Published in final edited form as:
Behav Brain Res. 2010 January 20; 206(2): 202–207. doi:10.1016/j.bbr.2009.09.013.

Reversal of learning deficits in hAPP transgenic mice carrying a mutation at Asp664: A role for early experience

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Abstract

In addition to the cleavages that generate amyloid-beta (Aβ), the Aβ-precursor protein (APP) is processed at Asp664, releasing a second toxic peptide (APP-C31). Transgenic mice otherwise identical to a well-characterized model of AD, PDAPP mice, but carrying a mutation that obliterates Asp664 show a reversal of AD-like deficits in memory and in non-cognitive components of behaviour in spite of accumulating high levels of Aβ. These results suggest that cleavage of APP at Asp664 plays a role in the generation of AD-like deficits, and that a major pathway of Aβ toxicity *in vivo*, or a pathway that crucially impinges on it, may depend on cleavage of APP at Asp664. Since young PDAPP(D664A) mice showed an akinetic phenotype when first required to swim, we trained a 3 month-old (mo) cohort to criterion (normal swimming), and briefly exposed it to the Morris water maze (MWM) environment prior to training at 7 mo, to avoid potentially confounding effects of the akinetic phenotype in MWM studies. Prior experience decreased floating in PDAPP(D664A) mice but not in PDAPP nor in non-Tg groups. While learning was restored in experienced PDAPP(D664A) mice, it was indistinguishable from both non-Tg as well as from PDAPP mice in naïve PDAPP (D664A) animals. Floating did not correlate with worse performance in naïve PDAPP(D664A) mice, suggesting that the contribution of prior experience to improved performance is related to its cognitive effects but not to non-cognitive components of behaviour. Our results suggest that early experience reduces the contribution of non-cognitive components of behaviour to performance, and may contribute to the restoration of learning at later ages in PDAPP(D664A) mice.

Keywords

Alzheimer's disease; Transgenic mouse model; Asp664 cleavage; Learning; Morris water maze; Non-cognitive components of behaviour

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly. Deficits in AD are believed to result in large part from the toxic effects of amyloid-beta (Aβ), a peptide released

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after proteolysis of the amyloid precursor protein (APP)([1]). In addition to the cleavages that generate Aβ, APP may also be cleaved intracytoplasmically at Asp664 by caspases ([2, 3]), releasing a cytotoxic carboxy (C)-terminal peptide, APP-C31([3, 4]), which is thought to activate apoptosis through an N-terminal Smac/DIABLO-like motif([5]). Various mechanisms mediating Aβ toxicity have been proposed on the basis of *in vitro* data, but genetic evidence for their relevance *in vivo* is still largely lacking. We recently showed([6, 7]) that transgenic mice otherwise identical to a well-characterized model of AD, PDAPP mice([8, 9]), but carrying a mutation that obliterates the Asp664 cleavage site in APP [PDAPP(D664A)mice], continue to produce and deposit Aβ, but do not develop AD-like deficits, suggesting that a significant component of Aβ toxicity *in vivo* may be dependent on the cleavage of APP at Asp664. One potential link between Aβ production and APP Asp664 cleavage has been described, with the demonstration that Aβ binds APP and induces APP multimerization([10–15]), leading in turn to cleavage of the APP cytosolic tail at Asp664([11–13]), followed by synaptic and neuronal damage ([12, 13]).

We previously showed ([6, 7]) that AD-like spatial learning and memory deficits, as well as synaptic impairments, were abolished in two independent PDAPP(D664A) transgenic mouse lines, in the moderate-expressing PDAPP(D664A)B21 line at least up to 12 mo of age (mo) and in the high-expressing PDAPP(D664A)B254 line at least until 7 mo([6, 7]). We also demonstrated that AD-like behavioural deficits, as well as aberrant patterns in non-cognitive components of behaviour, were largely absent from high-expressing PDAPP(D664A)B254 mice assayed longitudinally until late in life (13 mo), when Aβ-plaque deposition was increased by 50% and gliosis was similar to that in brains of the high-expressor PDAPP(J20) line([16]). These observations support the hypothesis that the Asp664 cleavage site significantly contributes to the generation of cognitive and non-cognitive aspects of AD-like behavioural deficits in the PDAPP transgenic mouse model until late in life, and suggest that additional, non-Asp664-dependent pathway(s) of Aβ toxicity may be operant that could underlie trends to poorer performance observed in aged Tg PDAPP(D664A) mice.

Young PDAPP(D664A) mice (2–2.5 mo), however, show a pronounced akinetic phenotype akin to the tonic phase of a seizure (immobility in stereotyped limb positions) when first exposed to swimming. In previous studies, cohorts were trained to criterion (normal swimming) at 3 mo and pre-exposed to the MWM environment at 5 mo, to rule out confounding effects (e.g., mistaken scoring as floating and increased latencies) in MWM studies. To determine whether swimming training-to-criterion and pre-exposure to MWM (‘experience’) affected cognitive and non-cognitive components of behaviour in PDAPP and PDAPP(D664A) mice, we compared behaviour in experienced and naive cohorts of 7 mo PDAPP and PDAPP(D664A) mice. We found that prior experience decreased floating in PDAPP(D664A) but not in PDAPP mice, nor in non-Tg littermates. While learning was restored to non-Tg levels in experienced PDAPP(D664A) mice, it was indistinguishable both from non-Tg littermates as well as from PDAPP mice in naïve PDAPP(D664A) animals. Suprisingly, floating did not correlate with worse performance in naïve PDAPP(D664A) mice, suggesting that the contribution of prior experience to improved performance in PDAPP(D664A) mice is a result of cognitive processes but not to effects related to non-cognitive components of behaviour such as increased helplessness. Our results suggest that cognitive effects of early experience contribute to the complete amelioration of learning deficits that arise at later ages in PDAPP(D664A) mice.

METHODS

Mice

A G-to-C mutation was introduced in the PDGF β-chain promoter-driven human APP minigene carrying the Swedish and Indiana mutations([8, 9]) that mutated Asp_{664} (APP_{695} numbering) to Ala (PDAPP(D664A)) to generate PDAPP(D664A) transgenic mice, as described([6])

(SAGANICH 2006). Mice from the high-expressing PDAPP(D664A)B254 transgenic line were used in this study. Mice from the high-expressing PDAPP(J20) line were kindly provided by Dr. Lennart Mucke, Gladstone Institutes for Neurological disease, San Francisco, CA. Non-Tg littermates were culled in equal proportions from both transgenic lines and used as controls. All transgenic lines were maintained by heterozygous crosses with C57BL/6J breeders (Jackson Laboratories, Bar Harbor, ME). All transgenic animals were heterozygous with respect to the transgene. After removal of animals showing no motivation to swim ("floaters") (see **Methods**, **Behavioural Testing**, below), experimental groups of animals that received swimming training-to-criterion and pre-exposure to MWM ("experienced") were: non-Tg, n=12; Tg PDAPP(D664A), n=10; Tg PDAPP, n=8. Experimental groups of naive animals were: non-Tg, n=10; Tg PDAPP(D664A), n=10; Tg PDAPP, n=10. All animals were 7 mo males. All procedures were conduced in accordance with the "Principles of laboratory animal care" (NIH publication # 85–23, revised 1996) and under protocol # A10010 approved by the Buck Institute IACUC.

Swimming training

Mice in all experimental groups were subjected to repeated 60 s swimming sessions at 2.5–3 mo in a 480 × 375 × 210(ht) mm rat cage that was filled with water at 24+/−1°C. Animals showing a pronounced akinetic phenotype akin to the tonic phase of a seizure (immobility in stereotyped limb positions) during swimming were lightly prodded to reinitiate swimming. Animals that did not respond to light prodding were immediately removed from the water. All animals were dried and returned to their home cages after each experience swim. Intervals between training swims ranged from 3 hours to 1 day. Training swims were repeated until each mouse reached criterion (2 consecutive 60 s sessions showing normal swimming). All animals reached criterion within 5 days.

Behavioural testing

The Morris water maze([17]) (MWM) was used to test spatial memory. All animals had normal motor and visual skills as determined by sensorimotor tasks performed prior to testing. Experienced and naive groups were assessed for swimming ability with a straight water alley (15 by 200 cm) containing a submerged (1 cm) 12 × 12 cm platform 2 days before testing. The procedure described by Morris et al. ([17]) was followed as described ([6, 16]). Animals were monitored daily and weights recorded weekly. Performance in all tasks was recorded by a computer-based video tracking system (HVS Image Analysis VP-200, HVS Image, Hampton, UK). Data were analyzed offline by using HVS Image, and processed with Microsoft EXCEL.

Pre-acclimation to MWM

All experimental animals were exposed to MWM environment conditions identical to those to be used during training (see **Behavioural Testing**) with one session comprising 3 swims administered on 2 consecutive days at 5 mo of age.

Statistical analyses

Statistical analyses were performed using GraphPad Prism (GraphPad, San Diego, CA). In two-variable experiments, repeated measures two-way ANOVA followed by Bonferroni's post hoc tests were used to evaluate the significance of differences between group means. When analyzing one-variable experiments with more than 2 groups, significance of differences between means was evaluated using one-way ANOVA followed by Tukey's post-hoc test. Values of $P < 0.05$ were considered significant. Slopes (m) of curves were calculated using the LINEST function in MS EXCEL. Pearson correlation coefficients between variables were calculated with regression analyses performed using GraphPad Prism (GraphPad, San Diego, CA).

RESULTS

We previously showed that PDAPP(D664A) transgenic (Tg) mice carrying the D664A mutation do not develop learning and spatial memory deficits in the Morris water maze test ([4, 7]), a widely used tool in the assessment of hippocampal-dependent learning in rodents([17]). In contrast to Tg PDAPP mice, impairments in cognitive and non-cognitive components of behaviour were absent from Tg PDAPP(D664A) mice assessed longitudinally until 13 mo, in spite of the accumulation of very high levels of soluble and deposited Aϐ that exceeded those of Tg PDAPP mice by approximately 50% ([7, 16, 18]).

We noticed that young PDAPP(D664A) mice (2–2.5 mo) showed a pronounced akinetic phenotype similar to the tonic phase of a seizure (immobility in stereotyped limb positions) when first exposed to swimming. To rule out confounding effects arising from this phenotype (e.g., mistaken scoring as floating and increased latencies) in MWM studies cohorts were trained-to-criterion (normal swimming) at 3 mo and pre-exposed to the Morris water maze (MWM) environment at 5 mo in our previous studies. To determine whether swimming training-to-criterion and pre-exposure to MWM (“experience”) affects cognitive and non-cognitive components of behaviour in PDAPP and PDAPP(D664A) mice, we then compared behaviour of experienced and naive cohorts of PDAPP and PDAPP(D664A) mice.

First, we examined groups of experienced Tg PDAPP(J20), Tg PDAPP(D664A)B254 (PDAPP and PDAPP(D664A) mice henceforth) and non-Tg littermates at 7 mo (Galvan et al. 2002). Seven mo Tg PDAPP, Tg PDAPP(D664A) and non-Tg littermates showed no deficiencies in swimming abilities, directional swimming or climbing onto a cued platform during pre-training and had no sensorimotor deficits, as determined with a battery of neurobehavioural tasks. Tg PDAPP mice but not the other experimental groups, however, consistently showed a higher tendency to float (swimming at speeds lower than 0.025 m/s), which was exacerbated in some animals. Tg PDAPP mice showing no motivation to swim (“floaters”) were taken out of the study.

Making no attempt to swim (floating) and swimming in close proximity to the pool wall (‘wall hugging’ or thigmotaxis) are frequent performance deficits to which certain strains of mice are prone, and are frequently more pronounced in mice that have been genetically manipulated ([19, 20]). These patterned behaviours are believed to be manifestations of emotional responses, such as anxiety and helplessness in stressful situations.

Percentage time spent floating in experienced transgenic PDAPP mice was not significantly different from non-Tg littermates but was significantly increased with respect to PDAPP (D664A) animals ($P<0.01$, Bonferroni’s post test applied to a significant effect of genotype on percent time floating, $F(2,56)=4.08$, $P=0.0280$, repeated measures two-way ANOVA) (Figure 1a). Overall, percentage time spent floating decreased along training for all experienced groups. In contrast, percent time spent floating increased along training for all naive groups [Figure 1b, significant effect of day number on percent time spent floating, $P<0.0001$, $F(3,96)=25.89$, repeated measures two-way ANOVA]. Interestingly, floating increased similarly for all naive groups during the first 3 days of training but stabilized on day 4 for both the non-Tg and the Tg PDAPP(D664A) groups, while it continued to increase for naive Tg PDAPP mice. Overall, percentage time spent floating was significantly increased for naïve Tg PDAPP mice with respect to both non-Tg and Tg PDAPP(D664A) groups [$P<0.001$ and $P<0.05$ with respect to the non-Tg and Tg PDAPP(D664A) groups, respectively, Bonferroni’s post test applied to a significant effect of genotype [$F(2,96)=3.69$, $P<0.03$], repeated-measures two-way ANOVA]. These observations suggest that experience (i.e., prior training-to-criterion and exposure to the MWM environment) has a marked effect on helplessness, abolishing a significant increase for the Tg PDAPP group with respect to non-Tg littermates and reversing a trend to increased

helplessness in all groups during training (Figures 1a and b). Overall, prior experience significantly decreased percent time floating for Tg PDAPP(D664A) but not for non-Tg nor for Tg PDAPP animals (Figure 1c). While floating was significantly increased for naïve Tg PDAPP and Tg PDAPP(D664A) mice with respect to non-transgenic littermates, experienced PDAPP(D664A) mice were indistinguishable from non-Tg littermates and significantly different from Tg PDAPP mice ($P<0.01$, Tukey's test applied to a significant effect of genotype on percent time floating, $P<0.0001$, Figure 1c). Thus, as we expected, training-to-criterion and preexposure to the MWM environment decreased floating in PDAPP(D664A) mice. Increased latencies were correlated with increased floating ($R^2= 0.2656$, $P=0.0015$) in all naive groups. No correlation between latencies and floating was observed, however, in experienced groups ($R^2=0.083$, $P=0.11$), suggesting that increased helplessness in naïve groups contributed to decreased performance levels. Floating, however, did not correlate with performance neither in naïve ($R^2=0.0015$, $P=0.9$) nor in experienced ($R^2=0.1692$, $P=0.2$) PDAPP(D664A) mice, suggesting that the contribution of prior experience to improved performance in PDAPP (D664A) mice is related to its cognitive effects but not to non-cognitive components of behaviour.

The percent of time that animals spent in thigmotactic swim (with swim paths restricted to distances ~10 cm from the tank wall) was significantly higher in experienced Tg PDAPP mice than in all other experienced groups (Figure 2a) and this effect was uniform across days of training [($P<0.001$ and $P<0.01$ with respect to non-Tg and Tg PDAPP(D664A) groups, respectively, using Bonferroni's post test applied to a significant effect of genotype on percent time spent in thigmotactic swim, $F(2,140)=11.69$, $P=0.0002$, repeated measures two-way ANOVA)]. Percent time spent in thigmotactic swim, however, decreased or stayed constant with day number for both experienced non-Tg and Tg PDAPP(D664A) mice respectively. Although genotype did not affect percent time spent in thigmotactic swim significantly for naïve groups, a trend to increased thigmotaxis that did not reach statistical significance was observed for naïve Tg PDAPP(D664A) and Tg PDAPP mice (Figure 2b). Percent time engaged in thigmotactic swim, however, decreased significantly with day number for all naïve groups during training [$F(3,96)=3.51$, $P=0.0181$, repeated measures two-way ANOVA]. Thus, prior experience has a negative effect on anxiety levels in Tg PDAPP, but not in non-Tg or in Tg PDAPP(D664A) mice.

Spatial learning was determined in naïve and experienced Tg PDAPP, Tg PDAPP(D664A) and non-Tg littermates at 7 mo. Seven mo naïve or experienced Tg PDAPP, Tg PDAPP (D664A) and non-Tg littermates showed no sensorimotor deficits as determined with a battery of neurobehavioural tasks performed prior to training. Experienced Tg PDAPP mice showed significant impairments in performance with respect to non-Tg littermates and to Tg PDAPP (D664A) animals during training at 7 mo [$P<0.001$ and $P<0.01$ respectively, as a result of Bonferroni's post-hoc test applied to a significant effect of genotype [($F(2,56)=10.96$; $P<0.0003$)), repeated measures two-way ANOVA] ([6, 7]). Performance of experienced 7 mo Tg PDAPP(D664A) mice during training, however, was indistinguishable from that of non-Tg littermates (Figure 3a). The interaction between day number and genotype was significant, thus genotype affected performance differently at different times during training. Although genotype affected performance differently on different training days, conclusions about genotype differences in performance could potentially be difficult to draw. However, the comparison of group mean latencies for days 1, 2 and 3 shows that improvements in performance during training occurred for non-Tg and Tg PDAPP(D664A) groups, but not for Tg PDAPP mice. These results indicate that learning impairments were present in experienced Tg PDAPP but not in experienced Tg PDAPP(D664A) mice at 7 mo ([6, 7]). In contrast to experienced animals, naïve non-Tg and Tg PDPP(D6664A) groups showed a biphasic pattern of performance, with decreased average latencies through the end of the second day of training, followed by increased latencies in the third day and a subsequent improvement (decreased

latencies) or stabilization for the Tg PDAPP(D664A) and non-Tg groups respectively (Figure 3b). Similar to our observations with experienced animals [([6]), Figure 3a], the performance of naive Tg PDAPP animals during training was significantly impaired with respect to that of non-Tg littermates [(P<0.001) as a result of Bonferroni's post-hoc test applied to a significant effect of genotype (F(2,87) = 9.43; P=0.0007; repeated measures two-way ANOVA)]. In contrast to the biphasic pattern observed for naïve non-Tg and Tg PDAPP(D664A) mice, latencies of naïve Tg PDAPP animals did not show a decrease until training day 4 (Figure 3a). While the performance of naïve Tg PDAPP(D664A) was indistinguishable from that of naïve non-Tg littermates, it was not significantly different from that of naïve Tg PDAPP mice either. This observation suggests that naïve PDAPP(D664A) animals show a trend to impairment that does not result in significant differences with respect to the control non-transgenic group. The interaction between genotype and swim number was significant during acquisition of naïve groups (F(6,87)=2.51; P=0.0273, repeated measures two-way ANOVA), thus genotype affected performance differently at different times during training. Session number significantly affected performance, with reduced latencies through the end of training for all naïve experimental groups (Figure 3b). Taken together, these data suggest that mild deficits in learning and a trend to a biphasic pattern of performance may be present in naïve Tg PDAPP (D664A) mice, but that these learning deficits may be modified by effects of prior experience.

DISCUSSION

We showed previously ([6, 7]) that mutation of Asp664 in the C-terminal domain of a FAD-hAPP (familial Alzheimer's disease-human APP) transgene abolished AD-like deficits in the PDAPP mouse model. AD-like deficits were absent in PDAPP(D664A) transgenic mice from the moderate-expressor B21 line up to 12 mo (mo)([7]) and in the high-expressor PDAPP (D664A) transgenic line B254 until 7 mo ([6]). Tg PDAPP(D664A)B254 mice express ~1.5X higher levels of the FAD-hAPP transgene and produce significantly higher levels of Aβ than Tg PDAPP(J20) mice ([6, 7]). Young (2–2.5 mo) PDAPP(D664A) mice, however, show a pronounced akinetic phenotype akin to the tonic phase of a seizure (immobility in stereotyped limb positions) when first exposed to swimming. In previous studies, cohorts were trained to criterion (normal swimming) at 3 mo and pre-exposed to the Morris water maze (MWM) environment at 5 mo, to rule out confounding effects (e.g., mistaken scoring as floating and increased latencies) in MWM studies. To determine whether swimming training-to-criterion and pre-exposure to MWM ("experience") affects cognitive and non-cognitive components of behaviour in PDAPP and PDAPP(D664A) mice, we compared behaviour in experienced and naive cohorts of 7 mo PDAPP and PDAPP(D664A) mice. We showed that prior experience significantly affects both cognitive and non-cognitive components of behaviour. Making no attempt to swim (floating) and swimming in close proximity to the pool wall ('wall hugging' or thigmotaxis) are patterned behaviours believed to be manifestations of emotional responses, such as anxiety and helplessness in the face of stress. Thigmotaxis is a pattern of behaviour frequently displayed by animals that do not learn well ([21]). Immobility, defined as the absence of active, escape-oriented behaviours such as swimming in the water tank, is considered indicative of depressive-like behaviour in experimental paradigms such as the forced swim test ([22, 23]).

Prominently, experience reversed a trend to increased percent time spent floating, a measure of helplessness, as training progressed in all experimental groups. Experience also abolished a significant increase in percent time spent floating for Tg PDAPP animals. Interestingly, experience did not improve but actually contributed to an increase in percent time spent in thigmotactic swim, a measure of anxiety, in the Tg PDAPP group while it did not significantly affect anxiety in non-Tg or in Tg PDAPP(D664A) mice. Thus, prior experience had a measurable effect on non-cognitive components of behaviour in all experimental groups.

While prior experience did not affect performance, a measure of learning, of non-Tg groups and did not ameliorate learning impairments observed in Tg PDAPP mice, prior training-to-criterion and exposure to the MWM environment had a significant effect on performance of Tg PDAPP(D664A) mice. Thus, while performance of experienced PDAPP(D664A) was indistinguishable from that of non-Tg Tg PDAPP(D664A) animals showed an intermediate phenotype, with performances indistinguishable from those of non-Tg littermates but also indistinguishable from those of Tg PDAPP animals. Thus, prior experience may contribute to improved learning in PDAPP(D664A) animals, suggesting that early experience may contribute to the complete amelioration of learning deficits in PDAPP(D664A) mice.

The results of the present study refine our previous findings ([6, 7, 16]) and indicate that the cleavage of hAPP at Asp664 by caspases (or possibly by a non-caspase protease or proteases), which does not overtly affect neither Aβ production, Aβ deposition, nor astrogliosis, is a critical step in the development of behavioural abnormalities in FAD-hAPP transgenic mice. The present studies suggest that the mechanism(s) by which stabilization of the C-teminal domain of APP in PDAPP transgenic mice abolishes the development of deficits in cognitive and non-cognitive components of behaviour may involve the activation of plasticity mechanisms dependent on prior experience. That prior experience results in a higher degree of tolerance to ongoing pathogenic processes in Tg PDAPP(D664A) mice, that have very high levels of soluble and deposited Aβ and show extensive astrogliosis ([16]), suggest that plastic processes may be activated in PDAPP(D664A) mice that are similar to those that underlie “cognitive reserve”, a notion arising from epidemiological data that suggests that prior experience (higher education and increased participation in intellectual, social and physical aspects of daily life) is associated with slower cognitive decline in healthy elderly and may reduce the risk of AD even in the presence of significant AD-asssociated pathology ([24]).

The C-terminal cleavage of APP by caspases truncates APP amino-terminally to sequences that are required for its interaction with motor proteins, components of the stress response, and transcriptional transactivators ([25–32]). Cleavage of APP by transiently activated caspases at neuronal terminals may therefore disrupt its interaction with several different protein complexes and thus alter the normal processing, turnover or function of the molecule. Thus, it is possible that protective mechanisms that partially compensate for Aβ toxicity may be upregulated when the C-terminal cleavage of APP is precluded in PDAPP(D664A) mice. Consistent with the hypothesis that upregulation of pathways that result in resistance to Aβ toxicity play a role in preventing the development of AD-like deficits in Tg PDAPP(D664A) mice, the requirement for prior experience for the complete prevention of cognitive deficits in PDAPP(D664A) mice suggests that these pathways might be upregulated by early learning or by environmental enrichment.

The results presented here point to a key role for early experience in the amelioration of functional AD-like deficits in a transgenic mouse model of AD by obliteration of the Asp664 cleavage site in APP. Furthermore, our data confirm and extend the previous conclusion that the intracytoplasmic domain of APP plays an important role in the pathogenesis of AD, and suggest that behavioural interventions may be used to complement therapies that may target the cleavage of APP at Asp664 in the treatment or prevention of AD.

Acknowledgments

We thank Ms. Katrine N. Krueger for excellent administrative assistance. Supported in part by Alzheimer’s Association NIRG 04-1054 to V.G., NIH R01 NS045093-05 to D.B., a Drown Foundation award to D.B., IIRG 06-27717 to D.B. and an award from S.D. Bechtel, Jr. to V.G.

Abbreviations

AD	Alzheimer's disease
APP	β-amyloid precursor protein
Asp664	aspartate 664
D664A	aspartate to alanine mutation at position 664 of APP
Tg	transgenic
Aβ	β-amyloid peptide
PDAPP mice	Platelet-derived growth factor β-chain promoter-driven amyloid precursor protein expressing transgenic mice

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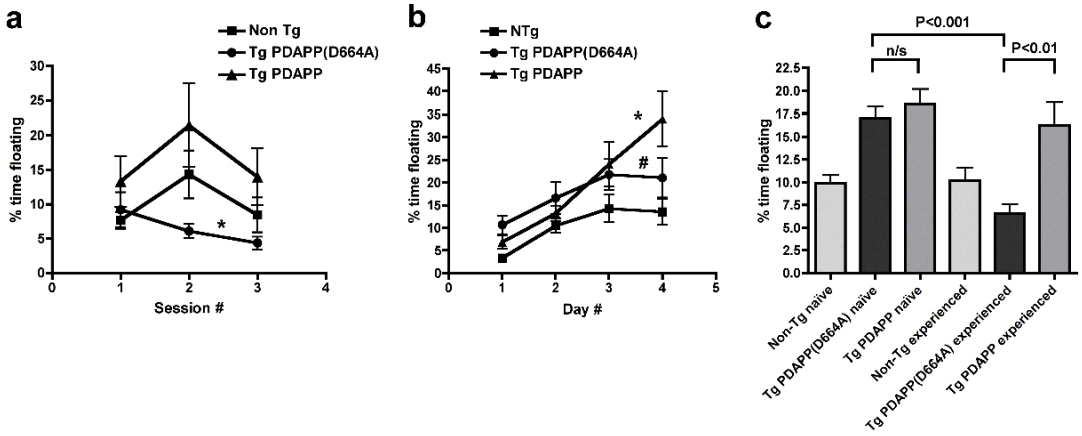

Figure 1. Effect of prior experience on percentage time spent floating (helplessness)

a. Experienced mice. Percent time spent floating decreased across sessions for experienced groups [significant effect of session number on percent time spent floating, $F(2,56)=3.38$, $P=0.04$, repeated measures one-way ANOVA]. Percent time spent floating for the experienced Tg PDAPP(D664A) group was indistinguishable from non-Tg littermates but significantly decreased with respect to Tg PDAPP animals [$^{*}P<0.01$, Bonferroni's post test applied to a significant effect of genotype, $F(2,56)=4.08$, $P=0.03$, repeated measures two-way ANOVA]. Percent time floating for experienced Tg PDAPP mice was indistinguishable fron non-Tg littermates. **b. Naïve mice.** Percent time spent floating significantly increased across sessions for all naïve groups [$P<0.0001$, $F(3,96)=25.89$, repeated measures two-way ANOVA]. Percentage time spent floating was significantly increased for naïve Tg PDAPP mice with respect to both non-Tg and Tg PDAPP(D664A) animals [$^{*}P<0.001$ and $^{\#}P<0.05$ respectively, Bonferroni's post test applied to a significant effect of genotype [$F(2,96)=3.69$, $P<0.03$], repeated-measures two-way ANOVA]. **c. Floating in experienced vs. naïve mice.** Prior experience significantly decreased percent time spent floating in Tg PDAPP(D664A) but not in non-Tg nor in Tg PDAPP mice ($P<0.001$, Tukey's post test applied to a significant effect of genotype on percent time spent floating, $P<0.0001$, one-way ANOVA). Floating in naïve PDAPP(D664A) mice was indistinguishable from Tg PDAPP mice and significantly increased with respect to non-Tg littermates ($P<0.001$, Tukey's post test, $P<0.0001$, one-way ANOVA). In contrast, floating in experienced Tg PDAPP(D664A) animals was indistinguishable from non-Tg littermates and significantly decreased with respect to Tg PDAPP mice ($P<0.01$, Tukey's post test applied to a significant effect of genotype on percent time spent floating, $P<0.0001$, one-way ANOVA). Overt floaters were excluded from all analyses. Data are means ± SEM.

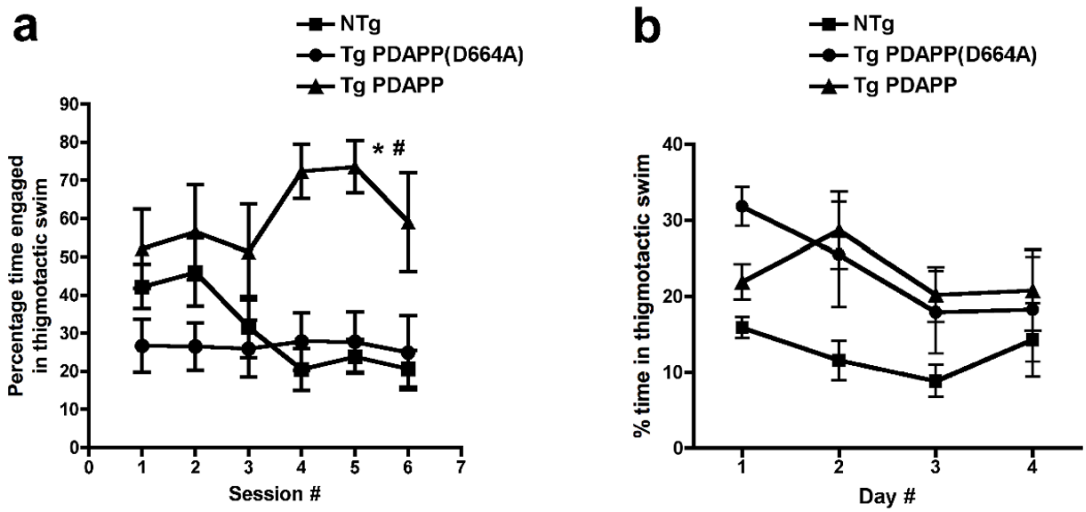

Figure 2. Effect of prior experience on percentage time engaged in thigmotactic swim (anxiety)

a. Experienced mice. Percent time spent engaged in thigmotactic swim remained constant or decreased for non-Tg and TgPDAPP(D664A) mice across training. In contrast, percent time swimming close to the tank wall increased across training and was significantly augmented for Tg PDAPP mice with respect to all other groups [$^{*}P<0.001$ and $^{\#}P<0.01$ with respect to non-Tg and Tg PDAPP(D664A) groups respectively, Bonferroni's post test applied to a significant effect of genotype, $F(2,140)=11.69$, $P=0.0002$, repeated measures two-way ANOVA]. **b. Naïve mice.** Time spent swimming close to the tank wall decreased significantly with day number for all groups of naïve mice during training [$F(3,96)=3.51$, $P=0.0181$, repeated measures two-way ANOVA]. No significant differences in percent time spent in thigmotactic swim were found between naïve experimental groups.

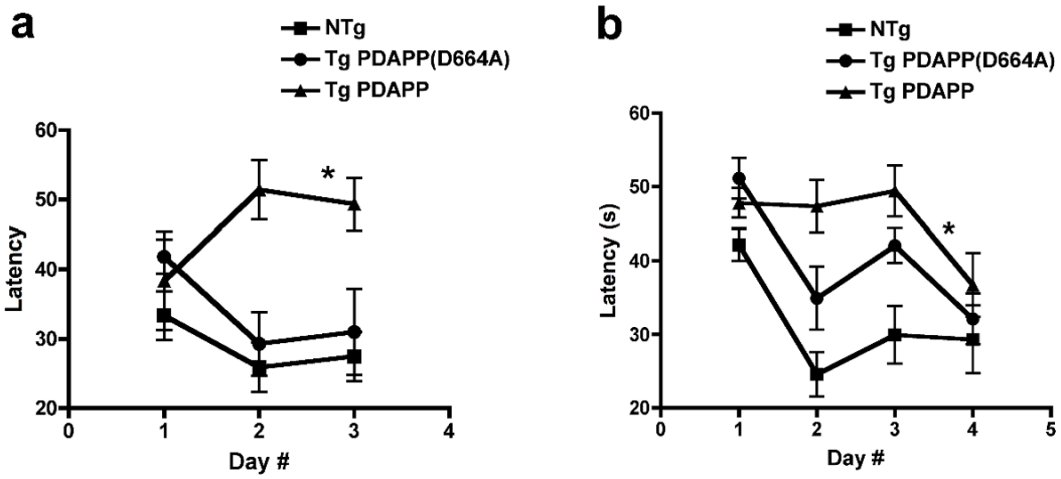

Figure 3. Effect of prior experience on spatial learning

a. Experienced mice. Mean latencies to reach a hidden platform were significantly increased for experienced Tg PDAPP mice with respect to non-Tg littermates and to Tg PDAPP(D664A) animals [**P*<0.01 for both comparisons as a result of Bonferroni's post-hoc test applied to a significant effect of genotype [(*F*(2,140)=10.96; *P*<0.0003), repeated measures two-way ANOVA]. No differences in performance were found between experienced PDAPP(D664A) mice and the non-Tg group. **b. Naïve mice.** Performance of naïve Tg PDAPP mice during training was significantly impaired with respect to that of non-Tg littermates [(**P*<0.001 as a result of Bonferroni's post-hoc test applied to a significant effect of genotype (*F*(2,87) = 9.43; *P*=0.0007; repeated measures two-way ANOVA]. Performance of naïve Tg PDAPP(D664A) was not significantly different from that of naïve non-Tg littermates nor from that of naïve Tg PDAPP mice.