Short Communication

Expression of the Neuronal Adaptor Protein X11α Protects Against Memory Dysfunction in a Transgenic Mouse Model of Alzheimer's Disease

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Handling Associate Editor: Thomas Shea

Abstract. X11α is a neuronal-specific adaptor protein that binds to the amyloid-β protein precursor (AβPP). Overexpression of X11α reduces Aβ production but whether X11α also protects against Aβ-related memory dysfunction is not known. To test this possibility, we crossed X11α transgenic mice with AβPP-Tg2576 mice. AβPP-Tg2576 mice produce high levels of brain Aβ and develop age-related defects in memory function that correlate with increasing Aβ load. Overexpression of X11α alone had no detectable adverse effect upon behavior. However, X11α reduced brain Aβ levels and corrected spatial reference memory defects in aged X11α/AβPP double transgenics. Thus, X11α may be a therapeutic target for Alzheimer's disease.

Keywords: Amyloid-β protein precursor, axonal transport, Mint1, protein trafficking, X11α

INTRODUCTION

Altered processing of the amyloid-β protein precursor (AβPP) to increase production of Aβ is believed to be a key pathogenic event in Alzheimer's disease [1]. Lowering Aβ production thus represents one of the favored routes for therapeutic intervention. X11α (also known as mint-1) is a neuronal-specific adaptor protein that binds to the intracellular domain of AβPP [2–4]. Overexpression of X11α decreases production of Aβ [5–10]. This finding has prompted the suggestion that modulating X11α function or X11α-AβPP interactions might provide therapeutic targets for Alzheimer's disease [11]. However, more formal support for this notion requires the demonstration that X11α not only reduces cerebral Aβ load but also rescues Aβ-related defects in cognition. To test this possibility, we studied the effect of X11α on memory function and brain Aβ levels by crossing transgenic mice that overexpress

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X11α [9] with AβPP-Tg2576 transgenic mice. AβPP-Tg2576 mice express a familial Alzheimer's disease "Swedish" mutant AβPP and develop age-related defects in spatial reference memory that correlate with increasing levels of brain Aβ [12–15].

Crossing of X11α and AβPP-Tg2576 mice produced the predicted ratio of genotypes (one quarter nontransgenic (NTg), AβPPswedish mutant (AβPPswe), X11α, AβPPswe/X11α). Mice were studied at 3–4 and 16–18 months of age. At 3–4 months, AβPP-Tg2576 have low levels of Aβ and normal memory function, but at 16–18 months, cerebral Aβ levels are elevated and the mice display memory dysfunction [14,15]. There were no significant differences in gender ratios between the four genotypes in either the 3–4 or 16–18 month age groups (chi-square analyses) and so males and females were pooled for analyses.

We initially tested animals for sensorimotor defects using the SHIRPA primary screen [16]. No significant differences were detected between the four genotypes at either age demonstrating that X11α overexpression has no obvious effect on sensorimotor function in either the absence or presence of AβPPswe (data not shown). We then studied the effect of X11α on spatial reference memory function using the Morris water maze. For these studies we performed visible platform training followed by hidden platform testing with three rounds of probe trials as described previously by us and others for AβPP-Tg2576 mice [15,17,18]. All genotypes in both age groups learned the location of the visible platform within 3 days of training (Fig. 1A, B) and were similarly proficient swimmers (data not shown). However, 16–18 but not 3–4 month old AβPPswe and AβPPswe/X11α mice both had increased latencies to reach the platform compared with NTg and X11α littermates in the first two training blocks on day 1 (Fig. 1A, B). This result is consistent with previous studies which also showed that older but not young AβPP-Tg2576 mice have an initial delay in escape latency to the visible platform [15,17]. Despite this lag in reaching the visible platform by 16–18 month AβPPswe and AβPPswe/X11α mice, our finding that there were no significant differences between any genotype on days 2 and 3 argues against sensorimotor deficits as a potential explanation for any defective performance in determining the location of the hidden platform. Others have formed the same conclusion with similar data on AβPP-Tg2576 mice [15,17]. In addition, our findings that there were no significant differences between NTg and X11α, or between AβPPswe and AβPPswe/X11α, at either time studied argues that the phenotype is due

to AβPPswe and is not influenced by the presence of X11α. We thus conclude that X11α has no effect on escape latency to reach the visible platform in the Morris water maze.

We then tested the abilities of the mice to reach the hidden platform in the Morris water maze. This involved analyses of escape latencies and of swimming patterns in probe trials where the platform was removed. At 3–4 months of age, all genotypes performed equally well in the trials (Fig. 1C and data not shown). However, at 16–18 months, AβPPswe mice displayed significant deficits compared to all other genotypes. This was revealed by an increase in escape latency to reach the hidden platform (Fig. 1D) and by reduced performance in the probe trials (Fig. 2). This age-dependent defect in the Morris water maze by AβPPswe transgenics is consistent with previous reports on AβPP-Tg2576 mice [15,17,18]. There were no detectable differences between X11α and NTg mice in any tests. However, 16–18 month AβPPswe/X11α mice displayed significant improvements in both escape latencies and mean probe trial scores compared to AβPPswe mice such that their performances were not significantly different from NTg or X11α mice (Figs 1D and 2).

Previous studies involving similar crossing of X11α with AβPP-Tg2576 transgenic mice have shown that overexpression of X11α reduces Aβ levels in the brains of 3 month old AβPPswe mice and reduces amyloid plaque numbers in older mice [9]. However, the effect of X11α on Aβ levels in older AβPPswe mice has not been reported. To determine whether the rescue of spatial memory function in 16–18 month old AβPPswe/X11α compared to AβPPswe littermate mice was associated with changes in Aβ, we analyzed $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the brains of these animals. In young AβPP-Tg2576 mice, Aβ levels are mainly soluble in aqueous buffers but in older mice Aβ levels rise and require solubilization in agents such as formic acid [14,17,19]. We thus prepared Tris-HCl-soluble and formic acid-soluble fractions for analyses. Levels of Tris-HCl-soluble and formic acid-soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ were significantly reduced in AβPPswe/X11α compared to AβPPswe littermates (Fig. 3). Thus, overexpression of X11α improves spatial reference learning and memory in AβPP-Tg2576 mice and this is associated with a decrease in cerebral Aβ load.

X11α decreased Aβ levels to approximately 60% and this produced a marked improvement in cognitive performance. This suggests that there is a threshold of
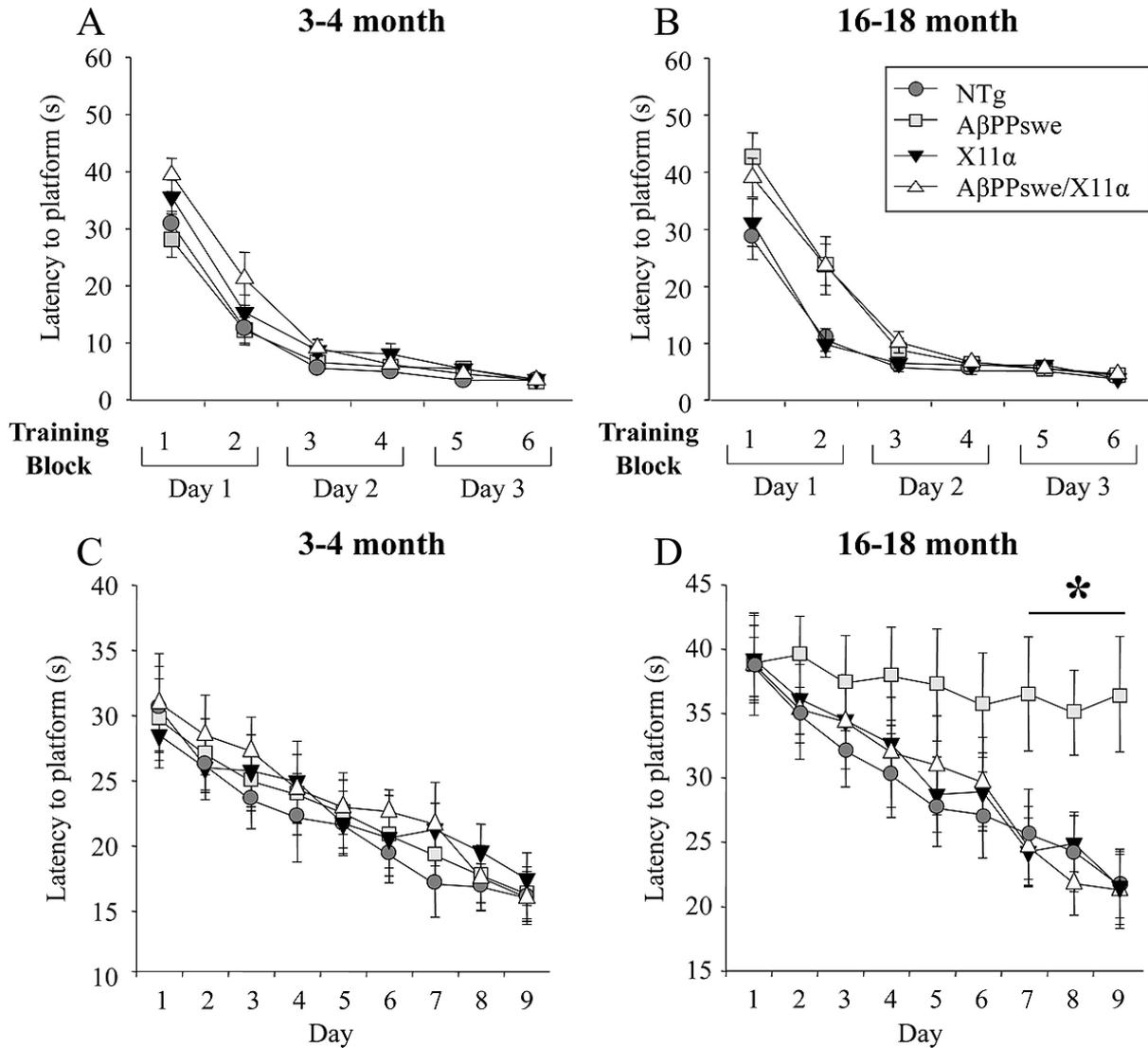
Fig. 1. X11α improves acquisition of platform location in aged AβPPswe mice in the Morris water maze. Escape latencies in seconds (s) for NTg, AβPPswe, X11α, and AβPPswe/X11α mice were measured during visible and hidden platform training. (A and B) show escape latencies to the visible platform in 3–4 month and 16–18 month mice. No significant differences were detected between any of the genotypes in visible platform training at 3–4 months of age (two-way ANOVA). In 16–18 month animals, both AβPPswe and AβPPswe/X11α transgenics showed increased latencies in the first two blocks (day 1) of visible platform training compared with both X11α and NTg ($P < 0.05$). No differences were detected between NTg and X11α and between AβPPswe and AβPPswe/X11α at any time point (two-way ANOVA). (C and D) show escape latencies to the hidden platform in 3–4 month and 16–18 month mice. No significant differences were detected between any genotype in 3–4 month mice (two-way ANOVA). However, in 16–18 month mice, latencies were specifically increased in AβPPswe compared to all other genotypes on days 7, 8 and 9 of testing and this effect was rescued in AβPPswe/X11α mice (two-way ANOVA; $P < 0.01$ day 7; $P < 0.001$ days 8 and 9). *indicates significant differences between AβPPswe and all other genotypes. $n = 15-21$ for 3–4 month mice; $n = 12-16$ for 16–18 month mice. Error bars are $\pm$ SEM.
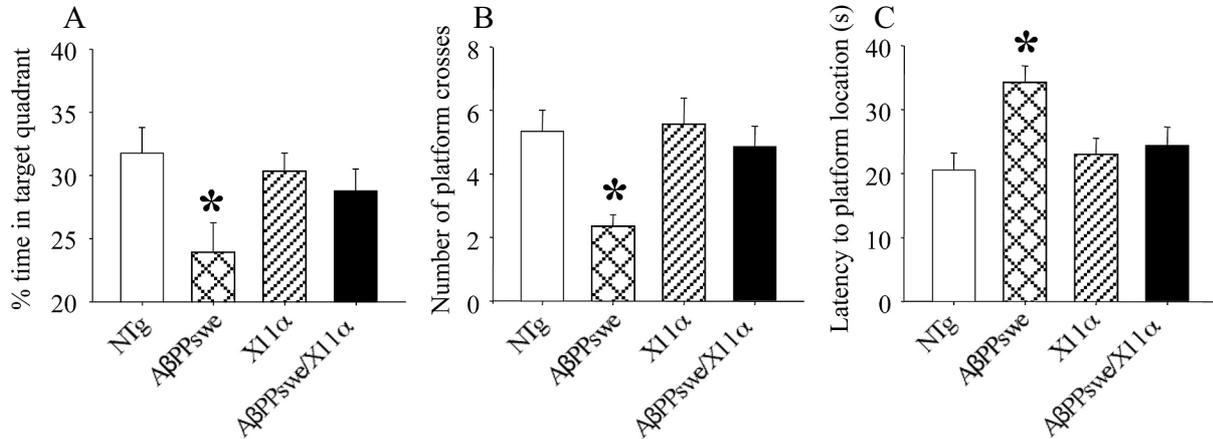
Fig. 2. X11α improves retention of platform location in 16–18 month AβPPswe mice in the Morris water maze. Spatial memory retention was assessed in probe trials which determined the % of time spent in target quadrant (A), mean number of platform crosses (B), and latency to platform location (C). AβPPswe mice had reduced performance in all measures and this effect was rescued in AβPPswe/X11α mice (one-way ANOVA $P < 0.05$). *indicates significant differences between AβPPswe and all other genotypes. Error bars are ± SEM.

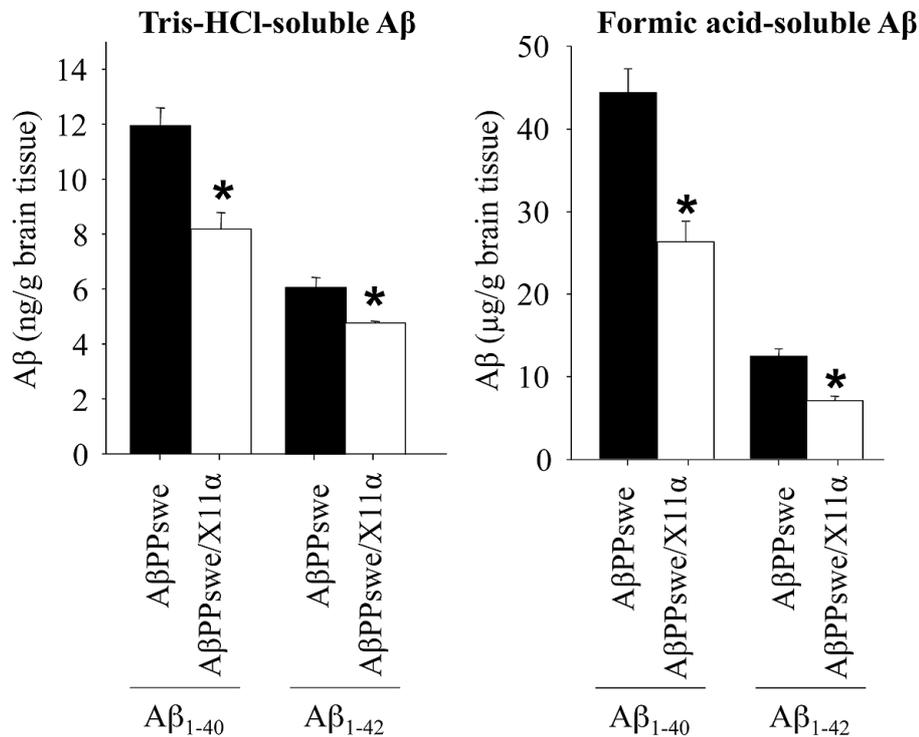Fig. 3. X11α reduces Aβ levels in total brain samples from AβPPswe mice. Histograms show Tris-HCl-soluble and formic-acid-soluble levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in 16–18 month AβPPswe and AβPPswe/X11α transgenic mice ($n = 12-16$). X11α significantly reduced the levels of both Aβ species. *indicates significant differences (t-test, $P < 0.05$). Error bars are ± SEM.

Aβ concentration that impacts on memory. Alternatively, X11α may positively influence cognition by additional Alzheimer's disease-related mechanisms that do not involve Aβ (e.g., modifying AβPP function). Indeed, the precise mechanisms by which X11α lowers Aβ are far from clear, and there is conflicting evidence on the effect of loss of X11α on AβPP processing [20–22]. X11α has both pre- and post-synaptic functions [23–26] which may involve trafficking of synaptic cargoes including AβPP but also NMDA receptors since X11α also binds kinesin KIF17 [27]. Altered protein trafficking and axonal transport is seen in Alzheimer's disease [28]. Finally, X11α binds to the copper chaperone for superoxide dismutase-1 and so may play a role in copper homeostasis [29]. Defective copper metabolism is also implicated in Alzheimer's disease [30].

The X11s comprise three family members (X11α, X11β, and X11γ) [11] and overexpression of X11β also inhibits Aβ production and corrects memory dysfunction in AβPP-Tg2576 mice [17,19]. However, X11α and X11β have different functions [31–33], and there is evidence that they inhibit Aβ production by different mechanisms [34,35]. As such it is important to determine whether different X11s similarly protect against Aβ-related memory dysfunction and whether there are isoform-specific differences in any protection. This is especially the case since X11α and X11β are differentially expressed in the brain which may provide a route for targeting different regions for therapy ci36. Our findings reported here that X11α lowers brain Aβ and protects against Aβ-related cognitive dysfunction thus validate X11α as a potential therapeutic target for Alzheimer's disease.

ACKNOWLEDGMENTS

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