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Nuclear receptor TLX stimulates hippocampal neurogenesis and enhances learning and memory in a transgenic mouse model

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The role of the nuclear receptor TLX in hippocampal neurogenesis and cognition has just begun to be explored. In this study, we generated a transgenic mouse model that expresses TLX under the control of the promoter of nestin, a neural precursor marker. Transgenic TLX expression led to mice with enlarged brains with an elongated hippocampal dentate gyrus and increased numbers of newborn neurons. Specific expression of TLX in adult hippocampal dentate gyrus via lentiviral transduction increased the numbers of BrdU^{+} cells and BrdU^{+}NeuN^{+} neurons. Furthermore, the neural precursor-specific expression of the TLX transgene substantially rescued the neurogenic defects of TLX-null mice. Consistent with increased neurogenesis in the hippocampus, the TLX transgenic mice exhibited enhanced cognition with increased learning and memory. These results suggest a strong association between hippocampal neurogenesis and cognition, as well as significant contributions of TLX to hippocampal neurogenesis, learning, and memory.

NR2E1 | neural stem cells | microRNAs

Adult neurogenesis is observed in two discrete areas of adult mammalian brains, the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (1). The process of adult neurogenesis includes proliferation, fate determination, and differentiation of neural progenitor cells, along with maturation of newborn neurons and integration of these neurons into the existing neural network (2). Each step of this process is subject to regulation by numerous intrinsic and extrinsic factors. Hippocampal neurogenesis begins with the proliferation of neural progenitor cells in the SGZ (3). New neurons arise from a local population of neural progenitor cells and eventually become granule neurons. Increasing evidence indicates that neurogenesis is important for hippocampal function (3).

TLX is an orphan nuclear receptor expressed in vertebrate forebrains (4). Mature TLX KO mice have significantly reduced cerebral hemispheres and exhibit increased aggressiveness and violent behavior (5–8). We previously showed that TLX is an essential regulator of neural stem cell self-renewal (6). TLX maintains adult neural stem cells in a self-renewable state, in part by complexing with the histone deacetylases and the histone demethylase LSD1 to repress the transcription of downstream target genes (9, 10). TLX also regulates transcription of the primary precursors of microRNAs (miRNAs), including miR-9 and miR-137, to repress the expression of these miRNAs (11, 12). In addition, TLX expression is also regulated by miR-9 and let-7 (11, 13, 14).

Moreover, we also have demonstrated that TLX activates Wnt signaling to stimulate adult neural stem cell proliferation and self-renewal (15). The TLX-positive cells in the hippocampal DG play an important role in spatial learning and memory (16), whereas the TLX-positive cells in the SVZ of adult brains represent the slowly dividing neural stem cells (15, 17).

In addition to its function in adult brains, TLX also plays an important role in neural development by regulating cell cycle progression in neural stem cells (18, 19). In a recently generated TLX transgenic mouse line that expresses a TLX transgene under the control of the TLX natural promoter, overexpression of TLX led to increased neural stem cell self-renewal in the SVZ (17). The effect of TLX overexpression on hippocampal neurogenesis has not yet been investigated, however, and the relationship between TLX overexpression in the brain and the behavioral output is unknown.

To investigate the role of TLX in hippocampal neurogenesis and behavioral output, we generated a TLX transgenic (Tg) mouse model in which the TLX transgene is under the control of the promoter of nestin, a well-characterized neural precursor marker (20). The nestin promoter-driven expression of TLX increased brain weight and size and led to expansion of the hippocampal DG. Accordingly, the TLX transgene stimulated hippocampal neurogenesis and enhanced learning and memory.

Results

Transgenic TLX Expression Leads to Enlarged Brains and Hippocampal DG. In this study, we took a gain-of-function approach to investigate the role of TLX in neural stem cell self-renewal, neurogenesis, and the behavioral output of the brain. We generated a TLX Tg mouse model that expresses a GFP-TLX fusion protein under the control of the nestin promoter (Fig. 1*A*), and established two founder lines that exhibit indistinguable phenotypes. We found that the

Significance

How do we learn new things and remember and recall episodes? An important region in the brain, the hippocampus, plays a critical role in learning and memory. In this study, we show that manipulating the expression of the *TLX* gene affects neurogenesis in the hippocampus of adult mammalian brains. Our data demonstrate that the expression of TLX in neural precursors is both necessary and sufficient for adult hippocampal neurogenesis. Moreover, the neural precursor-specific overexpression of TLX makes significant contributions to learning and memory. Because impaired learning and memory occur both with aging and in neurodegenerative diseases, increasing TLX expression provides a potential strategy for improving cognitive performance in the elderly and in patients with neurologic diseases.

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The authors declare no conflict of interest.

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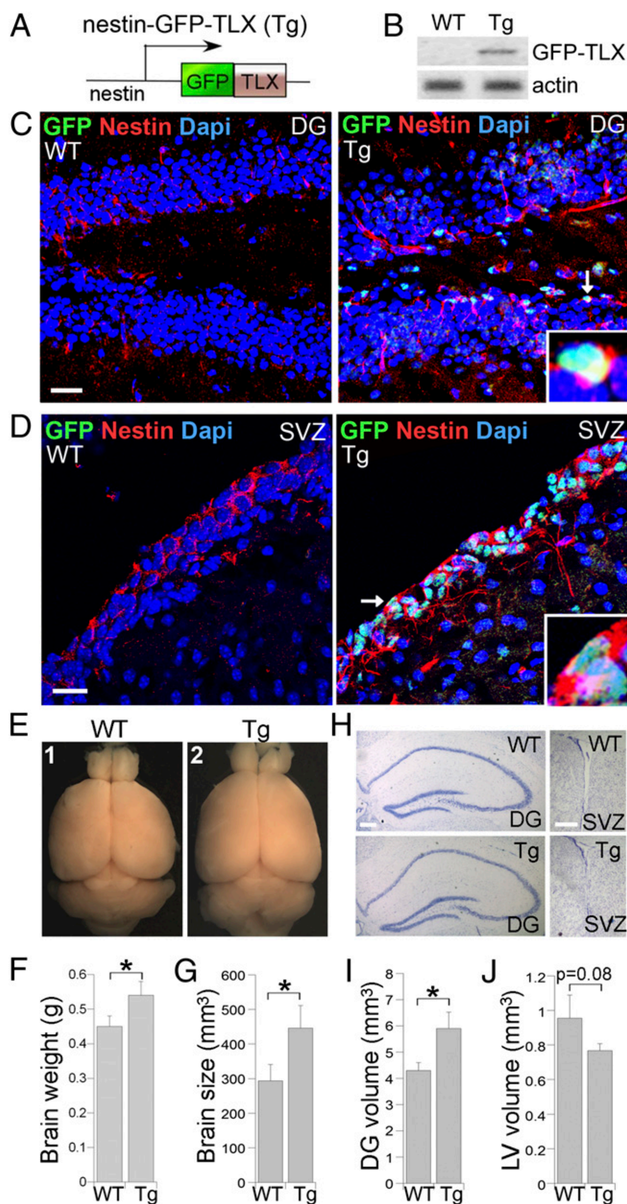

Fig. 1. The TLX Tg mice exhibited an enlarged hippocampal DG. (*A*) Schematic of the TLX transgene, a GFP-TLX fusion under the nestin promoter. (*B*) Expression of the TLX transgene (GFP-TLX) shown by RT-PCR. Actin served as a loading control. (*C* and *D*) Expression of the GFP-TLX transgene in hippocampal DG (*C*) and SVZ (*D*) of WT and Tg mice revealed by immunostaining with a GFP antibody. Nestin and DAPI staining are also shown. (*Insets*) Enlarged images of the cells denoted by arrows. (Scale bar: 50 μm.) (*E*) Images of WT and Tg brains. (*F* and *G*) Quantification of WT and Tg brain weight (*F*) and size (i.e., volume of the forebrain) (*G*). $^*P < 0.05$, Student *t* test. Error bars represent SD of the mean. $n = 8$ for both WT and Tg mice. (*H*) Tg mice displayed elongated hippocampal DG and slightly reduced lateral ventricles. (Scale bar: 200 μm.) All mice were 6-wk-old males. (*I* and *J*) Volumes of the hippocampal DG (*I*) and the lateral ventricles (*J*) measured stereologically. Error bars are s.d. of the mean. $^*P < 0.01$, Student *t* test in *I*. $n = 8$ for both WT and Tg mice in *I* and *J*. All mice were 6-wk-old males.

GFP-TLX transgene was expressed in brains of the Tg mice, but not those of their WT littermates (Fig. 1*B*). We also detected the GFP-TLX protein using a GFP antibody along the SGZ of the hippocampal DG and the SVZ, the two active adult neurogenic areas where endogenous nestin is expressed (Fig. 1 *C* and *D*). The Tg mice had enlarged forebrains with greater brain weight and size compared with their WT littermates (Fig. 1 *E*–*G*), although the two groups were of similar total body weight and length (Fig. S1). Histologically, the Tg mice exhibited an enlarged DG and slightly smaller lateral ventricles (Fig. 1 *H*–*J*).

TLX Transgene Expression Stimulates Neural Stem Cell Self-Renewal. The enlarged brains in the Tg mice prompted us to ask whether increased TLX expression led to enhanced neural stem cell self-renewal. To address this question, we isolated neural stem cells from the brains of 6-wk-old WT and Tg mice and cultured them in DMEM F12 medium supplemented with N2, epithelial growth factor (EGF), and fibroblast growth factor 2 (FGF-2). The self-renewal capacity of the WT and Tg neural stem cells was determined by clonal analysis. Increased TLX expression led to enhanced self-renewal of neural stem cells, as demonstrated by increases in both clonal size (sphere size and sphere cell numbers) and clonal rate (sphere formation rate) of primary (1st) and secondary (2nd) neurospheres (Fig. 2 *A*–*H*). Both WT and Tg neural stem cells were able to differentiate into Tuj1$^+$ neurons, GFAP$^+$ astrocytes, and O4$^+$ oligodendrocytes (Fig. S2), suggesting that both are multipotent. These results further

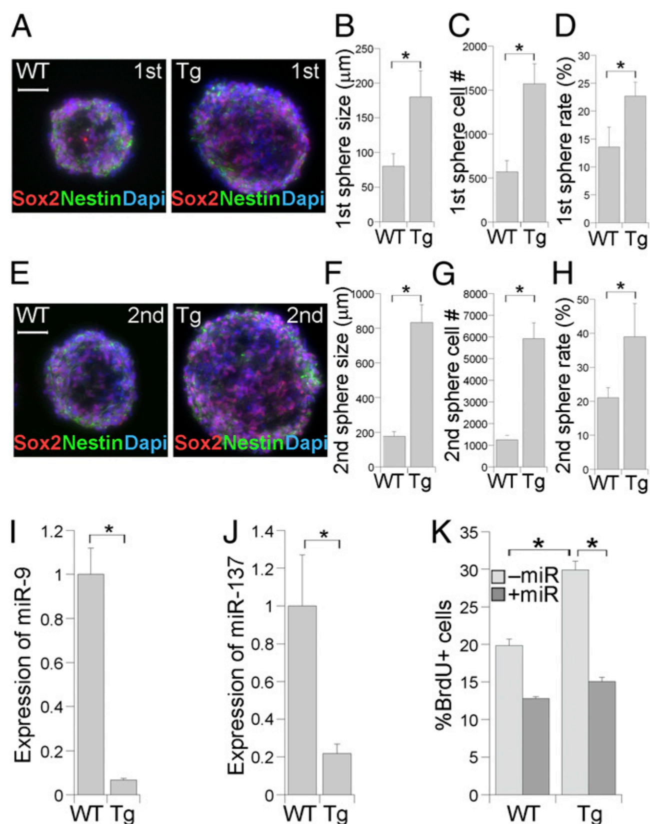

Fig. 2. Transgenic TLX expression enhanced neural stem cell self-renewal. (*A*) Clonal analysis of neural stem cells from WT and Tg mouse brains. Representative images of primary neurospheres are shown. (Scale bar: 50 μm.) (*B*–*D*) Quantification of primary neurosphere size, cell number, and sphere formation rate of WT and Tg neural stem cells. $^*P < 0.001$ (*B* and *C*); $^*P < 0.05$ (*D*), Student *t* test. (*E*) Representative images of secondary neurospheres are shown. (Scale bar: 50 μm.) (*F*–*H*) Quantification of secondary neurosphere size, cell number, and sphere formation rate of WT and Tg neural stem cells. $^*P < 0.001$, Student *t* test. (*I* and *J*) Relative expression of miR-9 (*I*) and miR-137 (*J*) in WT and Tg neural stem cells. $^*P < 0.001$ (*I*); $^*P < 0.005$ (*J*), Student *t* test. (*K*) Overexpression of miR-9 and miR-137 reversed elevated cell proliferation in Tg neural stem cells. WT and Tg neural stem cells were transfected with control RNA (−miR) or with miRNAs miR-9 and miR-137 together (+miR). Cell proliferation was monitored by BrdU labeling. $^*P < 0.001$, two-way ANOVA. Error bars represent SD of the mean. $n = 8$ for all quantifications.

support the idea that TLX plays an important role in neural stem cell self-renewal.

In previous work, we showed that TLX represses the expression of miRNAs miR-9 and miR-137 in neural stem cells, and that both miRNAs negatively regulate neural stem cell proliferation (11, 12). To determine whether these TLX downstream miRNAs play a role in regulating cell proliferation in Tg neural stem cells, we first examined the expression levels of miR-9 and miR-137 in WT and Tg neural stem cells. We found dramatically lower expression of miR-9 and miR-137 in Tg neural stem cells compared with WT cells (Fig. 2 *I* and *J*). Consistent with enhanced self-renewal in Tg neural stem cells (Fig. 2 *A*–*H*), we observed elevated cell proliferation in Tg neural stem cells compared with WT cells (Fig. 2*K*). Moreover, overexpression of miR-9 and miR-137 together reversed the elevated cell proliferation in Tg neural stem cells (Fig. 2*K*), suggesting that repression of miR-9 and miR-137 expression by TLX contributes to the phenotype of enhanced cell proliferation observed in Tg neural stem cells.

Adult Tg Mice Exhibit Increased Hippocampal Neurogenesis. To determine whether transgenic TLX expression regulates neural progenitor cell proliferation in adult brains, we performed BrdU labeling of adult mice for 5 d. Because the more prominent anatomic changes in Tg mice were seen in the hippocampal DG (Fig. 1 *H*–*J*), we focused on this brain region. Increased BrdU labeling was observed along the SGZ of the DG in Tg brains compared with WT brains (Fig. 3 *A* and *B*). In addition, increased staining of Ki67, a marker of proliferating progenitors, was observed in the DG of the Tg mice (Fig. 3 *A* and *C*). These results indicate enhanced neural progenitor cell proliferation in the Tg hippocampus.

We next examined whether TLX expression potentiates hippocampal neurogenesis in adult Tg brains. For this, 6-wk-old WT and Tg mice were treated with BrdU for 5 d, followed by a 3-wk survival period. Neurogenesis was assessed by double-labeling with BrdU and a neuronal marker, NeuN, in the SGZ of the DG. Quantification of the $BrdU^+NeuN^+$ cells revealed substantially increased hippocampal neurogenesis in the Tg mice compared with their WT littermates (Fig. 3 *D* and *E*). Together, these results indicate that the neural precursor-specific expression of the TLX transgene stimulates neural progenitor proliferation and neurogenesis.

To determine whether TLX directly regulates adult hippocampal neurogenesis, we overexpressed TLX in the DG of adult mouse brains by intracranial lentiviral delivery. A TLX-expressing lentivirus was injected into the DG of adult WT mouse brains by stereotaxic transduction. Viral expression of TLX was monitored by a coexpressed GFP reporter, and cell proliferation was monitored by BrdU labeling. Specific expression of TLX at the adult hippocampal DG led to a substantial increase in cell proliferation, as demonstrated by the increased percentage of $BrdU^+GFP^+$ cells in total GFP^+ cells (Fig. 4 *A* and *B*). When we treated the viral-transduced mice with BrdU for 5 d, followed by a 3-wk survival period, adult DG-specific expression of the TLX transgene also was able to enhance neurogenesis, as demonstrated by the increased percentage of $BrdU^+NeuN^+GFP^+$ cells (Fig. 4 *C* and *D*).

Transgenic TLX Expression Rescues Defects in Hippocampal Neurogenesis in TLX-Null Brains. To test whether the neural precursor-specific expression of the TLX transgene can rescue the neurogenic defects induced by loss of TLX expression, we bred the Tg mice with the $TLX^{-/-}$ mice (6) to obtain compound mice with the TLX transgene in a $TLX^{-/-}$ background ($TgTLX^{-/-}$) (Fig. S3*A*). The TLX transgene was specifically expressed in the $TgTLX^{-/-}$ mice, but not in the $TLX^{-/-}$ mice, and the expression level of the transgene was similar to that of endogenous TLX in WT mice (Fig. S3*B*). The nestin promoter-driven expression of TLX was able to substantially rescue the deficiencies in brain weight and size observed in $TLX^{-/-}$ mice (Fig. 5 *A*–*C*). Histologically, the $TgTLX^{-/-}$ mice exhibited

Fig. 3. Tg mice exhibited increased hippocampal neurogenesis in the DG. (*A*) The Tg mice showed increased neural progenitor cell proliferation in the DG of the hippocampus. WT and Tg mice were subjected to 5 d of BrdU labeling. Brain sections were stained for BrdU and Ki67. NeuN staining was included to show the structure of the DG. (Scale bars: 100 μm.) (*B* and *C*) Quantification of $BrdU^+$ (*B*) and $Ki67^+$ cells (*C*) per DG. $^*P < 0.005$, Student *t* test. $n = 11$ for both WT and Tg mice. (*D*) Tg mice exhibited increased numbers of $BrdU^+NeuN^+$ neurons. WT and Tg mice were subjected to 5 d of BrdU treatment, followed by 3 wk of survival. Brain sections were stained for BrdU and NeuN. An example of the $BrdU^+NeuN^+$ cells is shown in orthogonal planes (*Upper*) and at higher magnification (*Lower*). (Scale bars: 100 μm in *Upper*, 10 μm in *Lower*). (*E*) Quantification of $BrdU^+NeuN^+$ cells per DG. $^*P < 0.05$, Student *t* test. $n = 13$ for WT and Tg mice. Error bars represent SD of the mean for all quantifications. All mice were male.

considerably enlarged hippocampal DG and slightly smaller lateral ventricles compared with their $TLX^{-/-}$ littermates (Fig. 5*D*).

We next isolated neural stem cells from the forebrains of $TgTLX^{-/-}$ and $TLX^{-/-}$ littermate mice. The $TLX^{-/-}$ cells did not form neurospheres in DMEM F12 medium supplemented with N2, EGF, and FGF-2, but instead underwent spontaneous differentiation (Fig. 5*E*). In contrast, cells from the $TgTLX^{-/-}$ mouse brains cultured in the same medium were able to form neurospheres, similar to WT cells (Fig. S3*C*).

To determine whether the nestin promoter-driven expression of TLX could rescue neural progenitor cell proliferation in the hippocampal DG of $TLX^{-/-}$ mice, we treated adult $TgTLX^{-/-}$ and $TLX^{-/-}$ littermate mice with BrdU for 5 d. We found a substantial increase in $BrdU^+$ cells was observed in the DG of $TgTLX^{-/-}$ mice compared with their $TLX^{-/-}$ littermates (Fig. 5 *E* and *F*). We also noted increased Ki67 staining in the DG of $TgTLX^{-/-}$ mice (Fig. 5 *E* and *G*). These results indicate that the neural precursor-specific expression of the TLX transgene plays an important role in establishing the proliferative neural progenitor cell populations in adult brains. Expression of the

Fig. 4. Lentiviral expression of TLX increased neurogenesis in the hippocampal DG of adult brains. (*A*) Lentiviral transduction of TLX increased the number of $BrdU^+$ cells in the DG. CSC- or CSC-TLX–transduced mice were treated with BrdU for 5 d. The viral-transduced cells are shown in green, BrdU labeling is in red, and NeuN staining is in blue to show the structure of the DG. An example of the GFP^+BrdU^+ cells is shown in orthogonal planes (*Upper*) and at higher magnification (*Lower*). (Scale bars: 100 μm in *Upper*, 10 μm in *Lower*). (*B*) Quantification of the GFP^+BrdU^+ cells among the GFP^+ cells in the DG of viral-transduced brains. $*P < 0.005$, Student *t* test. (*C*) Lentiviral transduction of TLX increased the number of $BrdU^+NeuN^+$ cells in the DG. CSC- or CSC-TLX–transduced mice were treated with BrdU for 5 d, followed by a 3-wk survival. The viral-transduced cells are shown in green, BrdU labeling is in red, and NeuN staining is in blue. An example of the $GFP^+BrdU^+NeuN^+$ cells is shown in orthogonal planes (*Upper*) and at higher magnification (*Lower*). (*D*) Quantification of the $GFP^+BrdU^+NeuN^+$ cells among the GFP^+ cells in the DG of viral-transduced brains. $*P < 0.05$, Student *t* test. Error bars are SD of the mean. $n = 15$ for CSC- and CSC-TLX–transduced mice. All mice were male.

TLX transgene also was able to restore hippocampal neurogenesis in adult $TLX^{-/-}$ brains to a certain extent, as demonstrated by increased numbers of $BrdU^+NeuN^+$ neurons in the DG of adult $TgTLX^{-/-}$ mice compared with $TLX^{-/-}$ mice (Fig. 5 *H* and *I*).

Learning and Memory Are Enhanced in TLX Transgenic Mice. In adult brains, the hippocampus is crucial for spatial learning and memory (21). The Morris water maze has been used extensively to investigate spatial learning and memory. To compare the performance of the WT and Tg mice in the Morris water maze test, we first subjected the mice to the visible platform test. The WT and Tg mice exhibited similar swimming velocity, distance, and latency to the platform in this test (Fig. S4), suggesting comparable vision and motor stamina in the two groups of mice. We next subjected the mice to the hidden platform test, which determines spatial memory acquisition and retention. Specifically, WT and Tg mice were subjected to 10 trials daily for 3 d to evaluate spatial memory acquisition. Probe trials were performed after the end of the last training session (on day 3) to assess short-term memory retention and again at 1 wk after training (on day 10) to evaluate long-term memory retention (22) (Fig. 6*A*).

The Tg mice exhibited enhanced spatial memory acquisition, as indicated by their shorter latency to reach the platform throughout the trials (Fig. 6*B*) and shorter latency to reach the platform in each of the 3 d of training (Fig. 6*C*) compared with their WT littermates. Furthermore, the Tg mice demonstrated superior short-term memory retention by spending more time in the target quadrant, which had previously held the hidden platform (Fig. 6*D*), and entering the target quadrant at higher frequency (Fig. 6*E*). At 1 wk after training, the Tg mice also showed evidence of improved long-term memory retention by spending more time in the target quadrant and entering the target quadrant at higher frequency (Fig. 6 *F* and *G*). Together, these results suggest that expression of the TLX transgene in neural precursors made a significant contribution to spatial learning and memory.

The enhanced cognitive ability of Tg mice was further demonstrated using startle habituation and prepulse inhibition analyses (23), which provide a tool for studying fundamental properties of nervous system function, including cognition. Startle habituation

Fig. 5. Transgenic TLX rescued neurogenic deficits in the hippocampal DG of TLX-null brains. (*A*) Images of $TLX^{-/-}$ and $TgTLX^{-/-}$ mouse brains. (*B* and *C*) Quantification of brain weight (*B*) and size (i.e., volume of the forebrain) (*C*) in $TLX^{-/-}$ (1) and $TgTLX^{-/-}$ (2) mice. $*P < 0.05$, Student *t* test. $n = 8$ for both $TLX^{-/-}$ and $TgTLX^{-/-}$ mice. All mice were 6-wk-old males. (*D*) The $TgTLX^{-/-}$ mice displayed an enlarged DG and slightly smaller lateral ventricles. (Scale bars: 200 μm.) (*E*) The $TgTLX^{-/-}$ mice showed increased neural progenitor cell proliferation in the DG compared with $TLX^{-/-}$ mice. Mice were subjected to 5 d of BrdU labeling. Brain sections were stained for BrdU and Ki67. NeuN staining was performed to show the structure of the DG. (Scale bars: 100 μm.) (*F* and *G*) Quantification of $BrdU^+$ cells (*F*) and $Ki67^+$ cells (*G*) per DG in the SGZ of $TLX^{-/-}$ and $TgTLX^{-/-}$ mice. $*P < 0.05$ (*F*); $*P < 0.001$ (*G*), Student *t* test. (*H*) The $TgTLX^{-/-}$ mice had increased numbers of $BrdU^+NeuN^+$ neurons. Mice were subjected to 5 d of BrdU treatment, followed by a 3-wk survival. Brain sections were stained for BrdU and NeuN. (*Upper Left*) An example of the $BrdU^-NeuN^+$ cells in $TLX^{-/-}$ brains in orthogonal planes. (*Lower Left*) Higher-magnification view. (*Upper Right*) An example of the $BrdU^+NeuN^+$ cells in $TgTLX^{-/-}$ brains in orthogonal planes. (*Lower Right*) Higher-magnification view. (Scale bars: 100 μm in *Upper*, 10 μm in *Lower*.) (*I*) Quantification of $BrdU^+NeuN^+$ cells per DG in $TLX^{-/-}$ and $TgTLX^{-/-}$ mice. $*P < 0.05$, Student *t* test. $n = 11$ for $TLX^{-/-}$ and $TgTLX^{-/-}$ mice for *F*, *G*, and *I*. All mice were male. Error bars are SD of the mean for all of the quantifications.

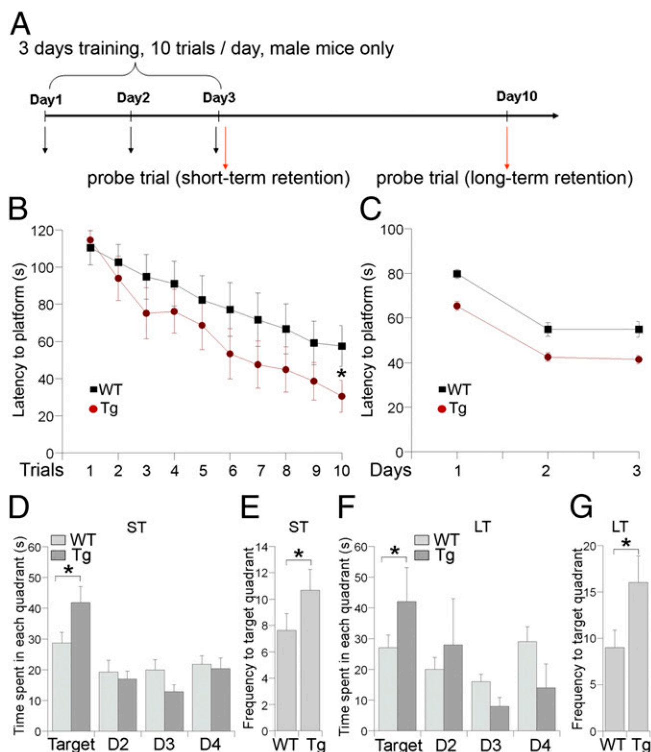

Fig. 6. The Tg mice exhibited enhanced learning and memory in the Morris water maze test. (*A*) Experimental scheme of the water maze test. All mice were 10-wk-old males in littermate pairs. (*B*) Memory acquisition in WT and Tg mice. Shown is the latency of WT and Tg mice to reach the hidden platform during the 10-trial training on day 1. $F(1, 11) = 36.4$; $P < 0.001$, repeated-measures ANOVA. $n = 12$ for both WT and Tg mice. (*C*) The average latency of 10 trials for WT and Tg mice to reach the hidden platform on days 1–3. $F(1, 11) = 6.827$; $P < 0.05$, repeated- measures ANOVA. (*D* and *E*) In the short-term (ST) memory retention test, Tg mice spent more time in the target quadrant (*D*) and entered the target quadrant at higher frequency (*E*). $*P < 0.05$, Student *t* test. (*F* and *G*) In the long-term (LT) memory retention test, compared with WT mice, Tg mice spent more time in the target quadrant (*F*) and entered the target quadrant at higher frequency (*G*). $*P < 0.05$ by Student *t* test. Errors bars are SEM for all of the quantifications.

represents the simplest form of learning. In a 36-trial session, startle habituation was assessed as the percent decrease in startle reactivity between the first block (trials 2–6) and the last block of trials (trials 32–36) (Fig. S5*A*). The Tg mice exhibited significantly greater startle habituation compared with their WT littermates (Fig. S5*B*).

Per-pulse inhibition (PPI) provides an operational measure of sensorimotor gating (24), and reduced PPI is a manifestation of cognitive deficits (25, 26). Tg mice subjected to three prepulses at 3, 6, and 12 dB above the 65 dB background noise, followed by a pulse stimulus at 120 dB, exhibited considerably enhanced PPI at all three prepulses compared with their WT littermates (Fig. S5*C*). These results, combined with the water maze data, strongly suggest that the Tg mice exhibit enhanced cognitive ability.

Discussion

In this study, we generated a nestin promoter-driven TLX Tg mouse model that allowed us to specifically overexpress TLX in neural precursor cells. Characterization of these mice revealed that the increased expression of TLX in neural precursors led to enhanced neural progenitor cell proliferation and hippocampal neurogenesis. Repression of the TLX downstream miRNAs miR-9 and miR-137 contributes to the phenotype of elevated cell proliferation in TLX Tg neural stem cells.

By expressing the TLX transgene in the TLX-null background, we showed that the neural precursor-specific expression of TLX could substantially rescue the neurogenic defects in the hippocampus of TLX-null mice. These results strongly support the idea that TLX expression in neural precursor cells is both necessary and sufficient to promote neural precursor proliferation and neuronal production in the hippocampal DG of adult mouse brains. This conclusion is further supported by clonal analysis, which evaluates the self-renewal ability of neural stem cells. In this study, clonal analysis revealed enhanced self-renewal ability in neurospheres isolated from the Tg mouse brains. In contrast, cells isolated from the $TLX^{-/-}$ mouse brains failed to form neurospheres. Introducing the TLX transgene to the $TLX^{-/-}$ cells restored the neurosphere-forming capability. These results further support the idea that TLX is both necessary and sufficient for neural stem cell self-renewal.

During the course of this study, another Tg mouse line was established by expressing TLX under the TLX promoter (17). These TLX promoter-driven TLX Tg mice exhibited increased neural progenitor proliferation in the SVZ of adult brains; however, the effect of TLX overexpression on hippocampal neurogenesis was not examined (17). Consequently, in the present study we conducted extensive studies to demonstrate that TLX plays an essential role in neural progenitor cell proliferation, neural stem cell maintenance, and neuronal production in the hippocampal DG.

In the present study, we used a neural precursor-specific nestin promoter to drive expression of the TLX transgene, which allowed us to uncouple the role of TLX in neural precursors from its neuronal function (16). This approach differs from that of Liu et al. (17), who used the TLX promoter to drive TLX expression in both neural precursors and TLX-expressing neurons. The present study provides clear evidence that the neural precursor-specific TLX expression is critical for hippocampal neurogenesis. In addition to overexpression of TLX in the Tg mice, we also specifically expressed TLX in the adult hippocampus by intracranial transduction of a TLX-expression lentivirus. The viral transduced cells were labeled by a GFP reporter. Quantification of the GFP^+BrdU^+ and $GFP^+BrdU^+NeuN^+$ cells revealed that the adult hippocampus-specific expression of TLX increased neural progenitor cell proliferation and hippocampal neurogenesis in a cell-autonomous manner.

The hippocampus is a crucial brain structure for spatial learning and memory (21). Enhanced hippocampal neurogenesis in the TLX Tg mice prompted us to study their learning and memory capacity. Using the Morris water maze test, we found that the Tg mice exhibited increased memory acquisition and retention, complementing previous studies using $TLX^{-/-}$ mice that showed reduced learning and memory (7, 16, 27). Thus, the present study reinforces the significance of TLX signaling in learning and memory. Most previous studies have shown deficits in memory acquisition and retention when neurogenesis is reduced by irradiation or genetic manipulation (16, 22, 28–31). This study demonstrates that increased neurogenesis leads to enhanced memory acquisition and retention, providing complementary evidence to strengthen the notion of a requirement for hippocampal neurogenesis in learning and memory.

Along with memory, hippocampus also modulates sensorimotor processes and PPI (32). For example, PPI disruption was observed when adult hippocampal neurogenesis was inhibited by irradiation (33). It is speculated that decreased hippocampal neurogenesis is the common cause of cognition disturbance and PPI disruption (34). PPI disruption also has been reported in cognitive disorders, including schizophrenia, obsessive compulsive disorder, and fragile X syndrome (35–37). Of interest, the TLX Tg mice exhibited dramatically enhanced PPI compared with their WT littermates. This result is consistent with the water maze data, lending further support to the concept of enhanced cognitive ability in TLX Tg mice.

Identifying molecules that increase hippocampal neurogenesis and improve cognition is pivotal to the development of therapeutic

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strategies for enhancing cognitive performance. Here we showed that the transgenic expression of TLX in neural precursor cells enhanced spatial learning and memory. Impaired learning and memory occur both during aging and in neurodegenerative diseases, such as Alzheimer's disease. This study identifies TLX as a potential therapeutic target for drug development aimed at improving cognitive performance in both populations.

Methods

Viral Production and Intracranial Lentivirual Infection. The CSC control vector and the CSC-TLX–expressing lentiviruses were generated as described previously (6). For intracranial lentiviral infection, 1 μL of 1×10^8 transduction unit/mL concentrated virus was injected into the hippocampal DG of 6-wk-old WT ICR mice by stereotaxic injection as described previously (15). At 2 wk after viral injection, the transduced mice were injected with BrdU for 5 d, followed by short-term BrdU labeling, or 3 wk of survival for long-term BrdU labeling. The coordinates for the DG of WT mice were anterior-posterior, −2.0 mm, mediolateral, ±1.7 mm; dorsoventral, −1.5; and −1.9 mm from the skull surface.

Morris Water Maze Test. The water maze test was conducted in a 1.5-m-diameter circular tank filled with opaque water with visible cues outside. The test consisted of 1 d of pretraining, 2 d of visible platform training, and 3 d of hidden platform training. For visible platform, the platform location was indicated by a colorful rod attached to the platform above the water. Mice were trained with 10 trials per day for two days. For the hidden platform test, the platform was placed in a constant position in quadrant 1, hidden 1 cm below the water surface. Mice were trained with 10 trials per day for three days. In each training trial, mouse was released, facing the wall of the tank, from quadrants 2–4. Each mouse was allowed to locate the platform within 2 min. If a mouse failed to find the platform within 2 min, it was guided onto the platform. The mouse was allowed to stay on the platform for 15 s after finding it.

After 10 trials on the last day of training, the platform was removed, and a probe trial was conducted as a test of short-term memory. For testing long-term memory, the probe trial was repeated at 1 wk after the final training trial. For both probe trials, each mouse was allowed to swim for 90 s. The procedure was recorded using a digital video camera system (San Diego Instruments). Additional experimental procedures are provided in *SI Materials and Methods*.

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