer object exploration, while negative values indicate more familiar object exploration. Zero indicates lack of preference.

2.7. Perfusion and tissue fixation

After the behavioral tests, animals under deep anesthesia (thiopental 50 mg/kg, i.p.) were transcardially perfused with approximately 100 mL PBS and 100 mL 4% PFA (paraformaldehyde). Brains were removed from the skull and kept in 4% PFA for 24h, being transferred to a hypertonic 30% sucrose solution for cryoprotection. Brains were frozen and sectioned coronally (40 μ m). Sections were stored in anti-freezing solution (500 mL PBS, 500 mL H₂O, 1.59g NaH₂PO₄, 5.47g Na₂HPO₄, 300 g sucrose, 300 mL ethylene glycol) at -20°C.

2.8. Immunohistochemistry

Sections containing the hippocampal region were washed through free-floating (10 minutes for 3 times), incubated with blocking buffer solution for 30 minutes. and left overnight with the primary antibodies: A β plaques (mouse anti-6-E10; 1:1,000; Covance) and microglial cells (rabbit anti-Iba-1; 1:1000, Wako Chemicals). Sections were then washed and incubated in secondary antibodies (anti-mouse, 1:600, Vector BA9200 or anti-rabbit, 1:600, Vector, BA1000) for 2h at room temperature, followed by ABC (Avidin/Biotinylated enzyme Complex Vectastain Elite, Vector) kit incubation for 1h30 and DAB for 5 minutes right after.

The microglia around the plaques were identified by immunofluorescence using an anti-6E10 (mouse anti-6-E10; 1:1,000; Covance) and anti-Iba-1 (rabbit anti-Iba-1; 1:1000, Wako Chemicals) antibodies. Briefly, sections were washed and incubated in the primary antibody overnight. On the following day, sections were incubated for 2 h with a fluorochrome-conjugated secondary antibody (Alexa 488 and Alexa 546) and washed in PBS. Slides were mounted and sealed with DPX and were subsequently analyzed by fluorescence microscopy using the Neurolucida capture software (MBF Bioscience).

2.9. Tissue cytometry

Hippocampal images were acquired with the TissueFAXS Cytometry (TissueGnostics GmbH, Vienna, Austria) system, coupled to a spinning disk confocal microscope (Zeiss). Data were analyzed with the StrataQuest software (TissueGnostics GmbH) as described elsewhere (24). 6-E10 and Iba-1 immunostaining was examined for the following parameters: number of cells and total area. Six hippocampal sections of 40 μ m were recorded, resulting in approximately 90 fields of view /section.

2.10. Statistical analysis

Statistical analysis was conducted using Graphpad Prism 8.0 software. Open Field and NOR tests were analyzed through Kruskal-Wallis test, followed by Dunn's post-test. Immunohistochemical quantitative analysis were performed using one-way ANOVA followed by Dunnett's post-test. Significance was assumed at $p < 0.05$.

Results

3.1 MSC and NSC reduced the numbers of hippocampal A β plaques but not the size of the plaques

After six weeks transplantation of NSC and MSC into the hippocampus of APP/PS1 mice the numbers and the size (area) of 6E10-stained A β plaques were analyzed. We found a significant decrease in the numbers of A β plaque in APP/PS1 + NSC (2.980 md $\pm$ 0.27) and APP/PS1 + MSC groups (2.605 md $\pm$ 0.63) when compared to APP/PS1 group (6.327 md $\pm$ 0.67) (Fig. 1A: $F_{3,20} = 28,99$; respectively $p = 0.0003$ and $p < 0.0001$). As expected, the APP/PS1 groups showed a significant increase in the number and size (area) of A β plaques in the hippocampus when compared with WT group (Fig. 1B: $F_{3,19} = 9,925$; $p < 0.0001$). No statistical significance was detected for the size (area) of A β plaques between APP/PS1 group (18.43 md $\pm$ 3.69) and APP/PS1 + NSC (19.32 md $\pm$ 2.50) or APP/PS1 + MSC groups (16.88 md $\pm$ 3.70) (Fig. 1B: $F_{3,19} = 9,925$; respectively $p = 0.9936$ and $p = 0.9642$).

3.2 APP/PS1 + NSC animals showed increased numbers of microglial cells and in total area occupied by the clusters of microglia in the hippocampus

The number of Iba-1 immune-stained cells was increased in APP/PS1 + NSC (2628 md $\pm$ 147) (Fig. 2A: $F_{3,16} = 15,16$; $p < 0.0001$), but not in APP/PS1 + MSC (1579 md $\pm$ 326.8) mice, when compared to APP/PS1 (891.1 md $\pm$ 248.7) and WT mice (915.8 md $\pm$ 108.2). Accordingly, total area occupied by the clusters of Iba-1 positive cells was also increased in APP/PS1 + NSC (9497 md $\pm$ 775.5) but not in APP/PS1 + MSC (4238 md $\pm$ 951.8) mice, when compared with APP/PS1 (1819 md $\pm$ 562.9) and WT groups (909.6 md $\pm$ 281.2) (Fig. 2B: $F_{3,17} = 32,17$; $p < 0.0001$). No statistical significance was detected for the number (Fig. 2A: $F_{3,16} = 15,16$; $p = 0.9996$) and total area occupied by the clusters of Iba-1 between WT and APP/PS1 groups (Fig. 2B: $F_{3,17} = 32,17$; $p = 0.7020$). As observed in Fig. 2C, the clusters of Iba-1 positive cells possibly correspond to microglial cells surrounding A β plaques (marked by 6E10 immune-staining).

3.3. NSC transplantation reverses the exacerbated locomotor activity of APP/PS1 mice in the Open Field test

Mice were tested in the Open Field for the locomotor activity analysis. The total number of entrances into any floor unit revealed that APP/PS1 mice showed a significant increase in locomotor activity when compared to WT animals (Fig. 3A: $H = 15,82$; $p = 0.0012$). Peripheral locomotion also increased in APP/PS1 and NSC transplantation was able to reverse such behavior to control levels (Figs. 3B: $H = 14,36$; $p = 0.0025$).

When mice were tested for the Novel Object Recognition (NOR) task, no statistically significant difference in DI between objects regarding the interaction time (time spent exploring the new object) (Fig. 3C: $H = 2,247$; $p = 0.5228$) and the number of interactions (number of contacts with the new object) was detected between groups, as shown in Fig. 3D ($H = 1.935$; $p = 0.5859$).

Discussion

The results of the present study indicated that the transplantation of NSC and MSC decreased the number of A β plaques in the hippocampus of APP/PS1 mice, although cell transplantation was not sufficient to diminish the size of plaques as no difference was observed between APP/PS1 groups when the plaque area was analyzed. In addition, NSC induced an increase in microglial cells in the hippocampus compared to the APP/PS1 group. Also, NSC were able to reverse the hyperlocomotion present in APP/PS1 animals, although both NSC and MSC cells did not affect the object recognition memory.

Even showing different characteristics, both cell types, NSC and MSC, were able to decelerate the buildup of new plaques reducing the amount of A β plaques. Considering that MSC were not found in the hippocampus six weeks after surgery (data not shown), it is plausible that the cell infusion produced an immunomodulatory effect by paracrine action, reducing neuroinflammation that, in turn, could inhibit the formation of new A β plaques. Reduced numbers of plaques caused by NSC transplantation has other underlying mechanisms, however. NSC express BACE2, a degrading enzyme of A β peptide able to cleave the existing A β proteins, destroying the molecule (25). In addition, NSC has been proposed to act as CNS immunomodulator, allowing homeostasis, maintaining and repairing tissue, not only structurally but also functionally (26). NSC transplantation in AD helps preparing the microenvironment, releasing immunoregulatory factors capable of modulating the progression of neuroinflammation (27).

Neuroinflammation was examined by the quantitation of microglial cells expressing Iba-1 protein. This marker belongs to a family of proteins with Ca²⁺ binding domains, with significant activity in cell signaling in the CNS. Iba-1 is found specifically in microglial cells, and in the case of inflammation there is an increase in expression due to cell activation. In fact, APP/PS1 + NSC group, together with the reduction in A β plaques, also showed a significant increase in hippocampal microglia. As the transplanted cells did not differentiate into microglia, it is reasonable to think that the number of microglia increased in NSC-treated mice as a consequence of an intensification of A β hippocampal levels, causing them to have a positive effect on the number but not on the size of the plaques in the hippocampus. Previous results corroborate our findings that microglial clusters around A β plaques may be benefic, suggesting that microglia could phagocyte the plaques, not allowing formation of new ones or effectively reducing their size (28, 29, 30). However, once the disease progresses, microglial function is reduced and, therefore, A β plaques continue to increase. Studies suggest that the phagocytic products of the plaques are not metabolized by microglia, which in turn enters an apoptotic process and releases the amyloid load back to the extracellular matrix, possibly forming new plaques (31). These high concentrations of microglia were preferentially found surrounding the A β plaques after NSC transplantation, which is in accordance with the suggestion that microglia are activated by the presence of A β proteins, clustering around it to secrete cytokines, neurotoxins and to phagocyte the plaques (32).

Different results were observed after MSC transplantation. Six weeks after intrahippocampal transplantation of MSC no alterations were observed in the number of microglial clusters (Iba-1-positive cells). It seems that MSC could attenuate A β deposition by modulating inflammatory response through a mechanism other than the microglial activation pathway and, considering that APP/PS1 animals have A β

plaques as a permanent condition, the plausible explanation is that microglia activation should be continuous due to the continuous formation of new plaques, even with lower numbers of A β plaques in transplanted groups. Interestingly, after cell transplantation into the brain a local neuroinflammation is supposed to occur due to the injection site lesion in brain tissue (33). However, even with no evident increase in microglia, MSC were capable of inhibiting the local inflammation caused in the injection site after the invasive procedure, possibly promoting tissue recovery and probably inducing the microglial phagocytic activity of A β plaques. Studies demonstrated that MSC can reduce the deposition of A β peptides, inhibiting the expression of APP-cleaving protein in the β site (BACE1), through immunomodulation and cytokine secretion (26, 34). Then, it is likely that the two cell types use different mechanisms of action for the regulation of A β plaques formation.

Seven-months old APP/PS1 mice showed hyperlocomotion in the Open Field test when compared to WT animals, and only NSC transplantation could reverse such behavior. APP/PS1 mice in the same age showed an increase in peripheral locomotion in the same test. Clinic observations already describe agitation and increase in locomotor activity in AD patients (35). Studies also describe the same behavior in 7 to 8-months old APP/PS1 mice both in Open Field and bright-dark tests.

Transplanted NSC are able to differentiate into neurons. Literature data show that newly formed neurons can increase connectivity and synaptic density (36, 37, 38, 39). Even though MSC are also capable of releasing neurotrophic factors and microglial and astrocytic modulators, increasing the tissue's ability to eliminate A β plaques in the hippocampus (40, 32, 41), these cells are not able to differentiate into neurons. These data may explain the NSC capability of reverting hyperlocomotion in the Open Field test (42).

APP/PS1 mice do not show impairment in recognition memory, as revealed by the NOR, being in agreement with previous reports (43, 44). Published studies already demonstrated that 9-months old APP/PS1 animals showed no neuronal loss in the hippocampus, since the amount of A β plaques were not yet capable of leading to neuronal death (45, 46). Therefore, our study was not able to evaluate whether stem cell transplantation could protect APP/PS1 animals from neuronal loss in later years, as demonstrated by (47), (48) and (49). Future research is needed to evaluate the same parameters in older animals, to identify whether NSC and MSC transplantation could protect recognition memory. The reduction of hyperlocomotion observed in APP/PS1 + NSC animals plays an important role, since it approaches APP/PS1 mice in their behavior to their WT counterparts. The fact that, 6 weeks after transplantation, MSC were not found in the hippocampus may account for the absence of significant differences observed in the hyperlocomotion between APP/PS1 and APP/PS1 + MSC animals.

Conclusion

Therefore, even though A β deposition can be handled by MSC, damage caused by A β however, tends to be worse in APP/PS1 animals treated with MSC than NSC, since our comparisons between NSC and MSC-treated animals revealed that NSC transplantation was more efficient at reducing A β plaques, in

association with increased microglia in the hippocampus, and reverting the peripheral hyperlocomotion activity displayed by APP/PS1 mice. However, although we could observe two distinct neuroprotective effect, it is not possible to detect the exact mechanism by which MSC and NSC act after hippocampal transplantation. The development of strategies that allow discriminating the effects of these two populations of cells should be considered in future studies.

As schematized in the Graphical Abstract, APP/PS1 transgenic mice have been largely used to study both neurobiology and new therapeutical approaches for the AD clinic. In AD patients, hallmarks include agitation and increased hippocampal A β plaques formation. Accordingly, APP/PS1 transgenic mice presented hyperlocomotion in the open field test along with enhanced hippocampal 6-E10 immune staining (A β plaque marker), which was reversed by NSC or MSC transplantation into the hippocampus. These results reinforce the possible beneficial effects of stem cell transplantation in AD treatment.

Declarations

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Authors' contributions

Henrique Correia Campos conducted the experiments and wrote the paper; Deidiane Elisa Ribeiro conducted the experiments, data analysis and wrote the paper; Debora Hashiguchi contributed in carrying out the experiments, data analysis and revised the paper; Deborah Y Hukuda contributed with the experiments and analysis; Christiane Gimenes helped conducting the experiments; Simone A A Romariz contributed conducting the experiments, statistics, figures and wrote the paper; Qing Ye contributed to data analysis and revised the paper; Yong Tang contributed to the construction and revised the paper; Henning Ulrich assisted with the use of the Tissue fax microscope and revised the paper, and Beatriz Monteiro Longo supervised the work, wrote the paper and revised the paper.

Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

All the procedures followed the Ethical Guidelines for the Use of Animals and were approved by the local commission (Comissão de Ética no Uso de Animais, Universidade Federal de São Paulo), process nº 9268250618, and the National Council for Scientific and Technological Development (CNPq).

Consent to Participate

Not applicable

Consent to Publish

Not applicable

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Figures

Figure 1

Quantification of Aβ-6E10-positive plaques in the hippocampus. The number of plaques (A) and total area (B) stained with 6E10 were quantified using tissue cytometry. Bars represent mean $\pm$ SEM; $n = 5-6$ animals/group. One-way ANOVA followed by Dunnett's post-test; $*p < 0,05$. Photomicrography showing Aβ plaques (arrows) in the hippocampus sections of APP/PS1 (C), APP/PS1+MSC (D) and APP/PS1+NSC (E), respectively. Scale bar = 40μm.

A. Number of Iba-1 positive cells

B. Cluster area of Iba-1 positive cells

Figure 2

Quantification of Iba-1-positive cells in the hippocampus of APP/PS1 and WT mice. The number (A) and total area of clusters (B) of Iba-1-positive cells were quantified using TissueFAXS. Bars represent mean $\pm$ SEM; n= 5-6 animals/group. One-way ANOVA followed by Dunnett's post-test; *p < 0,05.

Immunofluorescence showing A β plaques (6E10, green) and microglia (Iba-1, red) in the hippocampus of APP/PS1 (C-E), APP/PS1+MSC (F-H) and APP/PS1+NSC (I-K) mice, respectively. Observe in K the larger number of microglial cells in APP/PS1+NSC when compared to APP/PS1 (E) and APP/PS1+MSC (H) images. Scale bar = 100µm.

A. Total locomotion

B. Peripheral locomotion

C. Object interaction time

D. Number of contacts with the object

Figure 3

Locomotor activity analysis in APP/PS1 and WT mice subjected to the Open Field test. Total (A) and peripheral (B) locomotion was compared between the groups. Bars represent mean $\pm$ SEM; $n = 13-14$ animals/group. Kruskal-Wallis test, followed by Dunn's post-test; $*p < 0,05$. Discrimination index (DI) between APP/PS1 and WT mice subjected to NOR test is indicated in (C) and (D). Animals were subjected to NOR test for 5 minutes and interaction time (C), and the number of contacts with each object (D) were calculated using the Discrimination Index ($DI = ((TN - TF) / ((TN + TF)))$). Bars represent mean $\pm$ SEM; $n = 13-14$ animals/group. Kruskal-Wallis test, followed by Dunn's post-test; $*p < 0,05$.

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