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Life Extension Factor Klotho Enhances Cognition

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SUMMARY

Aging is the primary risk factor for cognitive decline, an emerging health threat to aging societies worldwide. Whether anti-aging factors such as klotho can counteract cognitive decline is unknown. We show that a lifespan-extending variant of the human *KLOTHO* gene, KL-VS, is associated with enhanced cognition in heterozygous carriers. Because this allele increased klotho levels in serum, we analyzed transgenic mice with systemic overexpression of klotho. They performed better than controls in multiple tests of learning and memory. Elevating klotho in mice also enhanced long-term potentiation, a form of synaptic plasticity, and enriched synaptic GluN2B, an N-methyl-D-aspartate receptor (NMDAR) subunit with key functions in learning and memory. Blockade of GluN2B abolished klotho-mediated effects. Surprisingly, klotho effects were evident also in young mice and did not correlate with age in humans, suggesting independence from the aging process. Augmenting klotho or its effects may enhance cognition and counteract cognitive deficits at different life stages.

INTRODUCTION

The world's population is aging rapidly, and preserving brain health has emerged as a major biomedical challenge. Without novel interventions, over 80 million people worldwide will suffer from memory problems resulting from aging and age-related disease by 2040 (Prince et al., 2013). Because aging, a process amenable to change (Guarente and Kenyon, 2000), is the primary risk factor for failing cognition, regulators of aging might be harnessed for the treatment and prevention of cognitive decline.

Like aging, cognition is modifiable. Learning and memory depend on networks spanning different brain regions, including the hippocampus and cortex (Ranganath and Ritchey, 2012; Wang and Morris, 2010), and involve coordinated activities of N-methyl-D-aspartate (NMDA) (Gladding and Raymond, 2011; Lee and Silva, 2009)- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Kerchner and Nicoll, 2008; Kessels and Malinow, 2009)-type glutamate receptors (NMDARs and AMPARs). Importantly, NMDAR- and AMPAR-mediated functions are disrupted by aging (Henley and Wilkinson, 2013; Magnusson et al., 2010) and age-related neurodegenerative disease (Chang et al., 2012; Li et al., 2011).

Whether factors that prolong life can also prevent, delay, or counteract neural dysfunction associated with aging and disease is a critical question with therapeutic implications. Klotho is an aging regulator that, when overexpressed, extends lifespan (Kurosu et al., 2005) and, when disrupted, accelerates aging phenotypes (Kuro-o et al., 1997). Higher klotho levels increase lifespan in mice (Kurosu et al., 2005) and nematodes (Château et al., 2010). In humans, a single allele of the KL-VS variant of the *KLOTHO* gene, which increases secreted klotho (Arking et al., 2002) and more strongly activates FGF23 signaling (Tucker Zhou et al., 2013) in cell culture, promotes longevity (Arking et al., 2002, 2005; Invidia et al., 2010) and diminishes age-related heart disease (Arking et al., 2005).

Klotho is a pleiotropic protein. Its transmembrane form (Shiraki-Iida et al., 1998) can be released by sheddases (Chen et al., 2007) and circulate throughout the body and brain (Imura et al., 2004; Kurosu et al., 2005). Klotho suppresses insulin (Kurosu et al., 2005) and wnt (Liu et al., 2007) signaling, regulates ion channel clustering (Chang et al., 2005) and transport (Imura et al.,

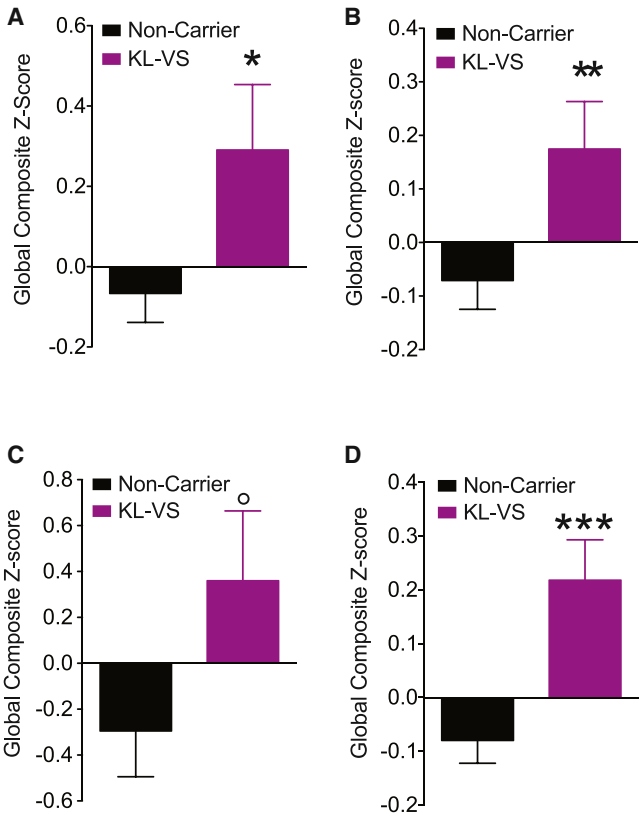

Figure 1. The KL-VS Allele Is Associated with Better Cognitive Performance in Three Independent Aging Populations without Dementia and in a Meta-Analysis of the Populations

Neuropsychological scores from tests spanning multiple cognitive domains (Table S4; Figure S1). Global composite *Z*-scores of 718 aging individuals (52–85 years of age) that were non-carriers (black; n = 530) or carriers (purple; n = 188) of a single KL-VS allele were obtained from three independent cohorts without cognitive impairments. In each cohort, an individual composite score was standardized and scaled to reflect performance as a measure of the number of SDs from the global average of that cohort (global composite *Z*-score). Higher scores indicate better cognitive performance. (A–C) Global composite *Z*-scores in (A) cohort 1 (179 non-carriers, 41 carriers), (B) cohort 2 (331 non-carriers, 135 carriers), (C) cohort 3 (20 non-carriers, 12 carriers), and (D) meta-analysis of the cohorts. All subjects had an MMSE score of 28 or greater and no dementia. Data were analyzed by linear models, accounting for effects of age, sex, and education and testing for effects due to KL-VS genotype. *APOE* ε4 carrier status had no significant effects (Tables S5 and S6). °p = 0.06, *p < 0.05, **p < 0.01, ***p < 0.001 versus non-carrier (linear regression t test). Data are mean ± SEM. See also Tables S2–S6 and Figure S1.

2007), and promotes FGF23 function (Urakawa et al., 2006). Although it regulates aging-dependent pathways (Kurosu et al., 2005; Liu et al., 2007), klotho also supports physiologic functions that are aging independent (Chang et al., 2005; Razzaque, 2009).

In mice, genetic klotho reduction during embryogenesis results in early postnatal death, hypomyelination (Chen et al., 2013), synaptic attrition (Shiozaki et al., 2008), and cognitive impairment (Nagai et al., 2003), suggesting that klotho is required for brain maturation. Because klotho circulates in serum and cerebrospinal fluid throughout life (Imura et al., 2004, 2007), and declines with aging (Duce et al., 2008; Semba et al., 2011, 2014), in parallel to the emergence of cognitive deficits, it is possible that klotho also fulfills important functions in the CNS at later life stages.

We therefore investigated whether klotho can impact physiological brain function and, more specifically, whether it can prevent or counteract cognitive decline in human aging. We demonstrate that the lifespan-extending variant of the *KLOTHO* gene, KL-VS, is associated with increased klotho levels in serum and enhanced cognition in aging people heterozygous for the allele in three cohorts and across multiple ages. We also analyzed transgenic mice with moderate systemic overexpression of klotho. Independent of age, these mice performed better in multiple tests of learning and memory than controls. Further investigation of potential underlying mechanisms revealed unexpected effects of klotho elevation on the functions of synapses and glutamate receptors.

RESULTS

KL-VS Genetic Variant of *KLOTHO* Is Associated with Enhanced Cognition in Three Independent Human Cohorts and in a Meta-Analysis

We first examined whether the KL-VS genetic variant of *KLOTHO* predicts healthy brain aging in humans in three independent populations, including the Hillblom Aging Study (cohort 1), the Memory and Aging Project (cohort 2), and the Normal Aging Cohort (cohort 3) (Table S1). Collectively, the cohorts comprised 718 individuals (52–85 years of age), primarily Caucasian, without dementia or cognitive complaints, and with a Mini-Mental State Exam (MMSE) score of 28 or greater (see Tables S2 and S3 for inclusion criteria and demographics). Twenty-six percent of individuals were heterozygous for the KL-VS allele, slightly above typical frequencies of 20%–25% (Arking et al., 2002). Three percent were homozygous for KL-VS, a rare genotype that for unknown reasons is associated with decreased lifespan and detrimental effects (Arking et al., 2002, 2005; Deary et al., 2005); they were excluded from the study.

In each cohort, cognitive abilities of heterozygous KL-VS carriers and non-carriers were analyzed using multiple neuropsychological tests (Table S4; Figure S1) and compiled into global composite *Z*-scores (Figures 1A–1C and S1). The scores represent a broad measure of cognition, including domains vulnerable to aging (Drag and Bieliauskas, 2010), and reflect the number of SDs from the global average. We used linear statistical models including KL-VS carrier status (zero or one allele), age, sex, and education, with or without *APOE* ε4, as predictors for performance (Table S5). *APOE* ε4, the main genetic risk factor for Alzheimer's disease (AD) (Verghese et al., 2011), did not contribute significant variance (Table S5), and including "cohort" as a covariate in meta-analysis did not alter results (data not shown). KL-VS carriers scored higher than non-carriers in each cohort and in meta-analysis of all cohorts (Figures 1A–1D; Table S5).

KL-VS Is Associated with Better Cognition Independent of Age, Sex, and *APOE* ε4 Allele Status

Meta-analysis showed that advancing age, male sex, and lower education decreased cognitive scores; KL-VS heterozygosity increased scores despite these effects (Table S5). Baseline

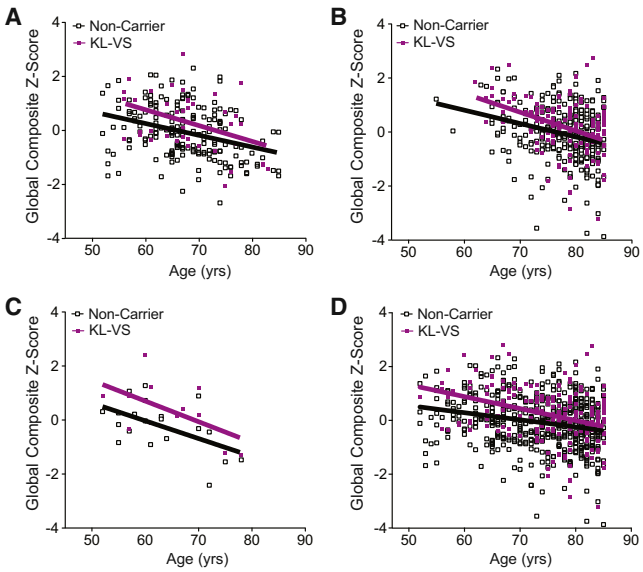

Figure 2. KL-VS-Associated Cognitive Enhancement Is Independent of Age

(A–C) Global composite *Z*-scores decreased as a function of age in non-carriers (empty squares) and carriers (purple squares) of the KL-VS allele in (A) cohort 1, (B) cohort 2, and (C) cohort 3.

(D) In meta-analysis of all cohorts, KL-VS-associated cognitive enhancement tended to decrease with advancing age (KL-VS:age interaction, $p = 0.10$ by ANOVA). Data were analyzed by linear models, accounting for effects of age, sex, and education and testing for effects due to an age by KL-VS interaction. *APOE* ε4 carrier status had no significant effects on these measures.

Data are mean ± SEM.

See also Tables S5 and S6.

cognition was not affected by *APOE* ε4, consistent with previous findings (Yaffe et al., 1997), though detrimental effects of *APOE* ε4 on cognitive aging might be revealed by longitudinal analysis (Deary et al., 2002; Yaffe et al., 1997). KL-VS did not differentially affect cognition by sex (Table S6), but there was a trend (Table S6) for its positive impact to decrease with advancing age (Figures 2A–2D).

KL-VS Increases Klotho Levels in Sera of Humans

Based on these findings, we concluded that KL-VS enhances baseline cognition and hypothesized that it does so, in part, by increasing klotho levels or activity. To begin to test this, we measured fasting, morning levels of klotho in serum by ELISA in individuals from cohort 1 with no or one KL-VS allele. Consistent with findings in cell culture (Arking et al., 2002), the KL-VS variant significantly increased levels of secreted klotho in the serum (Figure 3A; Table S7).

Elevation of Klotho Promotes Longevity and Enhances Cognition in Mice in an Age-Independent Manner

To test whether systemic increases in klotho levels enhance cognition, we analyzed heterozygous klotho (KL) transgenic mice that overexpress mouse klotho in plasma (Kurosu et al., 2005) and throughout the body and brain (Kuro-o et al., 1997), including the hippocampus (Figure 3B), which is critical to learning and memory. Klotho overexpression improved survival in mice, independent of age or time by proportional hazard analysis (Figure 3C). Similar to our human findings, these data suggest that klotho can engage mechanisms that do not depend on aging per se.

We tested spatial learning and memory in 10- to 12-month-old ("middle-aged") mice in the Morris water maze. KL mice performed better than nontransgenic (NTG) controls (Figure 3D). After hidden-platform training, the platform was removed, and spatial memory retention was assessed in a probe trial. KL mice reached the original platform location 2.5 times faster than NTG mice (Figure 3E). Klotho elevation also improved spatial learning and memory in 4- to 7-month-old ("young") mice (Figures 3F and 3G). Effects were independent of sex (Table S8). Of note, KL and NTG mice swam at equal speeds (Figures S2A and S2B) and located a cued platform equally well (Figures S2C and S2D). Thus, elevation of klotho enhances spatial learning and memory independent of sex and across different age groups. Because the effect of klotho on young mice was surprising and such mice are readily available and most suitable for electrophysiological analysis, we focused subsequent studies on this age group.

Klotho Elevation Improves Working and Fear Memory without Altering Other Behaviors in Young Mice

We tested whether elevation of klotho in mice enhances cognition in young mice (3–4 and 4–7 months of age) in other cognitive tasks. In the Y maze, KL mice showed more alternations than NTG controls (Figure 4A), an indication of superior working memory. In a fear-conditioning paradigm, KL mice showed better contextual memory than NTG mice (Figure 4B) but similar cued recall (Figures S3A and S3B). In contrast, klotho elevation did not affect exploratory and anxiety-related behaviors in the open field (Figure 4C) or elevated plus maze (Figure 4D). Thus, elevation of klotho enhances learning and memory in a range of tasks without altering other behaviors.

Klotho Elevation Increases Synaptic Levels of the GluN2B Subunit of NMDARs in Mouse Hippocampus and Cortex

To further explore the mechanism underlying beneficial effects of klotho on cognition, we turned our attention to NMDARs and AMPARs, whose functions are essential for learning and memory (Anggono and Huganir, 2012; Nakazawa et al., 2004). An initial screen of their subunit expression in hippocampus revealed that klotho elevation specifically increased total protein levels of the NMDAR subunit GluN2B (Figures S4A–S4E), without altering its mRNA (Figure S4F). Interestingly, transgenic overexpression of GluN2B in mice (Cao et al., 2007; Tang et al., 1999) and rats (Wang et al., 2009) enhances cognition, whereas dysfunction of this subunit in humans and mice contributes to cognitive decline in aging (Piggott et al., 1992; Zhao et al., 2009) and AD (Ittner et al., 2010; Li et al., 2011; Sze et al., 2001). In light of these findings and because the enhancement of learning and memory in KL mice resembled that in GluN2B-overexpressing mice, we investigated whether klotho modulates the GluN2B protein at synapses in the hippocampus and frontal cortex, regions directly involved in cognitive functions. Klotho

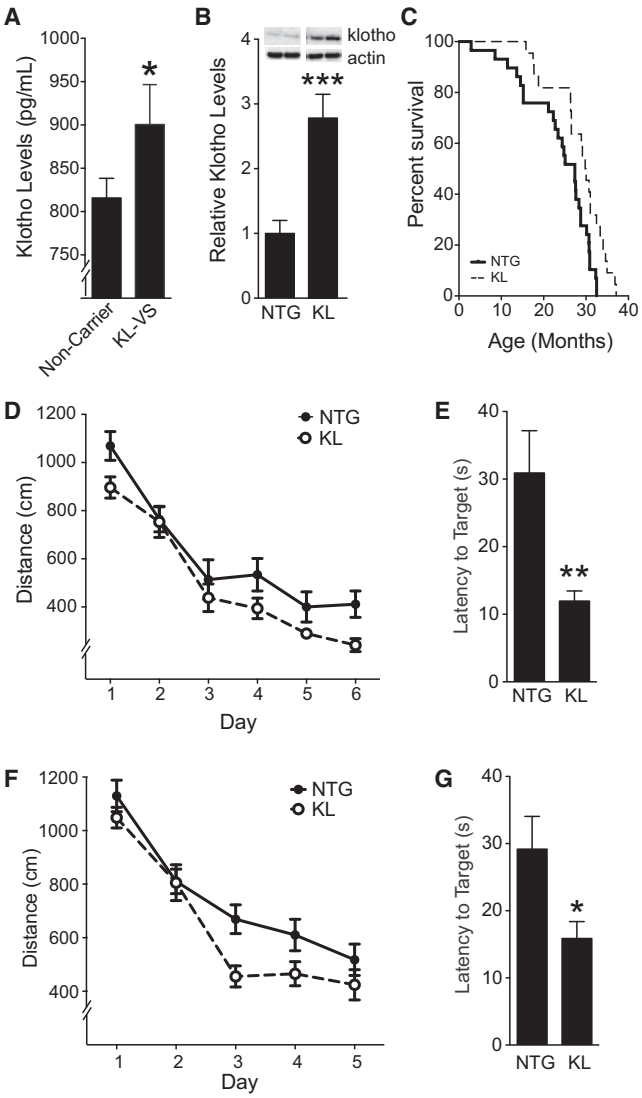

Figure 3. Elevation of Systemic Klotho Levels Occurs in Human KL-VS Carriers and Enhances Mouse Survival, Learning, and Memory Independent of Age

(A) Fasting morning serum klotho levels in individuals from cohort 1 (55–85 years of age) that were non-carriers (n = 118) or carriers (n = 38) of a single KL-VS allele. Data were analyzed by a linear model, accounting for effects of age, sex, and education and testing for effects due to KL-VS genotype. *APOE* ε4 carrier status had no effect (Table S7).

(B) Hippocampal levels of klotho in NTG and KL mice (n = 13–14 mice per genotype, age 3 months). Representative western blots for klotho and actin are shown above; images for each protein were from the same gel.

(C) Kaplan-Meier curves show increased survival of heterozygous KL mice from line 46 (Kuro-o et al., 1997; Kurosu et al., 2005) compared to NTG littermates (n = 22–29 mice per group; $p < 0.01$ by log rank test). Proportional hazard testing revealed that the KL effect was independent of age ($p = 0.76$).

(D and E) KL and NTG mice (n = 8–9 mice per genotype) were tested in the Morris water maze at 10–12 months of age.

(D) Spatial learning curves (platform hidden) are shown. Data represent the daily average of total distance traveled to the platform. KL effect, $p < 0.05$, by mixed-model ANOVA.

(E) Results of a probe trial (platform removed) 1 hr after completion of hidden-platform training show latency to reach the original platform location.

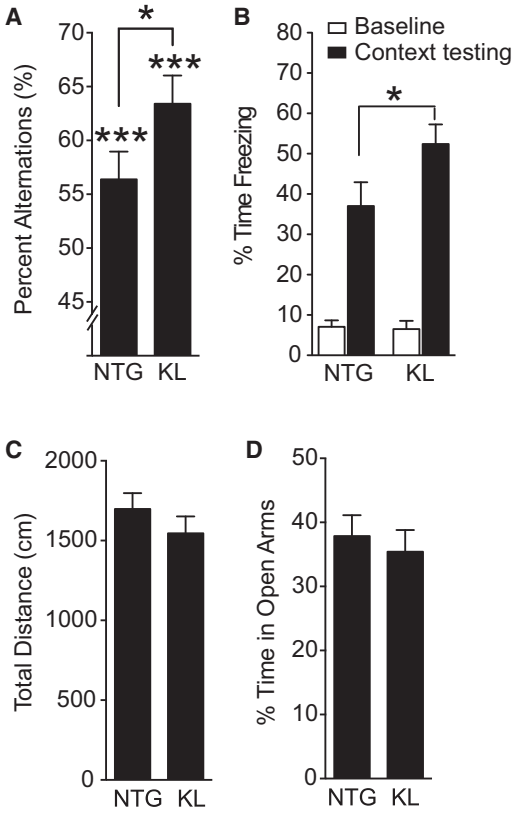

Figure 4. Klotho Elevation Also Improves Working and Context Memory without Altering Other Behaviors in Young Mice

(A) Percent alternations among arms by 3- to 4-month-old mice during exploration of a Y maze (n = 8–10 mice per genotype).

(B) Percent time 6-month-old mice spent freezing at baseline and 24 hr after context training in a fear-conditioning task (n = 6–7 male mice per genotype).

(C) Movements during exploration of an open field (n = 13–14 mice per genotype; $p = 0.30$ by two-tailed t test).

(D) Percent time spent exploring the open arms of an elevated plus maze (n = 14–15 mice per genotype; $p = 0.60$ by two-tailed t test).

*$p < 0.05$, ***$p < 0.001$ versus chance or as indicated by bracket (t test). Data are mean ± SEM. See also Figure S3.

elevation nearly doubled GluN2B, but not GluN2A or GluN2C, levels in postsynaptic density (PSD) fractions isolated from the mouse hippocampus (Figures 5A–5C). It did so without altering presynaptic (synaptophysin) and postsynaptic (PSD-95) markers in membrane fractions (Figures 5A, 5D, and 5E). We also found klotho-mediated increases in GluN2B in PSD-enriched fractions from the frontal cortex (Figures 5F and 5G). Collectively, these data suggest that klotho elevates synaptic GluN2B in a subunit-specific manner through posttranscriptional mechanisms.

(F and G) An independent cohort of mice (n = 17–19 mice per genotype) was tested in the water maze at 4–7 months of age.

(F) Spatial learning curves (platform hidden). KL effect, $p < 0.05$ by mixed-model ANOVA.

(G) Probe trial results.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (t test). Data are mean ± SEM. See also Tables S7 and S8 and Figure S2.

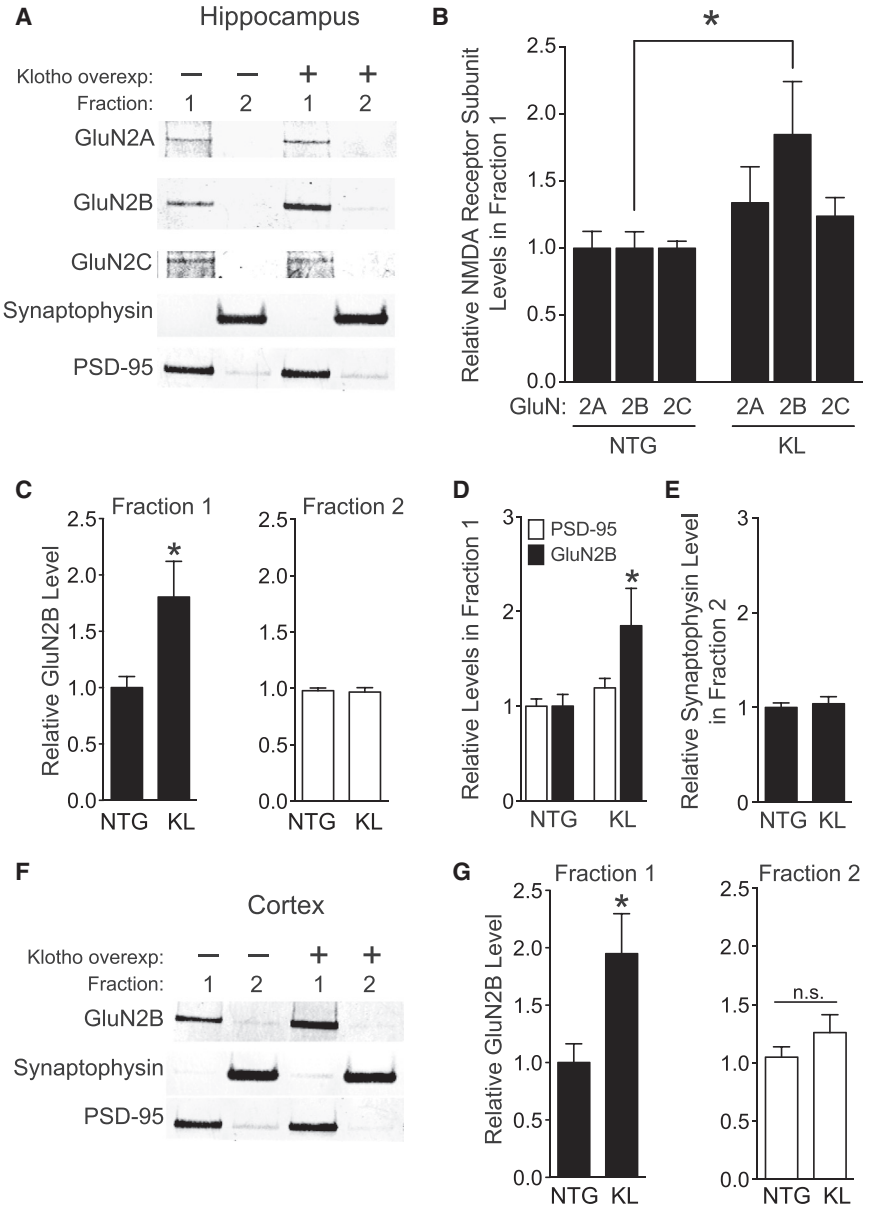

Figure 5. Klotho Overexpression Enhances Synaptic GluN2B Levels in the Hippocampus and Cortex

(A–G) Synaptic membrane Fraction 1 (PSD enriched) and Fraction 2 (non-PSD enriched) isolated from hippocampus or cortex of NTG and KL mice (n = 15–18 mice per genotype, age 3–4 months).

(A) Representative western blots of hippocampal fractions. Klotho overexp., Klotho overexpression.

(B–E) Quantitation of (B) GluN2A, GluN2B, and GluN2C levels in hippocampal Fraction 1, (C) GluN2B in hippocampal Fractions 1 (left) and 2 (right), (D) PSD-95 and GluN2B, and (E) synaptophysin levels.

(F) Representative western blots of cortical fractions isolated from frontal, motor, and somatosensory regions (bregma, 0–3.5 mm).

(G) Quantitation of GluN2B levels in cortical Fractions 1 (left) and 2 (right). For each fraction, protein levels are relative to NTG levels, arbitrarily defined as 1.0.

*$p < 0.05$ versus NTG (t test); n.s., not significant. Data are mean ± SEM. See also Figure S4.

Klotho Elevation Increases NMDAR-Dependent Gene Expression and Synaptic Plasticity

We next analyzed effects of klotho elevation on NMDAR-dependent gene expression and synaptic plasticity. We first examined *Fos*, an immediate early gene that is involved in memory consolidation (Kubik et al., 2007) and increased by NMDAR activation (Bading et al., 1993). We measured FOS-positive cells in the granular layer of the hippocampal dentate gyrus in 3- to 4-month-old KL and NTG mice after hidden-platform training and a probe trial in the water maze, tasks that engage the dentate gyrus and other regions of the hippocampus and cortex (Wang and Morris, 2010). Consistent with findings in mice at 10–12 and 4–7 months of age (Figures 3D–3G), KL mice also showed better learning and memory in the water maze at 3–4 months (data not shown). In the same cohort, FOS expression was more prominent in KL mice than NTG controls following the probe trial (Figures 6A and 6B), indicating that enhanced cognition in KL mice was associated with increased NMDAR-dependent gene expression.

NMDAR activation is also critical for long-term potentiation (LTP), a form of synaptic plasticity thought to underlie learning and memory (Morris et al., 1986; Nakazawa et al., 2004). To assess whether klotho enhances synaptic plasticity, we measured LTP in acute hippocampal slices at the medial perforant path to granule cell synapse, which is mostly mediated by NMDARs (Nguyen and Kandel, 1996). Elevation of klotho enhanced hippocampal LTP (Figure 6C) without changing AMPAR-mediated basal synaptic strength (Figures 6D and S5), as determined by field excitatory postsynaptic potential (fEPSP) and whole-cell excitatory postsynaptic current (EPSC) recordings.

Klotho Elevation Increases the GluN2B Portion of NMDAR Currents

We observed that klotho-mediated enrichment of synaptic GluN2B positively correlated with GluN1 levels (Figures S6A and S6B), suggesting an increase in functional GluN2B-containing NMDARs. To assess whether overexpression of klotho increases NMDAR currents, we measured the amplitude and kinetics of synaptic NMDAR-mediated currents by whole-cell patch-clamp recordings from dentate granule cells following perforant path stimulation. Because AMPAR-mediated synaptic transmission did not differ between NTG and KL slices (Figures 6D and S5),

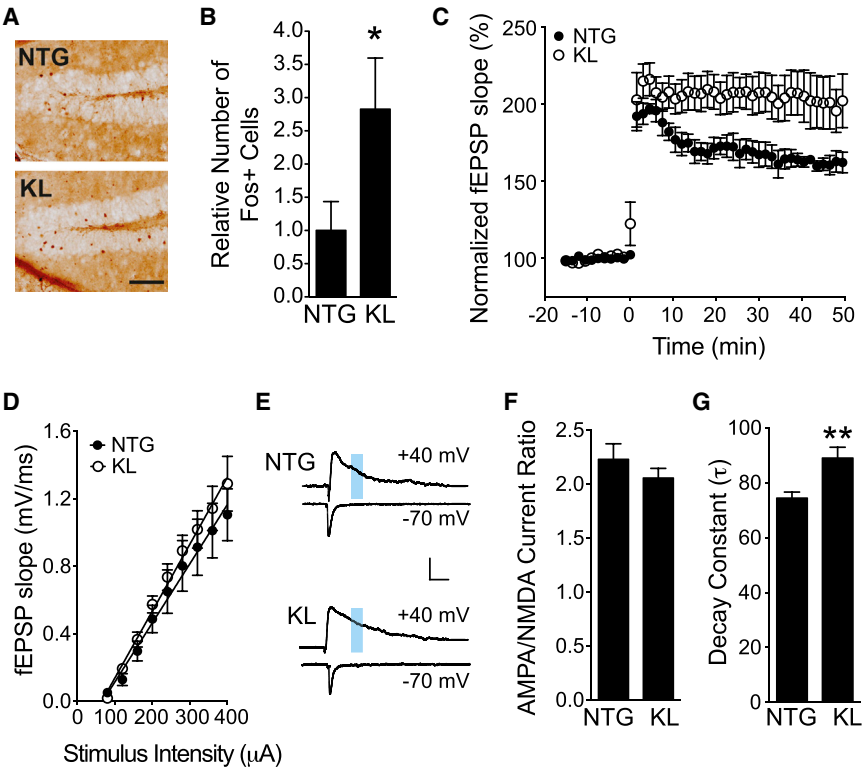

Figure 6. Klotho Overexpression Enhances NMDAR-, but Not AMPAR-, Dependent Functions

(A and B) FOS expression in the dentate gyrus of NTG and KL mice immediately following a probe trial in the water maze.

(A) Staining with antibodies to FOS revealed more immunoreactive granule cells in the dentate gyrus of KL (bottom) than NTG (top) mice. Scale bar, 100 μm.

(B) Quantitation of FOS-positive granule cells (n = 7 mice per genotype, age 3–4 months). The mean level in NTG controls was arbitrarily defined as 1.0.

(C) fEPSP recordings from acute hippocampal slices of 3.5- to 4.5-month-old NTG and KL mice. LTP induction and decay in the dentate gyrus were monitored for 45–50 min following theta burst stimulation of the medial perforant pathway. KL versus NTG genotype by time effect, $p < 0.01$ by mixed-model ANOVA. Four slices from four NTG mice and eight slices from six KL mice were analyzed.

(D) AMPAR-mediated basal synaptic transmission in acute hippocampal slices of 3.5- to 4.5-month-old mice at the medial perforant path to dentate granule cell synapse. Five slices from three NTG mice and nine slices from five KL mice were analyzed.

(E–G) Isolated NMDAR and AMPAR EPSCs measured by whole-cell patch-clamp recordings from dentate granule cells in acute hippocampal slices of 3- to 4-month-old mice.

(E) Representative traces of evoked EPSCs at +40 or −70 mV. NMDAR-mediated EPSCs were quantitated between 80 and 100 ms after stimulation (blue shading) in the top traces and AMPAR-mediated EPSCs at the nadir of the bottom traces. Scales, 100 pA and 50 ms.

(F) Quantitation of AMPAR/NMDAR EPSC ratios. Ten slices from three NTG mice and ten slices from three KL mice were analyzed. $p = 0.32$ (t test).

(G) Decay constant (τ) of isolated NMDAR EPSCs in the presence of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) (10 μM). Seven slices from three NTG mice and five slices from three KL mice were analyzed.

*$p < 0.05$, **$p < 0.01$ (t test). Data are mean ± SEM. See also Figure S5.

the amplitudes of NMDAR currents were normalized to AMPAR currents, revealing similar amplitudes in NTG and KL slices (Figures 6E and 6F). However, NMDAR currents decayed more slowly in KL slices than NTG slices, reflected by an increased decay constant (Figure 6G). Because NMDARs containing GluN2B deactivate slower than those containing GluN2A (Wang et al., 2008), the increased decay time of currents in KL slices suggests that GluN2B-containing NMDARs contributed more to currents in KL slices than in NTG slices. Consistent with this interpretation, treatment of slices with the GluN2B-specific antagonist ifenprodil (Mony et al., 2009) reduced NMDAR current amplitudes (Figures 7A and 7B) and decay constants (Figures 7C and 7D) more in KL slices than in NTG slices. Thus, klotho elevation probably increases the GluN2B component of synaptic NMDAR activity.

Acute Inhibition of GluN2B Blocks Klotho-Mediated Enhancement of Learning and Memory

We tested whether blocking GluN2B-containing NMDARs modulates effects of klotho elevation on context and working memory. We specifically tested if acute, transient, and broad blockade of GluN2B affects klotho-mediated enhancement of cognition without disrupting baseline cognitive functions. To this end, we used low doses of pharmacological GluN2B antagonists that minimally affect NTG mice (Mathur et al., 2009; Sotres-Bayon et al., 2007). Young mice were injected intraperitoneally (i.p.) with ifenprodil (5 mg/kg) or vehicle before training in fear conditioning and then tested 1 day later. Untreated KL mice showed better hippocampus-dependent contextual fear memory than untreated NTG mice (Figure 7E), consistent with their superior performance in other cognitive tasks (Figures 3D–3G, 4A, and 4B), enhanced hippocampal LTP (Figure 6C), and increased GluN2B function (Figures 7A and 7B). At the low dose used, ifenprodil did not affect fear conditioning in NTG controls (Figure 7E), consistent with previous results from Kojima et al. (2005) and Sotres-Bayon et al. (2007). In KL mice, ifenprodil blocked enhancement of context learning and memory (Figure 7E). At a higher dose, and with a lower number of shocks, ifenprodil suppressed learning and memory also in NTG mice (Figure S6C).

To assess effects of ifenprodil during another life stage and in another behavioral task, we injected middle-aged mice i.p. with ifenprodil or vehicle and tested them in the Y maze, which engages frontal cortical circuits. Untreated KL mice showed better working memory than NTG mice, as measured by percent alternations between maze arms (Figure 7F). In KL mice, ifenprodil blocked the enhancement of working memory (Figure 7F).

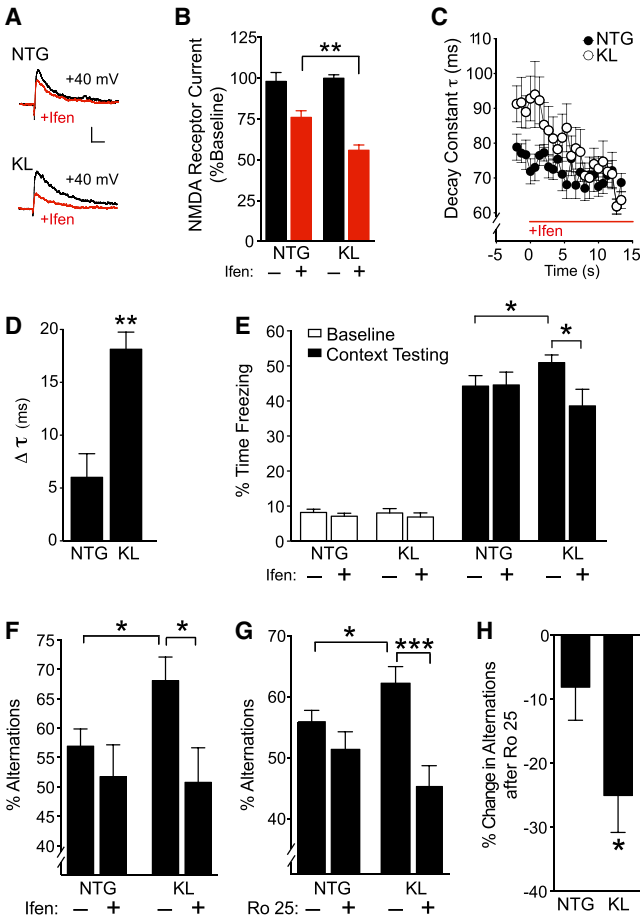

Figure 7. Treatment with GluN2B Selective Antagonists Blocks Klotho Effects on NMDAR Currents and Cognition

(A and B) Representative traces (A) and quantitation (B) of isolated NMDAR EPSCs in the presence of NBQX (10 μm) at baseline (black) and following perfusion with ifenprodil (Ifen; 3 μM) (red) in the same slices. Six slices from three NTG mice and five slices from three KL mice were analyzed. Two-way repeated-measures ANOVA: ifenprodil effect, $p < 0.0001$; ifenprodil by KL interaction, $p < 0.05$.

(C) Time course of NMDAR EPSC decay constant following ifenprodil perfusion in each genotype. Mixed-model ANOVA: $p < 0.0001$ for KL versus NTG genotype by time effect.

(D) Change in decay constant (τ) between 0 and 10 min after initiation of ifenprodil treatment in NTG and KL slices. Seven slices from three NTG mice and five slices from three KL mice were analyzed.

(E and F) Mice (n = 8–19 per group) received a single i.p. injection of vehicle (–) or ifenprodil (5 mg/kg) 30 min before training in a fear-conditioning paradigm or testing in the Y maze.

(E) Percent time mice (age 5–7 months) spent freezing at baseline and 24 hr after context training in a fear-conditioning task. Ifenprodil by KL interaction, $p < 0.05$ by two-way ANOVA.

(F) Percent alternations among Y maze arms that mice (age 10–12 months) showed during 3 min exploration. Ifenprodil by KL interaction, $p < 0.09$ by two-way ANOVA.

(G and H) Mice (n = 13–19 per group, age 3–5 months) received a single i.p. injection of vehicle (–) or Ro 25 (5 mg/kg) 10 min before testing in Y maze.

(G) Percent alternations among Y maze arms. Ro 25 by KL interaction, $p < 0.05$ by two-way ANOVA.

(H) Percent decrease in alternations following Ro 25 treatment in NTG and KL mice.

Thus, at a low dose, ifenprodil blocked klotho-mediated cognitive enhancement in young and middle-aged mice in two independent cognitive measures.

To further test and validate the role of GluN2B in klotho-mediated cognitive enhancement, we used Ro 25-6981 (Ro 25), a second-generation NDMAR blocker with 3,000-fold specificity to GluN2B relative to other subunits (Paoletti and Neyton, 2007). Young mice were injected i.p. with a low dose of Ro 25 (5 mg/kg) (Mathur et al., 2009) or vehicle and tested in the Y maze. As expected, vehicle-treated, but not Ro 25-treated, KL mice showed better working memory than NTG controls (Figures 7G and 7H). Altogether, these results are consistent with the synaptic enrichment of GluN2B in KL mice (Figure 5B) and the greater susceptibility of KL slices to ifenprodil-induced suppression of NMDAR currents (Figures 7A–7D).

DISCUSSION

Our genetic and neuropsychological data in humans combined with molecular, electrophysiological, pharmacological, and behavioral studies in transgenic mice reveal a role for the life extension factor klotho in enhancing cognition. In three independent human populations, the longevity-promoting KL-VS variant of the *KLOTHO* gene was associated with enhanced cognition in heterozygous individuals across all ages examined. As predicted, based on cell culture studies by Arking et al. (2002), KL-VS increased klotho levels in the sera of heterozygous individuals. In mice, systemic overexpression of klotho improved learning and memory in multiple cognitive tests, and this effect was independent of age. Klotho elevation enhanced NMDAR-related functions, including FOS expression following a learning and memory task and LTP in the dentate gyrus. It also resulted in postsynaptic enrichment of the NMDAR subunit GluN2B in the hippocampus and cortex, brain regions central to networks supporting cognition. Acute blockade of GluN2B abolished klotho-mediated effects on hippocampal NMDAR currents and on learning and memory. Taken together, these data support the hypothesis that, in addition to extending lifespan, klotho exerts beneficial effects on cognitive and synaptic functions through mechanisms that involve regulation of NMDARs and are uncoupled from aging per se.

Klotho and Better Cognitive Status: Effect Size, Relevance, and Caveats

Because cognition is a highly valued and central manifestation of brain function that diminishes with aging and disease, the potential to enhance it—even slightly—is of great relevance to the human condition. In human studies, we captured the magnitude of group differences in cognition between KL-VS carriers and noncarriers, by calculating an "effect size" with the widely used Cohen's *d* method (Ray and Shadish, 1996) to estimate the biologic significance of our findings. In studies of human cognition and behavior, an effect size of 0.25 on a scale of 0–1 is broadly

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus NTG or as indicated by brackets by t test (B, D, and H) or Bonferroni-Holm test (E–G). Data are mean ± SEM. See also Figure S6.

considered clinically significant (Rockwood, 2004; Smith et al., 2006). Our findings demonstrate an average Cohen's *d* score of 0.34 across three populations. This effect size exceeds that of *APOE* ε4, the most robust known genetic modifier of cognition in aging, calculated at 0.27 from longitudinal cognitive decline (Deary et al., 2002) or at 0.13–0.25 from select baseline scores in comparable aging individuals without dementia (Brown et al., 2011). It also exceeds effect sizes of Food and Drug Administration-approved treatments for AD, calculated between 0.15 and 0.28 (Rockwood, 2004; Smith et al., 2006). Determining if the klotho effect translates into advantages in daily life or greater reserve against neurodegenerative diseases is an important objective.

Because our findings were replicated in three independent human cohorts in three geographic regions, it is likely that our inferences are suitable at a population level, particularly because the populations included community-dwelling individuals. However, potential limitations in extrapolating our data worldwide include that most participants were Caucasian and that our studies were limited to the United States. Thus, it is possible that more diverse genetic or environmental influences could alter or mask the effect in other populations.

To our knowledge, the KL-VS variant has not been identified as a significant modulator in genome-wide association studies (GWASs) of human cognition in aging (De Jager et al., 2012; Luciano et al., 2011; Seshadri et al., 2007), including in a study of populations overlapping with the current study (De Jager et al., 2012). However, our hypothesis-driven query of a population previously studied by GWAS (De Jager et al., 2012) did, in fact, reveal a significant association of KL-VS heterozygosity with improved baseline cognition. Potential reasons for this include (1) previous outcome measures focused on cognitive decline (De Jager et al., 2012), which KL-VS did not prevent, and (2) benefits of KL-VS are limited to the heterozygous state and, thus, may be missed by conventional GWAS methodologies that assess additive effects of the minor allele.

Indeed, KL-VS homozygosity eliminates advantages in lifespan and health (Arking et al., 2002, 2005; Invidia et al., 2010; Majumdar et al., 2010) and similarly eliminated cognitive advantage in our discovery cohort (data not shown). The reasons for the paradoxical dose effect of the minor allele are unknown. Possibilities include adverse effects of high klotho levels maintained over decades in homozygous carriers or more complex dysregulation of klotho expression by the variant. For example, it is possible that the variant actually reduces klotho production, resulting in abnormally low levels of klotho in homozygotes, but causes a compensatory increase in expression of the wild-type allele in heterozygotes. Additional studies are needed to investigate these possibilities.

KL-VS homozygosity also decreased cognition in a previous study by Deary et al. (2005), consistent with our findings. However, the previous study did not detect differences in cognitive performance of KL-VS heterozygous individuals and noncarriers. The reasons for this discrepancy are unknown. It is worth noting, though, that we replicated our findings in three independent cohorts and that the current study focused on tests without maximum scores to increase the sensitivity of detecting cognitive enhancement.

Klotho-Mediated Cognitive Enhancement Is Independent of Aging

In light of klotho's role in aging, we were surprised that transgenic mice with global overexpression of klotho showed better learning and memory than NTG controls at all ages examined, including at 3 months, following entry into early adulthood. Furthermore, KL-VS-associated increases in serum klotho levels did not prevent aging-related cognitive decline in humans. Instead, the KL-VS variant enhanced cognitive functions over a wide age range, though there was a trend toward decreased effects at the most advanced ages.

In our opinion, a plausible interpretation of these findings is that the KL-VS variant, similar to global overexpression of klotho in transgenic mice, modulates baseline cognition and that this effect diminishes when the age-associated decline in secreted klotho levels (Duce et al., 2008; Semba et al., 2011) crosses a critical threshold. Longitudinal studies over a broader age span are needed to test this hypothesis. Although klotho elevation does not slow down cognitive aging per se, its age-independent cognition-enhancing effects could increase "cognitive reserve" and thereby augment one's ability to counter adverse effects of aging or related diseases, at least for a while.

Klotho and Mechanisms of Learning and Memory

In humans and mice, klotho effects were observed in cognitive tests that interrogate functions of diverse networks that include, but are not limited to, hippocampus and frontal cortex (Frankland and Bontempi, 2005; Wang and Morris, 2010). Klotho enhanced learning and memory in the water maze across several ages (Table S1). We conducted mechanistic studies in young mice because they are more readily available than older mice, yield high-quality electrophysiological recordings, and allowed us to address one of the most interesting findings of our studies—that klotho enhances cognition even in the young life stage. We focused on the hippocampus because of its well-characterized electrophysiological properties of learning and memory circuits.

Klotho elevation increased neuronal FOS expression during memory retrieval in the water maze, as well as LTP and the decay time of NMDAR currents. Collectively, these findings suggest a causal role for NMDARs and their GluN2B subtype in klotho-mediated cognitive enhancement.

The subunit composition of NMDARs dictates their functional properties (Yashiro and Philpot, 2008). Because GluN2B-containing NMDARs deactivate slower than GluN2A-containing NMDARs (Wang et al., 2008), the increased decay time of NMDAR currents in hippocampal slices from KL mice suggests that klotho elevation augments the contribution of GluN2B-containing NMDARs to NMDAR currents, a possibility that may extend to other brain regions. Consistent with this hypothesis, treatment of slices with the GluN2B-specific antagonist ifenprodil preferentially reduced NMDAR current amplitudes and decay constants in hippocampal slices from KL mice. We conclude that klotho elevation directly or indirectly increases the GluN2B component of synaptic NMDAR activity, which in turn facilitates induction of LTP (Foster et al., 2010; Yashiro and Philpot, 2008), a potential substrate of learning and memory. In support of this conclusion, blockade of GluN2B abolished klotho-mediated

increases in learning and memory. Because GluN2B is also involved in long-term depression, homeostatic plasticity, and metaplasticity (Brigman et al., 2010; Liu et al., 2004; Yang et al., 2012), it will be interesting to determine if and how klotho alters other forms of synaptic plasticity.

Although our initial screen of receptor subtype expression revealed that klotho increased total hippocampal protein levels of GluN2B, but not of other NMDAR or AMPAR subtypes, this does not exclude the possibility that klotho modulates other receptors in subcellular or submembrane compartments or under disease conditions. Furthermore, cognition and its underlying substrates are complex. Therefore, other klotho-related mechanisms may also contribute to enhanced cognition, including regulation or signaling of other ion channels (Imura et al., 2007), insulin (Chen et al., 2007; Kurosu et al., 2005), wnt (Liu et al., 2007), or FGF23 (Urakawa et al., 2006) in neuronal or nonneuronal cells. Klotho-mediated enrichment of GluN2B could intersect with one or more of these pathways.

Klotho elevated total and synaptic GluN2B protein levels in a subunit-specific manner without altering pre- and postsynaptic markers. How klotho elevates total levels of GluN2B protein and enriches GluN2B within synapses, directly or indirectly, remains to be determined but may involve regulating translation, posttranslational modification, recycling, or trafficking of the subunit. It also remains to be determined whether the effects of klotho elevation on GluN2B are mediated by the transmembrane or secreted form of klotho.

Our findings suggest that the KL-VS variant promotes cognition by increasing levels of secreted klotho. KL-VS carriers had higher serum klotho than non-carriers—and in both groups, higher klotho levels correlated or trended to correlate with better cognitive function on tests such as semantic fluency, category fluency, and modified trails ($p = 0.04–0.17$, linear regression t tests, cohort 1; $n = 153$). Furthermore, elevating klotho levels in another species, mice, also enhanced cognition. Nonetheless, it is important to note that the KL-VS variant may also alter the activities of klotho (Abraham et al., 2012; Arking et al., 2002; Tucker Zhou et al., 2013). Additional studies are needed to further explore the pleiotropic functions of this lifespan-extending and cognition-enhancing factor. Strategies that increase the level (Abraham et al., 2012; King et al., 2012) or activity of klotho or simulate its functions may improve cognition at different life stages and, possibly, even under pathological circumstances.

EXPERIMENTAL PROCEDURES

Cohorts

Several cohorts of humans and mice were utilized (Table S1).

Human Studies

Subjects were genotyped for the KL-VS variant. Cognitive data were collected blinded to genotype. Neuropsychological testing (Figure S1) spanned multiple cognitive domains (Table S4). Serum was analyzed for soluble α-klotho levels by ELISA (Immuno-Biological Laboratories) (Yamazaki et al., 2010). Studies were approved by committees on human research, and subjects provided written informed consent before participating.

Mice, Cognition, and Behavior

NTG C57BL/6 mice were crossed with hemizygous KL transgenic mice (line 46) (Kuro-o et al., 1997), which express mouse klotho under the EF-1α promoter. All studies were conducted in a blinded manner on age-matched and sex-balanced littermate offspring, unless indicated otherwise. Mice were tested in the Morris water maze, Y maze, fear-conditioning apparatus, elevated plus maze, and open field, as described by Cissé et al. (2011) and Harris et al. (2010). All animal studies were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco, and conducted in compliance with National Institutes of Health (NIH) guidelines.

Protein Analyses

Separation of synaptic membrane fractions (Goebel-Goody et al., 2009; Li et al., 2011) was performed as described with minor modifications. Western blot analyses and immunohistochemistry were performed as described (Palop et al., 2003). Protein input, antibody dilutions, and other details are described in Supplemental Experimental Procedures.

Electrophysiology

LTP was induced and measured from acute hippocampal slices using methods as described by Cissé et al. (2011) with minor modifications. AMPAR/NMDAR EPSC ratios and spontaneous EPSCs were recorded from dentate granule cells as described in detail in the Supplemental Experimental Procedures.

Statistical Analyses

Experimenters were blinded to the genotypes of humans and the genotypes and treatment of mice. Statistical analyses were performed using GraphPad Prism (5.0) for t tests, log rank tests, and repeated-measures ANOVA. R (nlme package) was used for mixed-model ANOVAs, post hoc tests, principle component analysis, linear models, and power analyses.

See Supplemental Experimental Procedures for further details on all experimental methods.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and eight tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.03.076.

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REFERENCES

Abraham, C.R., Chen, C., Cuny, G.D., Glicksman, M.A., and Zeldich, E. (2012). Small-molecule Klotho enhancers as novel treatment of neurodegeneration. Future Med. Chem. *4*, 1671–1679.

Anggono, V., and Huganir, R.L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. Curr. Opin. Neurobiol. *22*, 461–469.

Arking, D.E., Krebsova, A., Macek, M., Sr., Macek, M., Jr., Arking, A., Mian, I.S., Fried, L., Hamosh, A., Dey, S., McIntosh, I., and Dietz, H.C. (2002). Association of human aging with a functional variant of klotho. Proc. Natl. Acad. Sci. USA *99*, 856–861.

Arking, D.E., Atzmon, G., Arking, A., Barzilai, N., and Dietz, H.C. (2005). Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. Circ. Res. *96*, 412–418.

Bading, H., Ginty, D.D., and Greenberg, M.E. (1993). Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science *260*, 181–186.

Brigman, J.L., Wright, T., Talani, G., Prasad-Mulcare, S., Jinde, S., Seabold, G.K., Mathur, P., Davis, M.I., Bock, R., Gustin, R.M., et al. (2010). Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. J. Neurosci. *30*, 4590–4600.

Brown, J.A., Terashima, K.H., Burggren, A.C., Ercoli, L.M., Miller, K.J., Small, G.W., and Bookheimer, S.Y. (2011). Brain network local interconnectivity loss in aging APOE-4 allele carriers. Proc. Natl. Acad. Sci. USA *108*, 20760–20765.

Cao, X., Cui, Z., Feng, R., Tang, Y.P., Qin, Z., Mei, B., and Tsien, J.Z. (2007). Maintenance of superior learning and memory function in NR2B transgenic mice during ageing. Eur. J. Neurosci. *25*, 1815–1822.

Chang, Q., Hoefs, S., van der Kemp, A.W., Topala, C.N., Bindels, R.J., and Hoenderop, J.G. (2005). The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. Science *310*, 490–493.

Chang, P.K., Verbich, D., and McKinney, R.A. (2012). AMPA receptors as drug targets in neurological disease—advantages, caveats, and future outlook. Eur. J. Neurosci. *35*, 1908–1916.

Château, M.T., Araiz, C., Descamps, S., and Galas, S. (2010). Klotho interferes with a novel FGF-signalling pathway and insulin/Igf-like signalling to improve longevity and stress resistance in *Caenorhabditis elegans*. Aging (Albany, N.Y.) *2*, 567–581.

Chen, C.D., Podvin, S., Gillespie, E., Leeman, S.E., and Abraham, C.R. (2007). Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. Proc. Natl. Acad. Sci. USA *104*, 19796–19801.

Chen, C.D., Sloane, J.A., Li, H., Aytan, N., Giannaris, E.L., Zeldich, E., Hinman, J.D., Dedeoglu, A., Rosene, D.L., Bansal, R., et al. (2013). The antiaging protein Klotho enhances oligodendrocyte maturation and myelination of the CNS. J. Neurosci. *33*, 1927–1939.

Cissé, M., Halabisky, B., Harris, J.A., Devidze, N., Dubal, D.B., Sun, B., Orr, A., Lotz, G., Kim, D.H., Hamto, P., et al. (2011). Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. Nature *469*, 47–52.

Deary, I.J., Whiteman, M.C., Pattie, A., Starr, J.M., Hayward, C., Wright, A.F., Carothers, A., and Whalley, L.J. (2002). Cognitive change and the APOE epsilon 4 allele. Nature *418*, 932.

Deary, I.J., Harris, S.E., Fox, H.C., Hayward, C., Wright, A.F., Starr, J.M., and Whalley, L.J. (2005). KLOTHO genotype and cognitive ability in childhood and old age in the same individuals. Neurosci. Lett. *378*, 22–27.

De Jager, P.L., Shulman, J.M., Chibnik, L.B., Keenan, B.T., Raj, T., Wilson, R.S., Yu, L., Leurgans, S.E., Tran, D., Aubin, C., et al. (2012). A genome-wide scan for common variants affecting the rate of age-related cognitive decline. Neurobiol. Aging *33*, 1017.e1011–1017.e1015.

Drag, L.L., and Bieliauskas, L.A. (2010). Contemporary review 2009: cognitive aging. J. Geriatr. Psychiatry Neurol. *23*, 75–93.

Duce, J.A., Podvin, S., Hollander, W., Kipling, D., Rosene, D.L., and Abraham, C.R. (2008). Gene profile analysis implicates Klotho as an important contributor to aging changes in brain white matter of the rhesus monkey. Glia *56*, 106–117.

Foster, K.A., McLaughlin, N., Edbauer, D., Phillips, M., Bolton, A., Constantine-Paton, M., and Sheng, M. (2010). Distinct roles of NR2A and NR2B cytoplasmic tails in long-term potentiation. J. Neurosci. *30*, 2676–2685.

Frankland, P.W., and Bontempi, B. (2005). The organization of recent and remote memories. Nat. Rev. Neurosci. *6*, 119–130.

Gladding, C.M., and Raymond, L.A. (2011). Mechanisms underlying NMDA receptor synaptic/extrasynaptic distribution and function. Mol. Cell. Neurosci. *48*, 308–320.

Goebel-Goody, S.M., Davies, K.D., Alvestad Linger, R.M., Freund, R.K., and Browning, M.D. (2009). Phospho-regulation of synaptic and extrasynaptic N-methyl-d-aspartate receptors in adult hippocampal slices. Neuroscience *158*, 1446–1459.

Guarente, L., and Kenyon, C. (2000). Genetic pathways that regulate ageing in model organisms. Nature *408*, 255–262.

Harris, J.A., Devidze, N., Verret, L., Ho, K., Halabisky, B., Thwin, M.T., Kim, D., Hamto, P., Lo, I., Yu, G.Q., et al. (2010). Transsynaptic progression of amyloid-β-induced neuronal dysfunction within the entorhinal-hippocampal network. Neuron *68*, 428–441.

Henley, J.M., and Wilkinson, K.A. (2013). AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. Dialogues Clin. Neurosci. *15*, 11–27.

Imura, A., Iwano, A., Tohyama, O., Tsuji, Y., Nozaki, K., Hashimoto, N., Fujimori, T., and Nabeshima, Y. (2004). Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. FEBS Lett. *565*, 143–147.

Imura, A., Tsuji, Y., Murata, M., Maeda, R., Kubota, K., Iwano, A., Obuse, C., Togashi, K., Tominaga, M., Kita, N., et al. (2007). alpha-Klotho as a regulator of calcium homeostasis. Science *316*, 1615–1618.

Invidia, L., Salvioli, S., Altilia, S., Pierini, M., Panourgia, M.P., Monti, D., De Rango, F., Passarino, G., and Franceschi, C. (2010). The frequency of Klotho KL-VS polymorphism in a large Italian population, from young subjects to centenarians, suggests the presence of specific time windows for its effect. Biogerontology *11*, 67–73.

Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wölfing, H., Chieng, B.C., Christie, M.J., Napier, I.A., et al. (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell *142*, 387–397.

Kerchner, G.A., and Nicoll, R.A. (2008). Silent synapses and the emergence of a postsynaptic mechanism for LTP. Nat. Rev. Neurosci. *9*, 813–825.

Kessels, H.W., and Malinow, R. (2009). Synaptic AMPA receptor plasticity and behavior. Neuron *61*, 340–350.

King, G.D., Chen, C., Huang, M.M., Zeldich, E., Brazee, P.L., Schuman, E.R., Robin, M., Cuny, G.D., Glicksman, M.A., and Abraham, C.R. (2012). Identification of novel small molecules that elevate Klotho expression. Biochem. J. *441*, 453–461.

Kojima, N., Sakamoto, T., Endo, S., and Niki, H. (2005). Impairment of conditioned freezing to tone, but not to context, in Fyn-transgenic mice: relationship to NMDA receptor subunit 2B function. Eur. J. Neurosci. *21*, 1359–1369.

Kubik, S., Miyashita, T., and Guzowski, J.F. (2007). Using immediate-early genes to map hippocampal subregional functions. Learn. Mem. *14*, 758–770.

Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E., et al. (1997). Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature *390*, 45–51.

Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., Gurnani, P., McGuinness, O.P., Chikuda, H., Yamaguchi, M., Kawaguchi, H., et al. (2005). Suppression of aging in mice by the hormone Klotho. Science *309*, 1829–1833.

Lee, Y.S., and Silva, A.J. (2009). The molecular and cellular biology of enhanced cognition. Nat. Rev. Neurosci. *10*, 126–140.

Li, S., Jin, M., Koeglsperger, T., Shepardson, N.E., Shankar, G.M., and Selkoe, D.J. (2011). Soluble Aβ oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. J. Neurosci. *31*, 6627–6638.

Liu, L., Wong, T.P., Pozza, M.F., Lingenhoehl, K., Wang, Y., Sheng, M., Auberson, Y.P., and Wang, Y.T. (2004). Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science *304*, 1021–1024.

Liu, H., Fergusson, M.M., Castilho, R.M., Liu, J., Cao, L., Chen, J., Malide, D., Rovira, I.I., Schimel, D., Kuo, C.J., et al. (2007). Augmented Wnt signaling in a mammalian model of accelerated aging. Science *317*, 803–806.

Luciano, M., Hansell, N.K., Lahti, J., Davies, G., Medland, S.E., Räikkönen, K., Tenesa, A., Widen, E., McGhee, K.A., Palotie, A., et al. (2011). Whole genome association scan for genetic polymorphisms influencing information processing speed. Biol. Psychol. *86*, 193–202.

Magnusson, K.R., Brim, B.L., and Das, S.R. (2010). Selective vulnerabilities of N-methyl-D-aspartate (NMDA) receptors during brain aging. Front. Aging Neurosci. *2*, 11.

Majumdar, V., Nagaraja, D., and Christopher, R. (2010). Association of the functional KL-VS variant of Klotho gene with early-onset ischemic stroke. Biochem. Biophys. Res. Commun. *403*, 412–416.

Mathur, P., Graybeal, C., Feyder, M., Davis, M.I., and Holmes, A. (2009). Fear memory impairing effects of systemic treatment with the NMDA NR2B subunit antagonist, Ro 25-6981, in mice: attenuation with ageing. Pharmacol. Biochem. Behav. *91*, 453–460.

Mony, L., Kew, J.N., Gunthorpe, M.J., and Paoletti, P. (2009). Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. Br. J. Pharmacol. *157*, 1301–1317.

Morris, R.G., Anderson, E., Lynch, G.S., and Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature *319*, 774–776.

Nagai, T., Yamada, K., Kim, H.C., Kim, Y.S., Noda, Y., Imura, A., Nabeshima, Y., and Nabeshima, T. (2003). Cognition impairment in the genetic model of aging klotho gene mutant mice: a role of oxidative stress. FASEB J. *17*, 50–52.

Nakazawa, K., McHugh, T.J., Wilson, M.A., and Tonegawa, S. (2004). NMDA receptors, place cells and hippocampal spatial memory. Nat. Rev. Neurosci. *5*, 361–372.

Nguyen, P.V., and Kandel, E.R. (1996). A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. J. Neurosci. *16*, 3189–3198.

Palop, J.J., Jones, B., Kekonius, L., Chin, J., Yu, G.-Q., Raber, J., Masliah, E., and Mucke, L. (2003). Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. Proc. Natl. Acad. Sci. USA *100*, 9572–9577.

Paoletti, P., and Neyton, J. (2007). NMDA receptor subunits: function and pharmacology. Curr. Opin. Pharmacol. *7*, 39–47.

Piggott, M.A., Perry, E.K., Perry, R.H., and Court, J.A. (1992). [3H]MK-801 binding to the NMDA receptor complex, and its modulation in human frontal cortex during development and aging. Brain Res. *588*, 277–286.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., and Ferri, C.P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. *9*, 63–75.e2.

Ranganath, C., and Ritchey, M. (2012). Two cortical systems for memory-guided behaviour. Nat. Rev. Neurosci. *13*, 713–726.

Ray, J.W., and Shadish, W.R. (1996). How interchangeable are different estimators of effect size? J. Consult. Clin. Psychol. *64*, 1316–1325.

Razzaque, M.S. (2009). The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis. Nat. Rev. Endocrinol. *5*, 611–619.

Rockwood, K. (2004). Size of the treatment effect on cognition of cholinesterase inhibition in Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry *75*, 677–685.

Semba, R.D., Cappola, A.R., Sun, K., Bandinelli, S., Dalal, M., Crasto, C., Guralnik, J.M., and Ferrucci, L. (2011). Plasma klotho and mortality risk in older community-dwelling adults. J. Gerontol. A Biol. Sci. Med. Sci. *66*, 794–800.

Semba, R.D., Moghekar, A.R., Hu, J., Sun, K., Turner, R., Ferrucci, L., and O'Brien, R. (2014). Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. Neurosci. Lett. *558*, 37–40.

Seshadri, S., DeStefano, A.L., Au, R., Massaro, J.M., Beiser, A.S., Kelly-Hayes, M., Kase, C.S., D'Agostino, R.B., Sr., Decarli, C., Atwood, L.D., and Wolf, P.A. (2007). Genetic correlates of brain aging on MRI and cognitive test measures: a genome-wide association and linkage analysis in the Framingham Study. BMC Med. Genet. *8* (*Suppl 1*), S15.

Shiozaki, M., Yoshimura, K., Shibata, M., Koike, M., Matsuura, N., Uchiyama, Y., and Gotow, T. (2008). Morphological and biochemical signs of age-related neurodegenerative changes in klotho mutant mice. Neuroscience *152*, 924–941.

Shiraki-Iida, T., Aizawa, H., Matsumura, Y., Sekine, S., Iida, A., Anazawa, H., Nagai, R., Kuro-o, M., and Nabeshima, Y. (1998). Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein. FEBS Lett. *424*, 6–10.

Smith, M., Wells, J., and Borrie, M. (2006). Treatment effect size of memantine therapy in Alzheimer disease and vascular dementia. Alzheimer Dis. Assoc. Disord. *20*, 133–137.

Sotres-Bayon, F., Bush, D.E., and LeDoux, J.E. (2007). Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. Neuropsychopharmacology *32*, 1929–1940.

Sze, C., Bi, H., Kleinschmidt-DeMasters, B.K., Filley, C.M., and Martin, L.J. (2001). N-Methyl-D-aspartate receptor subunit proteins and their phosphorylation status are altered selectively in Alzheimer's disease. J. Neurol. Sci. *182*, 151–159.

Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., and Tsien, J.Z. (1999). Genetic enhancement of learning and memory in mice. Nature *401*, 63–69.

Tucker Zhou, T.B., King, G.D., Chen, C., and Abraham, C.R. (2013). Biochemical and functional characterization of the klotho-VS polymorphism implicated in aging and disease risk. J. Biol. Chem. *288*, 36302–36311.

Urakawa, I., Yamazaki, Y., Shimada, T., Iijima, K., Hasegawa, H., Okawa, K., Fujita, T., Fukumoto, S., and Yamashita, T. (2006). Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature *444*, 770–774.

Verghese, P.B., Castellano, J.M., and Holtzman, D.M. (2011). Apolipoprotein E in Alzheimer's disease and other neurological disorders. Lancet Neurol. *10*, 241–252.

Wang, S.H., and Morris, R.G. (2010). Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. Annu. Rev. Psychol. *61*, 49–79.

Wang, H., Stradtman, G.G., 3rd, Wang, X.J., and Gao, W.J. (2008). A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. Proc. Natl. Acad. Sci. USA *105*, 16791–16796.

Wang, D., Cui, Z., Zeng, Q., Kuang, H., Wang, L.P., Tsien, J.Z., and Cao, X. (2009). Genetic enhancement of memory and long-term potentiation but not CA1 long-term depression in NR2B transgenic rats. PLoS One *4*, e7486.

Yaffe, K., Cauley, J., Sands, L., and Browner, W. (1997). Apolipoprotein E phenotype and cognitive decline in a prospective study of elderly community women. Arch. Neurol. *54*, 1110–1114.

Yamazaki, Y., Imura, A., Urakawa, I., Shimada, T., Murakami, J., Aono, Y., Hasegawa, H., Yamashita, T., Nakatani, K., Saito, Y., et al. (2010). Establishment of sandwich ELISA for soluble alpha-Klotho measurement: age-dependent

change of soluble alpha-Klotho levels in healthy subjects. Biochem. Biophys. Res. Commun. *398*, 513–518.

Yang, K., Trepanier, C., Sidhu, B., Xie, Y.F., Li, H., Lei, G., Salter, M.W., Orser, B.A., Nakazawa, T., Yamamoto, T., et al. (2012). Metaplasticity gated through differential regulation of GluN2A versus GluN2B receptors by Src family kinases. EMBO J. *31*, 805–816.

Yashiro, K., and Philpot, B.D. (2008). Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology *55*, 1081–1094.

Zhao, X., Rosenke, R., Kronemann, D., Brim, B., Das, S.R., Dunah, A.W., and Magnusson, K.R. (2009). The effects of aging on N-methyl-D-aspartate receptor subunits in the synaptic membrane and relationships to long-term spatial memory. Neuroscience *162*, 933–945.