

Behavioral and Neurochemical Alterations in Mice Lacking the RNA-Binding Protein Translin

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Synapse-specific local protein synthesis is thought to be important for neurodevelopment and plasticity and involves neuronal RNA-binding proteins that regulate the transport and translation of dendritically localized transcripts. The best characterized of these RNA-binding proteins is the fragile X mental retardation protein (FMRP). Mutations affecting the expression or function of FMRP cause fragile X syndrome in humans, and targeted deletion of the gene encoding FMRP results in developmental and behavioral alterations in mice. Translin is an RNA-binding protein that regulates mRNA transport and translation in mouse male germ cells and is proposed to play a similar role in neurons. Like FMRP, translin is present in neuronal dendrites, binds dendritically localized RNA, and associates with microtubules and motor proteins. We reported previously the production of viable homozygous *translin* knock-out mice, which demonstrate altered expression of multiple mRNA transcripts in the brain and mild motor impairments. Here, we report that *translin* knock-out mice also exhibit sex-specific differences in tests of learning and memory, locomotor activity, anxiety-related behavior, and sensorimotor gating, as well as handling-induced seizures and alterations in monoamine neurotransmitter levels in several forebrain regions. Similar behavioral and neurochemical alterations have been observed in mice lacking FMRP, suggesting that both proteins may act within the same neuronal systems and signaling pathways. Our results in mice indicate that mutations in translin may contribute to fragile X-like syndromes, mental retardation, attention deficit hyperactivity disorder, epilepsy, and autism spectrum disorders in humans.

Key words: anxiety; attention; RNA trafficking; local translation; fragile X syndrome; norepinephrine

Introduction

Dendritic protein synthesis involves *cis*-acting targeting sequences in localized mRNAs and *trans*-acting RNA-binding proteins that regulate the storage, transport, translation, and degradation of these mRNAs. Genetically disrupting the targeting sequence of calcium/calmodulin kinase II α (CaMKII α) mRNA prevents its accumulation in hippocampal dendrites in mice and produces deficits in the Morris water maze and contextual fear conditioning, two hippocampus-dependent memory tasks (Miller et al., 2002). Mice lacking fragile X mental retardation protein (FMRP), the most extensively studied *trans*-acting factor, exhibit altered levels of dendritic mRNAs (Zalfa et al., 2003) and multiple behavioral phenotypes, including subtle water maze

deficits, hyperactivity, decreased anxiety-related behavior, mild motor impairments on the rotarod test, reduced acoustic startle responses, enhanced prepulse inhibition (PPI) of startle, and increased susceptibility to audiogenic seizures (Bakker et al., 1994; D'Hooge et al., 1997; Paradee et al., 1999; Peier et al., 2000; Chen and Toth, 2001; Mineur et al., 2002; Qin et al., 2002; Frankland et al., 2004). Mice lacking FXR2, a homolog of FMRP, exhibit water maze and contextual fear conditioning deficits, hyperactivity, rotarod impairments, and reduced prepulse inhibition (Bontekoe et al., 2002).

The current study explores the consequences of disrupting translin (also known as testis-brain RNA-binding protein), a protein thought to regulate mRNA transport and translation that shares many features with FMRP. Like FMRP, translin shows highest expression in the testis and brain (Han et al., 1995b; Hergersberg et al., 1995; Gu et al., 1998; Finkenstadt et al., 2001). In the testis, translin binds translationally repressed mRNAs containing *cis*-acting motifs termed Y and H elements and accompanies these transcripts along intercellular bridges between spermatids (Morales et al., 2002). In the brain, translin binds CaMKII α and the untranslated RNA BC1 (Kobayashi et al., 1998; Severt et al., 1999; Wu and Hecht, 2000), dendritically localized RNAs that also associate with FMRP (Zalfa et al., 2003). Both proteins exhibit a somatodendritic distribution in neurons (Feng et al., 1997; Kobayashi et al., 1998; Muramatsu et al., 1998; Wu et al., 1999;

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Finkenstadt et al., 2000), shuttle between the nucleus and cytoplasm (Feng et al., 1997; Morales et al., 2002), interact with microtubules and motor proteins (Han et al., 1995a; Wu and Hecht, 2000; Ohashi et al., 2002; Antar et al., 2005), repress translation (Kwon and Hecht, 1993; Lagerbauer et al., 2001; Li et al., 2001; Yang et al., 2003), and may be involved in RNA interference (Caudy et al., 2002; Ishizuka et al., 2002; Wang et al., 2004).

We reported previously the production of homozygous *translin* knock-out (KO) mice, which exhibit reduced growth, increased visceral adipose tissue, normal fertility but abnormal spermatogenesis, downregulation of multiple brain mRNAs, and mild impairments in the rotarod task (Chennathukuzhi et al., 2003). Given *in vitro* evidence that *translin* mediates the dendritic localization of CaMKII α (Severt et al., 1999), we hypothesized that *translin* KO mice would exhibit deficits in the Morris water maze and contextual fear conditioning. Considering the many similarities between *translin* and FMRP, we suspected that *translin* KO mice might show more subtle learning differences and alterations in activity, anxiety-related behavior, and sensorimotor gating.

Materials and Methods

Animals. The generation of *translin* KO mice and their genotyping by PCR amplification of tail DNA was described previously (Chennathukuzhi et al., 2003). *translin* KO mice were successively backcrossed to C57BL/6J inbred mice to minimize genetic variability. The C57BL/6J strain was chosen because it breeds well and exhibits average performance in tests of learning, activity, anxiety-related behavior, and sensorimotor gating (Crawley et al., 1997). After transmission of the targeted *translin* allele through three crosses to C57BL/6J mice, heterozygous N3 animals were mated to produce homozygous *translin* KO mice and wild-type (WT) littermates for all studies. Mice were segregated by sex, housed four to five to a cage, provided with water and rodent chow *ad libitum*, and maintained on a 12 h light/dark cycle. All experiments were performed during the light cycle using *translin* KO mice and WT littermate controls between 2 and 6 months of age and were conducted in accordance with the policies of the Institutional Animal Care and Use Committee of the University of Pennsylvania and the National Institutes of Health.

Water maze. Spatial learning and memory was tested in the water maze task originally developed by Morris et al. (1982) and adapted for mice as described previously (Lattal and Abel, 2001). Mice were trained to find a circular, submerged platform in a pool of water made opaque with nontoxic white tempera paint. The pool and the platform diameters were 117 and 11 cm, respectively. Distal spatial cues were placed around the room away from the edge of the pool, and the platform was placed in one of two locations on opposite sides of the pool. During acquisition training, each mouse received four trials per day on 6 consecutive days, with a maximum trial length of 60 s and intertrial intervals of 4–6 min. The latency to find the hidden platform during each trial was recorded. At the end of each trial, mice were left on the platform for 20 s before being returned to their home cages. During reversal testing, mice received four trials per day for 3 d, with the platform switched to the opposite side of the pool. During visible training, mice received four trials per day for 4 d, the platform was moved to one of four locations between each trial, and a marker extending above the pool surface indicated the platform position. Probe trials, in which the platform was removed and mice were allowed to search the pool for 60 s, were performed after acquisition and reversal training, on days 7 and 11 of testing, respectively. Each trial was recorded using an overhead CCD camera and video tracking software (HVS Image, Buckingham, UK), allowing for measurements of quadrant preference, swim speed, and thigmotaxis (swimming around the periphery of the pool within 6 cm of the wall).

Cued and contextual fear conditioning. Fear conditioning experiments were performed as described previously (Nie and Abel, 2001). Mice were trained to associate an auditory conditioned stimulus (CS) (white noise at 85 dB intensity) with an aversive unconditioned stimulus (US) (a 1.5

mA footshock). Mice were handled for 2 min in the conditioning room on each of 3 consecutive days before training. During training, mice were placed in the conditioning chamber for 3 min. After 2 min in the chamber, mice received a single CS–US pairing consisting of 30 s of white noise, coterminating with a 2 s footshock. Either 24 h (long-term test) or 1 h (short-term test) later, mice were returned to the same chamber for 5 min. One hour after testing in the original context, the animals were tested in a novel context, a separate chamber lacking a grid floor and with differing spatial, visual, and olfactory cues. After 2 min in the new chamber, mice were reexposed to the CS for 3 min. An observer blind to genotype manually assessed freezing behavior, defined as complete lack of movement except for respiration, in intervals of 5 s throughout training and testing trials. Groups of four mice were trained and tested simultaneously in four separate conditioning chambers. Each chamber was cleaned between animals with 75% ethanol during training and contextual testing or with dilute lemon-scented detergent during cued testing. To test shock sensitivity, mice were placed in a conditioning chamber for 2 min and given a series of footshocks of increasing intensity from 0.05 to 1.0 mA in 0.05 mA increments. Each shock was separated by 10 s. This procedure was repeated once, and results were averaged. Videotapes were scored for flinching (any noticeable reaction), vocalizing (any audible response), and jumping (at least two paws leaving the grid floor) during delivery of the shock. Different groups of mice were used in tests of long-term conditioning, short-term conditioning, and shock sensitivity.

Elevated zero maze. Activity and anxiety-related behavior were measured in the elevated zero maze test as described previously (Ecker et al., 2004). A circular variant of the elevated plus maze, the zero maze apparatus consists of a raised circular track divided into two open and two closed quadrants. The track had an internal diameter of 40.5 cm and a width of 5.1 cm and was elevated off the floor at a height of 40 cm. The closed quadrants had walls 11 cm high. Mice were housed individually for 1 h before testing and then placed into the center of a closed quadrant and observed for 5 min. Digitized video of each 5 min trial was scored manually and also analyzed using automated behavioral analysis software (Clever Systems, Reston, VA) to determine the percentage of time, total distance, and average speed of the animals in open and closed quadrants, the number of transitions between quadrants, the number of episodes of stretch-attend posture (SAP), and the number of head dips.

Light–dark exploration. Anxiety-related behavior was tested in the light–dark exploratory activity test as described previously (Miyakawa et al., 2001). The testing apparatus was a large plastic rodent cage (45.5 \times 25.6 \times 15.3 cm) modified so that two-thirds of the cage was open and brightly lit and the remaining one third was dark and enclosed. Passage between the light and dark compartments was possible via a small opening 7.5 cm wide and 5 cm high. Each mouse was placed in the center of the open compartment facing away from the dark compartment and videotaped for 10 min. Videotapes were scored manually to determine the latency to enter the dark compartment, the percentage of time in the open compartment, and the number of transitions between compartments.

Open field. Baseline locomotor activity was measured as described by Qin et al. (2002) in a 41 \times 41 cm square arena equipped with a 16 \times 16 grid of photobeam sensors spaced 2.54 cm apart (San Diego Instruments, San Diego, CA). A second array of 16 photobeam sensors at a height of 5 cm measured vertical activity such as rearing and jumping. Each mouse was placed in the center of the arena, and beam breaks were recorded in 1 min intervals over either 15 or 30 min. Activity levels were observed to plateau by 15 min, so all data were combined and only the first 15 min were analyzed. Light intensity in the square arena was 165 lux in the center and averaged 144 lux in the corners. Mice tend to keep to the walls of the open-field arena, and the percentage of beam breaks occurring in the center of the open area is commonly used as a measure of anxiety-related behavior (Simon et al., 1994). The center area was defined as the centermost 12 photobeams in each direction.

Acoustic startle responses and prepulse inhibition. Whole-body acoustic startle responses and PPI of the startle response were measured using the SR-Lab System (San Diego Instruments) as described previously (Gould et al., 2004). Testing was conducted in 35 \times 33 \times 38.5 cm sound-attenuating chambers, each ventilated with a small fan and illuminated

by a 15 W light bulb mounted in the ceiling. Mice were placed in clear Plexiglas cylinders (16 × 8.75 cm) mounted on Plexiglas frames (12.5 × 20.5 × 0.6 cm) elevated 2.75 cm above a 30 × 30 × 4 cm base by screws positioned at each corner. Acoustic stimuli were delivered through a speaker mounted in the ceiling of each chamber, 23 cm above the cylinders, and startle responses were detected by piezoelectric accelerometers mounted beneath each frame. Output signals were digitized, rectified, and recorded as consecutive 1 ms readings using San Diego Instruments Startle Reflex software. The average startle response and maximum amplitude of the startle response were recorded as San Diego Instruments startle units. Both startle responses and PPI were measured during a single session, and results from three such sessions conducted on 3 consecutive days were averaged for each mouse. Sessions started with a 5 min acclimation period of 65 dB background noise, followed by five 120 dB startle pulses. Startle trials used 40 ms pulses at 0 (no stimulus), 90, 95, 100, 105, 110, 115, and 120 dB. After startle testing, PPI was measured for prepulse intensities of 72, 76, and 84 dB or no stimulus. Each prepulse lasted 20 ms and was followed 100 ms later by a 40 ms startle stimulus at 120 dB. All startle and prepulse trials were administered five times each in a random order, with intertrial intervals randomized to 10–20 s. Data were collected as 60 1-ms voltage readings, which were averaged over the collection interval to give an average startle measure for each trial. Percentage of PPI for each prepulse intensity was calculated as $100 - 100 \times (\text{startle with prepulse} / \text{startle without prepulse})$.

HPLC. Levels of monoamine neurotransmitters were determined using HPLC essentially as described previously (Robinson et al., 1992). *translin* KO and WT mice of the same sex were killed and dissected in random order. After cervical dislocation, brains were removed from *translin* KO mice and WT littermates and rinsed briefly in cold PBS. Samples of the amygdala, hippocampus, cortex, prefrontal cortex (PFC), and striatum were rapidly dissected on ice, frozen in Eppendorf (Westbury, NY) tubes submerged in liquid nitrogen, and stored at -80°C . Samples were coded so that those performing the neurochemical analyses were blinded to the genotype. Each sample was diluted 1:10 directly into homogenizing buffer containing 3,4-dihydroxybenzylamine as an internal standard. Samples were sonicated on ice for 10 s and centrifuged at 14,000 rpm at 0°C for 20 min; HPLC analyses were generally performed on the same day. Approximately 30 μl of tissue sample was combined with 2.5 μl of ascorbate oxidase. Samples were injected onto a C18 reverse-phase column (ODS, 150 × 4.6 mm; 5 μm particles; Thermo Electron Corporation, Waltham, MA) by a Waters (Milford, MA) 717 Plus Autosampler, eluted using an isocratic system with a mobile phase (MD-TM; ESA, Bedford, MA), and pumped by a Waters 515 HPLC pump. Under these conditions, norepinephrine (NE), serotonin (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) can easily be resolved. The norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) elutes very near the injection front and was measured separately. External standards ranging from 0 to 10 pmol were injected every fifth specimen. The 10 pmol standard was injected twice (first and last) to determine whether there had been any drift during the analysis. A Coulochem II electrochemical detector equipped with a 5011 analytical cell (ESA) was used to detect the biogenic amines. Data were captured using the Empower Software system (Waters).

Order of testing. No mouse was exposed to either fear conditioning or shock threshold testing procedures more than once, and these tests were the last to be performed on any group of animals. Before fear conditioning or shock threshold testing, some mice were tested in the elevated zero maze or the light–dark box, the open-field test, the Morris water maze, or acoustic startle and prepulse inhibition in that sequence. Mice used for neurochemical measurements were entirely naive and were not subjected to any behavioral testing.

Statistical analysis. Data derived from water maze training trials, fear conditioning, open-field testing, acoustic startle testing, and prepulse inhibition were analyzed using three-way repeated-measures ANOVA with genotype, sex, and training day, testing condition, minute, acoustic stimulus intensity, or prepulse intensity as factors. Significant differences identified by ANOVA were further investigated using Tukey–Kramer *post hoc* multiple comparison procedures to account for different num-

bers of animals in each group. Data from the zero maze and the light–dark exploration test were analyzed using two-way ANOVA with genotype and sex as factors. When data initially failed tests of normality or equal variance, typically because of floor effects, nonparametric analyses were used. Cox proportional hazards regression models were used to compare the time to find the platform in the water maze between WT and KO mice over training days, stratified by sex and condition (acquisition, reversal, and visible). In this analysis, the hazard ratio represents the relative risk of finding the platform during a given period. The z -value is the ratio of each calculated regression coefficient to its SE. Interactions between day of trial and genotype were also tested. These models were adjusted for clustering by mouse across repeated measures using the robust variance estimator (Lin and Wei, 1989). Because many mice showed no thigmotaxis or no jumping behavior, logistic regression was used to compare the odds of any thigmotaxis versus no thigmotaxis and jumps versus no jumps between genotypes and over time, stratified by sex and condition. Generalized estimating equations methodology was used to adjust for clustering by mouse attributable to repeated measures over training days (Zeger and Liang, 1986). Again, interactions between day of trial and genotype were also tested. In several cases in which no jumps were recorded for WT mice, Fisher's exact test was used in place of the χ^2 test for calculating p values. All data are expressed as the group mean or percentage, with error bars signifying the SEM or percentage.

Results

Male *translin* KO mice exhibit performance deficits in the water maze but are able to learn the position of the hidden platform

To determine whether *translin* is required for spatial learning and memory in mice, we tested *translin* KO mice and WT littermates in the hippocampus-dependent, hidden platform version of the Morris water maze. After acquisition of the initial hidden platform position, reversal training was performed to assess the flexibility of spatial memory, and visible training was performed to detect deficits in nonspatial components of the task, such as motor and sensory ability, attention, or motivation. We trained *translin* KO mice ($n = 13$, 7 males and 6 females) and WT littermates ($n = 16$, 9 males and 8 females) to find the hidden platform using four trials per day over 6 d, followed by 3 d of reversal training and 4 d of training with the visible platform. Male and female mice of both genotypes significantly improved their performance across training days during acquisition ($z = 7.80$, $p < 0.001$ for males and $z = 8.03$, $p < 0.001$ for females for day 6 vs day 1); however, male *translin* KO mice exhibited significantly longer escape latencies than WT littermates overall (Fig. 1A) ($z = 2.82$; $p < 0.01$). Female *translin* KO mice exhibited somewhat longer relative latencies during the first 2 d of acquisition training, but this difference was not significant and disappeared over the remaining trials (Fig. 1B) ($z = 1.24$; $p = 0.22$). *translin* KO mice and WT littermates of both sexes showed significant improvements in performance across trials during reversal testing ($z = 6.71$, $p < 0.001$ for males and $z = 7.52$, $p < 0.001$ for females for day 3 vs day 1); however, male *translin* KO mice again exhibited longer escape latencies overall (Fig. 1A) ($z = 2.08$; $p < 0.05$), primarily because of poor performance on the third day of reversal training. In visible training, males and females of both genotypes again improved their performance over training days ($z = 3.37$, $p < 0.001$ for males and $z = 6.80$, $p < 0.001$ for females for day 4 vs day 1). The performance of female mice did not differ significantly by genotype, but there was a main effect of genotype (Fig. 1A) ($z = 3.84$; $p < 0.001$) and a significant interaction between genotype and training day (χ^2 test, $p < 0.05$) for male mice. Male *translin* KO mice exhibited significantly longer escape latencies than male WT littermates on each of the last 3 d of

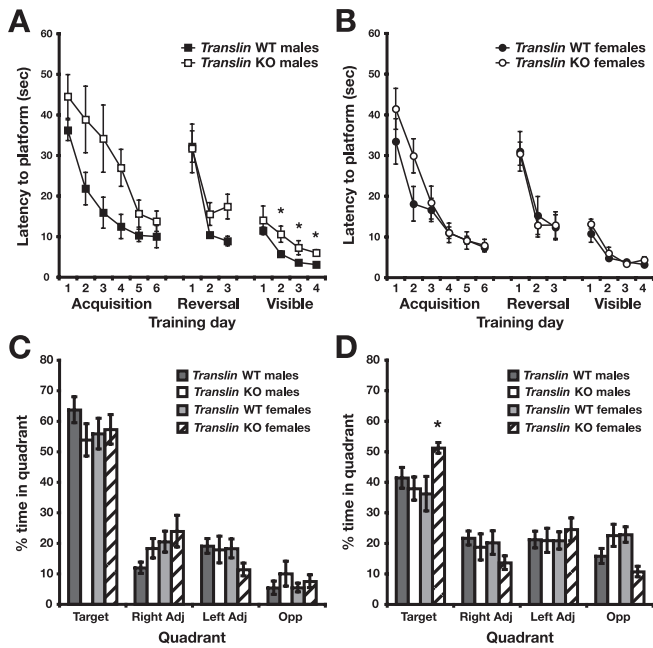


Figure 1. Male *translin* KO mice exhibit increased escape latencies during training in the Morris water maze, but spatial memory as assessed by probe trials is not impaired. *translin* KO mice ($n = 13$, 7 males and 6 females) and WT littermates ($n = 17$, 9 males and 8 females) were placed in a pool of water made opaque with white paint and trained using four trials per day over multiple days to find a submerged platform at an initial location (Acquisition; days 1–6), at a new location on the opposite side of the pool (Reversal; days 1–3), and at varying locations identified with a visible marker extending from the surface of the pool (Visible; days 1–4). Probe trials in which the platform was removed and mice were allowed to search the pool for 60 s were performed after acquisition and reversal training. **A**, Male *translin* KO mice exhibited significantly longer escape latencies than WT littermate controls during acquisition, reversal, and visible platform training. **B**, Escape latency did not differ significantly between female *translin* KO mice and WT controls. **C**, After 6 d of training with the platform in its initial location, *translin* KO mice and WT littermate controls of both sexes spent a significantly greater percentage of time in the quadrant that previously contained the hidden platform (target) relative to the other quadrants. **D**, After 3 d of training with the platform in a new location, all groups showed a significant preference for the new target quadrant. Female *translin* KO mice spent a significantly greater percentage of time in the target quadrant than male KO mice and WT controls. * $p < 0.05$. Opp, Opposite; Adj, Adjacent.

visible platform training (Fig. 1A) ($z = 2.78, 2.87, \text{ and } 3.84; p < 0.05$ for each day).

Male *translin* KO mice took longer to find the hidden platform during acquisition training, but their performance approached that of WT mice by the fifth and sixth days of training. Indeed, both *translin* KO mice and WT controls of both sexes showed a significant preference for the quadrant that previously contained the hidden platform in a probe trial performed on day 7 (Fig. 1C) ($F_{(3,78)} = 113.616; p < 0.0001$), indicating that all groups were able to learn the platform position. A probe test was also performed after reversal training (Fig. 1D). Despite the poor performance of male KO mice on day 3, all groups showed a significant preference for the new target quadrant ($F_{(3,78)} = 32.469; p < 0.001$). Surprisingly, female *translin* KO mice actually showed a greater preference for the new target quadrant than female WT mice or males of either genotype (quadrant \times genotype \times sex interaction, $F_{(3,78)} = 4.365, p < 0.01$; Tukey–Kramer test, $p < 0.05$ for target quadrant).

The small but significant increase in escape latency in male *translin* KO mice relative to WT littermates in the visible platform test indicates that there is a fundamental impairment in performance independent of differences in spatial learning. Deficits in motor

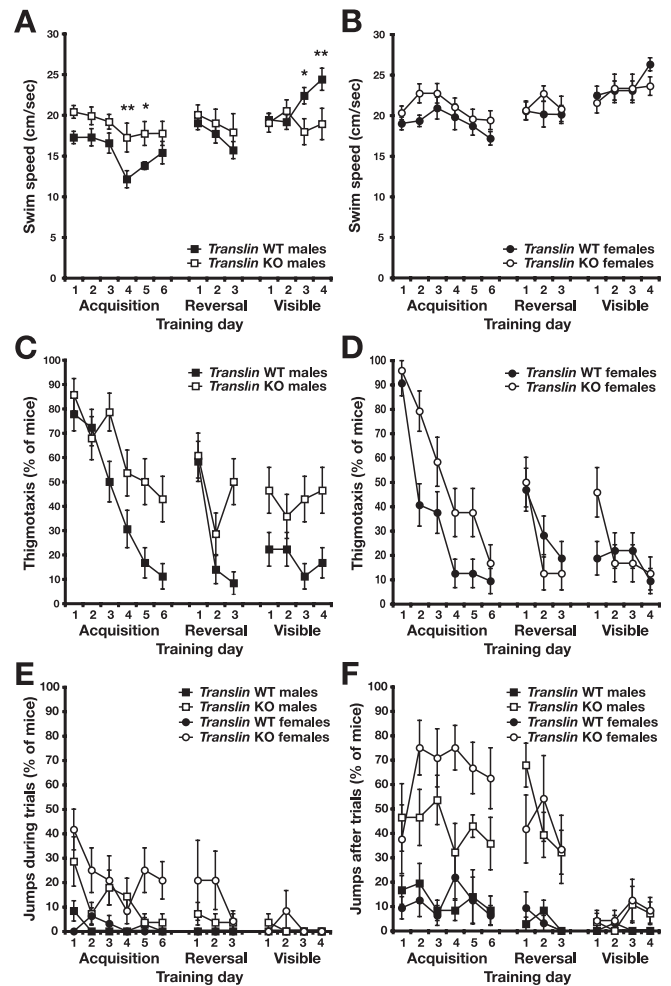


Figure 2. Increased escape latency in *translin* KO mice in the Morris water maze may be related to differences in swim speed, thigmotaxis, or jumping behavior. Swim speed, thigmotaxis, and jumping behavior of *translin* KO mice ($n = 13$, 7 males and 6 females) and WT littermates ($n = 17$, 9 males and 8 females) were recorded throughout acquisition, reversal, and visible platform training in the Morris water maze. **A**, Relative to male WT controls, the average swim speed of male *translin* KO mice was significantly greater during hidden platform training and slower during visible platform training. **B**, Although there was a significant difference in average speed between female *translin* KO mice and WT littermates overall, *post hoc* tests did not reveal significant differences on any given day. **C**, Swimming around the periphery of the pool, a behavior termed thigmotaxis, occurred in a significantly higher proportion of male *translin* KO mice than male WT littermates at the end of acquisition and during visible platform training. **D**, Female *translin* KO mice also exhibited significantly more thigmotaxis during acquisition but not during reversal or visible platform training. **E**, **F**, After locating the hidden platform during a given trial, mice are allowed to sit on it for 20 s before being returned to the home cage, but they occasionally jump back into the pool after finding the platform. *translin* KO mice of both sexes exhibited more of this jumping behavior than WT littermates immediately after finding the platform during acquisition and reversal training (**E**) and when we attempted to retrieve them from the platform throughout training (**F**). * $p < 0.05$; ** $p < 0.01$.

function could possibly limit the minimum escape latency achieved by male KO mice during training; however, male KO mice exhibited significantly higher swim speeds than male WT littermates during acquisition (Fig. 2A) (genotype \times trial, $F_{(14,196)} = 9.561, p < 0.001$; day 4, $p < 0.01$; day 5, $p < 0.05$), suggesting that motor ability is not impaired. Conversely, male KO mice exhibited slower average speeds than male WT mice in swimming to the visible platform (visible day 3, $p < 0.05$; day 4, $p < 0.01$). For females, there was also a significant interaction between trial and genotype, indicating higher swim speed in female KO mice overall (Fig. 2B) ($F_{(14,168)} = 1.805; p < 0.05$), but

post hoc testing did not reveal significant differences on any particular training day.

Several additional observations suggest alternative explanations for impaired escape behavior in *translin* KO mice. When first introduced to the water maze, most mice swim around the edge of the pool, a behavior called thigmotaxis. Much of the initial improvement in escape latency during acquisition training comes as the mice adopt a different search strategy and swim into the center of the pool (Schenk and Morris, 1985), and differences in thigmotaxis can account for significant behavioral variability between mutant and WT mice in the water maze (Wolfer et al., 1998). Male *translin* KO mice exhibited persistent thigmotaxis relative to WT controls that may have interfered with performance (genotype \times day, $p < 0.01$; genotype on day 6, $p < 0.01$). Persistent thigmotaxis may also explain the relatively higher swim speed of male KO mice during acquisition. Starting on day 4, there is a relative reduction and a subsequent recovery in swim speed in male WT mice that may reflect a progression from thigmotaxis to slower active searching to memory for the platform position. Male *translin* KO mice continued to show higher overall levels of thigmotaxis during reversal and visible platform training (main effects of genotype, $p < 0.05$ and $p < 0.001$, respectively). In females, the amount of thigmotaxis also decreased during acquisition training in both groups (Fig. 2D) (main effect of day, $p < 0.0001$) but was higher overall in *translin* KO mice (main effect of genotype, $p < 0.0001$). Thigmotaxis did not differ according to genotype during reversal or visible platform training in female mice.

Because most mice do not find the platform during early trials, initial improvements in water maze performance also require that mice cooperate in being guided to the platform. In training *translin* KO mice and their WT littermates, we noticed that many of the mice swam away rapidly and refused to be guided to the platform. Furthermore, on reaching the platform or being guided to it, mice would frequently jump off and continue swimming. During acquisition and reversal, this behavior occurred significantly more frequently in male and female *translin* KO mice (Fig. 2E) (acquisition, χ^2 test, $p < 0.0001$ for males and $p < 0.05$ for females; reversal, Fisher's test, $p < 0.05$ for males and $p < 0.0001$ for females). Throughout testing phases, male and female *translin* KO mice also displayed a significant tendency to jump off the platform when we attempted to return them to the home cage (Fig. 2F) (acquisition, χ^2 test, $p < 0.001$ for males, $p < 0.0001$ for females; reversal, χ^2 test $p < 0.0001$ for males, $p < 0.01$ for females; visible, Fisher's test, $p < 0.01$ for males, $p < 0.05$ for females). We previously observed the latter type of behavior, excessive jumping and running apparently in response to the threat of capture, when attempting to remove *translin* KO mice from their home cages. Interestingly, we also observed multiple episodes of tonic-clonic seizures in both male and female *translin* KO mice that seem to be provoked by such routine handling. Seizures were not observed during behavioral testing or in WT littermates.

Female *translin* KO mice exhibit enhanced conditioned freezing

To further assess learning and memory, we tested *translin* KO mice and WT littermates in cued and contextual fear conditioning. This task has the advantage that learning occurs in a single trial and that cued and contextual conditioning can dissociate dysfunction in different brain areas. Both types of learning require the amygdala, but contextual conditioning also depends on the hippocampus (Phillips and LeDoux, 1992). *translin* KO mice

($n = 23$, 13 males and 10 females) and WT littermates ($n = 23$, 12 males and 11 females) were conditioned to the shock context and the auditory cue and tested 24 h later. Conditioning had a significant effect on freezing responses (Fig. 3A) ($F_{(5,210)} = 122.388$; $p < 0.001$). Mice exhibited significantly increased freezing relative to baseline when reexposed to the context in which they received the shock 24 h after training ($p < 0.001$), indicating memory for the context. Both groups also demonstrated a small but significant increase in freezing when placed in a new context 24 h after training ($p < 0.01$), suggesting some degree of generalization of the US, and an additional significant increase in freezing in the new context during the period when the auditory stimulus was played ($p < 0.001$). There was also a main effect of genotype on the level of freezing responses ($F_{(1,210)} = 7.966$; $p < 0.01$), as well as a conditioning \times genotype \times sex interaction ($F_{(5,210)} = 2.434$; $p < 0.05$). Freezing levels did not differ at baseline in either context, but female *translin* KO mice showed significantly enhanced freezing relative to WT littermates when reexposed to either the context in which they received the shock or the auditory stimulus that accompanied the shock ($p < 0.05$, $p < 0.01$, respectively). In contrast, male *translin* KO mice did not exhibit enhanced freezing at 24 h but did exhibit significantly increased freezing during the 30 s period immediately after the shock on the training day ($p < 0.05$).

To assess short-term memory for contextual and cued conditioning, we trained a second cohort of *translin* KO mice ($n = 18$, 5 males and 13 females) and WT littermates ($n = 31$, 17 males and 14 females) using the same conditioning protocol, but this time we reexposed the mice to the context 1 h after training and the auditory cue in a new context 2 h after training. All groups again showed significantly increased freezing during contextual and cued testing (Fig. 3B) ($F_{(1,225)} = 135.264$; $p < 0.0001$). There were significant interactions between conditioning and genotype ($F_{(5,225)} = 2.335$; $p < 0.05$) and conditioning and sex ($F_{(5,225)} = 2.642$; $p < 0.05$). Female *translin* KO mice again demonstrated significantly enhanced freezing responses relative to WT littermates during cued testing in the novel context ($p < 0.01$), but the level of freezing during reexposure to the original context did not differ significantly for male or female mice. The level of freezing immediately after conditioning was not significantly higher in male *translin* KO mice in this experiment; however, combining data from the training day for both cohorts revealed a main effect of genotype ($F_{(1,182)} = 7.400$; $p < 0.01$), as well as interactions between conditioning and genotype ($F_{(2,182)} = 8.038$; $p < 0.001$) and conditioning and sex ($F_{(2,182)} = 5.836$; $p < 0.01$). Using data from both experiments, freezing behavior immediately after conditioning was significantly increased for male *translin* KO mice relative to WT littermates ($p < 0.01$).

Footshocks administered repeatedly or at higher current levels produce more robust freezing responses in rodents (Baldi et al., 2004). To test whether enhanced freezing in *translin* KO mice might be related to differences in shock sensitivity, we exposed a separate group of *translin* KO mice ($n = 14$, 8 males and 6 females) and WT littermates ($n = 17$, 8 males and 9 females) to a series of shocks of increasing intensity and determined the amount of current required to elicit various reactions from the mice. The average current thresholds required to produce flinching, vocalizing, and jumping behavior were not significantly different between *translin* KO mice and WT littermates (Fig. 3C), indicating that differences in shock sensitivity do not account for the enhanced freezing observed in the KO mice.

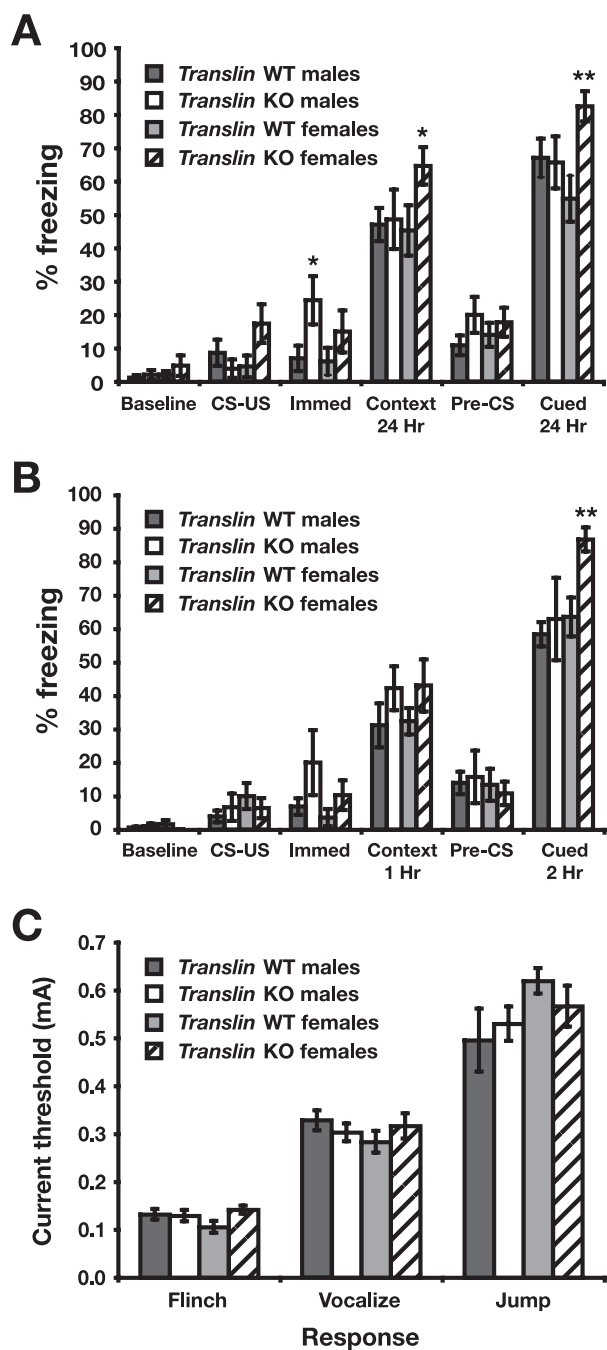


Figure 3. Female *translin* KO mice exhibit significantly enhanced freezing after contextual and cued fear conditioning. *translin* KO mice ($n = 23$, 13 males and 10 females) and WT littermates ($n = 23$, 12 males and 11 females) were placed in conditioning chambers in which they received an auditory CS (30 s of 85 dB white noise) coterminating with an US (2 s, 1.5 mA footshock). Freezing behavior was assessed in the chamber before conditioning (Baseline; 2 min), during the period of CS and US presentation (CS-US; 30 s), immediately after conditioning (Immed; 30 s), 24 h later in the same chamber (Context; 5 min), and in a new chamber representing a different context both before (Pre-CS; 2 min) and during (Cued; 3 min) CS presentation. **A**, All groups exhibited significant freezing responses relative to baseline when reexposed to the context or the CS 24 h after conditioning, but female *translin* KO mice showed significantly more freezing than female WT mice and males of either genotype. Male *translin* KO mice demonstrated significantly enhanced freezing immediately after training. **B**, In a separate experiment with a new group of *translin* KO mice ($n = 18$, 5 males and 13 females) and WT littermates ($n = 31$, 17 males and 14 females), female *translin* KO mice also exhibited enhanced freezing during presentation of the CS 2 h after conditioning. **C**, Shock sensitivity, as assessed by the average current level required to produce flinching, vocalizations, or jumping behavior, did not differ significantly between *translin* KO mice ($n = 14$, 8 males and 6 females) and WT littermates ($n = 17$, 8 males and 9 females). * $p < 0.05$; ** $p < 0.01$.

Male and female *translin* KO mice exhibit reduced anxiety-related behavior

Given their enhanced freezing after fear conditioning and jumping behavior during water maze training, we hypothesized that *translin* KO mice might also exhibit altered unconditioned responses in tests of anxiety-related behavior. Anxiety-related behavior is reduced in *fmr1* KO mice (Bakker et al., 1994; Peier et al., 2000; Qin et al., 2002; Mineur et al., 2002) and increased in mice lacking the untranslated dendritic RNA BC1 (Lewejohann et al., 2004). We used two related behavioral paradigms, the elevated zero maze test and the light–dark exploration test, to assess anxiety-related behavior in *translin* KO mice and WT littermates. In both tests, the source of anxiety is thought to be a conflict between the innate tendencies of rodents to explore novel environments while avoiding unfamiliar, exposed areas where they could be subject to predators (Treit et al., 1993a). Mice that spend more time in the open are considered less anxious, an interpretation supported by the finding that treatment with anxiolytic drugs increases time spent in open areas in both tests (Onaivi and Martin, 1989; Shepherd et al., 1994).

In the zero maze, *translin* KO mice ($n = 22$, 13 males and 9 females) and WT littermates ($n = 22$, 14 males and 8 females) were placed in the center of a closed quadrant and observed for 5 min. Both male and female KO mice spent a significantly greater percentage of time in the open quadrants than their WT littermates (Fig. 4A) ($F_{(1,40)} = 23.433$, $p < 0.0001$; males, $p < 0.01$; females, $p < 0.001$), indicating a reduction in anxiety-related behavior that was supported by several additional measurements. Mice often investigate the open quadrant with their nose and forepaws while keeping their hindquarters protected in the closed quadrant. The number of such entries into the open quadrants did not differ significantly between groups, but male *translin* KO mice ventured completely into the open quadrants significantly more often than WT controls (Fig. 4B) (main effect of genotype, $F_{(1,40)} = 5.818$, $p < 0.05$; males, $p < 0.05$; females, $p = 0.24$). Although the total distance traveled in the closed quadrants of the zero maze did not differ between genotypes, both male and female *translin* KO mice traveled significantly farther in the open quadrants of the maze (Fig. 4C) ($F_{(1,40)} = 25.835$, $p < 0.00001$; males, $p < 0.001$; females, $p < 0.01$). Average speed in both closed and open quadrants was significantly higher in *translin* KO mice compared with WT mice, primarily because of differences among male mice (Fig. 4D) (closed: genotype, $F_{(1,40)} = 6.63$, $p < 0.05$; genotype \times sex, $F_{(1,40)} = 2.55$, $p = 0.12$; males, $p < 0.01$; females, $p = 0.54$; open: genotype, $F_{(1,40)} = 7.55$, $p < 0.01$; genotype \times sex, $F_{(1,40)} = 3.70$, $p = 0.06$; males, $p < 0.001$; females, $p = 0.60$). This increase in average speed, particularly in closed quadrants, suggests a fundamental increase in locomotor activity in male *translin* KO mice. To separate anxiety from activity, we counted episodes of SAP and head dips, two ethological measures of anxiety-related behavior that are sensitive to pharmacological modulation (Shepherd et al., 1994). Male and female *translin* KO mice exhibited significantly fewer anxious SAPs than WT littermates (Fig. 4E) (data failed normality for males, rank-sum test, $p < 0.01$; t test, $p < 0.05$ for females). *translin* KO mice also showed significantly more exploratory head dips than WT littermates (Fig. 4F) ($F_{(1,40)} = 6.95$; $p < 0.05$). The difference was significant for male KO mice ($p < 0.05$) but did not reach significance for females ($p = 0.17$), probably because of the smaller group size. Overall, female mice exhibited significantly fewer head dips than male mice (30.0 ± 2.7 vs 40.0 ± 2.9 ; $F_{(1,40)} = 5.834$; $p < 0.05$). There was not a main effect of sex in any of the other measures of zero maze performance.

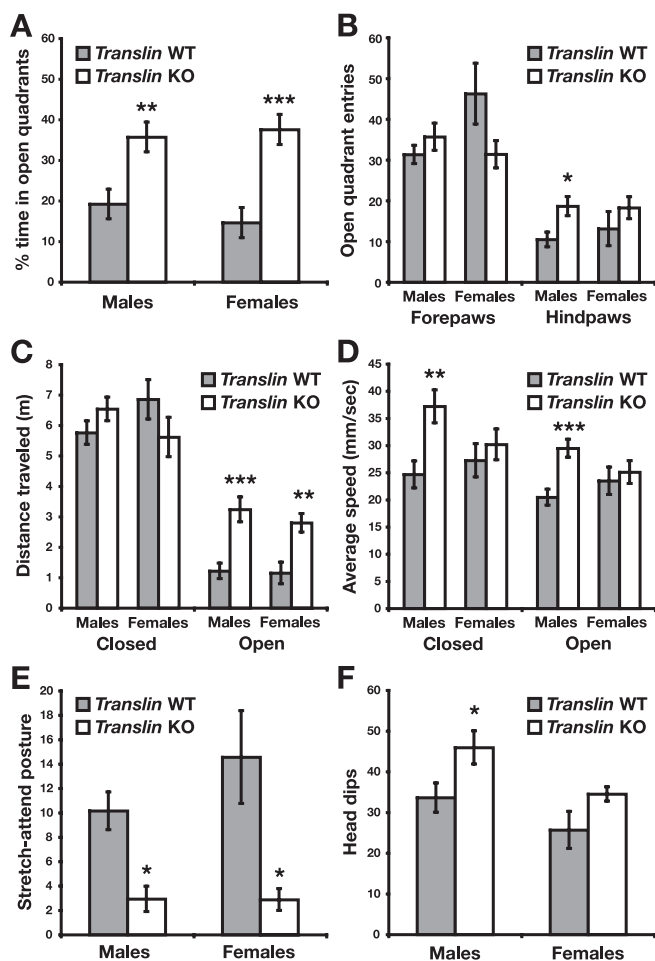


Figure 4. *translin* KO mice exhibit reduced anxiety-related behavior in the elevated zero maze test. *translin* KO mice ($n = 22$, 13 males and 9 females) and WT littermates ($n = 22$, 14 males and 8 females) were placed in the center of a closed quadrant of the elevated zero maze and observed for 5 min. **A**, Both male and female *translin* KO mice spent a significantly greater percentage of time in the open quadrants of the zero maze than WT littermates. **B**, The number of entries into the open quadrants with the head or forepaws did not differ significantly between genotypes, but, of these entries, male *translin* KO mice ventured completely into the open significantly more often. **C**, Both male and female *translin* KO mice traveled significantly farther in the open but not the closed quadrants of the zero maze. **D**, The average speed of male *translin* KO mice was significantly greater than WT controls in both the open and closed quadrants. **E**, Episodes of stretch-attend posture, a stereotyped behavior in which mice tentatively sample the environment by extending their snout and forepaws while keeping their hindpaws in place, were significantly reduced in *translin* KO mice. **F**, The number of head dips, an exploratory behavior in which mice extend their snout past the inside or outside edge of the open quadrants, was significantly greater in male mice overall and in male *translin* KO mice relative to male WT littermates. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

We tested a separate group of *translin* KO mice ($n = 15$, 9 males and 6 females) and WT littermates ($n = 20$, 14 males and 6 females) in the light–dark exploration test. In this case, there was a main effect of sex on the behavior of the mice; females spent more time in the dark compartment ($F_{(1,31)} = 8.701$; $p < 0.01$) and made fewer transitions between compartments ($F_{(1,31)} = 5.721$; $p < 0.05$). Accounting for sex differences, *translin* KO mice spent a significantly greater percentage of time in the light compartment over the 10 min trial (Fig. 5A) ($F_{(1,31)} = 5.328$; $p < 0.05$). The initial latency to enter the dark compartment after being placed in the light compartment was also significantly longer in *translin* KO mice (Fig. 5B) ($F_{(1,31)} = 4.730$; $p < 0.05$). The number of transitions between compartments did not differ

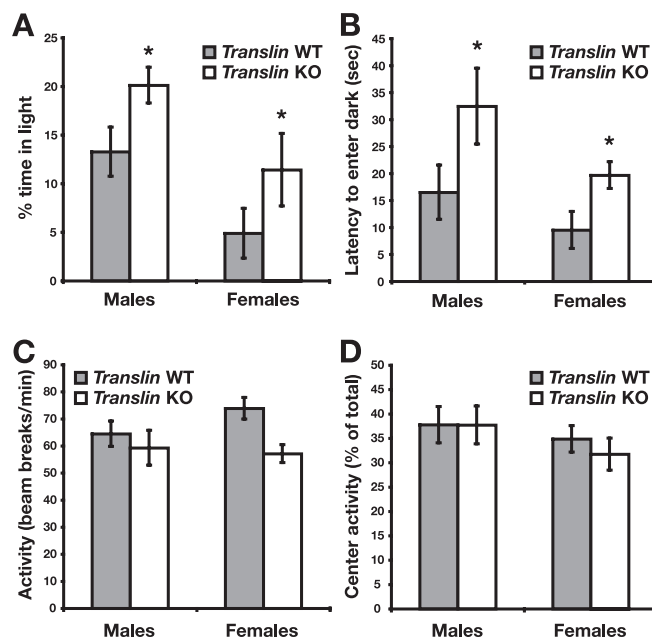


Figure 5. *translin* KO mice exhibit decreased anxiety-related behavior in the light–dark exploration test and normal baseline locomotor activity in the open field. **A, B**, *translin* KO mice ($n = 15$, 9 males and 6 females) and WT littermates ($n = 20$, 14 males and 6 females) were placed in the center of the open compartment of the light–dark box apparatus and observed for 10 min. **A**, Male mice spent a greater percentage of time in the open than female mice, and, accounting for sex differences, *translin* KO mice spent significantly more time in the open than their WT littermates. **B**, The latency to enter the dark compartment was also significantly longer in *translin* KO mice, accounting for differences attributable to sex. **C, D**, *translin* KO mice ($n = 33$, 26 males and 7 females) and WT littermates ($n = 43$, 34 males and 9 females) were placed in the center of the open-field arena, and activity was recorded over 15 min by arrays of photobeams. **C**, Locomotor activity did not differ significantly between *translin* KO mice and WT littermates. **D**, Anxiety-related behavior, as assessed by the percentage of total open-field activity that occurred in the central area of the arena, did not differ significantly between genotypes. * $p < 0.05$ for WT mice versus KO mice.

between groups (data not shown). One outlier, a male KO mouse, with a latency to enter the dark compartment of 487 s, was excluded from the analysis.

To determine whether baseline locomotor activity is altered in mice lacking *translin*, we tested *translin* KO mice ($n = 33$, 26 males and 7 females) and WT littermates ($n = 43$, 34 males and 9 females) in an automated open-field apparatus that records activity using a grid of photobeams. The total activity over 15 min in the open field as measured by photobeam breaks did not differ significantly between *translin* KO and WT animals (Fig. 5C). Because mice tend to stay close to the walls of the open-field chamber and avoid the center area, this test can also be used to measure anxiety-related behavior (Simon et al., 1994); however, despite our observations using the elevated zero maze and the light–dark box test, activity in the center of the open field versus the periphery did not differ significantly between *translin* KO mice and WT littermates (Fig. 5D). Male and female mice of both genotypes exhibited higher activity in the periphery, but the center of the chamber may not have been sufficiently anxiogenic to detect anxiety-related differences under the lighting conditions we used. Although we observed jumping behavior in *translin* KO mice during training in the Morris water maze, the amount of vertical activity recorded over 15 min in the open-field apparatus did not differ between genotypes (data not shown).

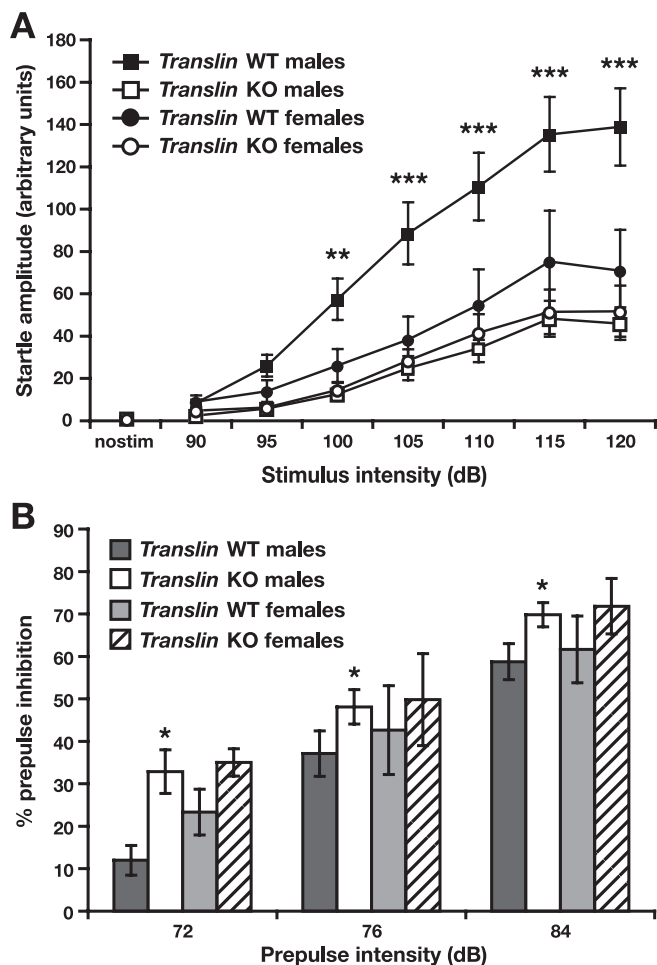


Figure 6. Acoustic startle responses are significantly reduced and prepulse inhibition of startle is significantly increased in *translin* KO mice. **A**, Male *translin* KO mice ($n = 16$) exhibit significantly reduced whole-body startle amplitudes relative to male WT littermates ($n = 20$) across a range of stimulus intensities from 100 to 120 dB. Startle responses of female *translin* KO mice ($n = 9$) did not differ significantly from female WT littermates ($n = 7$) or male KO mice. **B**, *translin* KO mice exhibit significantly enhanced prepulse inhibition of the startle response relative to WT littermates. There was a significant difference in percentage of prepulse inhibition between male *translin* KO mice and WT littermates at prepulse intensities of 72, 76, and 84. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for male WT mice versus male *translin* KO mice.

Acoustic startle responses are reduced and prepulse inhibition is enhanced in male mice lacking *translin*

fmr1 KO mice exhibit reduced startle responses and enhanced prepulse inhibition of the startle response (Chen and Toth, 2001; Nielsen et al., 2002; Frankland et al., 2004). We hypothesized that *translin* KO mice would exhibit similar alterations in sensorimotor gating. As expected, startle response amplitudes increased with greater stimulus intensities independently of genotype and sex (Fig. 6A) ($F_{(7,329)} = 53.463$; $p < 0.0001$); however, *translin* KO mice exhibited significantly reduced startle amplitudes relative to WT littermates at stimulus intensities from 95 to 120 dB ($F_{(1,47)} = 10.579$; $p < 0.01$). There were also significant stimulus intensity \times genotype and stimulus intensity \times genotype \times sex interactions ($F_{(7,329)} = 7.069$, $p < 0.0001$; $F_{(7,329)} = 2.602$, $p < 0.05$, respectively). Male *translin* KO mice ($n = 16$) exhibited significantly reduced average startle responses relative to male WT littermates ($n = 20$) at all stimulus intensities from 100 to 120 dB (100 dB, $p < 0.01$; 105–120 dB, $p < 0.001$). The average startle response of female *translin* KO mice ($n = 9$) was somewhat less than that of female littermates ($n = 7$) at each stimulus level, but

there was not a significant effect of genotype or a genotype by stimulus interaction when considering females alone. In fact, startle responses among females of both genotypes did not differ significantly from male *translin* KO mice, which is similar to what Qin et al. (2005) reported in a study including male and female *fmr1* KO mice. *translin* KO mice also exhibited significantly enhanced prepulse inhibition of the startle response relative to WT mice (Fig. 6B) ($F_{(1,47)} = 6.747$; $p < 0.05$).

As reported previously, *translin* KO mice are significantly smaller than their WT littermates until ~6 months of age (Chen-nathukuzhi et al., 2003). On average, both the male and female KO mice we tested weighed significantly less than their WT littermates (males, 23.1 ± 1.0 vs 26.4 ± 0.7 g; t test, $p < 0.05$; females, 16.9 ± 0.8 vs 22.4 ± 0.8 g, t test, $p < 0.001$). Startle measurements are partially dependent on mass but are unlikely to be affected by differences of this magnitude, and average startle amplitude was not correlated with weight by regression analysis. Hearing deficits could possibly account for reductions in startle amplitude; however, *translin* KO mice were trained specifically to the 85 dB white noise used in fear conditioning, and the tones used to evoke startle responses are considerably louder.

Monoamine neurotransmitter levels are altered in multiple forebrain areas in male and female *translin* KO mice

The monoaminergic neurotransmitters NE, 5-HT, and DA influence motor activity, anxiety-related behavior, learned fear, and sensorimotor gating in rodents. Differences in monoamine levels have been observed in the absence of FMRP in both mice and *Drosophila* (Gruss and Braun, 2001, 2004; Zhang et al., 2005). To determine whether differences in monoaminergic neurotransmission may underlie behavioral alterations in *translin* KO mice, we used HPLC to measure levels of monoamine neurotransmitters and their metabolites in the amygdala, hippocampus, and cortex in *translin* KO mice ($n = 12$, 7 males and 5 females) and WT littermate controls ($n = 14$, 7 males and 7 females). The amygdala and hippocampus were examined based on their role in mediating learning, fear, and anxiety-related behavior. The PFC and striatum were also analyzed in male mice because of the role of these regions in modulating exploratory behavior, motor activity, and acoustic startle responses.

Male and female *translin* KO mice exhibited significant differences in levels of the monoamine neurotransmitters and their metabolites in multiple brain regions. NE was significantly reduced in the hippocampus and cortex in male *translin* KO mice (Fig. 7A) (hippocampus, $p < 0.01$; cortex, $p < 0.05$). NE was reduced to a similar extent in the amygdala, but the apparent difference did not reach significance (Fig. 7A) ($p = 0.15$). NE was significantly reduced in the amygdala, hippocampus, and cortex in female *translin* KO mice compared with WT littermates (Fig. 7A) (amygdala, $p < 0.01$; hippocampus, $p < 0.001$; cortex, $p < 0.0001$). To determine whether reduced tissue levels of NE reflect decreased production or increased turnover, we also measured the amount of the NE metabolite MHPG in the hippocampus and amygdala in male mice. MHPG was significantly reduced in both brain regions in male *translin* KO mice (hippocampus, 0.290 ± 0.030 vs 0.186 ± 0.011 pmol/mg, $p < 0.01$; amygdala, 0.311 ± 0.044 vs 0.148 ± 0.014 pmol/mg, $p < 0.01$), but NE turnover as measured by the ratio of MHPG to NE was not significantly altered (hippocampus, 0.124 ± 0.012 vs 0.112 ± 0.007 ; amygdala, 0.14 ± 0.05 vs 0.09 ± 0.02), suggesting that NE synthesis is reduced in these brain regions.

Male *translin* KO mice also exhibited reductions in 5-HT in the hippocampus, cortex, and striatum relative to WT littermates

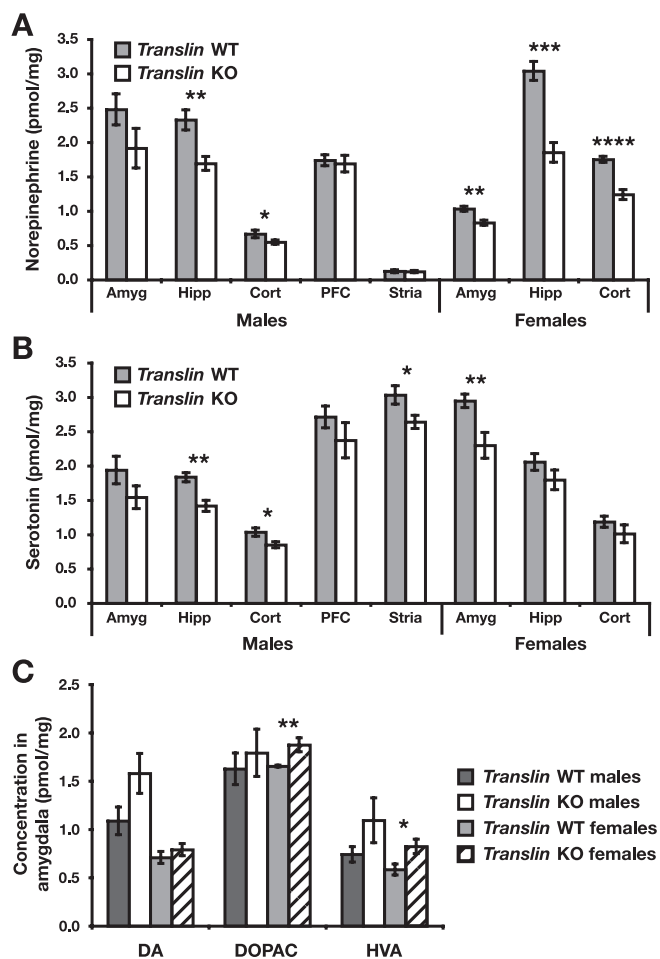


Figure 7. *translin* KO mice exhibit alterations in the level of monoamine neurotransmitters in the forebrain. HPLC was used to determine the concentration of the monoamine neurotransmitters NE, 5-HT, DA, and their metabolites in multiple brain regions in *translin* KO mice ($n = 12$, 7 males and 5 females) and WT littermate controls ($n = 14$, 7 males and 7 females). **A**, NE is significantly reduced in the hippocampus and cortex in male *translin* KO mice and in the amygdala, hippocampus, and cortex in female *translin* KO mice. **B**, 5-HT levels are significantly decreased in the hippocampus, cortex, and striatum in male *translin* KO mice and in the amygdala in female *translin* KO mice. **C**, Levels of DA in the amygdala do not differ between genotypes, but levels of the metabolites DOPAC and HVA are significantly increased in female *translin* KO mice. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

(Fig. 7B) (hippocampus, $p < 0.01$; cortex, $p < 0.05$; striatum, $p < 0.05$). Again, a similar reduction was observed in the amygdala, but the difference did not reach significance because of greater variability in this group of samples (Fig. 7C) ($p = 0.15$). In female *translin* KO mice, 5-HT was significantly reduced in the amygdala (Fig. 7B) ($p < 0.01$). We also determined the concentration of the 5-HT metabolite 5-HIAA in each brain area in male and female *translin* KO mice and WT littermates. The concentration of 5-HIAA was significantly reduced in the striatum in male *translin* KO mice (2.00 ± 0.07 vs 1.75 ± 0.08 pmol/mg; $p < 0.05$) but was not significantly altered in other brain areas or in female mice. Serotonin turnover, as measured by the ratio of 5-HIAA to 5-HT, did not differ significantly between male *translin* KO mice and WT littermates in any brain area. However, 5-HT turnover in the amygdala in female *translin* KO mice was significantly increased relative to WT littermates (1.37 ± 0.13 vs 1.74 ± 0.09 ; $p < 0.05$).

Levels of DA in the amygdala (Fig. 7C), cortex, PFC, and striatum did not differ between *translin* KO mice and WT litter-

mates, and DA was not reliably detected in the hippocampus; however, concentrations of the DA metabolites DOPAC and HVA were significantly increased in the amygdala in female *translin* KO mice (Fig. 7C) (DOPAC, $p < 0.01$; HVA, $p < 0.05$). DA turnover can be measured using the following ratios: DOPAC/DA, HVA/DA, and (DOPAC + HVA)/DA. In male *translin* KO mice, there was a trend toward decreased DA turnover in the amygdala, as measured by the ratio of DOPAC to DA (1.57 ± 0.17 vs 1.15 ± 0.11 ; $p = 0.055$). Increases in the average concentration of both DOPAC and HVA in the amygdala in female *translin* KO mice suggest an increase in DA turnover, but there was not a statistically significant increase in turnover in this or any other brain region in female mice using the above measures.

Discussion

We investigated the behavior of *translin* KO mice and WT littermates and observed multiple abnormalities, including increased escape latency in the Morris water maze in male KO mice, enhanced fear conditioning in female KO mice, reduced anxiety-related behavior in the zero maze and light–dark box in male and female KO mice, and reduced startle responses and increased prepulse inhibition of startle in male KO mice. *translin* KO mice also exhibit reductions in NE and 5-HT that may explain some of these behavioral differences. Our neurochemical findings indicate that *translin* may affect the synthesis of monoamine neurotransmitters or signaling pathways activated by monoamine receptor stimulation. Mice lacking FMRP exhibit several similar behavioral and neurochemical alterations, and current theories regarding the function of FMRP suggest a mechanism by which *translin* may influence monoamine signaling.

Behavioral and neurochemical alterations in *translin* KO mice

translin KO mice exhibit multiple, sex-dependent behavioral differences relative to WT littermates (Table 1). Overall, *translin* KO mice show decreased anxiety-related responses in the setting of potential threats but exaggerated fear responses and defensive behaviors (freezing, jumping, and escape) when exposed to actual threats. Exaggerated stress responses may lead to enhanced performance in fear conditioning in female KO mice but interfere with learning and impair performance in the Morris water maze, particularly in male KO mice. Stress-induced hyperactivity, inattention, or a failure to shift search strategies as indicated by persistent thigmotaxis may also contribute to poor performance in the water maze in male KO mice.

Both male and female *translin* KO mice exhibit reductions in NE and 5-HT, neurotransmitters with prominent roles in regulating fear and anxiety-related behavior. NE released from fibers arising in the locus ceruleus initiates fight-or-flight responses. NE depletion reduces anxiety-related behavior in the plus maze in rats (Lapiz et al., 2001), and mice lacking NE show similar trends in the plus maze and the light–dark box (Marino et al., 2005). 5-HT depletion also reduces anxiety-related behavior in rodents in the elevated plus maze (Treit et al., 1993b) and the light–dark box (Artaiz et al., 1998). In addition, 5-HT depletion increases impulsivity and hyperactivity (Winstanley et al., 2004), which may explain the jumping behavior and stress-induced hyperactivity we observed in *translin* KO mice. Interestingly, mice engineered to either lack or overexpress the 5-HT₃ receptor exhibit reduced anxiety-related behavior in the plus maze but increased fear conditioning (Harrell and Allan, 2003; Bhatnagar et al., 2004). Dopaminergic signaling in the amygdala supports both the formation and expression of conditioned fear (Pezze and Feldon, 2004), and increases in DOPAC and HVA suggest that

Table 1. Behavioral phenotypes of *translin* and *fmr1* KO mice

Behavioral test	<i>translin</i> KO	<i>fmr1</i> KO
Morris water maze		
Acquisition	Males impaired	Normal (1–4)
Reversal	Males impaired	Impaired (1, 2), normal (3)
Visible	Males impaired	Normal (1–3)
Quadrant preference	Normal	Normal (1–4)
Fear conditioning		
Immediate freezing	Males increased	Normal (3–5)
Contextual freezing	Females increased	Decreased (3), normal (4, 5)
Cued freezing	Females increased	Decreased (3), normal (4, 5)
Anxiety-related behavior		
Zero/plus maze	Reduced	Normal (6, 7)
Light–dark box	Reduced	Reduced (1–4)
Open field	Males normal Females normal	Reduced (4, 6, 8, 9), normal (7) Females normal (9)
Locomotor activity		
Zero/plus maze	Increased	Increased (6), normal (7)
Open field	Normal	Increased (4, 6, 8, 9), normal (7)
Accelerating rotarod	Mildly impaired (10)	Mildly impaired (4)
Acoustic startle	Males reduced Females normal	Males reduced (9, 11, 12) Females normal (8)
Prepulse inhibition	Males increased	Males increased (9, 11, 12)

Both *translin* and FMRP are thought to regulate mRNA localization and local translation in neurons. Our results reveal behavioral similarities and some differences in mice lacking these proteins. Listed phenotypes are the same for male and female *translin* KO mice unless otherwise specified. Results listed for *fmr1* KO mice are for male mice, unless otherwise specified. 1, Bakker et al., 1994; 2, D'Hooge et al., 1997; 3, Paradee et al., 1999; 4, Peier et al., 2000; 5, Van Dam et al., 2000; 6, Mineur et al., 2002; 7, Qin et al., 2002; 8, Qin et al., 2005; 9, Nielsen et al., 2002; 10, Chennathukuzhi et al., 2003; 11, Chen et al., 2001; 12, Frankland et al., 2004.

DA turnover is enhanced in female *translin* KO mice although it is relatively reduced in male KO mice.

NE also modulates attention, cognitive function, sensorimotor gating, and seizure susceptibility. NE is important for selective attention and behavioral flexibility (Aston-Jones and Cohen, 2005), and reduced hippocampal or cortical NE may contribute to water maze impairments in male *translin* KO mice. Notably, NE depletion in wild-type mice and overexpression of $\alpha 2C$ adrenoreceptors, which inhibit NE release, in transgenic mice increase thigmotaxis in the water maze (Bjorklund et al., 2000). Loss of locus ceruleus neurons is also associated with decreased startle responses (Von Coelln et al., 2004). Like male *translin* KO mice, mice overexpressing $\alpha 2C$ adrenoreceptors show reduced startle and increased PPI, whereas mice lacking these receptors exhibit the opposite effects (Sallinen et al., 1998). Finally, reduced NE levels may predispose *translin* KO mice to seizures because NE is thought to act as an endogenous anticonvulsant, and mice lacking NE exhibit enhanced seizure susceptibility (Szot et al., 1999).

The various sex-dependent behavioral alterations we observed probably reflect the influence of gonadal hormones and inherent sex differences in stress responses. Differences in learning, activity, and anxiety-related behavior between male and female mice from common inbred strains are well documented (Berger-Sweeney et al., 1995; Bolivar et al., 2001; Voikar et al., 2001; Jonasson, 2005). Notably, there are also behavioral and neurochemical differences between hemizygous male and homozygous female *fmr1* KO mice (Gruss and Braun, 2001, 2004; Qin et al., 2005).

Variability attributable to genetic background is always a concern in behavioral studies using genetically modified mice (Crawley et al., 1997). We tested *translin* KO mice alongside WT littermates to minimize effects of genetic and environmental variability. Although 129 mice perform better in learning tests, they exhibit increased anxiety-related behavior relative to C57BL/6J mice in multiple paradigms (Crawley et al., 1997);

thus, background effects cannot readily explain the decreased anxiety-related behavior we observed. Using conventional KO mice, we cannot determine whether phenotypes result from abnormal development or altered function of adult neurons. Neuroanatomy was indistinguishable from WT mice in all brain regions examined (Chennathukuzhi et al., 2003), but detailed neuronal connectivity has not been investigated. Furthermore, because the genetic manipulation is not restricted to a specific brain region or cell type, KO phenotypes may reflect the interplay of multiple regions and systems. Finally, the absence of *translin* in peripheral tissues, such as the adrenal glands, thyroid gland, or gonads, may affect behavior.

Similarities to *fmr1* KO mice and potential relevance to human disease

Translin and FMRP have many functional similarities at the cellular level, and our results reveal behavioral similarities in mice lacking these proteins (Table 1). Like *translin* KO mice, *fmr1* KO mice exhibit alterations in tests of activity and anxiety-related behavior, motor function, and sensorimotor gating. *fmr1* KO mice show combinations of hyperactivity and reduced anxiety-related behavior in the open-field test, the light–dark box, and the elevated plus maze (Bakker et al., 1994; Peier et al., 2000). *translin* and *fmr1* KO mice demonstrate nearly identical phenotypes in the accelerating rotarod test (Peier et al., 2000; Chennathukuzhi et al., 2003) and show similar reductions in startle responses and enhancements in prepulse inhibition (Chen and Toth, 2001; Nielsen et al., 2002; Frankland et al., 2004). *fmr1* KO mice also exhibit enhanced susceptibility to audiogenic seizures (Chen and Toth, 2001). We observed seizures in *translin* KO mice, but additional studies will be needed to quantitatively evaluate seizure susceptibility.

Mental retardation is a prominent component of fragile X syndrome, but learning deficits in the water maze and fear conditioning have been inconsistently observed in mice lacking FMRP and may be related to genetic background effects (Dobkin et al., 2000). Deficits have been reported recently in trace fear conditioning (Zhao et al., 2005) and the object recognition task (Ventura et al., 2004). In both cases, the poor performance of *fmr1* KO mice was attributed to impaired attention, which is consistent with attention deficits observed in fragile X syndrome in humans and a potential role for attention deficits in male *translin* KO mice. Abnormalities in monoamine signaling are implicated in attention deficit hyperactivity disorder (Faraone et al., 2005; Pliszka, 2005) and observed in both FMRP and *translin* KO mice. Although few alterations in monoamine neurotransmitters are detected in male *fmr1* KO mice at baseline (Gruss and Braun, 2001), they exhibit altered dopamine release after amphetamine treatment (Ventura et al., 2004). Female *fmr1* KO mice exhibit baseline reductions in 5-HT and DA and changes in 5-HT and DA turnover in multiple forebrain regions (Gruss and Braun, 2004). Unfortunately, there are no reports investigating NE levels in *fmr1* KO mice. Interestingly, fruit flies lacking the *Drosophila* homolog of FMRP have increased brain levels of both

5-HT and DA and increased expression and activity of enzymes involved in monoamine synthesis (Zhang et al., 2005).

Recent theories regarding neurotransmitter abnormalities in fragile X syndrome have focused on alterations in metabotropic glutamate receptor (mGluR) signaling (Bear et al., 2004) rather than monoamine neurotransmitters. However, Weiler et al. (2004) recently showed that FMRP acts downstream of protein kinase C (PKC) to regulate mGluR-dependent local translation, suggesting that the absence of FMRP may affect other neurotransmitter systems, including NE, 5-HT, and DA, with receptor subtypes that signal through PKC (Miyashiro and Eberwine, 2004). Like FMRP, *translin* may regulate local translation downstream of PKC activation, and misregulation in its absence may lead to compensatory changes in monoamine synthesis. Interestingly, *translin* is downregulated in the cortex of rats after chronic treatment with the NE and 5-HT reuptake inhibitor imipramine (Palotas et al., 2004), which may reflect a role for *translin* downstream of monoamine receptor stimulation.

Unfortunately, the relevant neuronal mRNA targets of FMRP and *translin* are not known. Studies using microarrays, RNA selection, and antibody-associated RNA amplification have identified numerous transcripts that may interact with FMRP (Brown et al., 2001; Darnell et al., 2001; Miyashiro et al., 2003), but these approaches have not yet been applied to *translin*. Direct evidence linking mutations in *translin* to disease in humans is also currently lacking. Mutations at the fragile X site are relatively common and exposed in the hemizygous condition, whereas mutations in *translin* would require homozygosity on human chromosome 2 and produce disease much more rarely. Nevertheless, our results in mice suggest that mutations affecting *translin* expression or function may contribute to behavioral and developmental disorders in humans, including fragile X-like syndromes, mental retardation, attention deficit hyperactivity disorder, epilepsy, and autism spectrum disorders.

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