

NR2B-Dependent Plasticity of Adult-Born Granule Cells is Necessary for Context Discrimination

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Adult-generated granule cells (GCs) in the dentate gyrus (DG) exhibit a period of heightened plasticity 4–6 weeks postmitosis. However, the functional contribution of this critical window of plasticity to hippocampal neurogenesis and behavior remains unknown. Here, we show that deletion of NR2B-containing NMDA receptors from adult-born GCs impairs a neurogenesis-dependent form of LTP in the DG and reduces dendritic complexity of adult-born GCs, but does not impact their survival. Mice in which the NR2B-containing NMDA receptor was deleted from adult-born GCs did not differ from controls in baseline anxiety-like behavior or discrimination of very different contexts, but were impaired in discrimination of highly similar contexts. These results indicate that NR2B-dependent plasticity of adult-born GCs is necessary for fine contextual discrimination and is consistent with their proposed role in pattern separation.

Introduction

The mammalian dentate gyrus (DG) generates new neurons throughout life (Altman and Das, 1965) that functionally integrate into the local circuitry (Toni et al., 2008) and can account for up to 10% of the granule cell (GC) population (Imayoshi et al., 2008). Studies in which adult neurogenesis has been manipulated indicate that this process contributes significantly to behavior, and it is widely hypothesized that functional differences between immature and mature GCs underlie the unique role adult-born neurons play in DG function (Zhao et al., 2008; Sahay et al., 2011b).

The development of adult-born GCs is a highly plastic process that recapitulates a number of aspects of early development, including the preferential expression of NMDA receptors that contain the NR2B subunit (Cull-Candy and Leszkiewicz, 2004). There is an initial critical period from 1 to 3 weeks postmitosis, during which the survival and integration of adult-born GCs is dependent on both GABAergic and glutamatergic input (Ge et al., 2006; Zhao et al., 2008). In particular, survival of adult-born GCs near the end of this time window requires NMDA receptor activation (Tashiro et al., 2006). Accumulating evidence indicates the existence of a second critical window between 4 and 6 weeks of cell age, when adult-born GCs exhibit enhanced synaptic plas-

ticity and impact specific behavioral functions (Ge et al., 2007; Denny et al., 2012). Consistently, ablation of neurogenesis prevents the induction of LTP evoked by medial perforant path (MPP) stimulation in the DG of slices with intact GABAergic transmission (ACSF-LTP), suggesting that this synaptic potentiation reflects a selective effect on adult-born GCs (Snyder et al., 2001; Saxe et al., 2006). This form of LTP is blocked by ifenprodil or Ro25-6981, NR2B subtype-specific antagonists, demonstrating that this NMDA subunit is essential for increased synaptic plasticity (Snyder et al., 2001; Ge et al., 2007). Together, these data indicate that during this 4–6 week window, adult-born GCs are hyperplastic and may as a result make unique contributions to DG circuits and hippocampal function. However, whether this period of enhanced plasticity influences the integration of young neurons into hippocampal circuitry, or influences behavior by other means, remains unknown. To test this, we selectively deleted the NR2B subunit of the NMDA receptor from adult-born GCs to study the functional impact of heightened synaptic plasticity and the requirement of NR2B for adult-born GC survival.

Materials and Methods

Mice

NestinCreERT2 and *NR2B^{fl/fl}* mice were generated as previously described (von Engelhardt et al., 2008; Dranovsky et al., 2011). Experimental male mice were generated by breeding *NestinCreERT2;NR2B^{fl/fl}* with *NR2B^{fl/fl}* mice. For labeling adult-born GCs, *NestinCreERT2;NR2B^{fl/fl}*; *ROSA26^{stopEYFP}/stopEYFP* were bred with *NestinCreERT2;NR2B^{fl/fl}* to generate *NestinCreERT2;NR2B^{fl/fl}*; *ROSA26^{stopEYFP/+}* and *NestinCreERT2;NR2B^{+/+}*; *ROSA26^{stopEYFP/+}* controls. Mice (8- to 10-weeks-old) were injected with 3 mg of tamoxifen [TMX; vehicle (VEH) solution of corn oil/10% ethanol] intraperitoneally once per day for 5 consecutive days. Previous work with this strain revealed that a brief pulse of TMX does not affect hippocampus-dependent behavior 6 weeks later (Sahay et al., 2011a). All experiments were approved by the Institutional Animal Care and Use Committee at Columbia University and the New York State Psychiatric Institute.

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Immunohistochemistry

For survival experiments, 48 h after the last TMX or VEH injection, BrdU was injected intraperitoneally (150 mg/kg). For all experiments, mice were perfused (4% paraformaldehyde), brains postfixed, cryoprotected, and sections (35 μ m) of the entire DG were labeled for BrdU, GFAP, NeuN, doublecortin (DCX), Ki67, or GFP [rat-anti-BrdU, 1:100 (Serotec); rabbit-anti-GFAP, 1:1500 (DAKO); mouse-anti-NeuN, 1:500 (Millipore Bioscience Research Reagents); goat-anti-DCX, 1:500 (Santa Cruz Bioscience); rabbit-anti-Ki67, 1:100 (Vector)], as previously described (Sahay et al., 2011a). Experimenters blind to treatment counted (at 20 \times) BrdU, DCX, and Ki67 cells in every sixth section throughout the DG, and DCX cells exhibiting tertiary dendrites were counted. For colabeling of BrdU/NeuN/GFAP, confocal scans (FluoView1000; Olympus) at 40 \times were taken of 60 BrdU cells/treatment across the anteroposterior (AP) axis of the DG. For enhanced yellow fluorescent protein (EYFP)/DCX counts, 120 DCX+ cells were imaged across the AP axis of the DG to determine colabeling with EYFP, excluding type-1 cells determined by their radial-glia morphology. Sholl analysis was conducted as previously described (Sahay et al., 2011a) and analyzed with the ImageJ Sholl Analysis plug-in (<http://www-biology.ucsd.edu/labs/ghosh/software/>).

Slice electrophysiology

Transverse hippocampal slices (400 μ m) were incubated in an interface chamber at 32°C and perfused with oxygenated ACSF (in mM: 11 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 26.2 NaHCO₃, 1 NaH₂PO₄, 11 glucose). Slices equilibrated for 2 h before a bipolar stimulating electrode was placed in the MPP and evoked field potentials were recorded in the molecular layer using a glass capillary microelectrode (1–3 M Ω) filled with ACSF. Isolation of the MPP was confirmed by assessing paired-pulse depression of the MPP/DG synaptic connection at 50 ms, which generated the highest level of depression (McNaughton, 1980). Input–output curves were obtained after 10 min of stable recordings. The stimulation intensity that produced one-third of the maximal response was used for the test pulses and tetanus. After 15 min of stable baseline (once every 20 s), LTP was induced with four trains of 1 s each, 100 Hz within the train, repeated every 15 s. Responses were recorded every 20 s for 60 min after LTP induction. The same protocol was used to elicit and record LTP of mature GCs except that 10 μ M bicuculline was added to the ACSF to block GABA_A-receptor-mediated inhibition.

Behavioral experiments

All behavioral experiments were conducted in male mice 14–16 weeks of age. For each test, time after TMX or VEH injection is noted in the figures or text.

Open field. Mice were placed in an acrylic open-field chamber 40 cm long \times 40 cm wide \times 37 cm high with illumination set to 16–20 lux (low lux) or 600 lux (high lux). Data were collected in 5 min bins during 60 min trials and analyzed for total distance traveled as well as time and distance traveled in center.

Forced-swim test, novelty suppressed feeding, and elevated plus maze. Forced-swim test (FST), novelty suppressed feeding (NSF), and elevated plus maze (EPM) were conducted as previously described (Dulawa et al., 2004; David et al., 2009; Sahay et al., 2011a).

Novel object recognition. Naive mice were placed in a storage container (45 \times 30 \times 30 cm) with woodchip bedding, and videotaped from above at a light intensity of 16–20 lux. Two objects were placed at either end of the arena (a slide box, a clear funnel, or a white ceramic shoe; randomized presentation). Mice explored two objects for four trials of 5 min, with a 3 min intertrial interval (during which the box would be wiped clean and the bedding changed), then one object was replaced with a novel object. An experimenter blind to the treatment condition analyzed videotapes, and total approaches, investigation time, and grid crossings were measured for each of the trials.

Fear conditioning. Conditioning took place in Coulbourn fear conditioning boxes that contained one clear Plexiglas wall, three aluminum walls, and a stainless steel grid floor. In the one-trial contextual fear conditioning, the training context (A) included a house light and fan, and anise scent was placed under the grid floor. After 180 s, mice received single 2 s foot shock of 0.75 mA. Mice were taken out 15 s after termina-

tion of the foot shock and returned to their home cage. The box was cleaned with Sanicloths (Professional Disposals International) between runs. Digital video cameras recorded the session; FreezeFrame and FreezeView software (Actimetrics) were used for recording and analyzing freezing behavior. For the dissimilar context, C, the grid floor was covered with a plastic panel and cage bedding. The chamber walls were covered and made circular using plastic inserts, the house fan and lights were turned off, and a mild lemon scent was placed below the grid floor. The chamber door was left ajar during testing. Ethanol (70%) was used to clean the chamber between runs.

Naive mice were used for contextual fear discrimination. The training context, A, was the same as used above for one-trial fear conditioning. The dissimilar context, C, was as described above, and the similar (no-shock) context, B, shared many features of the training context, including an exposed stainless steel grid floor, but differed in that two plastic inserts were used to cover the walls and make them circular, the house fan and lights were turned off, and the chamber door was left ajar during testing. A lemon scent was used as an olfactory cue, and 70% ethanol was used to clean the grids between runs. Mice were brought into the testing room in transport buckets by the same experimenter who had handled the mice for the training context. After 2 h, mice were placed in the similar context, in which they were left for 180 s and were never shocked. Measurement of the freezing levels in both the training context (3 min preshock) and the similar context (3 min) each day allowed the assessment of freezing in the two contexts. The order of presentation of contexts was randomized across days.

Results

Mice in which exon 9 of the *NR2B* gene was flanked by two loxP sites (*NR2B*^{2lox}) (von Engelhardt et al., 2008) were bred with *NestinCreER*^{T2} mice in which the TMX regulated Cre-recombinase (CreER^{T2}) is expressed under the control of a nestin promoter fragment (Dranovsky et al., 2011). TMX injection in adult mice excises sequences between the loxP sites selectively in neural stem cells and transit-amplifying progenitors to generate adult-born GCs lacking NR2B (*iNR2B*^{Nes}). Using EYFP as a surrogate for NR2B recombination and marker for adult-born GCs (Fig. 1A,B), we found that 71.9 \pm 7.2% of DCX-positive neurons expressed EYFP 6 weeks after TMX induction.

We next assessed whether NR2B is required for young GC survival. In animals expressing the EYFP reporter, young GC numbers did not significantly differ between *NR2B*^{fl/fl} and *NR2B*^{+/+} littermates (unpaired *t* test, *n* = 3/genotype, *t*₄ = 0.79, *p* = 0.49; Fig. 1C). In addition, BrdU pulse-chase experiments indicated that NR2B deletion did not affect survival of 2-, 4-, 6-, or 8-week-old neurons, as no difference was seen in number of BrdU-positive cells or phenotype of cells born after NR2B deletion (*n* = 3–7/treatment, unpaired *t* test, 2 weeks: *t*₄ = 1.3, *p* = 0.3; 4 weeks: *t*₄ = 0.6, *p* = 0.6; 6 weeks: *t*₇ = -1.4, *p* = 0.2; 8 weeks: *t*₁₁ = -1.7, *p* = 0.13; percentage of BrdU/NeuN: *t*₄ = 1.87, *p* = 0.14; Fig. 1E,F). There was also no difference between *iNR2B*^{Nes} and controls in progenitor proliferation as measured by Ki67+ cells (*n* = 5 TMX, 4 VEH, unpaired *t* test, *t*₇ = -1.5, *p* = 0.18; Fig. 1E) or in generation of immature DCX-positive neurons (*n* = 5 TMX, 4 VEH, unpaired *t* test, *t*₇ = -0.7, *p* = 0.52; Fig. 1E,I). We next assessed dendritic complexity of adult-born GCs lacking NR2B. Although within the DCX population, the number of cells that exhibited tertiary dendrites did not differ between TMX and VEH treatment (*n* = 5 TMX, 4 VEH, unpaired *t* test, *t*₇ = -0.8, *p* = 0.44; Fig. 1J), Sholl analysis on the dendrites of EYFP+ neurons from *NR2B*^{fl/fl} and *NR2B*^{+/+} littermates 6 weeks after TMX injection revealed a decrease in dendritic complexity after deletion of NR2B (*n* = 12–15 cells/3 mice/treatment, repeated-measures ANOVA, genotype \times distance interaction $F_{(28,700)} = 1.5$, *p* = 0.03; Fig. 1K).

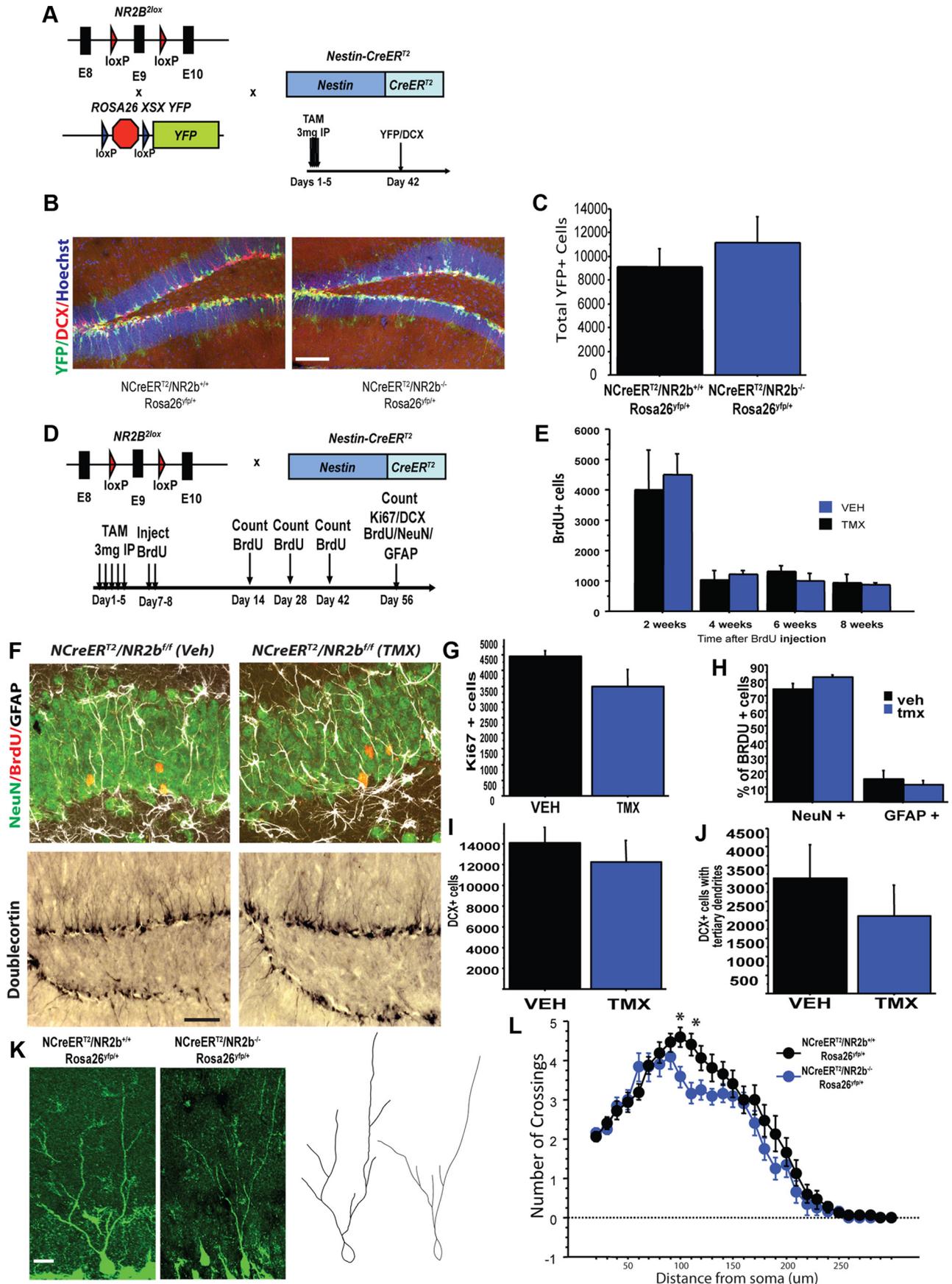


Figure 1. Deletion of NR2B in adult-born GCs does not affect neurogenesis in the DG. **A, B**, Three-mouse design using an inducible reporter, EYFP, to measure GC survival and TMX-induced recombination (**A**) with representative images of YFP and DCX expression 6 weeks after TMX injection (**B**). Scale bar, 100 μ m. **C**, Total number of adult-born GCs did not (*Figure legend continues*.)

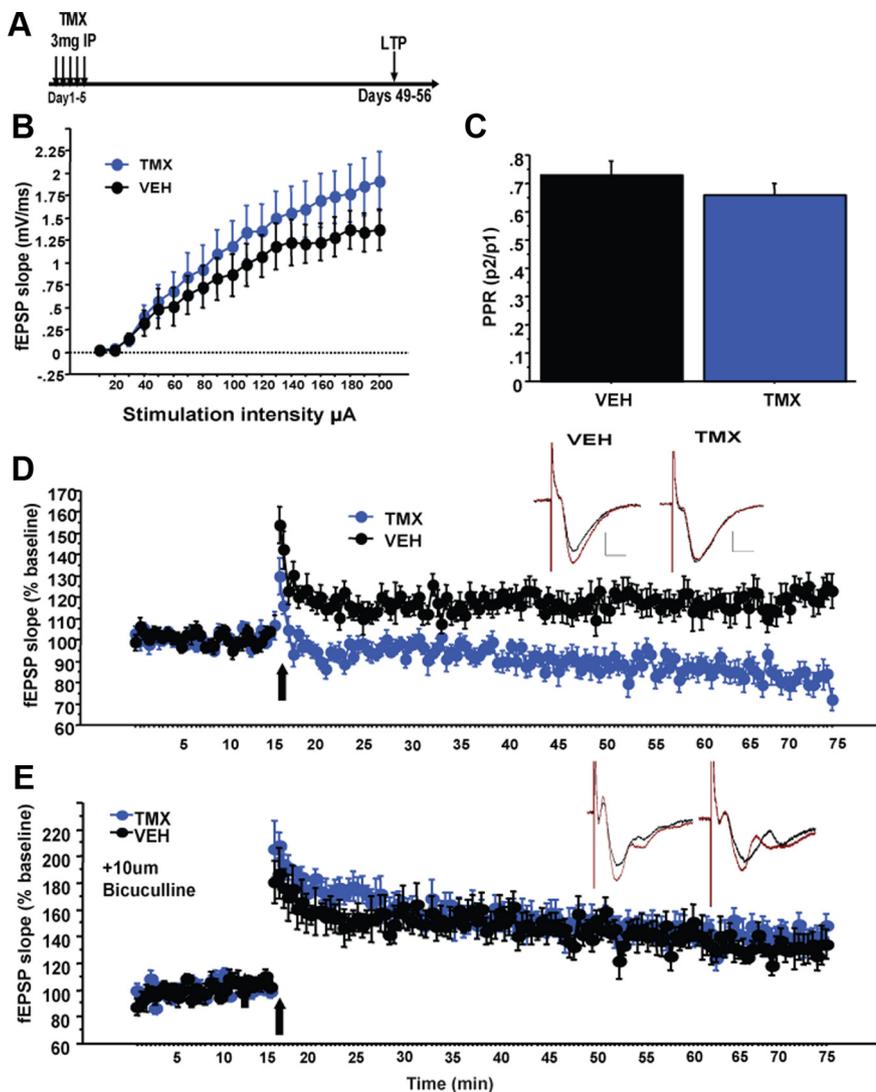


Figure 2. Impaired ACSF-LTP in the DG of *iNR2B^{Nes}* mice. **A**, Experimental timeline for electrophysiology experiments. **B**, **C**, No differences in input–output relationship (**B**) or paired-pulse depression (**C**; paired-pulse ratio (PPR), 50 ms interstimulus interval) of MPP inputs to the DG after deletion of NR2B. **D**, Significantly impaired ACSF-LTP in slices from *iNR2B^{Nes}* mice compared with controls. Inset, Representative average traces before and after HFS. Scale bars: verticle, 0.5 mV; horizontal, 5 ms. **E**, No difference in magnitude of LTP in slices in the presence of 10 μ M bicuculline. Data are mean \pm SEM.

To determine the consequence of NR2B deletion in adult-born neurons on synaptic plasticity in the DG, we measured neurogenesis-dependent ACSF-LTP in slices taken 6–7 weeks after VEH or TMX treatment. Deletion of NR2B did not disrupt the input–output relationship ($n = 12$ slices/genotype, repeated-measures ANOVA, treatment effect: $F_{(1,22)} = 0.85, p = 0.37$, treatment \times intensity interaction: $F_{(19,418)} = 1.1, p = 0.38$; Fig. 2B) or paired-pulse depression of fEPSPs evoked by MPP

(Figure legend continued.) differ between NR2B-deficient and WT littermate controls 6 weeks after TMX injection, as measured by total numbers of EYFP cells. **D**, Genetic and experimental timeline for neurogenesis measurement. **E**, No effect on cell survival as measured by BrdU number at specified times postinjection. **F**, Representative images for BrdU (green, NeuN; red, BrdU; white, GFAP) and DCX experiments. Scale bar: top, 30 μ m; bottom, 100 μ m. **G**, No effect of treatment on total Ki67-immunoreactive cells. **H**, No significant difference in phenotype of BrdU cells 8 weeks after induction. **I**, **J**, No significant difference in total number of DCX cells or DCX+ cells with tertiary dendrites. **K**, **L**, Sholl analysis revealed a decrease in dendritic complexity in adult-born GCs lacking NR2B. **K**, Representative images and tracings. Scale bar, 20 μ m. Data are mean \pm SEM.

stimulation ($t_{21} = 1.08, p = 0.29$; Fig. 2C). However, ACSF-LTP induction by high-frequency stimulation (four 1 s, 100 Hz trains every 15 s) was absent in *iNR2B^{Nes}* slices (repeated-measures ANOVA, last 10 min treatment effect: $F_{(1,22)} = 25.5, p < 0.001$; Fig. 2D). In contrast, LTP obtained in the presence of the GABA_A receptor antagonist bicuculline did not differ between groups ($n = 4$ VEH, 7 TMX, repeated-measures ANOVA, last 10 min treatment effect: $F_{(1,9)} = 0.9, p = 0.37$; Fig. 2E). These results suggest that the mature GCs exhibit normal LTP and that the deficit in ACSF-LTP is due to loss of NR2B in the immature neuron population. These *iNR2B^{Nes}* mice therefore provide a model to test the contribution of the enhanced synaptic plasticity of immature neurons to behavior.

We next tested *iNR2B^{Nes}* mice in a number of depression/anxiety-related behavioral assays. In neither low nor high lux open-field testing did the *iNR2B^{Nes}* mice differ from controls in locomotor activity, habituation, or percentage of time in center, but in the low lux condition they showed a trend toward less distance traveled in the center of the arena (low lux: $n = 13$ /treatment, total distance traveled: treatment effect: unpaired t test, $t_{24} = 0.15, p = 0.9$; treatment \times time interaction: $F_{(11,264)} = 1.19, p = 0.29$; percentage time in center: $t_{24} = -1.5, p = 0.15$; percentage center distance: $t_{24} = -2.03, p = 0.05$; high lux: total distance traveled: treatment effect: $t_{10} = 0.14, p = 0.9$; treatment \times time interaction: $F_{(10,110)} = 0.89, p = 0.55$; percentage time in center: treatment effect: $t_{10} = -0.5, p = 0.63$; percentage center distance: $t_{10} = -0.7, p = 0.9$; Fig. 3A–F). In addition, as seen in irradiated animals at baseline (Santarelli et al., 2003; David et al., 2009), *iNR2B^{Nes}* mice did not significantly differ from control mice in immobility time in the FST, in open arm time in the EPM, or latency to feed in the NSF ($n = 6$ /treatment, FST: treatment effect: $F_{(1,10)} = 0.01, p = 0.9$; treatment \times time interaction: $F_{(5,50)} = 0.6, p = 0.7$; EPM: $t_{10} = 1.9, p = 0.08$; NSF: $n = 12$ –13/treatment, Kaplan–Meier Survival analysis, Mantel–Cox log-rank test, $p = 0.6$, unpaired t test, $t_{23} = 1.2, p = 0.26$; Fig. 3G–I).

Recently, arresting adult neurogenesis has been shown to impact novelty exploration (Jessberger et al., 2009; Denny et al., 2012). To test whether impaired DG plasticity in *iNR2B^{Nes}* mice influences novelty exploration, mice explored two objects for 5 min for four consecutive trials, then one object was replaced with a novel object and number of approaches to the novel object were measured (Fig. 4A). *iNR2B^{Nes}* mice explored the objects and the arena less than control mice during early training trials, yet they habituated to arena and objects similar to control mice ($n = 12$ /treatment, total object approaches: repeated-measures ANOVA genotype effect: $F_{(1,22)} = 6.03, p = 0.02$, genotype \times trial interaction: $F_{(3,66)} = 0.34, p = 0.8$; arena grid crossings: ANOVA genotype effect: $F_{(1,22)} = 6.23, p = 0.021$; genotype \times trial inter-

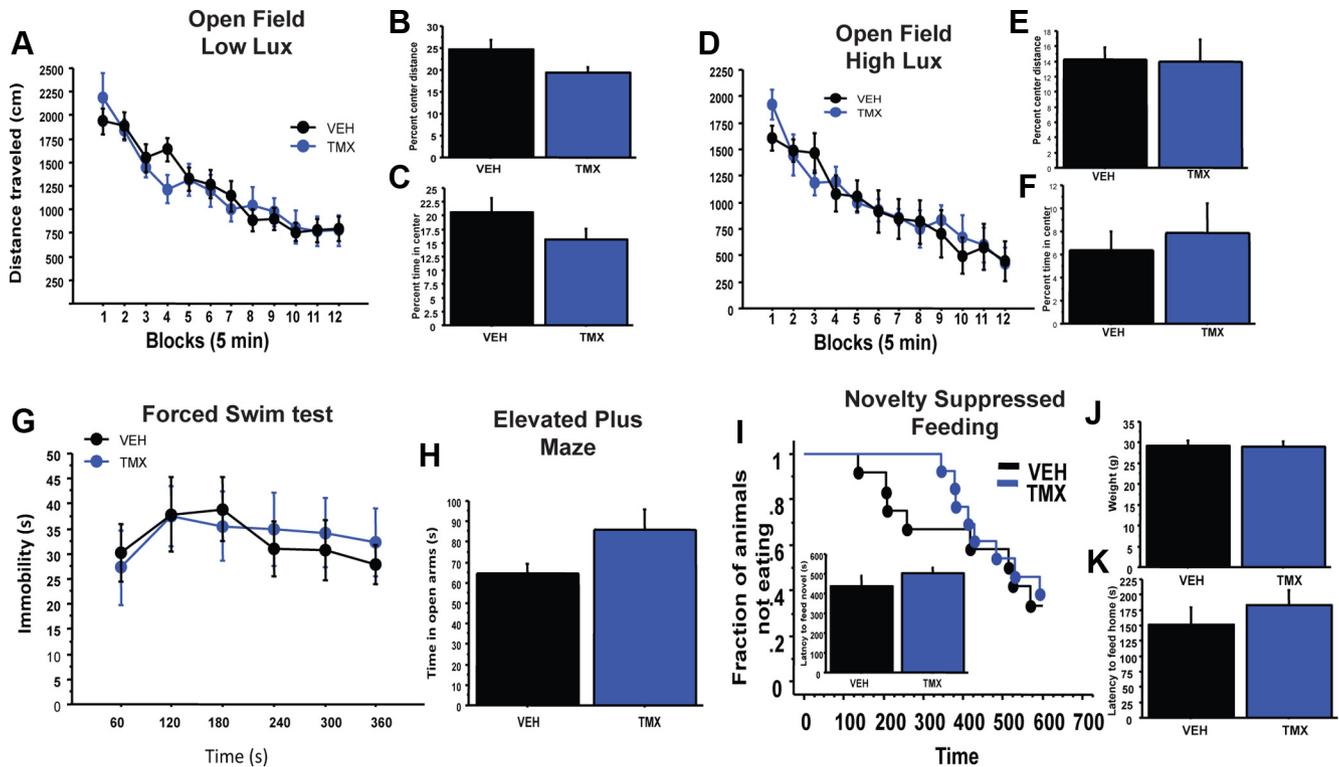


Figure 3. *iNR2B^{Nes}* mice in depression/anxiety-related behavioral assays. **A–F**, Open-field test under low and high lux conditions with total distance traveled (**A**, **D**), percentage center distance (**B**, **E**), and percentage center time (**C**, **F**). **G**, Forced-swim test. **H**, Elevated plus maze. **I**, Novelty suppressed feeding task. **J**, **K**, *iNR2B^{Nes}* mice did not differ in weight or latency to feed in their home cage. *iNR2B^{Nes}* mice did not differ from controls in FST, EPM, or NSF. Data are mean \pm SEM.

action: $F_{(4,88)} = 0.75$, $p = 0.56$; Fig. 4B). When the familiar object was replaced with a novel object, *iNR2B^{Nes}* did not discriminate between the two objects [repeated-measures ANOVA, genotype effect: $F_{(1,22)} = 5.6$, $p = 0.03$; genotype \times object interaction: $F_{(2,22)} = 12.2$, $p = 0.002$; discrimination ratio (novel – familiar/total): VEH = 0.495 ± 0.08 , TMX = 0.023 ± 0.13 , $p = 0.005$; Fig. 4C), further highlighting a deficit in exploration in *iNR2B^{Nes}* mice.

We next tested *iNR2B^{Nes}* in a one-trial contextual fear conditioning. *iNR2B^{Nes}* mice did not differ from control mice in time spent freezing in the shock context “A” 24 h after training, or in a significantly different context presented 48 h after training, suggesting that rapid encoding of contextual information was unaffected ($n = 25$ VEH, 24 TMX, repeated-measures ANOVA, treatment effect: $F_{(1,47)} = 0.8$, $p = 0.4$; treatment \times training effect: $F_{(1,47)} = 0.54$, $p = 0.46$; Fig. 4E). To assess contextual discrimination, *iNR2B^{Nes}* mice were tested in two fear discrimination tasks that required mice to either distinguish between two very different contexts (Fig. 4F, G) or two highly similar contexts (Fig. 4H, I). Both groups of mice could efficiently discriminate between the two different contexts ($n = 6$ /group, treatment effect: $F_{(1,20)} = 0.9$, $p = 0.4$; context effect: $F_{(1,20)} = 40.8$, $p < 0.001$; genotype \times day \times context interaction: $F_{(2,40)} = 0.04$, $p = 0.96$; Fig. 4G). Yet, when tested to discriminate between two highly similar contexts, freezing scores revealed that *iNR2B^{Nes}* mice took longer to learn the task, suggesting impaired fine discrimination in *iNR2B^{Nes}* mice ($n = 15$ – 17 /treatment, repeated-measures ANOVA, day \times genotype \times context interaction: $F_{(7,210)} = 2.39$, $p = 0.023$; VEH: context effect: $F_{(1,28)} = 7.68$, $p = 0.01$; day \times context interaction: $F_{(7,196)} = 7.23$, $p < 0.0001$; TMX: context effect: $F_{(1,32)} = 2.6$, $p = 0.12$; day \times context interaction: $F_{(7,224)} = 2.13$, $p = 0.04$; Fig. 4G). By day 4, control mice could efficiently

discriminate between the contexts, while *iNR2B^{Nes}* required 8 d to significantly discriminate between the contexts (day 4, repeated-measures ANOVA, treatment \times context interaction: $F_{(1,30)} = 5.6$, $p = 0.02$; Fig. 4H).

Discussion

Adult hippocampal neurogenesis, a unique form of DG plasticity, is regulated by behavioral experience, and when manipulated can have specific effects on behavior. Recent hypotheses have suggested that the enhanced synaptic plasticity exhibited by 4- to 6-week-old GCs allows them to uniquely contribute to hippocampal circuit function, and thus behavior, yet this has been difficult to test. Here we have examined the impact of this critical window of heightened plasticity on the function of young GCs. Deletion of the NR2B subunit from adult-born GCs resulted in an absence of ACSF-LTP similar to what is observed in neurogenesis ablation models, confirming that adult-born GCs are necessary for this form of plasticity via synaptic NR2B-containing NMDA receptors. Surprisingly, unlike NR1 deletion (Tashiro et al., 2006), deletion of NR2B did not affect cell survival, possibly due to residual non-NR2B-containing NMDA receptors. Deletion of NR2B did reduce dendritic complexity, highlighting the differential role of NR2B in dendritic patterning of developmentally and adult-born GCs (Espinosa et al., 2009).

Lesion and genetic studies have documented a role for the DG in novelty exploration (Hunsaker et al., 2008). The DG may play a role in both novelty encoding and context discrimination by contributing to the extraction of novel features of an environment to facilitate the storage of similar experiences as distinct representations. *iNR2B^{Nes}* mice showed a deficit in novelty exploration, which manifested as a decrease in novel object exploration. While arrest of adult neurogenesis has been shown to

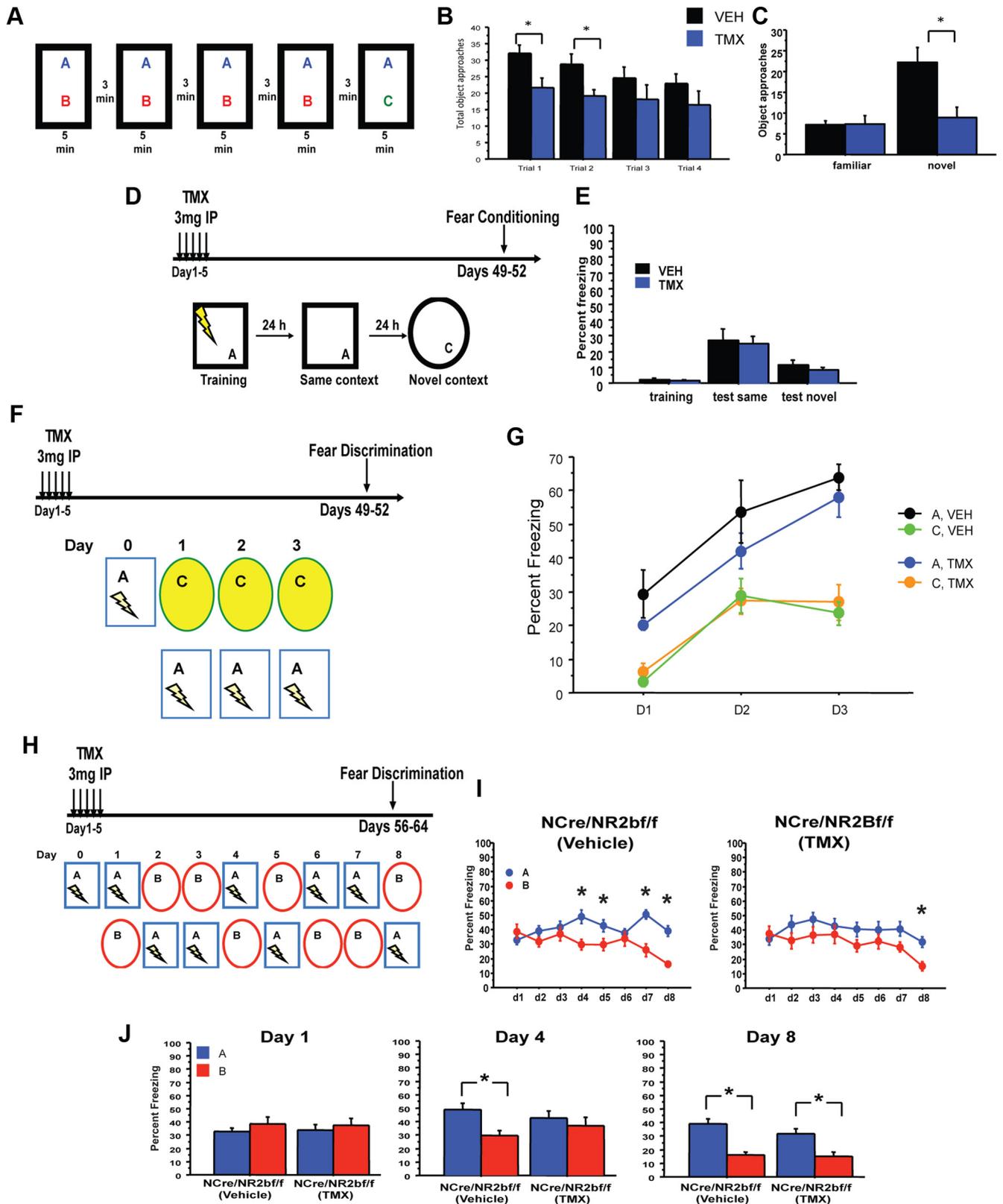


Figure 4. Impaired object exploration and contextual fear discrimination in *iNR2B^{Nes}* mice. **A**, Experimental design for novelty exploration. **B, C**, Total approaches to the objects during training trials and testing reveal *iNR2B^{Nes}* mice explored the novel objects significantly less than control mice. **D, E**, *iNR2B^{Nes}* mice did not differ from controls in a one-shock contextual fear conditioning protocol, and did not generalize freezing to a novel context. **F, G**, *iNR2B^{Nes}* mice did not differ from controls in discriminating between two different contexts (experimental design in **F**). **H, I**, *iNR2B^{Nes}* mice were slower to discriminate two similar contexts than VEH-treated controls (experimental design in **H**). **J**, VEH-treated controls could discriminate by the fourth day of training, while *iNR2B^{Nes}* could not discriminate until the eighth day of training. * $p < 0.05$. Data are mean \pm SEM.

impact novelty recognition in mice (Denny et al., 2012) and rats (Jessberger et al., 2009), a number of studies have specified a role for the perirhinal cortex in object recognition memory (Winters and Bussey, 2005), suggesting that hippocampus may only play a role in object memory when contextual cues are used to support recognition (Winters et al., 2004). As *iNR2B^{Nes}* mice were tested in the presence of extra-maze cues, this may have contributed to their decrease in novelty recognition. Alternatively, the decrease in object exploration during training may also have been responsible for their deficit in object recognition.

Multiple studies indicate that the DG, and specifically young GCs, play a role in pattern separation (Clelland et al., 2009; Sahay et al., 2011a; Nakashiba et al., 2012). Ablation of adult neurogenesis impairs appetitive spatial pattern separation as measured in a two-choice touch-screen task and a nonmatching to place radial arm maze task (Clelland et al., 2009). More recently, it has been shown that either increasing or decreasing levels of adult neurogenesis has bidirectional effects on the ability to distinguish between similar contextual representations in a fear discrimination task (Sahay et al., 2011a; Nakashiba et al., 2012). Here, we show that *iNR2B^{Nes}* mice are impaired in this task, similar to what was found in irradiated mice (Sahay et al., 2011a; Nakashiba et al., 2012). These results suggest that the critical window of heightened plasticity of young neurons is necessary for their contribution to fine contextual discrimination. Thus, these studies indicate that the main contribution of adult-born neurons to hippocampal function occurs during their critical 4–6 week period of heightened plasticity and that this heightened plasticity modulates the DG/CA3 circuit to facilitate pattern separation.

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