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Impaired conditioned taste aversion learning in *APP* transgenic mice

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Abstract

Cognition in transgenic mouse models of Alzheimer's disease (AD) has been predominantly characterized in explicit spatial orientation tasks. However, dementia in AD encompasses also implicit memory systems. In the present study a line of transgenic mice (TgCRND8) encoding a double mutated allele of the human amyloid precursor protein (*APP*) genes was evaluated in an implicit associative learning task of conditioned taste aversion (CTA). CTA is a form of Pavlovian classical conditioning, in which a mouse learns to avoid a novel taste of saccharine (conditioned stimulus) paired with an experimentally induced (systemic injection of lithium chloride) nausea (unconditioned stimulus). In contrast to conditioned non-Tg mice, TgCRND8 *APP* mice developed weaker aversion against saccharine and quickly increased its consumption in repeated tests. These results indicate that TgCRND8 mice show a significant impairment not only in explicit spatial memory, as has been previously shown [Nature 408 (2000) 979], but also in implicit memory. Control experiments confirmed that TgCRND8 and non-Tg mice had comparable taste sensitivities in response to appetitive as well as aversive tastes. The study suggests that the CTA paradigm can be a sensitive tool to evaluate deficits in implicit associative learning in *APP* transgenic mouse models of AD.

Keywords: Transgenic mouse; Amyloid; Alzheimer's disease; Associative learning; Taste aversion

1. Introduction

Alzheimer's disease (AD) is clinically diagnosed by a progressive loss of mental abilities, which coincides with selective dysfunction and damage of neurons in limbic and association cortices critical for cognition [29,36,47]. Pathologically AD is characterized by extra-cellular senile plaques containing aggregates of highly fibrillogenic β-amyloid peptide ($A\beta_{42}$), by intra-cellular neurofibrillary tangles (NTF) containing aggregates of hyperphosphorylated microtubule-associated protein tau, and by a wide spread of synaptic and neural loss [16,32,41,46]. The first signs of cognitive decline which is observed in AD include difficulties with the acquisition of new information and memory dysfunction [1]. This memory impairment is correlated with a significant amyloid deposition and atrophy, initially in the cortical and hippocampal regions [8,10,21], and at later stages of the disease, in other parts of the brain, including cerebellum and brain stem [4].

Recently developed transgenic (Tg) mouse models of AD express familial AD-related mutated amyloid precursor protein (*APP*) or presenilin (*PS1* or *PS2*) genes. These models replicate many features of AD pathology including the development of extra-cellular Aβ deposits, initially identified in the hippocampus and the forebrain [6,50], and later in most parts of the brain, including cerebellum [7,17,23]. Cognitive deficits in these transgenic mice has been studied using predominantly hippocampally-dependent explicit spatial orientation tasks, such as Morris water maze, radial arm water maze, or an object recognition task ([15,22,27] for reviews). However, not only spatial memory but almost every learning and memory system is affected in AD, including short-term as well as long term memory which can be studied as explicit as well as implicit memory [11–13,24,37,44,52]. Thus, it is important to establish whether other than hippocampally-dependent forms of learning and memory are compromised in transgenic mouse models of AD. Such confirmation of cognitive impairment across different memory systems in existing mouse models would increase their validity and provide a more powerful experimental framework for behavioural characterization of future models and screening of potential therapeutics.

Thus, in the present study we investigated possible deficits of *APP* transgenic mice in a hipocampally-independent

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implicit associative learning tasks. Transgenic mice (TgCRND8), expressing human mutated *APP* genes implicated in AD, with an early-onset of AD pathology were used. These mice exhibit an increasing number of Aβ deposits and levels of sodium dodecyl sulphate (SDS) soluble Aβ by 12 weeks of age which coincides with significant deficits in explicit spatial reference [7,26], and working [25] memory. The implicit memory task selected for the present study was conditioned taste aversion (CTA) paradigm, a form of Pavlovian conditioning that can be observed in many different species including humans [5,19]. CTA is an adaptive specialization of learning which defends an organism against repeated ingestion of toxic foods causing gastrointestinal malaise [18,19,40,43]. When acquiring a CTA, an animal learns to associate the specific taste of a novel food (conditioned stimulus, CS) with experimentally induced nausea (unconditioned stimulus, US). As a result, a long lasting avoidance of food with this specific taste develops. The brain areas implicated in the CTA include the agranular insular cortex, the parvicellular thalamic ventral posteromedial nucleus, and the parabriachial nucleus of the pons, which are part of the gustatory pathway [28,42], and the amydgala [30,31]. The anatomy and cellular processes implicated in CTA, including details on CS and US neural processing are reviewed by Welzl et al. [51]. In our studies, we used 49-week-old TgCRND8 mice which at that age show a widespread amyloid deposits in the brain, including the areas implicated in CTA, the cerebellum and brain stem [7]. Here we report impaired long-term memory of CTA in 49-week-old TgCRND8 *APP* mice as compared to non-transgenic littermates.

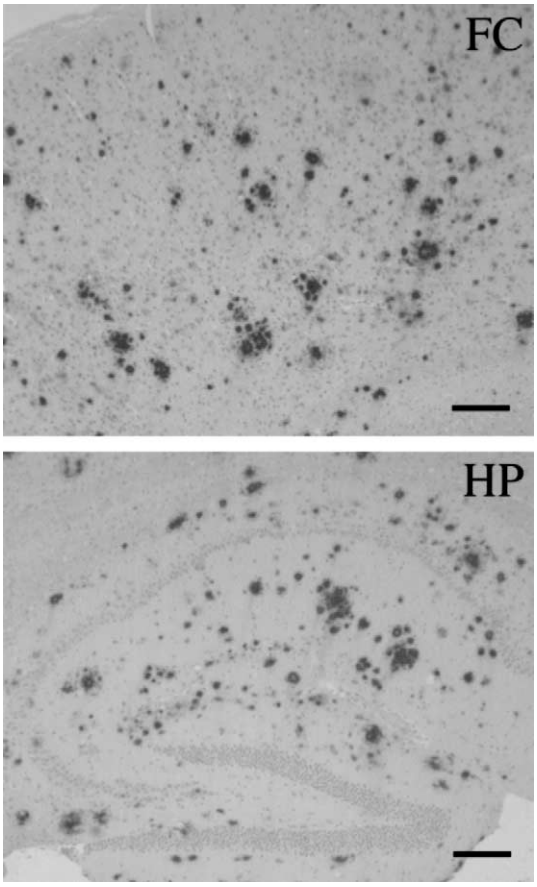

Fig. 1. A sagittal section of an 46-week-old TgCRND8 mouse brain immunostained with 6F/3D antibody (DAKO) illustrating amyloid deposits in the hippocampus (HP) and frontal cortex (FC). Scale bars: 200 μm.

2. Methods

2.1. Animals

Transgenic (TgCRND8) mice encoding a double mutated allele of the human *APP* genes implicated in AD (Swedish; *KM670/671NL* + Indiana; *V717F*) under the control of hamster PrP gene promoter [7] were maintained on a hybrid genetic background (C57BL/6/C3H). To obtain mice for the experiments, TgCRND8 males were crossed with C57BL/6 wild type female mice. Twenty-seven mice (15 TgCRND8 and 12 non-Tg littermates), gender and weight balanced, at an average age of 49 weeks (49 ± 1.3, mean ± S.E.M.; 49, median) were used. They were housed in groups of 2–4 under standard laboratory conditions (12 h:12 h light/dark cycle with lights on at 06:00 h) with a room temperature of 21 °C. The TgCRND8 mice used in the study showed profound burden accumulation of $A\beta_{40}$ and $A\beta_{42}$ with amyloid deposits in limbic and cortical areas of the brain readily evident by immunohistochemistry (Fig. 1). All experimental manipulations were performed during the light-on phase of the cycle in accordance with institutional and CCAC guidelines.

2.2. Conditioned taste aversion test

One week before the onset of an experiment mice were transferred to individual cages in which they had ad libitum access to food, but restricted (from 09:00 to 16:00 h) access to water presented in two 15-ml bottles. Water intake of mice during the first 30-min drinking interval (09:00–09:30 h) was recorded separately. At the end of a 6-day adaptation phase, mice reliably consumed more than 1 ml of water during the initial 30-min interval. Under this water deprivation regimen mice maintained body weight between 99 and 102% of the pre-experimental value of 30.1 ± 1.5 g.

On the conditioning day, mice were allowed to drink only 0.5% saccharin solution (CS) (2,3-dihydro-3-oxobenzisosulfonazole, Sigma Chemical Co.) provided in one 15-ml bottle during the initial 30-min interval. One hour after exposure to the CS, mice in the conditioned group ($N_{Tg} = 9$, $N_{non\text{-}Tg} = 6$) were injected via an intra-peritoneal (i.p.) route with lithium chloride (LiCl; 0.14 M, 2% body weight) as a nausea inducing agent (US). Mice in the unconditioned, control group ($N_{Tg} = 6$, $N_{non\text{-}Tg} = 6$) were injected with corresponding amount of saline. Behavioural signs of malaise (unconditioned response), like "lying-on-belly" [34,49],

"freezing", "chin rubbing" [48], and other behaviours like, grooming, locomotion, and eating were recorded for 20 min following LiCl administration using instantaneous sampling method (sampling intervals of 30 s) [33]. During the remaining time of the day (09:30 to 16:00 h) mice had free access to water. After one recovery day (water from 09:00 to 16:00 h) and two days after CS–US conditioning all mice were given a two-bottle choice test between water presented in one bottle and 0.5% saccharin solution presented in another bottle during the initial 30-min drinking interval. Placement of saccharine bottles with reference to the water bottles was random. Saccharine intake was expressed as the percent of saccharine consumed of the total fluid intake (ml saccharine/(ml water + ml saccharine) × 100). The choice test was repeated on days 8, 15, and 22 after the CS–US pairing to determine the degree of CTA extinction. To determine the basic taste sensitivity and unconditioned taste aversion in response to a bitter tasting substance TgCRND8 mice and non-Tg littermates were given a 30-min two-bottle choice test with a quinine solution (quinine monohydrochloride dihydrate 90%, Aldrich Chemical Co., at a concentration of 0.02%) in one bottle and water in another bottle 27 days following conditioning.

2.3. Data analysis

A factorial model analysis of variance (ANOVA) was employed with the Genotype (Tg versus non-Tg littermates) and Treatment (saline-injected control mice, LiCl injected conditioned mice) as between subject factors, and Tests as repeated measure factor. When necessary, degrees of freedom were adjusted by Greenhouse-Geisser epsilon correction for the heterogeneity of variance. Comparisons between genotypes or experimental treatment of a single variable were performed using the Student's *t*- test. The critical α level was set to 0.05 for all statistical analyses, and all values in the text and figures represent means ± S.E.M. Data analyses were done using SPSS statistical program version 6.1 for Apple Macintosh computer.

3. Results

Pairing of the novel taste of saccharine with nausea significantly reduced saccharine intake in all mice (Treatment: $F(1, 23) = 59.7$, $P < 0.001$). However, the conditioned aversion was significantly attenuated in TgCRND8 mice compared to non-Tg littermates (genotype: $F(1, 23) = 15.4$, $P < 0.01$). Genotype selectively affected the strength of conditioning but not the natural preference for saccharine as seen in unconditioned control mice (Genotype × Treatment: $F(1, 23) = 5.8$, $P < 0.05$; Genotype × Treatment × Tests: $F(2, 69) = P < 0.01$). To elucidate the nature of these interactions the data were analysed separately within each of the experimental conditions. Conditioned TgCRND8 mice showed a significant impairment in associative learning of taste aversion ($F(1, 13) = 13.8$, $P < 0.01$; Fig. 2A). Already during the first choice test the Tg mice consumed more saccharine than non-Tg littermates (37 ± 8.9% versus 8.8 ± 2.1%, respectively; $t(9) = 3.1$, $P < 0.02$) which indicates that long-term memory for taste aversion was impaired by the *APP* transgene. The difference in strength of taste aversion between Tg and non-Tg mice changed during the experiment (Genotype × Tests: $F(3, 39) = 17.04$, $P < 0.001$). The saccharine intake of Tg mice increased linearly in consecutive re-tests ($F(1, 8) = 59.6$ $P < 0.001$, ANOVA linear trend analysis), (Fig. 2A), and 22 days after the CS–US conditioning their taste aversion was almost completely absent, i.e., the TgCRND8 mice consumed saccharine at comparable levels to their saline-injected counterparts ($t(13) = 2.0$, $P = 0.07$; compare scores of Tg mice on day 22 in Fig. 2A and B). In contrast, conditioned non-Tg mice strongly avoided the saccharine solution during all four choice tests (Fig. 2A; none of the polynomial components in trend analysis was significant). In control, unconditioned (saline-injected) conditions both TgCRND8 mice and non-Tg littermates showed a strong preference for the saccharine solution in the tests (Fig. 2B).

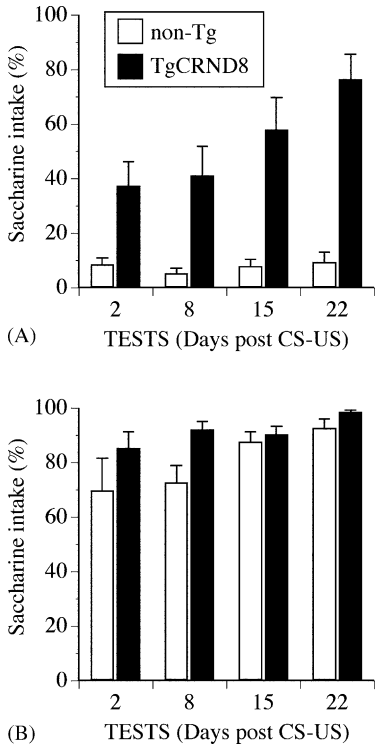

Fig. 2. Conditioned TgCRND8 mice consumed more saccharine solution showing a significantly weaker taste aversion than non-Tg mice ($P < 0.02$, ANOVA), and a significantly faster extinction of taste aversion ($P < 0.01$, ANOVA) during the whole testing period (A). In control (saline injected) conditions, both TgCRND8 mice and their non-Tg littermates showed strong preference for saccharine throughout the test (B).

The naïve to saccharine taste TgCRND8 mice showed a comparable to non-Tg littermates response to a novel taste of

saccharine, and the intake of saccharine during CS–US conditioning session was 1.3±0.12 and 1.2±0.1 for TgCRND8 and non-Tg mice, respectively. Also, mice in conditioned and unconditioned groups consumed comparable amounts of saccharine during a CS–US session (a post hoc ANOVA analysis; none of the factors nor interactions between them were found significant). Although, we found that the signs of malaise after administration of LiCl were much less consistent in mice than reported in rats [34], and some mice did not show any observable changes in their behaviour, the proportions of TgCRND8 and non-Tg littermates which showed clear signs of malaise were comparable (data not shown). Also, the amount of fluid intake (water plus saccharin) during re-tests did not differ between genotypes under both treatment conditions (Genotype: $F(1, 25) = 1.31$, $P = 0.26$); Genotype × Tests: $F(3, 15) = 0.62$, $P = 0.61$). Comparable initial consumption of the saccharine solution by Tg and non-Tg mice during conditioning session, and comparable intake of saccharine by unconditioned mice of both genotypes (Fig. 2B) indicates that sensitivity to the sweet taste of saccharine was similar in Tg and non-Tg mice. Further, taste sensitivity and rejection of a naturally aversive bitter tasting quinine solution (0.02%) was not different between unconditioned TgCRND8 and unconditioned non-Tg mice (Fig. 3A) indicating comparable general gustatory propensities in these mice. In conditioned Tg and non-Tg mice, however, non-Tg mice avoided quinine more than the Tg littermates ($t(13) = 2.73$, $P < 0.02$, Fig. 3B). This difference is likely due to increased neophobia induced by the conditioning procedure in non-Tg mice.

Inspection of individual scores of saccharine intake revealed that unconditioned Tg and non-Tg mice showed little variance in their preference of saccharine (Fig. 4A and B, respectively) and, correspondingly, conditioned non-Tg mice showed little variance in their avoidance of saccharine (Fig. 4C). On the other hand, conditioned TgCRND8 mice showed considerable variance in their response to saccharine (Fig. 4D), ranging from strong avoidance (6%) to a strong preference (81%) during the first choice test carried out on day 2 after CS–US pairing. Furthermore, all the conditioned TgCRND8 mice showed increase of saccharine intake over the course of testing, regardless of the strength of their response to saccharine in the first test, which suggests a compromised long-term memory function in these mice. This stable across re-tests variability in the acquisition of taste aversion in TgCRND8 mice indicates that the CTA paradigm may be suitable to associate the individual

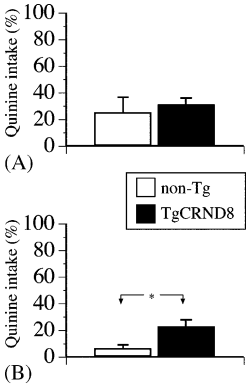

Fig. 3. At the end of the experiment (day 27), the mice were given 30-min choice test between a bitter tasting quinine solution (0.02% quinine monohydrochloride dihydrate 90%, Aldrich Chemical Co.) and tap water. TgCRND8 and non-Tg mice avoided a novel bitter taste of quinine in the unconditioned (A) and conditioned groups (B). $^*P < 0.02$.

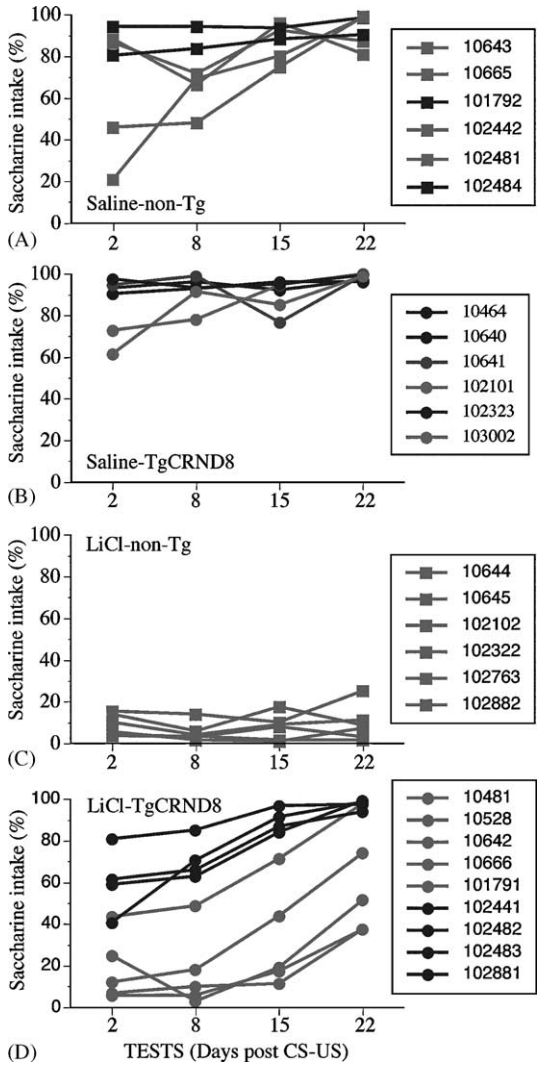

Fig. 4. Control, unconditioned (saline-injected) non-Tg mice (A) and TgCRND8 mice (B) showed a little variance in their preference for a saccharine solution throughout all tests. Correspondingly, conditioned non-Tg mice showed homogenous avoidance of saccharine (C). In contrast, conditioned TgCRND8 mice showed considerable variation in their development and maintenance of taste aversion (D). While some mice (IDs: 10481, 10528, 10642) avoided saccharine at the level comparable to their non-Tg mice (panel C for comparison), other Tg mice showed no evidence of taste aversion learning. All TgCRND8 mice, however, showed increased preference for saccharine solution as testing progressed.

differences in learning abilities with neuropathology of TgCRND8 mice.

4. Discussion

The present results expand the list of known cognitive deficits in transgenic *APP* mouse models of AD. Not only explicit, hipocampally-dependent spatial memory but also a form of implicit memory, classically conditioned taste aversion, are severely compromised in our line of *APP* TgCRND8 mice. These results further strengthen the similarity between pathological profile of behavioural deficits of the present mouse model of AD and the profile of implicit memory impairment seen in AD patients [37,52]. Since the impairment in TgCRND8 mice appeared not only in acquisition but also in maintenance of the learned taste aversion, the paradigm has potential to selectively investigate processes of acquisition (short-term memory) and/or consolidation (long-term memory) in transgenic mouse models of AD. Since the experimental design of the present study did not separate the two processes, the parsimonious interpretation of the results is that implicit long-term memory of taste aversion is impaired in the TgCRND8 mice. Moreover, the increased saccharine intake by TgCRND8 mice during longitudinally administered re-tests may indicate an increased extinction of taste aversion in these mice. However, since the TgCRND8 mice showed a significantly weaker than the conditioned non-Tg littermates taste aversion memory during the first test (Fig. 2A), such interpretation is not unequivocally supported by the present results. Some support for an increased extinction rate in TgCRND8 mice comes from inspection of individual scores of the conditioned TgCRND8 mice which showed a comparable to their non-Tg counterparts avoidance of saccharine during the first test (Fig. 4D). These mice, unlike the conditioned non-Tg mice, showed a steady increase in saccharine intake in later re-tests. However, a small sample size of this sub-cohort of Tg mice prevented a reliable analysis to substantiate this hypothesis statistically.

Overall, our findings extend further demonstrations of impairment in implicit learning in lines of transgenic *APP* mice. Other studies which addressed the issue of implicit associative learning in *APP* transgenic mice focused mainly on contextual fear conditioning. Gerlai et al. [20] investigated PDAPP mice, which express mutated human *APP* gene (*V717F*) under the platelet derived growth factor (hence PDAPP) [17], in a contextual fear conditioning (CFC) test including both context and cue dependent fear conditioning components. Detailed analysis of fear-induced freezing response, along with other behaviours performed during the test, revealed that TgPDAPP mice showed some reduction in fear response in a cue-dependent conditioning. This result was confounded, however, by increased locomotor and exploratory reactivity and/or activity of Tg mice. The authors concluded that the transgene effect was not limited to cognitive aspects of behaviour but likely influenced other behavioural systems. In the second group of studies, a different line of transgenic mice, overexpressing the Swedish mutation of human *APP* gene (*KM670/671NL*), known as Tg2576 mice [23] was used. These mice were tested in hippocampally-dependent T-maze alternation task and in contextual fear conditioning [9]. The results clearly demonstrated an impairment in T-maze alternation, but surprisingly contextual as well as auditory fear conditioning was intact. Only when the salience of the context was decreased, old Tg2576 mice showed attenuated hippocampally-dependent associative learning of contextual discrimination, but not tone conditioning. Similar results of impaired hipocampally-dependent associative learning of contextual fear conditioning were found in mice co-expressing *APP* and *PS1* (Tg2576 × Tg(A246E)PS1) mice [14].

Conditioned taste aversion is a form of implicit memory that can be acquired even after massive damage to the hippocampus. Selective, excitotoxic or electrolytic, lesions of the hippocampus did not impair the acquisition and consolidation of CTA [38,39]. Lesions increased, however, the sensitivity to latent inhibition (attenuation of conditioning after CS-pre-exposure). Thus, the impairment of CTA in TgCRND8 mice suggests that—in addition to the hippocampus—other brain sites are functionally impaired by the progressive accumulation of Aβ deposits in the brain. Whether learning deficits in CTA are correlated with specific neuropathological markers should be determined in future studies.

The contextual fear conditioning studies demonstrated that, first, other non-specific effects of the *APP* transgene may affect the main variable of a cognitive task [20]. Second, it is possible that chosen experimental parameters of the test may render the test not sensitive enough to differentiate the effect of a genotype [9], or to identify age-dependent emergence of impairment [14]. In this light, our study which demonstrated a clear impairment in *APP* transgenic mice in CTA test revealed several practical advantages of this paradigm in detecting cognitive differences due to transgene expression. The CTA test minimises experimental stress since the test is carried out in a home cage, and it does not depend heavily on locomotor ability. The association between novel taste (CS) and nausea (US) is rapidly established during a single CS–US pairing, and the memory of this association (amount of saccharine consumed in later tests, CR) can be easily measured. Future studies targeting cohorts of mice at different stages of amyloid brain pathology should reveal the potential of CTA paradigm in identifying the onset and developmental progression of impairment. Nevertheless, besides a potential use for screening therapeutics, since the paradigm has been well studied with regard to underlying neural mechanisms ([2–3,35], and see [45,51] for reviews) it may provide a useful behavioural tool in future experiments delineating neuropathological mechanisms of cognitive impairment in *APP* transgenic mice.

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