Supplemental data

Files in this Data Supplement:

- supplemental material - S3. Table Summarizing the Memory Deficits and Emotional Alterations in BACE1 Knockout Mice.
- supplemental material - S1. The effect of strain background on Morris water maze performance in 16-18 month-old mice. Distance to reach the hidden platform in A. Non-transgenic (NTG, females represent 47%) and APPswe/PS1dE9 double transgenic mice (females 40%) on congenic C57B6 background. B. NTG (females 40 %) and APPswe/PS1dE9 (females 57%) mice on F2 C57B6/129sv background. An analysis of the effect of strain background in a three-way ANOVA (background x genotype x day) yielded significance for the effects of strain background ($F(1,70)=18.52$, $p<0.0001$), genotype ($F(1,70)=46.11$, $p<0.0001$), day of training ($F(3,210)=117.04$, $p<0.0001$) and for genotype by day interaction ($F(3,210)=12.38$, $p<0.0001$). Importantly, strain background by genotype interaction was not significant ($F(1,70)=0.45$, $p>0.51$) indicating that hybrid genetic background used in our study of 16-18 mo-old mice did not significantly affect our ability to detect genotype-related differences.
- supplemental material - S2. The effect of strain background on Morris water maze performance in 16-18 month-old mice. One of the well-known behavioral characteristics of 129sv strain that may affect the performance in the Morris Water maze is a relatively high floating time (Wolfer, Lipp, 1997). We compared Time spent floating in our cohorts of F2 C57B6/129sv mice. To test whether the genotypes used in our study differ in this behavior. Suppl. Fig. 2 shows an average floating time (%) in consecutive days of training in the Morris Water maze (hidden platform version). The same mice as in Suppl. Fig. 1 are shown plus groups of age-matched BACE1-/- (females 36%), APPswe/PS1dE9/BACE1-/- (females 50%) and APPswe/PS1dE9/BACE1+/- (females 41%). Two-way (genotype x day) ANOVA for time spent floating during probe trials revealed no significant effect of genotype ($F(4,63)=0.49$, $p>0.62$) or genotype by day interaction ($F(12,189)=1.01$, $p>0.21$). As shown in Suppl. Fig. 2, mice of different genotypes spent similar time floating. There were no significant differences between genotypes in another behavioral trait of 129sv mice, time spent in proximity to the wall (Thigmotaxis, data not shown). These data indicate that effects of 129sv alleles on behavioral phenotypes appeared to be well balanced between different subgroups.
- supplemental material - S4. Absence of Aβ and APP β-CTF Generation in Brains of APPswe;PS1ΔE9 Mice lacking BACE1. Protein extracts from brains of littermate APPswe;PS1ΔE9;BACE1+/+, APPswe;PS1ΔE9;BACE1+/-, and APPswe;PS1ΔE9;BACE1-/- mice were subjected to: (A) immunoblot analysis (50 µg each) with antisera against APP, APP-CTF, BACE1, PS1, and actin; note that APP β-CTF was detected in APPswe;PS1ΔE9;BACE1-/- mice, but significant increases of full-length APP, BACE2 and α-secretase derived APP-CTFs (X-CTF) were observed in these APPswe;PS1ΔE9;BACE1-/- animals; (B) Aβ ELISA analysis (10 µg each) and note that soluble Aβ was not detected in APPswe;PS1ΔE9;BACE1-/- mice while a significantly reduction of Aβ (31%, $n=6$, $p<0.002$, student t-test) was observed in APPswe;PS1ΔE9;BACE1+/- animals; and (C) mass spectrometric analysis (10 µg each), note that Aβ 1-40 was not detected in APPswe;PS1ΔE9;BACE1-/- mice using Ciphergen ProteinChip system coated with 6E10 antibody. Sagittal brain sections (10 µm) from 12 (panels D-I) month old APPswe;PS1ΔE9;BACE1+/+ (panels D,F and H) and APPswe;PS1ΔE9;BACE1-/- (panels E, G and I) mice were subjected to Hirano silver staining (D and E), immunostaining with 4G8 (F and G), and thioflavin staining (H and I). Note that Aβ deposition observed in APPswe;PS1ΔE9;BACE1+/+ mice was

not detected in APPswe;PS1ΔE9;BACE1-/- mice. Sagittal brain sections (10 μm) from 20 month old APPswe;PS1ΔE9;BACE1+/+ (J) and APPswe;PS1ΔE9;BACE1-/- (K) mice subject to Bielschowsky silver staining. No Aβ deposition was observed in APPswe;PS1ΔE9;BACE1-/- mice.

- supplemental material - S5. Deletion of BACE1 Prevents Neuropathology in a mouse model of Aβ Amyloidosis. Glial responses, including astrocytosis, and microgliosis, often accompany Aβ deposits in brains of individuals with AD as well as in mutant APP mice (Price and Sisodia, 1998). To confirm whether amyloid plaques are directly associated with neuritic damage, we examined the morphological changes of neurites in 12-month old APPswe;PS1ΔE9;BACE1-/- mice as compared to age-matched littermate controls (Fig. S3A and S3D). Many enlarged neurites are present adjacent to the amyloid deposits in APPswe;PS1ΔE9 mice (Fig. S3G), but there were no neuritic abnormalities in APPswe;PS1ΔE9;BACE1-/- mice lacking amyloid aggregates (Fig. S3H). Consistent with our findings that BACE1 is present in presynaptic terminals, BACE1 immunoreactivities appeared in the dystrophic neurites surrounding the Aβ deposits in APPswe;PS1ΔE9 mice (Fig. S3I); these lesions were absent in APPswe;PS1ΔE9;BACE1-/- animals (Fig. S3J). To confirm whether Aβ or its aggregates trigger glial responses, we examined the extent of microgliosis and astrocytosis in brains of 12-month old mutant APPswe;PS1ΔE9 mice with or without BACE1. Whereas APPswe;PS1ΔE9 mice showed extensive astrogliosis (Fig. S3A), we found no evidence of astrogliosis in brains of APPswe;PS1ΔE9;BACE1-/- mice (Fig. S3B) as compared to non-transgenic animals (Fig. S3C). In addition, the microglial activation, as revealed by the F4/80 immunostaining, observed in brains of APPswe;PS1ΔE9 (Fig. S3D) was also prevented in APPswe;PS1ΔE9;BACE1-/- mice (Fig. S3E). Thus, these results confirmed that the accumulation of Aβ induces microgliosis and astrocytosis in the brains of transgenic mice and deletion of BACE1 prevents both of these glial inflammatory responses.
- supplemental material - S6B. Down regulation of BACE1 mRNA by shRNA. Lentiviral vectors expressing only GFP (PLL), GFP plus SH1-BACE1, or GFP plus SH2-BACE1 were transiently transfected into N2A cells. Three days later, total RNAs were extracted and 1 μg of each sample was subjected to RT-PCR analysis with primers (5′ CTGGTGAAGCAGACCCACATTC and 5′ TGCGGAAGGACTGATTGGTGAC) for BACE1 that spanned exon 4 to exon 7. Actin was used as a positive control. Samples were analyzed after 25 cycles of PCR. S6C. Level of BACE1 protein is reduced by shRNA. Lentiviral vectors expressing only GFP (PLL), GFP plus SH1-BACE1, or GFP plus SH2-BACE1 were transiently transfected into N2A cells. Three days later, 50 μg of protein lysates from cells transfected with PLL (lanes 3-5), SH1 (lanes 6-80) and SH2 (lanes 9-11) were subjected to protein blot analysis. Lanes 1 and 2 are positive (transfected hBACE) and negative (BACE1 null fibroblast cells) controls for the specificity of the BACE1 antibody. S6D. shRNA for BACE1 inhibits Aβ secretion. Lentiviral vectors expressing only GFP (PLL), GFP plus SH1-BACE1, or GFP plus SH2-BACE1 were transiently transfected into N2A cells. 24 hours later, the cells were infected with adenoviral vector expressing APPswe. Three days after the transfection, the conditioned media were subjected to Aβ1−40 analysis by ELISA. SH1- and SH2-BACE1 inhibited secretion of Aβ1-40 by 73% and 72%, respectively.