Functional Impairment of Microglia Coincides with Beta-Amyloid Deposition in Mice with Alzheimer-Like Pathology

Grietje Krabbe1*, Annett Halle2,3*, Vitali Matyash1, Jan L. Rinnenthal2, Gina D. Eom2, Ulrike Bernhardt2, Kelly R. Miller2, Stefan Prokop2, Helmut Kettenmann1*, Frank L. Heppner2*

1 Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany, 2 Department of Neuropathology, Charité-Universitätsmedizin Berlin, Berlin, Germany, 3 Center of Advanced European Studies and Research (caesar), Bonn, Germany

Abstract
Microglial cells closely interact with senile plaques in Alzheimer’s disease and acquire the morphological appearance of an activated phenotype. The significance of this microglial phenotype and the impact of microglia for disease progression have remained controversial. To uncover and characterize putative changes in the functionality of microglia during Alzheimer’s disease, we directly assessed microglial behavior in two mouse models of Alzheimer’s disease. Using in vivo two-photon microscopy and acute brain slice preparations, we found that important microglial functions - directed process motility and phagocytic activity - were strongly impaired in mice with Alzheimer’s disease-like pathology compared to age-matched non-transgenic animals. Notably, impairment of microglial function temporally and spatially correlated with Aβ plaque deposition, and phagocytic capacity of microglia could be restored by interventionally decreasing amyloid burden by Aβ vaccination. These data suggest that major microglial functions progressively decline in Alzheimer’s disease with the appearance of Aβ plaques, and that this functional impairment is reversible by lowering Aβ burden, e.g. by means of Aβ vaccination.

Introduction
Microglial cells execute important functions in the brain. They constantly survey their surrounding and react to acute tissue injuries [1,2]. In the healthy brain, microglia contact synapses for seconds, which can be prolonged up to one hour upon acute injury and eventually leads to the disappearance of the contacted neuronal structure [3,4]. Microglia are also involved in shaping adult hippocampal neurogenesis through phagocytosis of apoptotic newborn neuroblasts [5]. Together, these data highlight that so-called “resting” microglia in the healthy brain are able to modify their environment, e.g. by their intrinsic phagocytic activity [6].

In Alzheimer’s disease (AD), microglia are attracted to β-amyloid (Aβ) plaques, produce elevated levels of proinflammatory cytokines and reactive oxygen species, and exhibit a change in morphology [7–11]. These phenotypical and morphological changes of microglia are commonly interpreted as an “activated” state [12,13]. However, microglial activation is not an all-or-none process and the morphology of microglial cells does not necessarily match their functional state [14,15].

A number of in vitro studies have not only shown that administration of Aβ stimulates the production of inflammatory cytokines and activation markers in microglial cells [8] but also triggers its uptake by receptor-mediated phagocytosis [16]. Further studies found that microglial cells internalize soluble, protofibrillar and fibrillar forms of Aβ [9,17,18] in vitro and in vivo by several mechanisms such as macropinocytosis or endocytosis, highlighting the general aptitude of microglia to remove Aβ. However, the question of whether resident microglia restrict Aβ plaque growth in AD has remained controversial, as the recruitment of microglia to Aβ plaques does not seem to result in their degradation [13,18]. More importantly, we have recently found that temporary ablation of microglia has no effect on the formation and maintenance of Aβ plaques [19]. Interestingly, expression of receptors and enzymes involved in microglial Aβ-uptake and degradation is progressively downregulated in a transgenic mouse model of Alzheimer’s disease [20]. Thus, it has been suggested that microglia are possibly less efficient in removing and degrading Aβ at later stages of Alzheimer’s disease and become dysfunctional [13,20].

In order to characterize putative progressive AD-associated changes in microglia not only at an expression level, but also...
functionally, we directly assessed and quantified microglial phagocytic and directed motile function in AD mouse models using two-photon microscopy and acute cerebral slice preparations. Furthermore, we reduced Aβ plaque burden in an Alzheimer’s disease mouse model by administering the Aβ-specific antibody Ab9 and assessed whether this interventional treatment restores AD-associated functional changes in microglia. Our data obtained from two different mouse models of Alzheimer’s disease reveals further insight into specific changes of microglial behavior during Alzheimer’s disease and suggest that Aβ plaque deposition and microglial function are closely interrelated.

**Materials and Methods**

**Ethics Statement**

All procedures involving handling of living animals were performed in accordance with the German animal protection law and were approved by the regional offices for health and social services in Berlin.

**Mouse Models**

APPPS1 mice, a transgenic mouse model of cerebral amyloidosis expressing human APP with the Swedish mutation (KM670/671NL) and human mutated PS1 (PS1-L166P) [21] were kindly provided by Mathias Jucker, University of Tübingen, Germany. APP23 mice, which express APP with the Swedish mutation [22] were provided by Novartis, Basel, Switzerland. For two-photon imaging experiments Cx3cr1GFP/eGFP mice [23] were crossbred to APPPS1 mice and resulting offspring heterozygous for Cx3cr1GFP and/or for the APPPS1 transgene was used for imaging experiments. Cx3cr1GFP/eGFP mice were kindly provided by Frank Kirchhoff, University of Saarland, Germany. All mice were kept under standard housing conditions with a 12 h light/dark cycle and access to standard food and water *ad libitum*. Throughout this study transgene-negative littermates were used as controls except when using APP23 mice, where age-matched wildtype animals served as controls. Female and male mice were included in the experiments in an about equal distribution.

**Primary Microglial Cell Culture**

Microglial cultures were prepared from cerebral cortex of 1–3 day old APPPS1 and wildtype mice as previously described [24] and cultured in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. After establishment of an astrocytic monolayer, medium was additionally supplemented with 30% L929-conditioned DMEM (M-CSF-secreting mouse fibroblast cell line) to stimulate microglial proliferation. After separating microglia from cell. The percentage of cells in each group was multiplied by the coverage of respective brain area (cortex, hippocampus, cerebellum) by Thiazine Red-positive plaques was quantified by scanning cerebral sections with a Nikon Ti Epsilon microscope using a TRITC FL-filter set and fixed acquisition settings. Large images of whole coronal brain section were obtained by stitching single images using NIS Elements software. Coverage of respective brain area (cortex, hippocampus, cerebellum) by Thiazine Red-positive plaques was quantified by analyzing images using Image J with a fixed intensity threshold. Staining artifacts were manually removed prior to analysis. 3–4

**Confocal Microscopy and Quantification of Phagocytosis**

Confocal laser scanning microscopy was performed on Leica SPE and ZEISS LSM5 Exciter confocal microscopes with LAS AF and ZEN 2008 software, respectively. In brain sections derived from acute brain slices z-stacks of 20 μm thickness were performed using a 40x objective with a step size of 1 μm beginning from the top of the slice, where the microspheres are located. Beads per cell were counted using Image J MacBiophotonics cell counter plugin ensuring that only beads inside a cell were counted as positive. The phagocytic index was determined by assessing the percentage of cells which contained 0, 1–4, 5–7, 8–10 and >10 microspheres per cell. The percentage of cells in each group was multiplied by the corresponding grade of phagocytosis (1–4: 1–4; 5–7: 5–7; 8–10: 8–10; >10: >10). The sum of the products in each group was then termed and displayed as phagocytic index [25]. 4–15 ROIs (i.e. fields of view) were analyzed per animal.

**Plaque Load Assessment**

Brain sections were incubated in 0.001% Thiazine Red (Sigma) solution in 0.1 M PB, thereby labeling the β-sheet structure of dense core plaques [26]. Thiazine Red-positive plaques were quantified by scanning cerebral sections with a Nikon Ti Epsilion microscope using a TRITC FL-filter set and fixed acquisition settings. Large images of whole coronal brain section were obtained by stitching single images using NIS Elements software. Coverage of respective brain area (cortex, hippocampus, cerebellum) by Thiazine Red-positive plaques was quantified by analyzing images using Image J with a fixed intensity threshold. Staining artifacts were manually removed prior to analysis.
whole coronal brain sections were analyzed per animal and respective brain region.

**Two-photon Imaging of Acute Brain Slices**

300 µm thick acute coronal slices were prepared from 10 month old mice (3 mice per genotype) and stained with 0.001 % Thiazine Red in aCSF for 10 min before imaging experiment. Imaging was performed with a two-photon laser scanning microscope directly coupled to a Chameleon ultrafast laser (Coherent). In order to create a discrete laser lesion, the laser was focused at −55 µm depths from the surface of the z-stack at a wavelength of 800 nm until autofluorescence was visible. In **APPPS1-Cx3cr1+/gfp** mice laser lesions were placed next to an Aβ plaque with a maximum distance to a neighboring Thiazine Red-positive plaque of 80 µm. For monitoring microglial responses 60 µm z-stacks were imaged with a step size of 3 µm covering a field of 307.2×307.2 µm every minute for 60 min. Recordings were analyzed with Image J, as adapted from Davalos et al. 2005 [2]. For calculating the average microglial response 7 ( **Cx3cr1+/gfp** ) or 8 ( **APPPS1-Cx3cr1+/gfp** ) ROIs from 4 mice per genotype were analyzed.

**In vivo Imaging**

For intravital imaging, β-amyloid plaques were labeled with methoxy-XO4 (10 mg/kg) as described [27]. Methoxy-XO4 was kindly provided by William Klunk, University of Pittsburgh, USA. Before surgery mice were anesthetized by i.p. injection of a xylazine and ketamine mix in physiological saline (9.2 and 131 mg/kg body weight respectively). At approximately the center of the parietal bone a small cranial window with a 1.5–2 mm diameter (corresponding to an area of 1.8–3 mm²) was prepared using a high-speed dental drill. A circular bone fragment was carefully removed and the dura mater was left intact. Focal lesion and time-lapse recording was started 30 min after the surgical procedure to ensure absence of bleeding. All **in vivo** recordings were made in a time interval between 30 min and 8 h after the surgical procedure. The skull was fixed to the microscope stage and aCSF was placed into the chamber to allow 30 min and 8 h after the surgical procedure. The skull was fixed to the microscope stage and aCSF was placed into the chamber to allow imaging using a water-immersion objective (20x, NA 0.95, WD 2 mm; Olympus, Germany). Imaging was performed by a commercially available multiphoton imaging system (TiM Scope I, LaVision BioTec, Germany), equipped with a Ti:Sa laser (Chameleon Ultra II, Coherent). Fluorescence (GFP excitation wavelength 920 nm, methoxy-XO4 excitation wavelength 800 nm) was collected by using three non-descanned PMT-detectors using dichroic mirrors and three interference filters (593/40 nm, 525/50 nm and 447/40 nm). XYZ-Stacks were collected every minute with a z-plane distance of three non-descanned PMT-detectors using dichroic mirrors and methoxy-XO4 excitation wavelength 800 nm) was collected by Coherent. Fluorescence (GFP excitation wavelength 920 nm, BioTec, Germany), equipped with a Ti:Sa laser (Chameleon Ultra II, Coherent). In order to create a discrete laser lesion, the laser was focused at −55 µm depths from the surface of the z-stack at a wavelength of 800 nm until autofluorescence was visible. In **APPPS1-Cx3cr1+/gfp** mice laser lesions were placed next to an Aβ plaque with a maximum distance to a neighboring Thiazine Red-positive plaque of 80 µm. For monitoring microglial responses 60 µm z-stacks were imaged with a step size of 3 µm covering a field of 307.2×307.2 µm every minute for 60 min. Recordings were analyzed with Image J, as adapted from Davalos et al. 2005 [2]. For calculating the average microglial response 7 ( **Cx3cr1+/gfp** ) or 8 ( **APPPS1-Cx3cr1+/gfp** ) ROIs from 4 mice per genotype were analyzed.

**Statistical Analysis**

Data sets were tested for normality by Shapiro-Wilks Test using SPSS. Two-sided levels of significance were determined by using the non-parametric Mann-Whitney-U- Test or the parametric T-Test according to the distribution and are depicted as *p<0.05, **p<0.01, ***p<0.001. Data are presented as mean ± s.e.m. For statistical analyses matching regions of interest (ROI) of multiple acute brain slices or cells cultures derived from various mice per experimental group were assessed as indicated and analyzed in a standardized fashion.

**Results**

**Directed Microglial Process Motility Towards Acute Tissue Lesion is Impaired in Transgenic AD Mice**

Microglia respond to defined tissue injuries by extending their processes towards the lesion in an ATP-dependent fashion [2]. We used this canonical function of microglia as a measure to evaluate their functionality in an AD mouse model **in vivo** by intravital time-lapse two-photon microscopy. Notably, the ability of microglia for directed extension of their processes towards a lesion was largely reduced in Aβ plaque carrying **APPPS1-Cx3cr1+/gfp** mice (Fig. 1A and B and Videos S1 and S2), which were obtained by crossbreeding **APPPS1** mice, a mouse model of cerebral amyloidosis [21] with **Cx3cr1+/gfp** mice [23], a mouse model that allows visualization of microglia. Aβ plaques were labeled by intraperitoneal injection of Methoxy-XO4 [27]. Whereas microglia from 8 month old **Cx3cr1+/gfp** control animals moved their processes towards the laser-induced injury with an average peak response of 2.44±0.6 arbitrary units (a.u.), we only detected a sparse response of microglia in 8 month old **APPPS1-Cx3cr1+/gfp** mice, which harbor a substantial Aβ burden at that age (average peak response of 0.44±0.22 a.u., Fig. 1A and B). Since **in situ** experiments using acute cerebral slices allow stringent quantification of microglial response in a high number of experiments, we additionally conducted two-photon microscopy studies in acute coronal cerebral slices. Here, microglia exhibited slightly slower but otherwise similar process motility towards the lesion as compared to **in vivo** experiments. Importantly, microglial response towards the laser lesion in acute cerebral slices from 10 month old Aβ plaque-carrying **APPPS1-Cx3cr1+/gfp** mice were significantly reduced compared to their **Cx3cr1+/gfp** littermates (Fig. 1C and D, Videos S3 and S4). Together, these data indicate that lesion-directed process extension as a typical functional feature of microglia is severely impaired in **APPPS1** mice that harbor Aβ plaques.

**Phagocytic Activity of Cortical Microglia is Impaired in **APPPS1** and **APPP23** Mice**

We next aimed to investigate another key function of microglia - their phagocytic capacity. Since directed microglial process motility was similarly impaired when assessed intravittally or in acute cerebral slices derived from **APPPS1** mice, we reasoned that defined microglial functions are adequately reflected in acute cerebral slice preparations. As microglia in different brain regions, including cerebellum and hippocampus, can be readily evaluated with this **in situ** method, we used cerebral slice preparations to investigate microglial phagocytic capacity and quantified uptake of fluorescent polystyrene microparticles by microglia of **APPPS1** mice as described previously [25]. Likewise to the impaired directed process extension, microglial phagocytic activity in 9 month old **APPPS1** mice was significantly reduced when compared to wildtype littermates (Fig. 2A).

Mutations of PS1 have been suggested to influence the phagocytic activity of cells [28,29]. To prove that functional decline of microglia is a general feature of AD pathology, and to exclude an impact of the PS1 transgene on microglia in **APPPS1** mice unrelated to amyloid pathology, we assessed microglial phagocytic activity in **APPP23** mice, a transgenic mouse model of cerebral amyloidosis that is based on transgenic expression of APP with the Swedish mutation [22], but not of mutated PS1. Again, using **in situ** quantification of microglial phagocytosis, we detected a drastic impairment of microglial phagocytic activity in **APPP23** mice (Fig. 2B). While we formally cannot exclude an – overall rather unlikely - impact of the APP-transgene on the performance of microglia from **APPP23** mice, these data indicate that microglial dysfunction occurs in cerebral amyloidosis mouse models irre-
spective of the transgenic strain used and, thus, is dependent on cerebral amyloidosis.

Impairment of Microglial Phagocytic Capacity Correlates Temporally and Spatially with the Appearance of Plaques

To test whether the observed impairment of microglial phagocytosis depends on Aβ plaque deposition, we compared microglial phagocytosis in 7–9 week old APPPS1 mice that had not yet developed detectable amyloid plaque burden to 7–9 week old APPPS1 mice that showed first cortical amyloid plaques (Fig. 3A and Fig. S1), as detected by 4G8 immunohistochemistry or Thiazine Red staining. Importantly, in slices prepared from 7–9 week old APPPS1 mice lacking cortical plaque load, microglial phagocytosis was as efficient as in wildtype littermates. However, we observed a significant reduction in phagocytosis in acute slices from 7–9 week old APPPS1 mice that showed first cortical amyloid plaques (Fig. 3A and Fig. S1). As a result, we observed a significant reduction in phagocytosis in acute slices from 7–9 week old APPPS1 mice that showed first cortical amyloid plaques (Fig. 3A) and APPPS1-Cx3cr1<sup>−/+</sup> mice that showed significant cortical plaque load (Fig. 3A and Fig. S1). Overall, relative microglial phagocytic activity and cortical Aβ plaque burden exhibited a significant inverse correlation with a Spearman’s correlation coefficient of \( \rho (\rho) = -0.75 \) (p = 0.0014, Fig. 3B).

To further establish the interconnection of Aβ plaque appearance and microglial dysfunction, we investigated microglial phagocytosis in various brain regions of APPPS1 mice differing in the onset of plaque deposition [21]. In plaque-bearing cortices of 7–9 week old APPPS1 mice, phagocytic activity was impaired, whereas it was normal in still plaque-free hippocampi of the same APPPS1 animals (Fig. 3C and Fig. S1). Moreover, plasmin capacity was impaired in hippocampi from 4 month old APPPS1 mice, when plaques were present in that region. Importantly, in the cerebellum, which is devoid of plaques even in aged APPPS1 mice (21) and Fig. S1, phagocytic capacity remained unaltered also at 4 months of age (Fig. 3D).

Aβ-plaque Load Reduction by Anti-Aβ Antibody Administration Restores Microglial Phagocytic Capacity in APPPS1 Mice

Finally, we investigated whether the functional impairment of microglia in plaque-bearing AD mice is reversible by interventions aimed at lowering Aβ burden. One way to counteract progressive Aβ plaque deposition is Aβ vaccination resulting in reduced plaque burden and increased number of Aβ-laden microglia in the cortex of AD patients [30]. Additionally, clearance of amyloid...
Figure 2. Phagocytic capacity of cortical microglia is impaired in two mouse models of cerebral amyloidosis. (A) Representative images (left) and microglial phagocytic index (in arbitrary units, a.u., right) of 9 month old APPPS1 mice and wildtype littermate controls (3 mice per genotype; p<0.001). Images show microglia (Iba-1, red), Aβ (4G8, blue) and fluorescent microspheres (green). Orthogonal views of z-stack images are shown in the bottom panel. (**p<0.01, right) of 20 month old mice per genotype; p<0.001). Images show microglia (Iba-1, red), Aβ (4G8, blue) and fluorescent microspheres (green). Orthogonal views of z-stack images are shown in the bottom panel. (B) Representative images (left) and microglial phagocytic index (in arbitrary units, a.u., right) of 3 month old APP23 and age-matched control mice (3 mice per genotype, p<0.001, right) are shown. Data are mean±s.e.m. Scale bars: 10 μm.

deposits and increase in microglial response was observed by in vivo imaging after stimulation with Aβ-specific antibodies when administered directly to an open skull preparation in a mouse model of AD [31].

Amyloid plaque burden was therefore reduced in 5 month old plaque-carrying APPPS1 mice by passive Aβ vaccination administering the anti-Aβ antibody Ab9 [32] biweekly for six weeks. This treatment significantly diminished Thiazine Red-stained dense core plaques in the hippocampus of APPPS1 mice, while there was no significant reduction of amyloid in the cortex when compared to control IgG-treated APPPS1 littermates [Fig. 4A left, 4A left]. Strikingly, in accordance with the regional reduction in cerebral amyloid plaques, microglial phagocytic activity, measured in acute cerebral slices, was restored to levels found in Ab9- or IgG-treated wildtype mice exclusively in the hippocampus [Fig. 4B right]. In contrast, microglial cells in the cortex, which lacked a substantial decrease in amyloid plaque burden, were still dysfunctional [Fig. 4A right]. Thus, microglial dysfunction is not irreversibly impaired by Aβ plaque deposition, but can be restored during the course of disease.

Discussion

The Aβ plaque-associated functional decline of microglia we have shown here may explain why temporary ablation of microglia for up to 30 days in APPPS1 mice does not change the formation and maintenance of Aβ burden [19]. Our findings may also give an explanation as to why microglia fail to sufficiently remove Aβ plaques in AD in vivo [18,20], and would support the notion of a dystrophic rather than an activated phenotype of microglia at late stages of AD [33].

Our methodological approach to quantify phagocytosis extends existing data on the function of AD-associated microglia during disease progression. It allowed us to overcome restrictions of current approaches including (i) the ex vivo analysis of isolated microglia whose functionality could be disturbed by the isolation procedure per se, (ii) post-mortem studies, which are stationary, typically restricted to defined time points, and do not provide direct information about cellular functionality or (iii) in vivo imaging studies that are typically limited to superficial cortical brain areas.

Mechanistically, Aβ may directly affect microglial function, as we were able to detect a significant inverse correlation between Aβ plaque burden and microglial phagocytic activity. Further studies will be required to address the question, of what kind of Aβ species may be responsible for inducing microglial dysfunction.

A large number of in vivo studies have shown that microglial cells release a battery of proinflammatory mediators, including nitric oxide and tumor necrosis factor alpha (TNFα), when stimulated with Aβ peptides [34–36]. Such chronic production of inflammatory molecules by microglia and/or constant exposure to a proinflammatory microenvironment within plaque-bearing AD brains may present another explanation of why microglial function may be influenced negatively [15,37,38]. This hypothesis is also supported by the finding that microglia stimulation with the proinflammatory cytokine TNFα leads to a downregulation of receptors involved in Aβ binding and degradation and reduces phagocytosis of Aβ in vivo [20].

To investigate a second biologically relevant microglial function we studied process motility by assessing process extensions towards an acute injury induced by a laser lesion [1,2]. Our results obtained from in vivo observations were similar to data retrieved by us in acute brain slices with respect to speed and quantity of lesion-directed process movements. The significant decrease in the amount of microglial processes sent towards the injury site, which we consistently found both in vivo and in situ, supports a report on impaired baseline dynamics of plaque-associated microglia [27]. However, another detailed analysis of baseline microglial process turnover in APPPS1 mice has shown that turnover speed of processes in microglia near plaques (within 50 μm from the plaque surface) was similar compared to microglia in non-transgenic animals. Only process speed in microglia directly on Aβ plaques was slightly but significantly reduced [9].

Notably, we show that functional impairment of microglial phagocytosis can be reversed to non-diseased aptitude by reducing amyloid by Aβ vaccination. This speaks against microglial senescence as the main underlying mechanism for the decline in microglial function demonstrated herein. Microglial senescence has been described in the human brain during aging and in Alzheimer’s disease and is associated with telomere shortening and reduced telomerase activity [39]. In contrast to the microglial phenotype reported here, cellular senescence is irreversible, at least in lymphocytes and at late stages of cellular senescence [40]. However, similar mechanisms might be involved in inducing microglial dysfunction and have to be investigated in future...
studies. Furthermore, microglial senescence may play an important role in humans during aging.

These data highlight the interrelation of plaque deposition and microglial behavior, thus expanding the possible modes of action of other amyloid-reducing approaches to rescuing microglial function. Such view is in line with a recent report on bexarotene-mediated reduction of \( \text{A}\beta \) burden, which resulted in an improvement in neuronal function and, importantly, increased the number of \( \text{A}\beta \)-laden microglia [41].

Taken together, our data demonstrate that microglial dysfunction develops early in the course of AD in an \( \text{A}\beta \)-dependent fashion and can be restored by interventional anti-\( \text{A}\beta \) approaches, such as \( \text{A}\beta \) vaccination. Our findings therefore suggest that novel treatment strategies aimed at maintaining or increasing microglial

**Figure 3.** Impairment of microglial phagocytosis in \( \text{APPSS1} \) mice correlates with \( \text{A}\beta \) plaque deposition. (A) \( \text{A}\beta \) plaque load (brain area covered by Thiazine red-positive plaques) and relative microglial phagocytic activity normalized to corresponding wildtype littermate in the cortex of 7–9 week, 4 and 9 month old \( \text{APPSS1} \) mice. 7–9 week old mice were sub-classified according to apparent 4G8 positive plaque deposition as with (+) or without (−) detectable plaque load. (B) Correlation between extent of plaque load and relative microglial phagocytic activity in the cortex of \( \text{APPSS1} \) mice. (C, D) Thiazine red-covered area and relative phagocytic activity of microglia in the hippocampus of 7–9 week and 4 month old mice (C) and in the cerebellum of 4 month old \( \text{APPSS1} \) mice (D). Absolute values of microglial phagocytic indices from \( \text{APPSS1} \) mice were normalized to wildtype littermate controls (3–4 mice per age group and genotype, **\( p < 0.001 \)). (E) Phagocytic index (3 independent experiments, \( p = 0.181 \)) and representative images of primary microglial cultures from wildtype and \( \text{APPSS1} \) mice. Microglia (Iba-1, red), nuclei (DRAQ5, blue) and microspheres (green). All data are mean ± s.e.m, *\( p < 0.05 \), **\( p < 0.01 \). a.u. = arbitrary units. Scale bars: 10 \( \mu \)m.

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function may represent an attractive therapeutic approach even at advanced stages of AD.

Supporting Information

Figure S1 Age- and brain area-dependent Aβ plaque load in APPPS1 mice. Representative confocal images of fixed and stained acute coronal brain slices from APPPS1 mice of the indicated age showing cortex (a), hippocampus (b) or cerebellum (c). Microglia (Iba-1, red), Aβ plaques (Thiazine Red, green) and nuclei (Hoechst 33258; blue). Scale bars: 50 μm.

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