1.1 Supplementary material

Results

Behavioural tests

Open field (OF)

We used the OF to measure the effect of the AD-like pathology on explorative and anxiety-related behavior. In the open field, locomotor activity (walk distance & walk velocity) and active exploration parameters (walking, sitting, wall leaning, rearing) and grooming were scored for 30 minutes. AβPP/PS1 mice seemed to be more active in the OF than their WT littermates. AβPP/PS1 mice walked more (*Fig. 1C*, $F(1,15)=4.0$, $p<0.063$) and sat less (*Fig. 1C*, $F(1,15)=3.6$, $p<0.078$) than their WT littermates. This resulted in a larger distance walked (*Fig. 1D*, $F(1,15)=3.5$, $p<0.080$) and increased walk velocity (*Fig. 1F*, $F(1,15)=3.7$, $p<0.074$) in our AD model mice than in their non-transgenic controls. AβPP/PS1 mice exhibited more explorative behaviour against the walls of the open field like wall leaning (*Fig. 1C*, $F(1,15)=5.5$, $p<0.033$) than their WT littermates. Both AβPP/PS1 and WT mice spent more time in both corners (*Fig. 1E*, $F(1,30)=220.6$, $p<0.001$) and periphery (*Fig. 1E*, $F(1,30)=285.0$, $p<0.001$) than in the center of the open field. Especially, the AβPP/PS1 mice (*Fig. 1E*, $F(1,12)=5.4$, $p<0.039$) stayed longer in the corners than in the periphery indicating an increased anxiety in these transgenic mice.

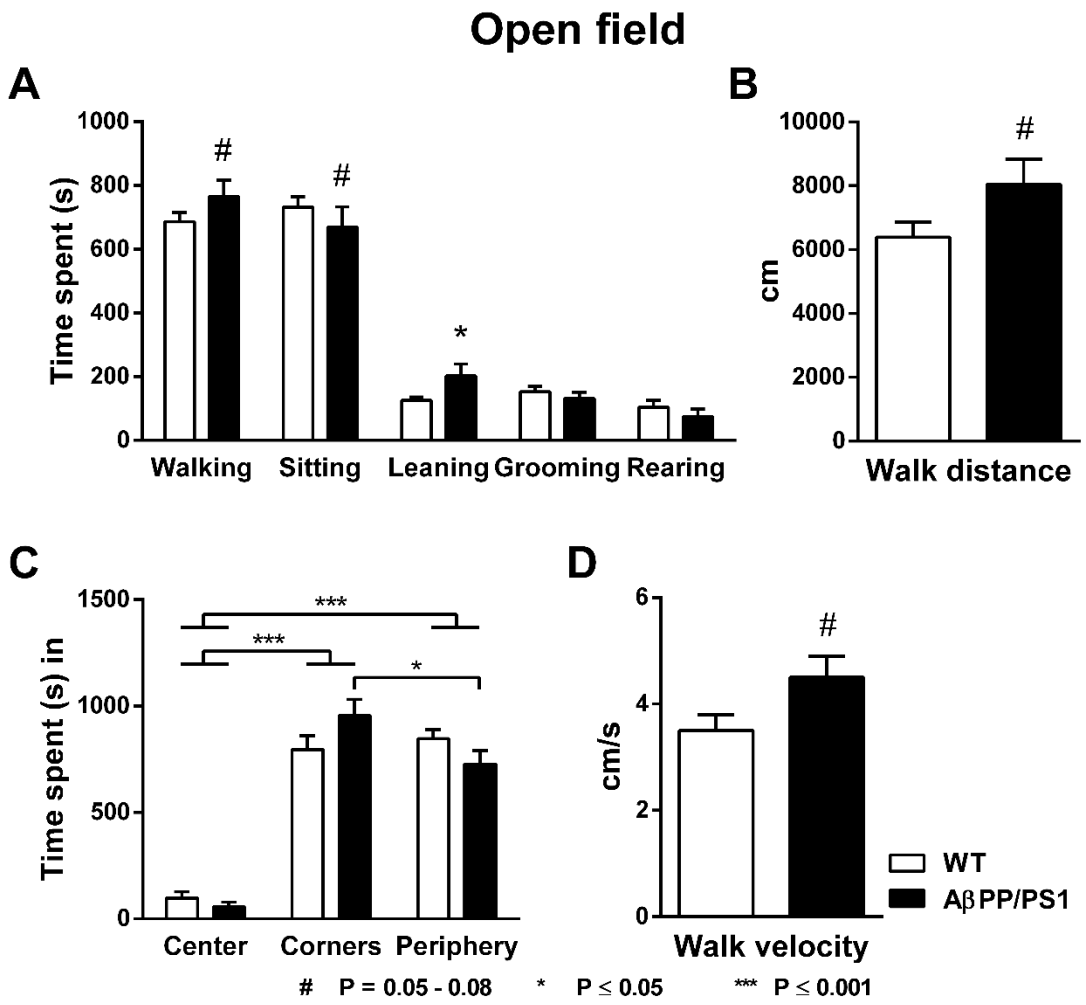

Figure 1 Explorative and anxiety-related behavior, and locomotor activity (C-F) was measured in the open field (OF) in 17-month-old AβPP/PS1 and wild-type (WT) mice. (A) In the OF AβPP/PS1 mice walked more ($p<0.063$) and sat less ($p<0.078$) than their WT littermates. AβPP/PS1 mice exhibited more wall leaning ($p<0.033$) than their WT littermates. (B+D) An increased walk distance ($p<0.080$) and walk velocity ($p<0.074$) was found in our AD model mice. (C) Both AβPP/PS1 and WT mice spent more time in both corners ($p<0.001$) and periphery ($p<0.001$) than in the center of the open field. Notably, only AβPP/PS1 mice ($p<0.039$) stayed longer in the corners than in the periphery indicating an increased anxiety in these transgenic mice.

Morris water maze (MWM)

We used the MWM to investigate the impact of the AD-like pathology on spatial learning abilities. During the acquisition phase AβPP/PS1 mice demonstrated a longer latency time to reach the hidden platform than their WT littermates (*Fig. 2A*, $F(1,15)=8.6$, $p<0.011$). Moreover, these AD mice swam a larger distance than their WT littermates to find the hidden platform during acquisition (*Fig. 2B*, $F(1,15)=5.5$, $p<0.033$), while only at acquisition days 2 and 3 the AD model mice swam faster than their WT littermates (*Fig. 2C*, Day 2: $F(1,15)=3.6$, $p<0.078$; Day 3: $F(1,15)=7.1$, $p<0.018$).

During the probe phase no significant genotype differences were found in the mean number of platform area crossings (*Fig. 2F*, F(1,15)=1.1, p<0.310), the swim distance (*Fig. 2D*, F(1,15)=0.2, p<0.677) and the swim velocity (*Fig. 2E*, F(1,15)=0.2, p<0.684).

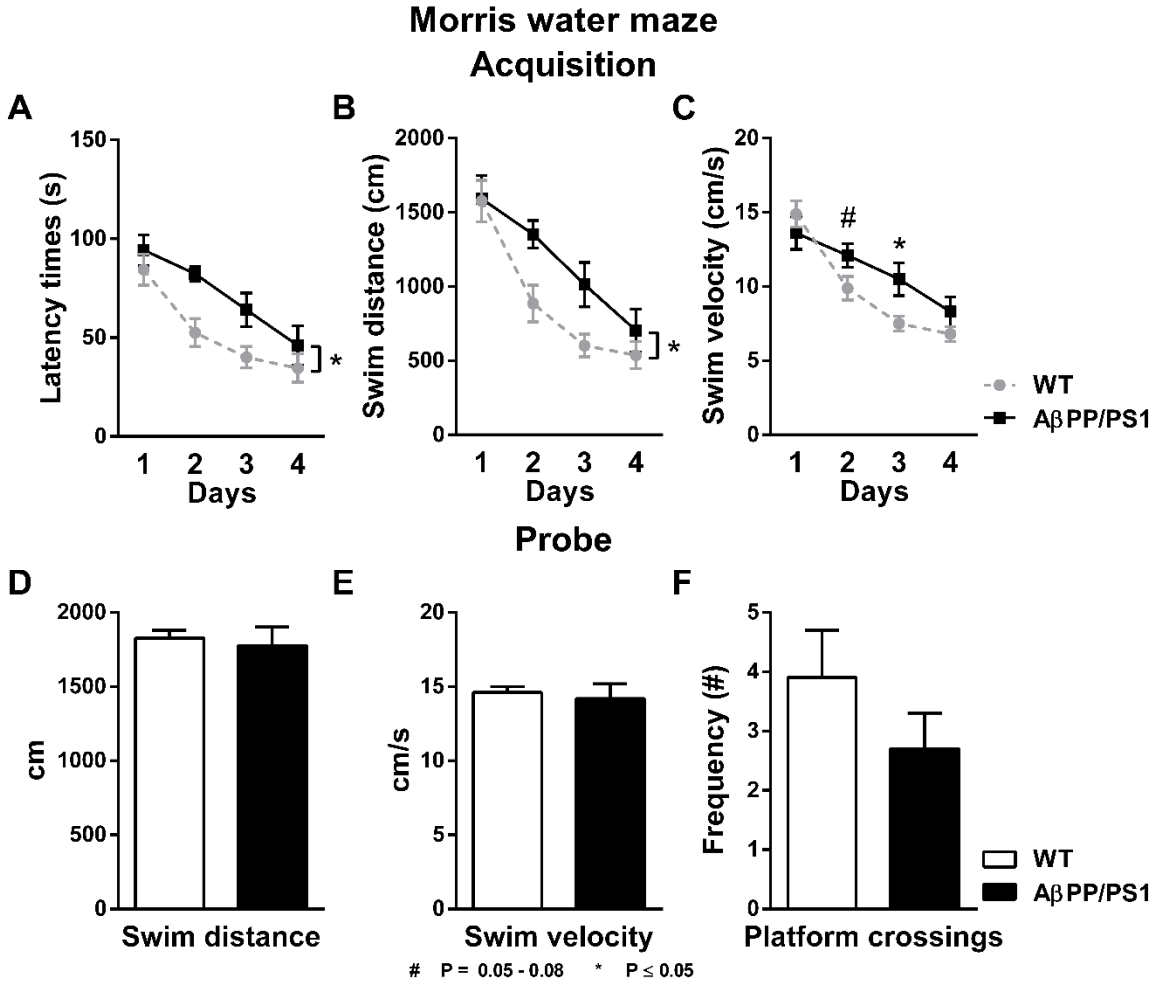

Figure 2 Morris water maze learning and memory in 18-month-old AβPP/PS1 and wild-type (WT) mice. (A) Determining the latency to find a hidden platform in the North-East (NE) quadrant in a 4-day acquisition phase, spatial learning was measured. (A) During all four acquisition days, AβPP/PS1 mice reached the hidden platform slower than their WT littermates (p<0.011). (B) AβPP/PS1 mice showed a larger swim distance during all acquisition days compared to their WT littermates (p<0.033). (C) During the second (p<0.078) and third (p<0.018) acquisition day, all AβPP/PS1 mice swam faster than their WT littermates. (D+E+F) Spatial memory was tested in the probe phase measuring the frequency of crossing the former platform location. During the probe phase no significant genotype differences were found in the frequency crossing the platform area, the swim distance and swim velocity.

Immunohistochemical procedures

PSD-95

Postsynaptic density (PSD) was stained with a polyclonal antibody against PSD-95 reflecting synaptic function. PSD was measured in the visual (V1) and somatosensory cortex (SSC), and in several hippocampal subregions: cornu ammonis (CA)2, stratum lucidum (SL) of CA3, stratum radiatum (SR) of CA1, outer molecular layer (OML) of the dentate gyrus (DG), and inner molecular layer (IML) of DG (Fig. 3A+B). In the V1, SSC, CA2, SL of CA3, and IML of DG no genotype effects were found. In the SR of the CA1 ($F(1,14)=5.1$, $p<0.041$) and OML of the DG ($F(1,14)=16.5$, $p<0.002$), AβPP/PS1 mice had less PSD95+area than their WT littermates revealing a decreased PSD in these transgenic mice.

DCX

Immature neurons were visualized in all mice with a polyclonal antibody against doublecortin (DCX). As a measure for neurogenesis, DCX+ cells were counted in the subgranular zone of the dentate gyrus (Fig. 3C+D). In the hippocampus of AβPP/PS1 mice less DCX+ neurons were counted than in the hippocampus of their WT littermates ($F(1,15)=7.3$, $p<0.017$) indicating a decreased neurogenesis in these AD model mice.

IBA-1

Brain sections of all mice were immunohistochemically stained against ionized calcium-binding adapter molecule 1 (IBA-1). IBA-1 is a marker for active and resting microglia, but also a marker for phagocytes in general (monocytes and macrophages). Here, we measured the relative area of the total section area being stained for IBA-1 (for results see the supplementary material) and the number of IBA-1+ cells in the cortex, hippocampus, and thalamus (Fig. 3E-G). Only for the number of IBA-1+ cells in the cortex a genotype effect was found. In detail, AβPP/PS1 mice had more IBA-1+-cells in the cortex (Fig. 3F, $F(1,15)=6.5$, $p<0.023$) than their WT littermates revealing an increased inflammation in these transgenic mice. No results were detected for the relative area of the total section area being stained for IBA-1 (Fig. 3G).

GLUT-1

All brains were processed for immunohistochemical staining with glucose transporter-1 (GLUT-1, *Fig. 3H-J*) antibody as well. In order to reveal the changes in total amount of GLUT-1, we measured the relative area of the total section area being stained for GLUT-1 in the cortex, hippocampus, and thalamus (*Fig. 3J*). Furthermore, we also measured vascular density represented by the number of GLUT-1+ blood vessels (*Fig. 3I*). For both amount of GLUT-1 and vascular density, no genotype effects were found.

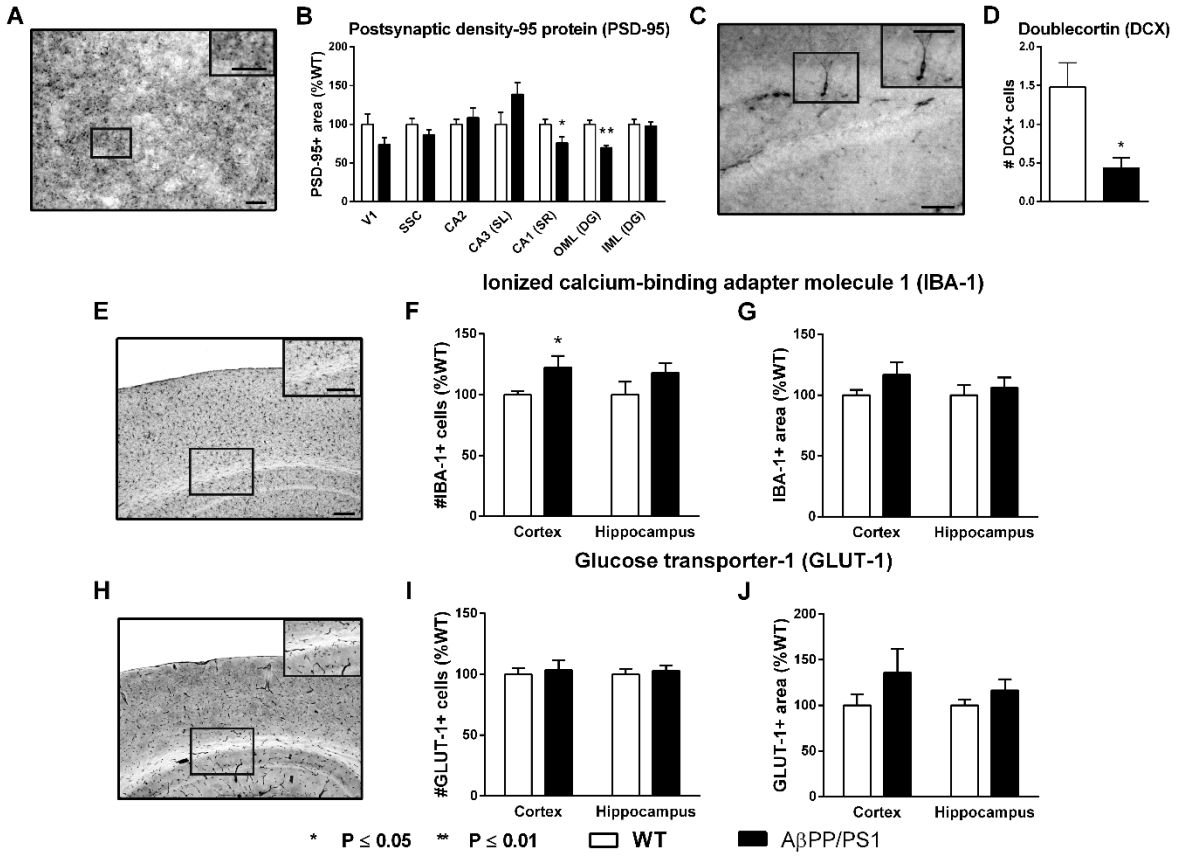

Figure 3 Immunohistochemical stainings for Postsynaptic Density-95 Protein (PSD-95, A+B), for doublecortin (DCX, C+D), for ionized calcium-binding adapter molecule 1 (IBA-1, E-G), and for glucose transporter-1 (GLUT-1, H-J) performed on brains of 18-Month-old AβPP/PS1 and wild-type (WT) mice. (A+B) PSD-95 was measured in the visual (V1, representative photo + magnified photo A, scale bar = 10µm) and somatosensory cortex (SSC), and in several hippocampal subregions: cornu ammonis (CA)2, stratum lucidum (SL) of CA3, stratum radiatum (SR) of CA1, outer molecular layer (OML) of the dentate gyrus (DG), and inner molecular layer (IML) of DG. In the SR of the CA1 ($p<0.041$) and OML of the DG ($p<0.002$), AβPP/PS1 mice had less PSD95+-area than their WT littermates revealing a decreased PSD in these transgenic mice. (C+D) In the hippocampus (DG, representative photo + magnified photo C, scale bar = 50µm), AβPP/PS1 mice had less DCX+ neurons than their WT littermates ($p<0.017$) indicating a decreased neurogenesis in these AD model mice. (E-G) IBA-1 is specifically expressed in activated microglia. Here, we measured the number of IBA-1+ cells (F) and the relative area of the total section area being stained for IBA-1 (G) in the cortex, hippocampus, and thalamus (representative photo + magnified photo E, scale bar = 200µm). AβPP/PS1 mice had more IBA-1+-cells in the cortex ($p<0.023$) than their WT littermates revealing an increased inflammation in these transgenic mice. In contrast, for the relative area of the total section area being stained for IBA-1 no genotype effects were detected. (H-J) We measured the vascular density via the number of GLUT-1+ blood vessels (I) and the total amount of GLUT-1 being stained for GLUT-1 (J) in the cortex, hippocampus, and thalamus (representative photo + magnified photo C, scale bar = 200µm). For both, amount of GLUT-1 and vascular density no genotype effects were found.

Table 1 Relative brain fatty acid content represented in average ± SEM for each experimental group. (Used abbreviations: PA=Palmitic acid; SA=Stearic acid; SFA=Saturated fatty acid; OA=Oleic acid; MUFA=Mono-unsaturated fatty acid; AA=Arachidonic acid; Ω-6=Omega-6; DHA=Docosahexaenoic acid; Ω-3=Omega-3; Ω 3/6=Omega 3/6); # P = 0.05 - 0.08, * P ≤ 0.05, ** P ≤ 0.05

Brain fatty acid content										
	PA	**SA**	**SFA**	**OA**	**MUFA**	**AA**	**Ω-6**	**DHA**	**Ω-3**	**Ω 3/6**
Genotype	**Relative fatty acid content (%)**									**Ratio**
WT	24,3±0,2	18,1±0,2	46,5±0,1	14,2±0,1	21,4±0,1	9,6±0,0	14,0±0,1	17,3±0,1	18,1±0,1	1,3±0,0
AβPP/PS1	23,8±0,3	18,7±0,3 *	46,6±0,2	14,4±0,2	21,8±0,3	9,8±0,1 *	14,3±0,2 #	16,6±0,3 **	17,3±0,3 **	1,2±0,0 *

Table 2 Plasma sterol levels represented in average ± SEM for each experimental group.

Plasma sterol levels																
	Cholestanol	**Lathosterol**	**Campesterol**	**Campestanol**	**Stigmasterol**	**Sitosterol**	**Sitostanol**	**Avenasterol**	**Brassicasterol**	**Lanosterol**	**Desmosterol**	**Dihydro-Lanosterol**	**24OH Cholesterol**	**7aOH Cholesterol**	**27OH Cholesterol**	**Cholesterol (GC)**
Genotype	**[mg/dl]**	**[mg/dl]**	**[mg/dl]**	**[µg/dl]**	**[µg/dl]**	**[mg/dl]**	**[µg/dl]**	**1000*Ratio(EPI)**	**[µg/dl]**	**[µg/dl]**	**[mg/dl]**	**[µg/dl]**	**[ng/ml]**	**[ng/ml]**	**[ng/ml]**	**[mg/dl]**
WT	2,2±0,4	0,0±0,0	3,5±0,2	56,4±7,5	19,7±1,9	1,4±0,1	16,5±2,7	4,2±0,4	10,6±1,1	24,6±6,6	0,1±0,0	1,4±0,2	46,3±2,2	14,1±4,5	77,5±6,5	185,2±13,3
AβPP/PS1	1,9±0,3	0,0±0,0	3,1±0,5	44,7±8,9	16,5±0,7	1,2±0,2	12,1±1,6	3,1±0,5	9,6±1,6	17,4±1,9	0,1±0,0	1,2±0,1	40,4±2,7	8,2±0,8	66,3±8,0	176,6±27,9

Table 3 Brain sterol levels represented in average ± SEM for each experimental group. * P ≤ 0.05

Brain sterol levels											
	Cholestanol	**Lathosterol**	**Campesterol**	**Stigmasterin**	**Sitosterol**	**Lanosterol**	**Dihydro -Lanosterol**	**Desmosterol**	**24OH Cholesterol**	**27OH Cholesterol**	**Cholesterol (GC)**
Genotype	**[ng/mg]**	**[ng/mg]**	**[ng/mg]**	**[ng/mg]**	**[ng/mg]**	**[ng/mg]**	**[ng/mg]**	**[ng/mg]**	**[µg/mg]**	**[ng/mg]**	**[µg/mg]**
WT	361,6±14,1	77,0±2,3	187,4±14,5	2,3±0,1	32,4±2,3	22,5±1,4	0,2±0,0	156,1±6,1	0,1±0,0	2,0±0,2	84,5±2,4
AβPP/PS1	377,6±10,3	76,5±2,4	195,3±10,3	2,3±0,1	31,7±1,5	21,1±1,0	0,3±0,0 *	151,4±8,1	0,1±0,0	2,0±0,0	84,3±2,0