Experimental Neurology **175,** 398–406 (2002)
doi:10.1006/exnr.2002.7915

Object Recognition Memory and Cholinergic Parameters in Mice Expressing Human Presenilin 1 Transgenes

E. Vaucher,*[,1] P. Fluit,† M. A. Chishti,† D. Westaway,† H. T. J. Mount,† and S. Kar*[,2]

*Douglas Hospital Research Center, Department of Psychiatry, McGill University, Verdun, Québec H4H 1R3, Canada; and †Center for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada

Received September 5, 2001; accepted March 13, 2002

Most autosomal dominant forms of Alzheimer disease (AD) are related to missense mutations in the human presenilin (PS) 1 gene. Although the underlying mechanisms associated with pathophysiology of AD have yet to be clearly established, pathogenic mutations in the PS1 gene influence the processing of β-amyloid precursor protein, leading to increased production and deposition of highly fibrillogenic amyloid $\beta_{1\text{-}42}$ peptide in the brains of AD patients. As cognitive dysfunction in AD is associated with a dramatic loss of cholinergic innervation particularly in the hippocampus and neocortex, we investigated learning and cholinergic neurochemistry in transgenic mice expressing pathogenic mutant L286V or wild-type (wt) human PS1 transgenes. Relative to wt, the L286V PS1 transgenic mice exhibited reduced sensorimotor activity and marked deterioration of object memory between 3 and 5 h after the first encounter. Activity of the biosynthetic enzyme choline acetyltransferase was not altered in the hippocampus, frontoparietal cortex, or striatum of mutant transgenic mice relative to wt transgenic or littermate nontransgenic controls. No differences in the densities of M1/[^{3}H]pirenzepine, M2/[^{3}H]AF-DX 384, or α_7 nicotinic/^{125}I-α-bungarotoxin receptor binding sites were evident in any brain regions among L286V PS1 transgenic, wt PS1 transgenic, and littermate nontransgenic controls. These results suggest that overexpression of a mutated PS1 gene induces a subtle alteration in object memory without affecting cholinergic neurochemistry.

Key Words: **acetylcholine; Alzheimer disease; choline acetyltransferase; memory; muscarinic receptors; nicotinic receptor; object recognition; presenilin.**

INTRODUCTION

Although most cases of Alzheimer's disease (AD)[2] occur sporadically after the age of 60–65 years, a small proportion of cases correspond to the early onset (<60 years) autosomal dominant form of the disease. To date, mutations in three genes—the β-amyloid precursor protein (APP) gene on chromosome 21, the presenilin 1 (PS1) gene on chromosome 14, and the presenilin 2 gene on chromosome 1—have been identified as the cause of a large proportion of early onset familial AD (24, 39, 44, 47, 49). Pathogenic mutations in the PS1 gene increase the level of β-amyloid$_{1\text{-}42/43}$ ($A\beta_{1\text{-}42/43}$) in the brain, possibly by enhancing cleavage of APP at a γ-secretase site (5, 9, 10, 13). Evidence from *in vitro* studies further indicates that PS1 itself may either act as γ-secretase or mediate the catalytic activity of the enzyme (11, 34, 38, 45). In addition to increased Aβ levels and deposition, the development of a central cholinergic deficit is considered to be a neuropathological hallmark of AD. Cholinergic hypofunction correlates with progressive memory impairment associated with the disease and may result from loss or atrophy of basal forebrain cholinergic neurons that innervate the hippocampus and neocortex (3, 19, 40, 41, 52).

Transgenic (Tg) mice expressing human APP and PS1 genes have been created to study neuropathologic mechanisms of AD and to model some of the AD-associated cognitive deficits. Some of these mice exhibit molecular, cellular, and/or cognitive phenotypes that replicate symptomatology of AD (8, 22, 23, 27, 36, 48). Tg mice with "Swedish" mutations (K670N and M671L) in human APP show elevation of $A\beta_{1\text{-}42/43}$, neuritic amyloid deposits, and deficits in correlative memory (27). Mice overexpressing mutated PS1 have elevated $A\beta_{1\text{-}42/43}$ levels, but do not show overt AD-like pathology or spatial learning deficits in the Morris

[1] Present address: Optometry School, Université de Montréal, Montreal, Quebec, Canada.

[2] To whom correspondence should be addressed. E-mail: satyabrata.kar@mcgill.ca.

[3] Abbreviations used: ACh, acetylcholine; Aβ, amyloid β-peptide; AD, Alzheimer disease; APP, amyloid precursor protein; ChAT, choline acetyltransferase; mut, mutant; PrP, prion protein; PS1, presenilin 1; Tg, transgenic; wt, wild type.

0014-4886/02 $35.00

water maze test (5, 9, 13, 23, 28). Coexpression of mutated human APP and PS1 genes accelerates the development of Aβ deposits in the brain and also induces deficits in spatial memory, suggesting that mutations in PS1 may act synergistically with mutant APP to cause pathology reminiscent of the AD brain (1, 4, 8, 23, 25, 26, 35). Although Aβ deposits have been well characterized in mutant APP and PS1 Tg mice, very little information is available on the cholinergic system in these animals (23, 35, 39). Studies on two lines of APP Tg mice have demonstrated dystrophic cholinergic fibers or elevated density of cholinergic synapses in the cortex. Mice expressing both APP and PS1 mutant transgenes display an extensive loss of cholinergic synapses in the frontal cortex and hippocampus—two areas consistently affected in AD brains (6, 53). In the present study, we investigated sensorimotor activity and nonspatial working memory of L286V mutant PS1 Tg and wild-type (wt) human PS1 Tg lines expressing human PS1 protein at levels (8) similar to those of littermate non-Tg mice. Choline acetyltransferase (ChAT) activity and cholinergic receptor binding sites were also studied in selected brain regions of these mice.

MATERIALS AND METHODS

Subjects

L286V mutant PS1 Tg, wt PS1 Tg, and non-Tg littermate control mice were used in the study. These were established in the FVB/N background and used in the second backcross to C57Bl/6J strain for these studies. Studies were performed on 12- to 16-month-old mice. To examine the age dependence of our observations, some experiments were repeated on younger (3–7 months) animals. All animals were maintained in a pathogen-free colony with a 12-h light/dark photoperiod and food and water *ad libitum* as per the guidelines of the Canadian Council for Animal Care. Human PS1 transgenes with or without Leu → Val mutation at codon 286 (L286V) were constructed under the control of the prion (PrP) promoter (43), which allowed expression of the transgene in many neuronal types and, to a lesser extent, in systemic tissues (8, 9). Animals were genotyped by PCR of tail biopsy genomic DNA or with transgene-specific probes positioned in the 3′ untranslated region of the PrP promoter (9).

Behavioral Testing

Open-field activity. Motor activity was assessed during the light cycle. Mutant PS1 Tg (n = 5), wt PS1 Tg (n = 8), and non-Tg control (n = 14) mice were placed individually in a clear Perspex box (42 cm L × 20 cm W × 20 cm H) that was uniformly lighted. Odors were controlled by wiping the floor and walls of the box with a mild ethanol solution between tests. Motor behavior was recorded over a 5-min test session. Duration of walking and rearing (forepaws elevated from the floor) was analyzed as indices of spontaneous locomotor activity.

Sensorimotor function. Following open-field testing, sensorimotor function was examined with an Economex accelerating rotarod (Columbus Instruments, Columbus, OH). The rod was covered with fine sandpaper (120 grade) to facilitate traction. Animals were placed on a slowly revolving (5 rpm) rod. As each animal began to walk, the rod was set to accelerate at a rate of 0.2 rpm/s. Latency to fall was recorded in four daily trials, performed at 30-min intervals. After 1 day of training, mice received 3 consecutive days of testing and the daily score for each mouse was the summed latency to fall, over the four test trials.

The object recognition test. L286V PS1 Tg (n = 8), wt PS1 Tg (n = 6), and non-Tg (n = 8) mice were tested for object recognition in clear plastic small animal cages (36 cm L × 22 cm W × 25 cm H) as described elsewhere (14, 46, 51). For each animal, one pair of objects was selected at random from a set of four objects that differed in shape, surface color, contrast, and texture. The four objects were selected from a larger pool of objects on the basis of the criterion that mice would spend approximately equal time exploring each of the objects. Mice were habituated to the environment over seven daily sessions of 15 min. On the test day, two objects were placed on the center line of the long axis of the floor, 5 cm from each cage end. Mice were allowed to explore the two objects for 10 min and exploratory activity (i.e., time spent in object-directed exploration during the initial period) was recorded. After a delay of 3 h, mice were reexposed for 5 min to one of the original objects and one member of a second pair of objects. Time spent exploring each object was recorded. At 5 h following the initial exposure, mice were allowed to explore the other member of the original sample object pair and the remaining member of the second pair for 5 min. Again, time spent exploring the objects was recorded. A mouse was considered to be engaging in exploratory behavior if the animal touched the object with its forepaw or nose or sniffed at the object within a distance of 1.5 cm. Testing was performed by an experimenter who was blind to the genotype of the mice. After each exposure, the objects and the cage were wiped with 70% ethanol to eliminate odor cues. A memory index (MI) was calculated for each mouse, wherein "t_o" represents time exploring an object during the original exposure and "t_n" represents time spent exploring an object that is novel on the reexposure: $\mathrm{MI} = (t_n - t_o)/(t_n + t_o)$ (14, 46, 51).

Choline Acetyltransferase Activity

L286V PS1 Tg (n = 11), wt PS1 Tg (n = 6), and non-Tg control (n = 6) mice were decapitated, and selected regions of the brain (i.e., caudate nucleus,

hippocampus, and frontoparietal cortex) were dissected according to the atlas of Franklin and Paxinos (20). Tissues were frozen in 2-methylbutane at −40°C and stored at −80°C. ChAT activity was measured by the method of Fonnum (18). In brief, tissues were homogenized in 40 mM phosphate buffer containing 0.5% Triton and then incubated 20 min at 37°C in the presence of 0.25 mM [^{14}C]acetyl coenzyme A (2.25 Ci/mol; New England Nuclear, Boston, MA), 0.2 mM eserine salicylate, and 12.5 mM choline chloride (Sigma, St. Louis, MO). The reaction was terminated by addition of 400 μl acetylcholine chloride (0.2 mM; Hoffman Laroche, Basel, Switzerland) and then all acetylcholine (ACh) was extracted with tetraphenylboron in 3-heptanone (15 g/L; Aldrich, Milwaukee, WI) as described earlier (30, 51). ChAT activity was expressed in nmol radioactive ACh formed/mg tissue/h.

In Vitro Receptor Autoradiography

The animals used for the object recognition test and some additional mice from three different groups [i.e., L286V PS1 Tg (n = 12), wt PS1 Tg (n = 12), and non-Tg (n = 12) mice] were subsequently processed for receptor binding assays. In brief, mice were decapitated and their brains were removed, frozen in 2-methylbutane at −40°C and stored at −80°C. Coronal brain sections (20 μm) were cut at −18°C on a cryostat, thaw-mounted onto gelatin-coated slides, desiccated overnight at 4°C, and stored at −80°C until use.

M1 and M2 receptor binding sites. Muscarinic M1 and M2 binding sites were visualized with [^{3}H]pirenzepine (79.5 Ci/mmol) and [^{3}H]AF-DX 384 (106 Ci/mmol) (New England Nuclear), respectively, as described in detail elsewhere (2, 51). Brain sections from different regions were first equilibrated to room temperature and preincubated for 15 min in Krebs buffer (NaCl, 120 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; glucose, 5.6 mM; $NaHCO_3$, 25 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM, pH 7.4). Sections were then incubated for 1 h at 22°C in fresh Krebs buffer containing either 10 nM [^{3}H]pirenzepine or 2 nM [^{3}H]AF-DX 384. Alternate sections were incubated with 1 μM atropine (a nonselective muscarinic antagonist; Sigma) for the determination of nonspecific binding. Slides were transferred sequentially through three rinses (4 min each) in Tris–HCl buffer (50 mM, pH 7.4) at 4°C, followed by a rapid dip in cold distilled water. Sections were dried and juxtaposed to Hyperfilm (Amersham, Ontario, Canada) alongside with ^{3}H-labeled standards for 2 (^{3}H-pirenzepine) or 3 (^{3}H-AF-DX 384) weeks.

^{125}I-α-Bungarotoxin/α_7 nicotinic receptor binding sites. The α_7 nicotinic receptor binding sites were detected with ^{125}I-α-bungarotoxin (2000 Ci/mmol; New England Nuclear) as described earlier (2, 42). Brain sections were equilibrated to room temperature and incubated for 2 h at 22°C in Tris–HCl buffer (50 mM, pH 7.4) containing 2.5 nM ^{125}I-α-bungarotoxin. Nonspecific binding was determined in the presence of 1 μM unlabeled α-bungarotoxin. Sections were washed six times (10 min each) in cold Tris–HCl buffer (50 mM, pH 7.4), dried, and exposed to Hyperfilms with ^{3}H-labeled standard for 1 week.

All autoradiograms were quantified densitometrically with a computerized image analysis system (MCID Image Analysis; St Catharine, Ontario) as described earlier (29, 51). In brief, a standard curve was generated from the ^{3}H microscale standard, which was coexposed with radiolabeled sections. The optical density values of the standard curve were then converted to femtomoles per milligram of tissue based on the specific activity and radioactive decay of the various ligands. Referencing this curve, the optical densities obtained from different regions of five consecutive total (i.e., in the absence of the unlabeled atropine or α-bungarotoxin) and nonspecific (i.e., in the presence of the unlabeled atropine or α-bungarotoxin) brain sections were converted into fmol/mg tissue. Specific binding for each receptor was then calculated by subtracting nonspecific from total binding. Data from selected brain regions such as caudate nucleus, frontoparietal cortex, occipital cortex, perirhinal cortex, and hippocampus are reported herein.

Statistical Analysis

A two-way ANOVA followed by post hoc multiple comparisons (Tukey HSD) test was performed to examine interactions between the transgene expression and the timing of the object recognition test. For locomotor activity and sensorimotor performance a one-way ANOVA followed by Fisher post hoc analysis was used to evaluate the significance of differences between L286V PS1 Tg, wt PS1 Tg, and non-Tg littermate groups. For autoradiographic study, a one-way ANOVA followed by post hoc multiple comparisons (Tukey HSD) was used to evaluate differences for each brain region among three littermate groups. In all analyses $\alpha = 0.05$.

RESULTS

Open-Field Activity and Rotarod Performance

Wt and L286V mutant PS1 transgenes produced opposite effects on sensorimotor behavior. These effects were not influenced by aging, as similar responses were observed in young (3–7 months) adult and aged (12–16 months) animals. Young wt PS1 Tg mice exhibited enhanced locomotor activity relative to age-matched non-Tg and L286V PS1 Tg animals [$F(2, 15) = 4.52$, $P = 0.03$, Fig. 1A]. Aged wt PS1 Tg mice showed a similar tendency that did not achieve significance [$F(2, 25) = 2.74$, $P = 0.08$]. Aged L286V PS1 Tg mice exhibited less rearing than wt PS1 Tg mice [$F(2, 25) = 4.32$, $P = 0.02$, Fig. 1B]. Differences in rearing between

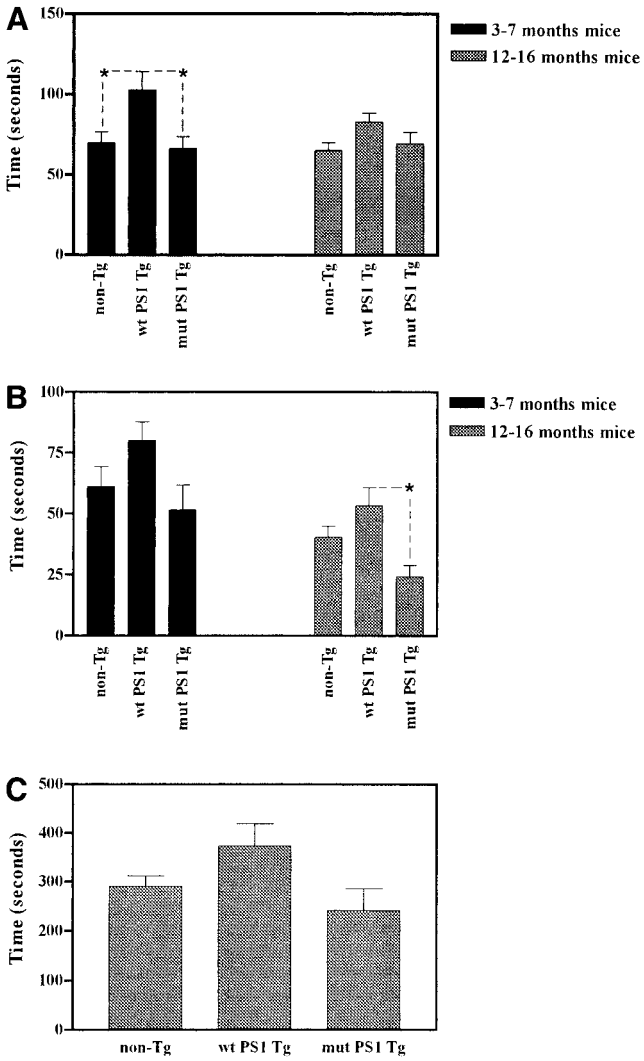

FIG. 1. Open-field activity and sensorimotor function in non-transgenic (non-Tg), wild-type PS1 transgenic (wt PS1 Tg), and L286V mutant PS1 transgenic (mut PS1 Tg) mice. Young and aged animals were respectively 3–7 months and 12–16 months of age. Data represent means ± SEM. (A) Spontaneous locomotor activity was enhanced in 3- to 7-month young wt PS1 Tg mice compared to non-Tg and mut PS1 Tg mice. (B) Rearing was significantly decreased in 12- to 16-month old mut PS1 Tg mice relative to wt PS1 Tg mice. (C) Rotarod performance, as measured by latency to fall from an accelerating rotarod, was significantly lower in 12- to 16-month old mut PS1 Tg mice compared to wt PS1 tg mice. *$P < 0.05$.

groups of young mice were less marked [$F(2, 15) = 1.83$, $P = 0.18$]. For aged mice, intergroup differences in latency to fall from an accelerating rotarod approached but did not achieve significance [$F(2, 23) = 3.09$, $P = 0.06$, Fig. 1C]. However, the aged wt PS1 Tg mice did perform better than their L286V PS1 Tg cohorts (Fisher' protected LSD $P = 0.03$). No significant difference in body weight was discerned between Tg and non-Tg mice. Tg mice did not show any spontaneous abnormal behaviors when observed in their home cages.

Object Recognition Test

An object recognition test was used to evaluate the cognitive performance because AD patients exhibit deficits in object recognition (17, 21). Previously we determined that PS1 Tg mice do not differ from non-Tg littermates in the Morris water maze, a task that tests spatial memory (28). We also found that PS1 Tg mice swam faster than non-Tg littermates (28). In the present study, the two-way ANOVA indicated that the memory of a previously encountered object deteriorated between 3 and 5 h after the initial exposure [main effect, $F(1, 43) = 21.494$, $P \leq 0.001$] and showed that the groups differ in the rate at which memory declines [interaction $F(2, 43) = 4.3$, $P \leq 0.02$]. The genotype did not affect the level of object recognition across trial per se [main effect, $F(2, 43) = 1.2$, ns]. Tukey test ($\alpha = 0.05$ HSD) analysis showed that memory index was significantly ($P \leq 0.05$) decreased at 5 h compared to 3 h after the initial exposure only in the mutant PS1 Tg mice group. Memory for the previously encountered object was not significantly affected in wt or non-Tg mice between 3 and 5 h after the initial exposure. Moreover, our results on the object recognition test indicated that performance of mutant PS1 Tg mice did not differ from that of wt PS1 Tg mice (3 h, 0.52 ± 0.07 vs 0.33 ± 0.07) but improved significantly compared to non-Tg littermate mice (3 h, 0.52 ± 0.07 vs 0.23 ± 0.05, $P \leq 0.05$, $\alpha = 0.05$ HSD) at 3 h after the initial exposure of the object (Fig. 2). No significant changes of the memory index were seen between groups during the 5-h trial. There were also no significant differences in exploratory activity between groups during the initial exposure period [mutant PS1, 146 ± 26; wt PS1, 129 ± 22; and non-Tg 161 ± 15; main effect $F(2, 43) = 0.74$, ns] or between trials [3 h, 160 ± 17, or 5 h, 130 ± 18, main effect $F(1, 43) = 1.43$, ns].

Cholinergic Neurochemistry

ChAT enzyme activity levels in different brain regions (i.e., caudate nucleus, hippocampus and frontoparietal cortex) were similar in L286V PS1 Tg, wt PS1 Tg, and littermate non-Tg mice (Table 1). The distribution of M1/[^{3}H]pirenzepine binding sites in the murine brain was in accord with previous results (2, 51). High concentrations of [^{3}H]pirenzepine sites were seen in various cortical areas and the striatum, as well as in the CA1 subfield and dentate gyrus of the hippocampal formation (Fig. 3A, Table 2). No differences in the density of [^{3}H]pirenzepine binding sites were apparent among L286V PS1 Tg, wt PS1 Tg, and non-Tg control mice (Figs. 3A–3D, Table 2). A high density of M2/[^{3}H]AF-DX 384 binding sites in littermate control mice was observed in the striatum, whereas moderate binding sites were evident in most cortical areas and the hippocampal formation (Fig. 3E, Table 3). No significant alteration in [^{3}H]AF-DX 384 binding sites was apparent in any brain region between the three groups

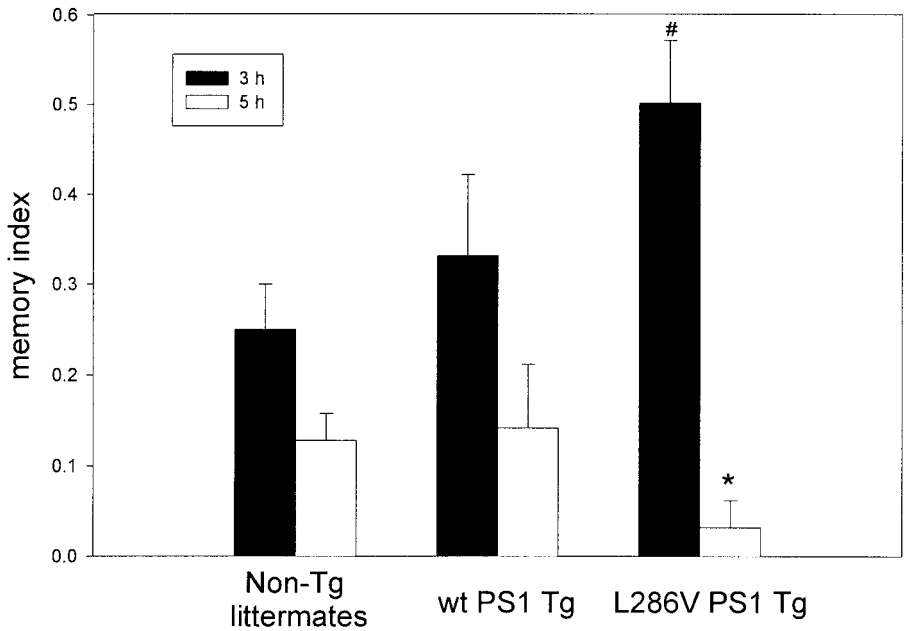

FIG. 2. Memory of wild-type PS1 transgenic (wt PS1 Tg), L286V mutant PS1 transgenic (mut PS1 tg), and non-Tg mice at 3 and 5 h in the object recognition task. Performance of mut PS1 Tg mice was better than that of non-Tg mice at 3 h, but memory of the previously encountered object was deteriorated significantly in these mice compared to non-Tg and wt PS1 Tg mice. #$P < 0.05$ mut PS1 Tg vs non-Tg; *$P < 0.05$ 5 h vs 3 h in mut PS1 Tg mice.

of mice studied (Figs. 3E–3H, Table 3). In keeping with earlier reports (2, 42), high levels of α_7 nicotinic/^{125}I-α-bungarotoxin binding sites in littermate control mice were evident particularly in cortical areas and thalamus, whereas a lower density of sites was found in the hippocampus. Striatum displayed an even lower density of binding sites (Fig. 3I). Compared to non-Tg controls, no significant difference in α_7 nicotinic/^{125}I-α-bungarotoxin binding sites was apparent in any brain region of L286V PS1 or wt PS1 Tg mice (Figs. 3I–3L, Table 4).

DISCUSSION

We found that L286V PS1 Tg mice exhibited reduced sensorimotor activity and deterioration of object memory between 3 and 5 h after initial exposure compared to wt PS1 Tg or non Tg mice. Wt PS1 Tg mice displayed a relative increase in locomotor activity but no alteration in object memory. Neither the activity of ChAT enzyme nor the density of M1/[^{3}H]pirenzepine, M2/[^{3}H]AF-DX 384, or α_7 nicotinic/^{125}I-α-bungarotoxin receptor binding sites was altered in any brain regions of Tg mice compared to non-Tg littermates. These results, taken together, suggest that expression of the human PS1 transgene alters locomotor activity and induces subtle differences in object memory, without affecting cholinergic parameters.

TABLE 1

Choline Acetyltransferase Activity in Selected Brain Regions of Non-Tg, Wt PS1 Tg, and L286V PS1 Tg Mice

Brain region	Non-Tg littermates (n = 4)	Wt PS1 Tg (n = 6)	L286V PS1 Tg (n = 11)
Caudate nucleus	429 ± 40	409 ± 24	431 ± 14
Frontoparietal cortex	81 ± 9	91 ± 8	90 ± 6
Hippocampus	125 ± 7	120 ± 4	124 ± 5

Note. Values represent mean activities ± SEM expressed in nmol of acetylcholine synthesized/mg tissue/h.

Memory Recall Is Decreased in L286V PS1 Tg Mice

The object recognition test is based on the spontaneous, differential exploration of familiar and novel objects and does not depend on spatial cues. This test allows assessment of subtle changes in nonspatial working memory (7, 14, 46). Our results indicate that object recognition memory was not significantly altered over a 5-h test period in wt PS1 Tg mice and non-Tg controls. Relative to wt PS1 Tg mice, L286V PS1 Tg mice displayed a significant deterioration in object memory between 3 and 5 h after initial exposure. Given that memory for a familiar object in this paradigm usually lasts 6 h in control C57Bl/6J mice (see 51), it seems that long-term retention is impaired in L286V PS1 Tg mice compared to wt PS1 Tg mice. The deficit in object recognition might be attributed to an increased level of basal memory ability in PS1 mice, as the 3-h performance was found to be better for this group compared to non-Tg mice. Whether this is related to a reduced rearing activity or other motor dis-

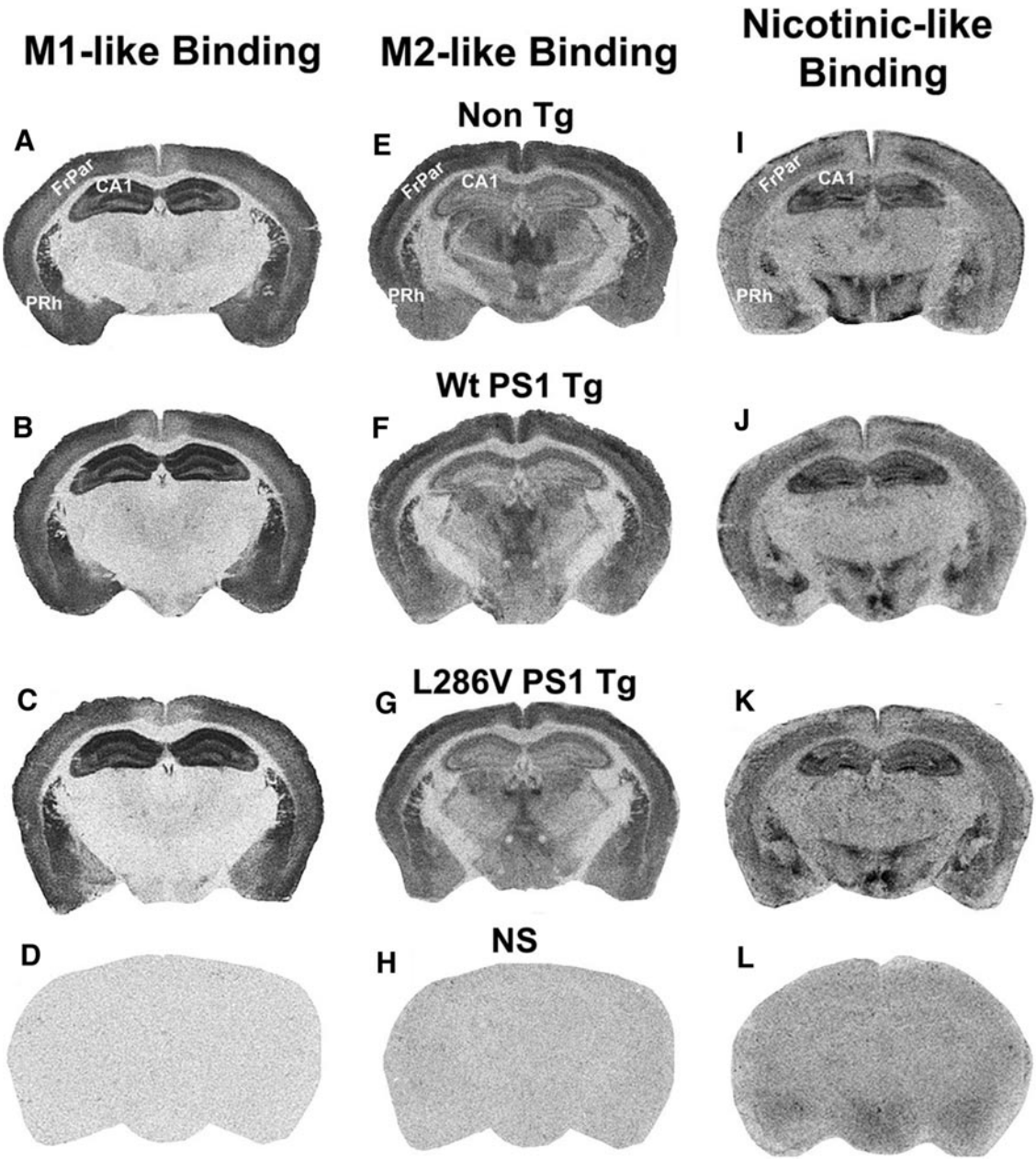

FIG. 3. Photomicrographs of [^{3}H]pirenzepine (A–C), [^{3}H]AF-DX 384 (E–G), and ^{125}I-α-bungarotoxin (I–K) binding sites in brain regions through hippocampal formation in non-Tg (A, E, I), wt PS1 Tg (B, F, J), and L286V mut PS1 Tg (C, G, K) mice. (D, H, and L) Nonspecific binding of [^{3}H]pirenzepine (D), [^{3}H]AF-DX 384 (H), and ^{125}I-α-bungarotoxin (L). No significant differences between animal groups were apparent in the distribution profiles or in densities of [^{3}H]pirenzepine, [^{3}H]AF-DX 384, or ^{125}I-α-bungarotoxin binding. FrPar, frontoparietal cortex; PRh, perirhinal cortex; Th, thalamus; DG, dentate gyrus.

turbances in mutant PS1 Tg mice remains unclear but seems unlikely as mutant PS1 Tg mice exhibit normal exploratory activity during the initial exposure period. It is possible that PS1 mutation may alter the consolidation processes of memory traces.

Mutant PS1 Tg mice, which are reported to be spared on a spatial discrimination paradigm (28), are found to exhibit deficits in long-term retention of object recognition memory. PDAPP mice, which overexpress V717F hAPP, on the other hand, manifest impairments both in the spatial discrimination and in the object discrimination paradigms (12). A series of recent data from rodents with lesion of specific brain regions indicates a significant role for hippocampus in spatial memory, whereas integrity of medial temporal lobe structures such as the perirhinal and entorhinal cortices is more critical for object recognition than for spatial memory performance (7, 15, 16, 31, 46). Thus overexpression of the mutant PS1 transgene may possibly elicit an influence on the functioning of the medial

TABLE 2

Levels of Specific [^{3}H]Pirenzepine/M1 Binding Sites in Non-Tg, Wt PS1 Tg, and L286V PS1 Tg Mice

Brain region	Non-Tg littermates (n = 18)	Wt PS1 Tg (n = 12)	L286V PS1 Tg (n = 12)
Caudate nucleus	260 ± 9	280 ± 9	275 ± 12
Frontoparietal cortex	209 ± 7	221 ± 7	213 ± 8
Occipital cortex	204 ± 6	229 ± 8	221 ± 8
Perirhinal cortex	187 ± 6	192 ± 4	184 ± 5
Hippocampus	349 ± 9	370 ± 9	359 ± 13

Note. Values represent means ± SEM of M1 binding sites expressed in fmol/mg tissue wet wt.

TABLE 4

Levels of Specific ^{125}I-α-Bungarotoxin/α_7 Nicotinic Receptor Binding Sites in Non-Tg, Wt PS1 Tg, and L286V PS1 Tg Mice

Brain region	Non-Tg littermates (n = 8)	Wt PS1 Tg (n = 6)	L286V PS1 Tg (n = 6)
Caudate nucleus	62 ± 5	63 ± 6	69 ± 9
Frontoparietal cortex	57 ± 8	61 ± 7	52 ± 5
Occipital cortex	52 ± 4	62 ± 8	60 ± 5
Perirhinal cortex	18 ± 1	18 ± 1	18 ± 1
Hippocampus	245 ± 27	269 ± 33	317 ± 36

Note. Values represent means ± SEM of α_7 nicotinic receptor binding sites expressed in fmol/mg tissue wet wt.

temporal lobe, which leads to a deficit in long-term retention of object memory.

Cholinergic Neurochemistry in PS1 Tg Mice

L286V PS1 or wt PS1 overexpression did not alter ChAT enzyme activity in any region of the brain, thus indicating that synthesis of ACh remains unaffected in Tg mice. These findings are in contrast with a previous *in vitro* study (37), which showed that PC12 cells transfected with mutant human PS1 gene displayed reduced ChAT activity compared to untransfected cells. However, a parallel decrease in ChAT protein levels implicated decreased ChAT expression rather than direct effect on the enzyme activity (37). The densities of muscarinic M1 and M2 and nicotinic α_7 receptor binding sites were also unaltered in PS1 Tg mice compared to non-Tg controls. Changes in cholinergic activity are usually associated with alteration in cholinergic receptor levels (33, 50) and/or cholinergic fibers density (32). Neither cholinergic function nor ACh synthesis was altered in L286V PS1 or wt PS1 Tg mice. Given the evidence that ChAT activity was determined in tissue homogenates and cholinergic receptor levels were measured using film autoradiography, the methods which offer limited cellular resolution, it remains possible that cholinergic abnormalities might be detected by approaches with better cellular and/or synaptic specificity. However, a recent study showed no significant differences in size or number of cholinergic nerve terminals in the hippocampus or cortical areas of mice overexpressing M146L PS1 (53). Conversely, studies from two different lines of APP transgenic mice demonstrated dystrophic cholinergic fibers in the vicinity of neuritic plaques and elevation in density of cholinergic synapses in the hippocampus and cortical areas. Double transgenic mice overexpressing both mutant APP and mutant PS1 have been found to display extensive loss of cholinergic synapses in the frontal cortex and hippocampus—suggesting that overexpression of human Aβ peptide combined with a shift toward longer forms of Aβ terminating at residues 42 or 43 due to mutation of PS1 is required to elicit cholinergic deficits in mice (6, 53). Thus in summary, mutation of the human PS1 gene may alter sensorimotor activity and long-term retention of object recognition memory but not ChAT enzymatic activity or cholinergic receptor binding sites. Whether this cognitive deficit is due to the alteration in APP processing of the endogenous mouse APP holoprotein by the L286V PS1 mutation or relates to altered functions of other neurotransmitter(s) remains to be examined.

TABLE 3

Levels of Specific [^{3}H]AFDX-384/M2 Binding Sites in Non-Tg, Wt PS1 Tg, and L286V PS1 Tg Mice

Brain region	Non-Tg littermates (n = 18)	Wt PS1 Tg (n = 12)	L286V PS1 Tg (n = 12)
Caudate nucleus	153 ± 4	163 ± 5	151 ± 4
Frontoparietal cortex	73 ± 2	76 ± 2	76 ± 2
Occipital cortex	69 ± 2	68 ± 4	63 ± 2
Perirhinal cortex	47 ± 1	45 ± 1	44 ± 5
Hippocampus	51 ± 1	56 ± 2	55 ± 2

Note. Values represent means ± SEM of M2 binding sites expressed in fmol/mg tissue wet wt.

ACKNOWLEDGMENTS

This study was supported by grants from the CIHR to S.K., H.T.J.M., and D.W. E.V. is a recipient of a postdoctoral fellowship from the Alzheimer Society of Canada.

REFERENCES

1. Arendash, G. W., D. L. King, M. N. Gordon, D. Morgan, J. M. Hatcher, C. E. Hope, and D. M. Diamond. 2001. Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res.* **891:** 42–53.
2. Aubert, I., W. Rowe, M. J. Meaney, S. Gauthier, and R. Quirion. 1995. Cholinergic markers in aged cognitively impaired Long-Evans rats. *Neuroscience* **67:** 277–292.

3. Bartus, R. T., R. L. Dean, B. Beer, and A. S. Lipa. 1982. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217:** 408–417.
4. Borchelt, D. R., T. Ratovitski, J. van Lare, M. K. Lee, V. Gonzales, N. A. Jenkins, N. G. Copeland, D. L. Price, and S. S. Sisodia. 1997. Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* **19:** 939–945.
5. Borchelt, D. R., G. Thinakaran, C. B. Eckman, M. K. Lee, F. Davenport, T. Ratovitsky, C. M. Prada, G. Kim, S. Seekins, D. Yager, H. H. Slunt, R. Wang, M. Seeger, A. I. Levey, S. E. Gandy, N. G. Copeland, N. A. Jenkins, D. L. Price, S. G. Younkin, and S. S. Sisodia. 2000. Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ142/1-40 ratio in vitro and in vivo. *Neuron* **17:** 1005–1013.
6. Bronfman, F. C., D. Moechars, and F. Van Leuven. 2000. Acetylcholinesterase-positive fiber deafferentation and cell shrinkage in the septohippocampal pathway of aged amyloid precursor protein London mutant transgenic mice. *Neurobiol. Dis.* **7:** 152–168.
7. Buckley, T. J., and D. Gaffan. 1998. Perirhinal cortex ablation impairs visual object identification. *J. Neurosci.* **18:** 2268–2275.
8. Chishti, M. A., D.-S. Yang, C. Janus, A. L. Phinney, P. Horne, J. Pearson, R. Strome, N. Zucker, J. Loukides, J. French, S. Turner, G. Lozza, M. Grilli, S. Kunicki, C. Morrisette, J. Paquette, F. Gervais, C. Bergeron, P. E. Fraser, G. A. Carlson, P.-S. St George-Hyslop, and D. Westaway. 2001. Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of APP695. *J. Biol. Chem.* **276:** 21562–21570.
9. Citron, M., D. Westaway, W. Xia, G. Carlson, T. Diehl, G. Levesque, K. Johnson-Wood, M. Lee, P. Seubert, A. Davis, D. Kholodenko, R. Motter, R. Sherrington, B. Perry, H. Yao, R. Strome, I. Lieberburg, J. Rommens, S. Kim, D. Schenk, P. Fraser, H.-P. St George, and D. J. Selkoe. 1997. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat. Med.* **3:** 67–72.
10. Czech, C., G. Tremp, and L. Pradier. 2000. Presenilins and Alzheimer's disease: Biological functions and pathogenic mechanisms. *Prog. Neurobiol.* **60:** 363–384.
11. De Strooper, B. 2000. Alzheimer's disease. Closing in on gamma-secretase. *Nature* **405:** 627–629.
12. Dodart, J.-C., H. Meziane, C. Mathis, K. R. Bales, S. M. Paul, and A. Ungerer. 1999. Behavioral disturbances in transgenic mice overexpressing the V717F β-amyloid precursor protein. *Behav. Neurosci.* **113:** 982–990.
13. Duff, K., C. Eckman, C. Zehr, X. Yu, C. M. Prada, J. Perez-tur, M. Hutton, L. Buee, Y. Harigaya, D. Yager, D. Morgan, M. N. Gordon, L. Holcomb, L. Refolo, B. Zenk, J. Hardy, and S. Younkin. 1996. Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature* **383:** 710–713.
14. Ennaceur, A., and J. Delacour. 1988. A new one-trial test for neurobiological studies of memory in rats. 1. Behavioral data. *Behav. Brain Res.* **31:** 47–59.
15. Ennaceur, A., and J. P. Aggleton. 1997. The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behav. Brain Res.* **88:** 181–193.
16. Ennaceur, A., N. Neave, and J. P. Aggleton. 1996. Neurotoxic lesions of the perirhinal cortex do not mimic the behavioral effects of fornix transection in the rat. *Behav. Brain Res.* **80:** 9–25.
17. Fleischman, D. A., J. D. Gabrieli, S. L. Reminger, C. J. Vaidya, and D. A. Bennett. 1998. Object decision priming in Alzheimer's disease. *J. Int. Neuropsychol. Soc.* **4:** 435–446.
18. Fonnum, F. 1975. A rapid radiochemical method for determination of choline acetyltransferase. *J. Neurochem.* **24:** 407–409.
19. Francis, P. T., A. M. Palmer, M. Snape, and G. K. Wilcock. 1999. The cholinergic hypothesis of Alzheimer's disease: A review of progress. *J. Neurol. Neurosurg. Psychiatry* **66:** 137–147.
20. Franklin, K. B. J., and G. Paxinos. 1997. *The Mouse Brain in Stereotaxic Coordinates.* Academic Press, San Diego.
21. Fujimori, M., T. Imamura, H. Yamashita, N. Hirono, and E. Mori. 1997. The disturbances of object vision and spatial vision in Alzheimer's disease. *Dement. Geriatr. Cognit. Disord.* **8:** 228–231.
22. Games, D., D. Adams, R. Alessandrini, R. Barbour, P. Berthelette, C. Blackwell, T. Carr, J. Clemens, T. Donaldson, and F. Gillespie. 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* **373:** 523–527.
23. Guenette, S. Y., and R. E. Tanzi. 1999. Progress toward valid transgenic mouse models for Alzheimer's disease. *Neurobiol. Aging* **20:** 201–211.
24. Hardy, J. 1997. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* **20:** 154–159.
25. Holcomb, L., M. N. Gordon, E. McGowan, X. Yu, S. Benkovic, P. Jantzen, S. I. Wright, R. Mueller, D. Morgan, S. Sanders, C. Zehr, K. O'Campo, J. Hardy, C. M. Prada, C. Eckman C., S. Younkin, K. Hsiao, and K. Duff. 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med.* **4:** 97–100.
26. Holcomb, L. A., M. N. Gordon, P. Jantzen, K. Hsiao, K. Duff, and D. Morgan. 1999. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: Lack of association with amyloid deposits. *Behav. Genet.* **29:** 177–185.
27. Hsiao, K., P. Chapman, S. Nilsen, C. Eckman, Y. Harigaya, S. Younkin, F. Yang, and G. Cole. 1996. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* **274:** 99–102.
28. Janus, C., S. D'Amelio, O. Amitay, M. A. Chishti, R. Strome, P. Fraser, G. A. Carlson, J. C. Roder, P. George-Hyslop, and D. Westaway. (2000) Spatial learning in transgenic mice expressing human presenilin 1 (PS1) transgenes. *Neurobiol. Aging* **21:** 541–549.
29. Kar, S., J. G. Chabot, and R. Quirion. 1993. Quantitative autoradiographic localization of [^{125}I]insulin-like growth factor I, [^{125}I]insulin-like growth factor II and [^{125}I]insulin receptor binding sites in developing and adult rat brain. *J. Comp. Neurol.* **333:** 375–397.
30. Kar, S., A. M. Issa, D. Seto, D. S. Auld, B. Collier, and R. Quirion. 1998. Amyloid β-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. *J. Neurochem.* **70:** 2179–2187.
31. Kornecook, T. J., T. E. Kippin, and J. P. J. Pinel. 1999. Basal forebrain damage and object-recognition in rats. *Behav. Brain. Res.* **98:** 67–76.
32. Levey, A. I., S. M. Edmunds, V. Koliatsos, R. G. Wiley, and C. J. Heilman. 1995. Expression of m1–m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. *J. Neurosci.* **15:** 4077–4092.
33. Levin, E. D., and B. B. Simon. 1998. Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berlin)* **138:** 217–230.
34. Li, Y. M., M. Xu, M. T. Lai, Q. Huang, J. L. Castro, J. DiMuzio-Mower, T. Harrison, C. Lellis, A. Nadin, J. G. Neduvelil, R. B. Register, M. K. Sardana, M. S. Shearman, A. L. Smith, X. P. Shi, K. C. Yin, J. A. Shafer, and S. J. Gardell. 2000. Photoacti-

vated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* **405:** 689–694.

35. McGowan, E., S. Sanders, T. Iwatsubo, A. Takeuchi, T. Saido, C. Zehr, X. Yu, S. Uljon, R. Wang, D. Mann, D. Dickson, and K. Duff. 1999. Amyloid phenotype characterization of transgenic mice overexpressing both mutant amyloid precursor protein and mutant presenilin 1 transgenes. *Neurobiol. Dis.* **6:** 231–244.
36. Nalbantoglu, J., G. Tirado-Santiago, A. Lahsaini, J. Poirier, O. Goncalves, G. Verge, F. Momoli, S. A. Welner, G. Massicotte, J. P. Julien, and M. L. Shapiro. 1997. Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature* **387:** 500–505.
37. Pedersen, W. A., Q. Guo, B. K. Hartman, and M. P. Mattson. 1997. Nerve growth factor-independent reduction in choline acetyltransferase activity in PC12 cells expressing mutant presenilin-1. *J. Biol. Chem.* **272:** 22397–22400.
38. Pigino, G., A. Pelsman, H. Mori., and J. Busciglio. 2001. Presenilin-1 mutations reduce cytoskeletal association, deregulate neurite growth, and potentiate neuronal dystrophy and tau phosphorylation. *J. Neurosci.* **21:** 834–842.
39. Price, D. L., and S. S. Sisodia. 1998. Mutant genes in familial Alzheimer's disease and transgenic models. *Annu. Rev. Neurosci.* **21:** 479–505.
40. Quirion, R. 1993. Cholinergic markers in Alzheimer's disease and the autoregulation of acetylcholine release. *J. Psychiatry Neurosci.* **18:** 226–234.
41. Roberson, M. R., and L. E. Harrell. 1997. Cholinergic and amyloid precursor protein metabolism. *Brain Res. Rev.* **25:** 50–69.
42. Schulz, D. W., R. H. Loring, E. Aizenman, and R. E. Zigmond. 1991. Autoradiographic localization of putative nicotinic receptors in the rat brain using ^{125}I-neuronal bungarotoxin. *J. Neurosci.* **11:** 287–297.
43. Scott, M. R., R. Kohler, D. Foster, and S. B. Prusiner (1992) Chimeric prion protein expression in cultured cells and transgenic mice. *Protein Sci.* **1:** 986–997.
44. Selkoe, D. J. 1999. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **399:** A23-A31.
45. Selkoe, D. J., and M. S. Wolfe. 2000. In search of gamma-secretase: Presenilin at the cutting edge. *Proc. Natl. Acad. Sci. USA* **97:** 5690–5692.
46. Steckler, T., W. H. I. M. Drinkenburg, A. Sahal, and J. P. Aggleton. 1998. Recognition memory in rats. II. Neuroanatomical substrates. *Prog. Neurobiol.* **54:** 313–332.
47. St George-Hyslop, P. H. (1999) Molecular genetics of Alzheimer disease. *Semin. Neurol.* **19:** 371–383.
48. Sturchler-Pierrat, C., D. Abramowski, M. Duke, K. H. Wiederhold, C. Mistl, S. Rothacher, B. Ledermann, K. Burki, P. Frey, P. A. Paganetti, C. Waridel, M. E. Calhoun, M. Jucker, A. Probst, M. Staufenbiel, and B. Sommer. 1997. Two amyloid precursor protein transgenic mouse models with Alzheimer's disease-like pathology. *Proc. Natl. Acad. Sci. USA* **94:** 13287–13292.
49. Tandon, A., E. Rogaeva, M. Mullan, and P. H. St George-Hyslop. 2000. Molecular genetics of Alzheimer's disease: The role of beta-amyloid and the presenilins. *Curr. Opin. Neurol.* **13:** 377–384.
50. Van der Zee, E. A., and P. G. Luiten. 1999. Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: A review of immunocytochemical localization in relation to learning and memory. *Prog. Neurobiol.* **58:** 409–471.
51. Vaucher, E., I. Raymond, R. Najaffe, S. Kar, R. Quirion, M. M. Miller, and K. B. J. Franklin. 2002. Estrogen effects on object memory and cholinergic receptors in young and old female mice. *Neurobiol. Aging* **23:** 87–95.
52. Whitehouse, P. J., D. L. Price, R. G. Struble, A. W. Clark, J. T. Coyle, and M. R. Delon. 1982. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* **215:** 1237–1239.
53. Wong, T. P., T. Debeir, K. Duff, and A. C. Cuello. 1999. Reorganization of cholinergic terminals in the cerebral cortex and hippocampus in transgenic mice carrying mutated presenilin-1 and amyloid precursor protein transgenes. *J. Neurosci.* **19:** 2706–2716.