Amyloid-β Deposition Is Associated with Decreased Hippocampal Glucose Metabolism and Spatial Memory Impairment in APP/PS1 Mice

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Abstract. In Alzheimer disease (AD) patients, early memory dysfunction is associated with glucose hypometabolism and neuronal loss in the hippocampus. Double transgenic (Tg) mice co-expressing the M146L, presenillin 1 (PS1) and K670N/M671L, the double “Swedish” amyloid precursor protein (APP) mutations, are a model of AD amyloid-β deposition (Aβ) that exhibits earlier and more profound impairments of working memory and learning than single APP mutant mice. In this study we compared performance on spatial memory tests, regional glucose metabolism, Aβ deposition, and neuronal loss in APP/PS1, PS1, and non-Tg (nTg) mice. At the age of 2 months no significant morphological and metabolic differences were detected between 3 studied genotypes. By 8 months, however, APP/PS1 mice developed selective impairment of spatial memory, which was significantly worse at 22 months and was accompanied by reduced glucose utilization in the hippocampus and a 35.8% dropout of neurons in the CA1 region. PS1 mice exhibited a similar degree of neuronal loss in CA1 but minimal memory deficit and no impairment of glucose utilization compared to nTg mice. Deficits in 22 month APP/PS1 mice were accompanied by a substantially elevated Aβ load, which rose from 2.5% ± 0.4% at 8 months to 17.4% ± 4.6%. These findings implicate Aβ or APP in the behavioral and metabolic impairments in APP/PS1 mice and the failure to compensate functionally for PS1-related hippocampal cell loss.

Key Words: Alzheimer disease; Amyloid-β; Behavioral testing; Presenilin-1; Regional brain glucose metabolism; Transgenic mice.

INTRODUCTION

Early impairment of memory and learning are the most characteristic clinical features of Alzheimer disease (AD). In humans these symptoms correspond well to glucose hypometabolism within the hippocampus, which is present even at early stages of the disease when only mild cognitive impairment is evident clinically (1, 2). Later, in fully symptomatic AD, decreased metabolism can also be detected in association areas of the cerebral cortex (3). In order to model AD pathology, transgenic (Tg) mice expressing wild type or mutated forms of the human amyloid precursor protein (APP) have been generated (4, 5). For example, a Tg line overexpressing the double “Swedish” K670N/M671L APP mutation develop Aβ deposits in the brain parenchyma and leptomeningeal vessels at the age of 9 to 11 months and demonstrate significant behavioral impairment (6). Pedigrees with familial AD related to presenilin (PS) 1 or 2 mutations exhibit increased Aβ deposition (7–9) and several Tg lines expressing mutant human PS1 were also generated, for example, PS1M146L(10) and PS1p17L(11). Although Tg mice expressing mutant human PS1 do not develop Aβ deposits, cross-breeding them with Tg mice expressing mutant APP results in the generation of a double Tg line that has an accelerated AD phenotype (9). Aβ deposits can be first detected in APPK670N/M671L/PS1M146L mice as early as 3 months of age (12), and by 6 months of age the Aβ load is comparable to that of 12-month-old single APPK670N/M671L Tg mice (13). Both APP and APP/PS1 mice demonstrate behavioral impairment that has an age-dependent emergence and progression. The most striking deficit has been documented for working memory evaluated on the Y-maze and spatial memory tested on the Morris water maze (9, 14, 15). The behavioral deficit in lines expressing PS1 mutations has not yet been analyzed in a great detail.

Although 18-month-old APPK670N/M671L or APPV717F mice show a profound learning impairment and their Aβ load may be as high as 28%, they do not have overt neuronal loss (16, 17). In the absence of neuronal loss, a possible increased density of presynaptic terminals (18) and/or Aβ related toxicity (19, 20) have been suggested as explanations for the behavioral impairment. One study of neuronal counts in APPK670N/M671L/PS1M146L mice at the age of 12 months also failed to demonstrate significant neuronal loss (21); however, this finding is at odds with the reported effect of PS1 mutations on neurogenesis and age-dependent neuronal loss in Tg mice (22, 23).

In this study we analyzed the progression of spatial memory deficit in APPK670N/M671L/PS1M146L (APP/PS1) and PS1M146L (PS1) mice up to the age of 22 months and tried to elucidate mechanisms responsible for the memory impairment. Since the hippocampus plays a pivotal role in
were estimated at the ages of 2, 8, and 22 months. A
performed on 8- and 22-month-old mice. Glucose utilization
hour light/dark cycle was maintained. Behavioral testing was
mice. Food and water were available ad libitum and a 12/12-
prevent episodes of aggression frequently observed in APP/PS1
housed in cages individually to simplify identification and to
were genotyped, labeled, and separated. All animals were
offspring were either non-transgenic (nTg), APP Tg, PS1 Tg,
from a Swiss-Webster/B6D2F1
background, whereas the M146L PS1 animals were derived
Tg2576 (K670N/M671L) line (4) with M146L PS1 mice (30).
The Tg 2576 mice were derived from a C57B6/SJL
3
Tg2576 (K670N/M671L) line (4) with M146L PS1 mice (30).

learning (24), hippocampal glucose metabolism was studied using the
14C-labeled 2-Deoxy-D-Glucose (14C-2DG) method and the absolute number of pyramidal neurons in
the CA1 sector of the cornu Ammonis was estimated with the fractionator method (25, 26). The CA1 pyramidal
neurons are the most sensitive subpopulation of hippocampal neurons to AD pathology (27). Their numbers
decline in AD, but not in normal aging, (28) and correlate with duration disease (29). The regional Aβ burden was
also evaluated.

MATERIALS AND METHODS

Animals

Double Tg animals were generated by mating the APP
Tg2576 (K670N/M671L) line (4) with M146L PS1 mice (30).
The Tg 2576 mice were derived from a C57B6/SJL × C57B6
background, whereas the M146L PS1 animals were derived from a Swiss-Webster/B6D2F1, × B6D2F1, background (14).
Offspring were either non-transgenic (nTg), APP Tg, PS1 Tg,
or expressed both transgenes. At the age of 4 weeks, animals
were genotyped, labeled, and separated. All animals were
housed in cages individually to simplify identification and to
prevent episodes of aggression frequently observed in APP/PS1
mice. Food and water were available ad libitum and a 12/12-
hour light/dark cycle was maintained. Behavioral testing was
performed on 8- and 22-month-old mice. Glucose utilization
was evaluated in 2- and 22-month-old mice. Neuronal counts
were estimated at the ages of 2, 8, and 22 months. Aβ load
was measured in 8- and 22-month-old APP/PS1 mice (Table 1).

Locomotor and Coordination Testing

Altered complex motor behavior is frequently observed in
aging and may be associated with extensive AD pathology (31).
In order to exclude locomotor and/or coordination performance
deficits as an explanation for changes in behavioral test scores,
a mouse-adapted sensorimotor task battery was administered
(32). Locomotor activity of Tg and nTg mice was assessed using
a mouse photoactometer (BRS/LVE) that consisted of a 45-
cm-diameter arena enclosed by 40-cm-high walls, covered by
a wooden lid. Movement was detected by 4 sets of photobeams
situated in the walls at 1 cm above floor level. The session
duration was 5 min and photobeam interruptions were automa-
tically recorded. Coordination was tested using the traverse
beam test. This task has been adapted for mice from a test
originally described for rats (33). Mice were trained to walk
across a narrow beam (8-mm-wide and 60-cm-long). At one
end, a 60 W lamp was positioned 20 cm above the beam and
an enclosed box measuring 15 × 5 × 5 cm was attached to the
other end (34). Mice were placed under the lamp and trained
to run to the enclosed box to escape the bright light. Latency
to cross and the number of foot slips were recorded by video
camera. Mice were given 2 trials a day for 2 consecutive days.

Learning and Memory Testing

Working memory and spatial learning was assessed using the
Morris water maze test (35–37). The mouse-adapted Morris wa-
ter maze was a circular polyethylene tank (75-cm-diameter and
13.5-cm-deep), filled with water made opaque with the addition
of a non-toxic paint. The water temperature was maintained at
32°C. A circular glass platform 7.5 cm in diameter was sub-
merged 1 cm below the water level. Prominent extra maze cues
surrounded the maze. For adaptation to the maze procedure,
mice were placed in the water inside a 9-cm-wide and 20-cm-
high channel that extended across the diameter of the pool. The
walls of the channel prevented animals from viewing the pool
during adaptation. The glass platform was placed at one end of
the channel and mice were trained to swim to the platform and
remain there for 10 seconds. Acquisition training for the hidden
platform task began 24 hours following adaptation. Mice were
placed in the water facing the wall in 1 of the 4 quadrants and
allowed to find the platform. Mice who failed to locate the
platform within 2 min were gently pushed towards it. All ani-
mals were given 5 trials a day, with each trial beginning from a
randomly selected quadrant. The inter-trial interval was 1 hour. During the interval, mice were placed on a dry towel in
a cage under a heating lamp. On the following day, the position
of the platform was changed and mice were given 5 trials to
find the platform in the new location. Mice were tested for 4
consecutive days to explore all possible locations of the plat-
form. The time to locate the platform and length of the swim
path were recorded at each trial using a SMART Video tracking
system (San Diego Instruments, San Diego, CA). Statistical
analysis was performed by a 2-way repeated measures ANOVA.

Table 1

The Number of Animals Used for Each Type of Analysis at Different Time Points

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>2 months</th>
<th>8 months</th>
<th>22 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nTg</td>
<td>PS1</td>
<td>APP/PS1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behavioral</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glucose utilization</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Aβ load</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Neuronal count</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

At the age of 2 months, the neuronal count was determined on the same animals on which glucose utilization was evaluated. At 8 months, the neuronal count was determined on 6 PSI and 6 APP/PSI animals used for behavioral studies and the Aβ load was measured on the same APP/PSI animals used for neuronal determination. At the age of 22 months, glucose utilization was evaluated on animals previously assessed behaviorally, which were later used for the determination of neuronal counts and Aβ load.
Fig. 1. Locomotor activity and sensorimotor coordination was tested at 8 and 22 months of age. Results at the age of 22 months are shown. No significant changes were noted in each of the 3 tested murine genotypes. APP/PS1 mice scored slightly worse when tested using the photoactometer (A) and had a slightly longer latency crossing the traverse beam (B); however, the differences were not statistically significant.

followed by a Tukey-Kramer means analysis in cases where the ANOVA was significant using Statistica software (version 6.1, StatSoft Inc., Tulsa, OK).

The Hebb-Williams maze is a more challenging test than the Morris water maze and is designed to examine problem solving abilities based on spatial memory (38–41). The test was performed on a rectangular field, 60-cm square with 9-cm-high walls, divided into thirty-six 9.5-cm × 9.5-cm squares and covered by a clear Plexiglas top. A goal box and a start chamber, each 11 cm × 16 cm at the base and 9-cm-high, were situated at diagonal corners of the field. Prior to testing, mice were adapted to a food restriction schedule that maintained body weight at 90% to 92% of ad libitum levels. For mice weighing 25 to 30 grams this was achieved by feeding 2.5 grams of lab chow daily after testing. Mice were given 2 adaptation sessions prior to the beginning of testing. In the first session all animals were fed in the goal box for 10 min. In session 2 they were placed in the start chamber and permitted to explore the field and to enter the goal box where half of a fruit loop cereal piece was available. When mice were running reliably from the start chamber to the goal box they were given 3 practice sessions on simple problems where 1 or 2 barriers were placed in different positions in the field to obstruct direct access to the goal box. Formal testing consisted of 6 problems graded in difficulty. One problem per day was presented and mice were given 5 trials on each problem with an inter-trial interval of 5 min. Performance was scored in terms of errors (i.e. entries and re-entries into designated error zones) and by the time to complete each trial. Statistical analysis was performed using ANOVA followed by Newman-Keuls post hoc analysis.

Analysis of Regional Brain Glucose Utilization

To identify metabolic deficiencies in Tg mice the $^{14}$C-2-deoxy-glucose ($^{14}$C-2DG) method was used, as previously described (42–44). After overnight fasting, animals received an intraperitoneal injection of 120 $\mu$Ci/kg $^{14}$C-2DG (Perkin Elmer, Wellesley, MA). To increase the injection volume, the isotope was diluted and administered in 0.4 ml of 0.9% NaCl. Mice were killed 45 min later. Brains were swiftly removed from skulls and flash-frozen in isopentane cooled to $-30^\circ$C with dry ice and stored in a freezer at $-80^\circ$C (42). Blood samples were collected, centrifuged, and the serum glucose level was assessed. Serum radioactivity was measured by adding 5 ml of scintillation fluid to 5 $\mu$l of serum and recording the number of counts emitted per minute using a Beckman-Coulter LS 6500 scintillation counter. The obtained values were converted into number of counts per minute per milliliter. Quantitative autoradiography was performed as described elsewhere (42–44). Briefly, coronal brain sections (40-μm-thick) were cut on a
Fig. 3. Results of the Hebb-Williams test at the ages of 8 and 22 months. A: A significant genotype effect (ANOVA $p = 0.004$) at the age of 8 months was observed. On post hoc analysis there was a significant difference between APP/PS1 and nTg mice ($p < 0.05$). PS1 mice scored worse than nTg mice, but better than APP/PS1 mice (non-significant trend). At the age of 22 months (B), APP/PS1 mice scored distinctly worse than either PS1 or nTg mice. No difference between PS1 and nTg mice was observed at that age.

Fig. 4. This figure shows the regional brain glucose utilization (BGU) index in 22-month-old mice. Optical density ratios were color-coded. Black and blue represent the lowest, whereas red corresponds to the highest optical density ratio. In APP/PS1 mice but not in PS1 mice, the BGU was significantly reduced in the hippocampus (*) comparing to nTg control ($p < 0.05$). A non-significant trend toward decreased BGU was also noticed in the cortex of APP/PS1 mice (arrows).
buffer. The brain was removed from the skull, cryoprotected using increasing concentrations of sucrose, and cut on a cryostat as described above. For Aβ immunohistochemistry, sections were washed 3 times in distilled water and endogenous peroxidase activity was quenched in 3% H2O2 for 30 min. Aβ deposits were detected using monoclonal antibody 6E10 at 1:1,000 dilution (49, 50), followed by an anti-mouse IgG biotinylated secondary antibody at a 1:2,000 dilution and avidin-horseradish peroxidase complex (Vector Elite staining kit, Vector Laboratories, Burlingame, CA). Sections were developed using a 3,3′-diaminobenzidine kit with or without nickel ammonium sulfate (Vector Laboratories).

Aβ Load

Aβ load (i.e., percentage of test area occupied by Aβ) was measured on 6 coronal plane sections in the cortex and in the hippocampus. They were selected 800 μm apart for the cortex and 400 μm apart for the hippocampus starting from the first randomly chosen section. All measurements were performed in the right hemisphere. The profile of the whole cortex from the corpus callosum on the medial aspect of the hemisphere to the rhinal sulcus on the lateral side was traced using the Bioquant image analysis system (R&M Biometrics Inc., Nashville, TN). Test areas (320 μm × 240 μm) were randomly selected by applying a grid (800 μm × 800 μm) over the traced contour. This gave on average 6 to 8 test areas per cross-section of the cortex and 3 to 4 areas per cross-section of the hippocampus. Images of the test areas were captured and a threshold optical density was obtained that discriminated staining from background. Objects smaller than 170 μm² (average cross-sectional area of a hippocampal pyramidal neuron plus 2 standard deviations) (23, 51) were filtered out and the total area of remaining objects was summed and divided by the area of the test field (0.0768 mm²). If needed, artifacts such as non-specific meningeal or vascular staining were eliminated manually.

Neuronal Counting

CA1 pyramidal neurons are a subpopulation of hippocampal neurons that are the most sensitive to nociceptive insult (52) and to AD pathology (27). The absolute number of CA1 pyramids was assessed using the fractionator method (25, 26) on serial hippocampal sections 200 μm apart. Using the Bioquant image analysis system, a contour of the CA1 pyramidal layer was traced and a grid (100 μm × 100 μm) was randomly applied to selected points for disector sampling. The sampling disector box was 15 μm × 15 μm at the base and 15-μm-high. Only neurons with visible nucleoli were counted by focusing a 100× oil immersion lens throughout the specimen (25, 53). Measurements were started 5 μm below the upper surface of the specimen. The thickness of each section was measured by focusing the lens throughout the whole specimen. It ranged from 25 μm to 28 μm. On average, 2 to 4 neurons per disector were counted and 60 to 80 dissectors per animal were assessed (26, 53). The coefficient of error for a single animal was less than 0.06 (26, 54, 55). Measurements of the absolute neuronal number were performed by one investigator (J.P.) blinded to the animals’ genotype. Statistical analysis between groups was performed by means of the one-tailed unpaired t-test.

RESULTS

Behavioral Analysis

Locomotor Activity: The PS1 and APP/PS1 mice did not show significant abnormalities in the locomotor activity compared to nTg mice when tested in a mouse photoactometer at the ages of 8 and 22 months (Fig. 1A). Similarly, all 3 genotypes did not differ in latency (Fig. 1B) and in the number of foot faults when tested on a traverse beam at comparable ages.

Learning and Memory Testing: Eight-month-old APP/PS1 mice showed significant impairment when tested on the Morris water maze. They scored worse than PS1 mice (p < 0.01 by ANOVA; Fig. 2A) and age-matched nTg controls (p < 0.001). PS1 mice were worse than age-matched nTg controls (p < 0.05). When animals were tested on the Morris water maze at the age of 22 months, APP/PS1 mice scored significantly worse than both PS1 and nTg mice (p < 0.05; Fig. 2B) but no significant differences between PS1 and nTg groups were noticed at this time point. Comparisons between the 8- and 22-month-old groups revealed a more significant age-related deterioration in the performance of nTg mice compared to PS1 mice, resulting in a decreasing discrepancy between these 2 groups at the age of 22 months.

A statistically significant genotype effect was seen (ANOVA p = 0.004) in 8-month-old animals tested on the Hebb-Williams maze. APP/PS1 mice made significantly more errors than nTg mice (p < 0.05). PS1 mice scored worse than nTg mice and better than APP/PS1 mice, but the differences lacked statistical significance (Fig. 3A). At the age of 22 months, differences between APP/PS1 mice and both nTg mice (p < 0.001) and PS1 mice (p < 0.001) become more distinct (Fig. 3B). At this time point, PS1 mice scored similar to nTg mice. When comparing mice performance between 8 and 22 months, a significant age effect on the Hebb-Williams maze was seen in APP/PS1 mice but not in PS1 mice or nTg mice. APP/PS1 mice made significantly more errors at the age of 22 months compared to their performance at the age of 8 months (p < 0.01).

Assessment of Brain Metabolism Using 14C-2DG

There were no significant differences in regional BGU index between APP/PS1, PS1, and nTg mice at the age of 2 months (Table 2). At the age of 22 months, the BGU index in the hippocampus of APP/PS1 mice was reduced by 26.6% compared to nTg mice (Fig. 4; p < 0.05). In subdivisions of the hippocampus, the reductions in BGU index were as follows: the dentate gyrus—25.9% (p < 0.05); the CA3 sector—25% (p < 0.05); the CA1 sector—21.6% (p = 0.067). A non-significant trend for decreased glucose metabolism was also noticed in the cortex of APP/PS1 mice. The BGU index was reduced by
18.3% in the cingulate cortex, by 14.4% in the somatosensory cortex, and by 13.3% in the motor cortex compared to the control group values. In PS1 mice the BGU index was comparable to that of nTg mice, with numerical differences not exceeding 5%.

Neuronal Counting in the CA1 Sector

Total Number of Neurons in the CA1 Sector of the Cornu Ammonis: There were 215,000 ± 28,000 neurons (mean ± standard deviation) in the CA1 sector in 2-month-old nTg mice and 195,000 ± 21,000 neurons in 22-month-old nTg mice. This 9.3% age-related dropout was not statistically significant (Fig. 5A). The total number of neurons in 2-month-old PS1 and APP/PS1 mice was calculated to be 219,000 ± 31,000 and 204,000 ± 37,000, respectively (non-significant difference vs nTg). At 8 months, PS1 mice had on average 167,000 ± 38,000 pyramidal neurons in the CA1 sector (23.7% dropout from the original number), whereas APP/PS1 mice had 157,000 ± 20,000 neurons (29.8% dropout). At 22 months of age, the total number of neurons in the CA1 sector declined further (Fig. 5B–E). In PS1 mice it was 149,000 ± 30,000 (31.9% age related dropout), whereas in APP/PS1 mice the number was 131,000 ± 27,000 (35.8% dropout). At 22 months, differences in the total number of CA1 neurons were statistically significant when comparing nTg and PS1 (p < 0.05) and nTg and APP/PS1 (p < 0.001). The difference between PS1 and APP/PS1 mice was not significant.

Amyloid-β Load

As previously reported, PS1 mice do not form Aβ deposits (10). No Aβ deposits were detected in 2-month-old APP/PS1 mice, whereas at 8-months the Aβ load was calculated to be 2.5% ± 0.4% and 2.1% ± 0.5% in the cortex and hippocampus, respectively (Fig. 6A). At 22 months the Aβ load rose to 25.2% ± 5% in the cortex and 17.4% ± 4.6% in the hippocampus (Fig. 6B).

DISCUSSION

APP and APP/PS1 mice have been generated as a model to study neurodegeneration related to Aβ deposition (4, 5). Similar to human subjects with AD, these mice demonstrate deficits in working memory, as assessed on the Y-maze and spatial memory on Morris water maze or radial arm water maze (9, 14, 56–58). Our Morris water maze results are confirmatory of previous observations. However, we were able to also show that APP/PS1 mice have a progressive impairment on the Hebb-Williams maze, which, in addition to spatial and working aspects of memory, is designed to test the ability to solve a specific problem. Impairment worsened with increasing age on the Hebb-Williams maze. Similarly, problems with recall of newly acquired information and solving specific visuo-spatial tasks has been documented in middle-stage AD patients (59–62).

Unlike APP and APP/PS1 mice, the behavior of PS1 mice has not been studied in great detail. Holcomb et al (9, 14) reported no behavioral impairment in 6- and 9-month-old PS1M146L mice. On the contrary, our 8-month-old PS1 mice scored significantly worse on the Morris water maze than age-matched nTg mice. They also had worse scores on the Hebb-Williams maze, but these were not statistically significant. At the age of 22 months, no differences between PS1 and nTg mice were noticed. This can be explained by the more profound behavioral deterioration between the ages of 8 and 22 months in nTg mice compared to PS1 Tg mice. PS1 mice were significantly better than APP/PS1 mice both at the age of 8 and 22 months.

We correlated the performance of APP/PS1 and PS1 mice with hippocampal metabolism. The hippocampus is pivotal for the integrity of memory function and spatial memory in particular (24). No metabolic impairment was found in PS1 and APP/PS1 mice at the age of 2 months. At this time point there were no differences in the total

APP/PS1 (p

TABLE 2

Regional Brain Glucose Utilization (BGU) Index in nTg, PS1, and APP/PS1 Mice at 2 Months and 22 Months

<table>
<thead>
<tr>
<th>Brain region</th>
<th>2 months (nTg)</th>
<th>2 months (PS1)</th>
<th>2 months (APP/PS1)</th>
<th>22 months (nTg)</th>
<th>22 months (PS1)</th>
<th>22 months (APP/PS1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>1.12 ± 0.36</td>
<td>1.07 ± 0.08</td>
<td>1.22 ± 0.45</td>
<td>0.79 ± 0.18</td>
<td>0.76 ± 0.14*</td>
<td>0.58 ± 0.19*</td>
</tr>
<tr>
<td>CA1</td>
<td>1.19 ± 0.38</td>
<td>1.10 ± 0.09</td>
<td>1.23 ± 0.39</td>
<td>0.74 ± 0.17</td>
<td>0.72 ± 0.10*</td>
<td>0.58 ± 0.20*</td>
</tr>
<tr>
<td>CA3</td>
<td>1.04 ± 0.34</td>
<td>0.98 ± 0.07</td>
<td>1.29 ± 0.42</td>
<td>0.72 ± 0.16</td>
<td>0.70 ± 0.11*</td>
<td>0.54 ± 0.17*</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>1.14 ± 0.38</td>
<td>0.96 ± 0.10</td>
<td>1.35 ± 0.47</td>
<td>0.77 ± 0.18</td>
<td>0.73 ± 0.14*</td>
<td>0.57 ± 0.20*</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>1.70 ± 0.37</td>
<td>1.50 ± 0.27</td>
<td>1.72 ± 0.38</td>
<td>0.87 ± 0.17</td>
<td>0.91 ± 0.19*</td>
<td>0.71 ± 0.26*</td>
</tr>
<tr>
<td>Motor cortex</td>
<td>2.02 ± 0.50</td>
<td>1.91 ± 0.68</td>
<td>2.13 ± 0.58</td>
<td>1.12 ± 0.27</td>
<td>1.14 ± 0.28*</td>
<td>0.97 ± 0.43*</td>
</tr>
<tr>
<td>Somatosensory cortex</td>
<td>1.98 ± 0.41</td>
<td>1.82 ± 0.28</td>
<td>2.36 ± 0.67</td>
<td>1.11 ± 0.22</td>
<td>1.12 ± 0.26*</td>
<td>0.95 ± 0.42*</td>
</tr>
</tbody>
</table>

Values are given as a mean ± standard deviation. Groups were compared using a one-tailed unpaired t-test. No significant differences between nTg, PS1, APP/PS1 animals were detected at the age of 2 months. At 22 months, statistically significant differences were found comparing the CA1, CA3, and the dentate gyrus of APP/PS1 mice to the same regions of nTg mice. PS1 differences between nTg, PS1, APP/PS1 animals were detected at the age of 2 months. At 22 months, statistically significant differences were found comparing the CA1, CA3, and the dentate gyrus of APP/PS1 mice to the same regions of nTg mice. APP/PS1 mice were not statistically different from nTg mice. ns, non-significant; * p < 0.05.
At the age of 2 months no differences in the number of CA1 pyramidal neurons was detected between nTg, PS1 and APP/PS1 mice (A). There was a significant neuronal dropout detectable at the age of 8 months in PS1 and APP/PS1 mice, which continued to progress at 22 months. At this time point, the differences between nTg and either PS1 or APP/PS1 mice, but not between PS1 and APP/PS1 mice, were statistically significant (*p < 0.05, **p < 0.001, respectively). The pyramidal layer of the CA1 sector of the cornu Ammonis, stained with cresyl violet, in 22-month-old nTg (B, D) and APP/PS1 mice (C, E). Scale bars: B, C = 50 μm; D, E = 10 μm.

Fig. 5.
IMPAIRED GLUCOSE METABOLISM IN APP/PS1 MICE

Fig. 6. Anti-Aβ immunohistochemistry demonstrates increasing Aβ deposition in APP/PS1 mice between 8 months (A) and 22 months (B) of age. Abbreviations: C, cortex; H, hippocampus. Scale bar = 400 μm.

At 2 months, all analyzed animal groups (PS1, APP/PS1 and nTg) had similar numbers of pyramidal CA1 neurons. Consistent with previously published observations (63), our nTg mice showed only minor, statistically insignificant age-related neuronal drop-out. Unlike nTg mice, both Tg lines demonstrated significant age related neuronal loss already detectable at the age of 8 months. This was slightly more profound in the APP/PS1 mice compared to the PS1 mice. Some previously published studies did not demonstrate neuronal loss in 16-month-old Tg mice expressing the “Swedish” APP<sub>K670N/M671L</sub> mutation (16, 17). Interestingly, the same mutation was studied by Calhoun et al, who demonstrated a 20% age-related neuronal loss in the hippocampus but not in the cortex (63). Neuronal loss in APP/PS1 mice has been studied less extensively. Takeuchi et al reported a statistically non-significant lower number of cortical and CA1 pyramidal neurons in 12-month-old APP<sub>K670N/M671L</sub>/PS1<sub>M146L</sub> mice (21). This study utilized only 4 animals per group and neurons were counted in only 25 disectors per animal. In our study the fractionator method was used, which allows for unbiased sampling of the whole structure in 3 dimensions. Neurons were counted in more disectors, greatly improving the sampling scheme and resulting in a lower coefficient of error (64, 65). Previous stereological studies performed on PS1 mice have demonstrated that expression of the PS1 mutant protein alone has a negative impact on neuronal number, an effect which worsens with increasing age (22, 23). This may be related to the well-characterized interaction between PS1 and the Notch 3 signaling system (11), as well as with the induction of apoptosis by PS1 mutants (22).

At the age of 22 months, there were on average 195,000 neurons in the CA1 sector of the cornu Ammonis in nTg mice and 149,000 in PS1 mice, although no significant behavioral or metabolic differences were observed between these 2 genotypes. This suggests that the brain possesses mechanisms capable of compensating for some degree of neuronal loss. One of such mechanism is dendritic sprouting and formation of new synapses by surviving neurons (66, 67). In fact, presynaptic nerve terminals, rather than neuronal bodies, account for most of the glucose uptake in the brain and, therefore, the majority of the 14C signal comes from synapses (68–70). Likewise, it is possible that the regional level of metabolism could be maintained despite neuronal dropout through the maintenance of normal synaptic density (71).
Although APP/PS1 mice showed only slightly greater neuronal loss than PS1 mice (statistically non-significant), they had significant behavioral and metabolic impairment. Progressive deposition of Aβ was the major feature distinguishing these 2 Tg lines. Synaptotoxicity of Aβ oligomers and fibrils has been well documented in vitro and in vivo and appears through several mechanisms including oxidative-stress (69, 72, 73), metabolic impairment (74), calcium overload (75), downregulation of cholinergic receptor (76), and disturbances of ionic homeostasis necessary for proper synaptic transmission (77). It is plausible that Aβ in APP/PS1 mice impairs the metabolic rate of existing synapses and prevents the sprouting response of surviving neurons. Electron microscopy studies have confirmed abnormal synaptic formation in the vicinity of Aβ deposits in AD Tg mice (78). This could explain why 14C-2DG and behavioral impairment are seen only in APP/PS1 mice, despite them having a similar neuronal loss to mice expressing the single PS1 transgene. In fact, measurements of presynaptic bouton density demonstrated no differences between APP or PS1M146L single Tg lines and wild type mice, but showed a marked reduction in the density of presynaptic boutons in double APP or PS1M146L Tg mice that was associated with the appearance of Aβ deposits (79).

In some AD Tg lines, overexpression of wild type or a mutated variant of APP produces synaptic dysfunction well before the development of microscopically detectable Aβ deposits (80). For example, 1130H Tg mice overexpressing the human wild type APP show an impaired glucose utilization at both 3 and 15 months, although this line does not form Aβ deposits (42). In our study, APP/PS1 mice did not demonstrate an impairment of glucose utilization at the age of 2 months, which is before the first Aβ deposits develop. The metabolic deficit detected at the age of 22 months was associated with a significant Aβ load. This suggests a causative relationship between Aβ deposition and impaired glucose utilization in APP/PS1 mice, although, the late emergence of the APP transgene effect cannot be absolutely excluded.

PS1 is a protein involved in multiple intracellular functions. Familial AD-linked PS1 mutations have an effect on Aβ deposition by influencing γ-secretase function, as well as having a direct role on neuronal pathology and neuronal loss. Single Tg APP mice with Aβ deposition, neuritic degeneration, and synaptic pathology, but not with overt neuronal loss, are considered to model early AD pathology (81). In contrast, we document that aged, double Tg APP/PS1 mice represent a model for AD that recapitulates many features of the advanced human disease, including regional cerebral hypometabolism, neuronal loss, Aβ deposition, and profound behavioral changes.

REFERENCES


