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Hippocampal Adult Neurogenesis Is Maintained by Neil3-Dependent Repair of Oxidative DNA Lesions in Neural Progenitor Cells

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SUMMARY

Accumulation of oxidative DNA damage has been proposed as a potential cause of age-related cognitive decline. The major pathway for removal of oxidative DNA base lesions is base excision repair, which is initiated by DNA glycosylases. In mice, Neil3 is the main DNA glycosylase for repair of hydantoin lesions in single-stranded DNA of neural stem/progenitor cells, promoting neurogenesis. Adult neurogenesis is crucial for maintenance of hippocampus-dependent functions involved in behavior. Herein, behavioral studies reveal learning and memory deficits and reduced anxiety-like behavior in *Neil3*$^{-/-}$ mice. Neural stem/progenitor cells from aged *Neil3*$^{-/-}$ mice show impaired proliferative capacity and reduced DNA repair activity. Furthermore, hippocampal neurons in *Neil3*$^{-/-}$ mice display synaptic irregularities. It appears that Neil3-dependent repair of oxidative DNA damage in neural stem/progenitor cells is required for maintenance of adult neurogenesis to counteract the age-associated deterioration of cognitive performance.

INTRODUCTION

One of the major challenges in understanding behavior has been to demonstrate a causal relationship between cognitive performance and the constitutive genesis and functional integration of new neurons (Deng et al., 2010). The progressive cognitive decline associated with age-dependent neurodegeneration is proposed to be caused by accumulation of oxidative damage to macromolecules (Barja, 2004; Harman, 1956, 1981). New neurons face a lifetime of potential threats to the integrity of their DNA, and accordingly, efficient mechanisms for DNA repair or neuronal replacement are required to maintain homeostasis.

In adult brains, neurogenesis is restricted largely to the subventricular zone (SVZ) of the lateral ventricles and to the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampal formation (Landgren and Curtis, 2011). The hippocampus contributes to learning, memory, and spatial navigation, and is also implicated in anxiety-related functions (Engin and Treit, 2007). Signal transmission within the hippocampal formation, as elsewhere in the central nervous system (CNS), is relayed through excitatory and inhibitory synapses. Glutamate is the main excitatory neurotransmitter in the CNS, activating the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors within the excitatory synapses. The AMPA receptor provides the fastest excitatory synaptic transmission in the brain, which underlies synaptic strength and plasticity (Krugers et al., 2010). NMDA receptors are mainly modulatory, inducing long-term potentiation (LTP) and long-term depression (LTD) of excitatory transmission in the hippocampus. Additionally, the NMDA receptor plays a role in the regulation of neurogenesis (Hardingham and Bading, 2010). The main inhibitory neurotransmitter, γ-aminobutyric acid (GABA), activates the GABA receptors in the inhibitory synapses. The $GABA_A$ receptor mediates the majority of the inhibitory signaling in the brain, but can also function as a trophic factor by influencing proliferation, differentiation, migration, and synapse maturation (Farrant and Nusser, 2005; Jacob et al., 2008; Owens and Kriegstein, 2002).

Hippocampal synaptic plasticity is modulated by reactive oxygen species (ROS) (Serrano and Klann, 2004). In particular, it has been shown that ROS can serve as a cellular messenger during LTP. ROS have important roles in cell signaling and

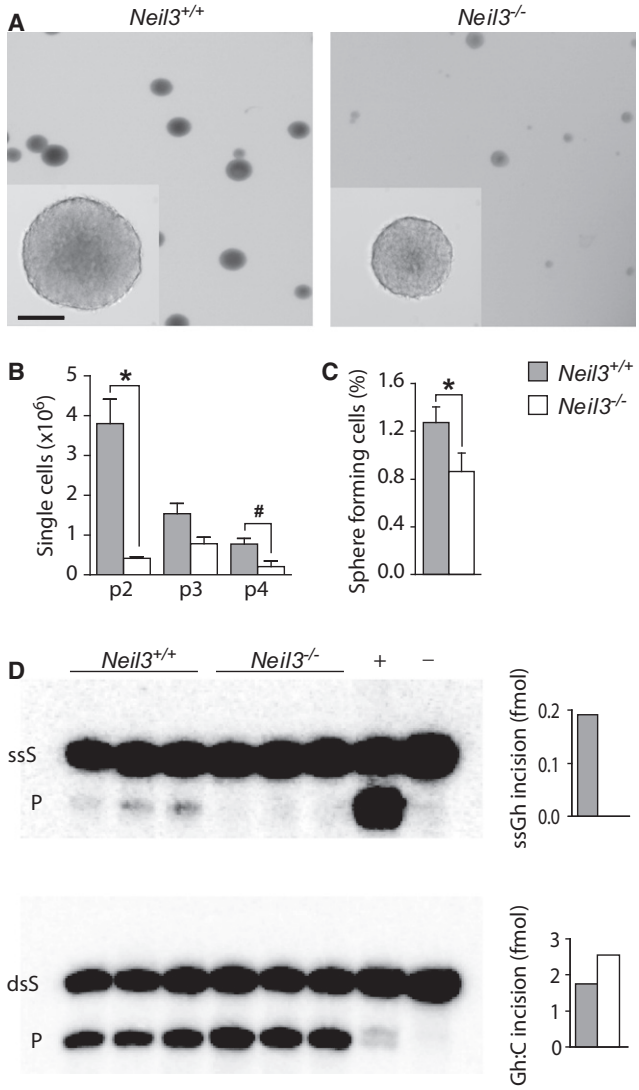

Figure 1. Reduced Proliferation and DNA Repair Activity of *Neil3*$^{-/-}$ NSPCs In Vitro

(A) Representative images illustrating the density and morphology of neurospheres from the SVZ of aged mice in a colony-forming assay with 2,000 single cells in culture. Scale bar: 100 μm.

(B) Total number of cells at passages 2–4 (p2–p4), day 7. *p = 0.005; #p = 0.046.

(C) Percentage of spheres formed from 2,000 plated cells at p3, day 10. *p = 0.007.

In (B) and (C), n = 3 independent experiments from different animals per genotype are shown. Data are shown as the mean ± SEM.

(D) Cleavage of guanidinohydantoin-containing DNA by cell extracts from neurospheres. Representative gels show triplicates from each genotype, summarized in bar graphs. ssS, single-stranded substrate; dsS, double-stranded substrate; P, product; +, purified core catalytic domain of human NEIL3; C, cytosine; Gh, guanidinohydantoin.

homeostasis, but due to their highly reactive nature, they may cause damage to cell structures, including DNA. Deficiencies in the DNA repair pathways have been linked to neurodegeneration, but a causal relationship with age-dependent decline in cognitive performance remains to be established (Englander, 2008; Jeppesen et al., 2011). The base excision repair (BER) pathway maintains genomic integrity by removing DNA base lesions caused by oxidation, alkylation, and deamination. BER is initiated by specialized DNA glycosylases interrogating the surface of DNA by flipping bases out of the helix and subsequently removing those that are recognized as unduly modified (Dalhus et al., 2009; Hegde et al., 2008). The resulting apurinic/apyrimidinic (AP) site is processed by an AP endonuclease, and repair synthesis is completed by gap filling and ligation.

Neil3 is one of several mammalian DNA glycosylases specific for removal of oxidized base lesions. Although most DNA glycosylases are ubiquitously expressed across neuronal populations and brain regions, *Neil3* displays a discrete expression pattern in the rodent SVZ and SGZ (Hildrestrand et al., 2009; Rolseth et al., 2008). The substrate specificity of Neil3 was described recently (Liu et al., 2010; Morland et al., 2002; Takao et al., 2009; Torisu et al., 2005). Neil3 is the main DNA glycosylase for removal of hydantoin lesions in single-stranded DNA (ssDNA) of neural stem/progenitor cells (NSPC), and newborn *Neil3*$^{-/-}$ mice lack the normal increase in proliferation of NSPCs after hypoxic-ischemic stroke in the forebrain, suggesting that Neil3 promotes neurogenesis (Sejersted et al., 2011).

To elucidate a possible role of Neil3 in adult hippocampal neurogenesis and maintenance of cognitive performance, we examined mice with targeted disruption of the *Neil3* gene. Our results implicate the DNA glycosylase Neil3 in the modulation of hippocampal composition, synaptic plasticity, and associated behaviors.

RESULTS

Impaired Proliferation of Neurospheres Derived from Aged *Neil3*$^{-/-}$ Mice

Our previous data demonstrated that Neil3 is required for increased neurogenesis after stroke in newborn mice (Sejersted et al., 2011). To examine the self-renewal capacity of NSPCs in aged animals, we applied the neurosphere assay. In vitro expansion of cells derived from aged mice revealed that *Neil3*$^{-/-}$ SVZ NSPC cultures retained only 60% of the capacity to form spheres observed in *Neil3*$^{+/+}$ cultures (Figure 1). The *Neil3*$^{-/-}$ spheres tended to be smaller in size than the *Neil3*$^{+/+}$ spheres, although the difference was not statistically significant (Figure 1A). Passaging was performed four times, and the single cell counts from each passage showed a consistent reduction in *Neil3*$^{-/-}$ cultures compared with *Neil3*$^{+/+}$ (Figure 1B).

Expansion of neurospheres from aged animals presented a challenge. Not one hippocampus from the *Neil3*$^{-/-}$ mice produced neurospheres, whereas expansion was successful for one-third of the *Neil3*$^{+/+}$ animals. By contrast, we were able to expand neurospheres from SVZ tissue of both genotypes. It thus appeared that hippocampal and SVZ growth of NSPCs was strongly impaired in aged *Neil3*$^{-/-}$ mice, suggesting a function of Neil3 in continuous neurogenesis.

To investigate the NSPC capacity for repair of DNA lesions, we performed nicking assays on ssDNA and double-stranded DNA oligos containing guanidinohydantoin (Gh) (Figure 1D). In line with previous results obtained with neurospheres derived from juvenile mice (Sejersted et al., 2011), the SVZ-derived

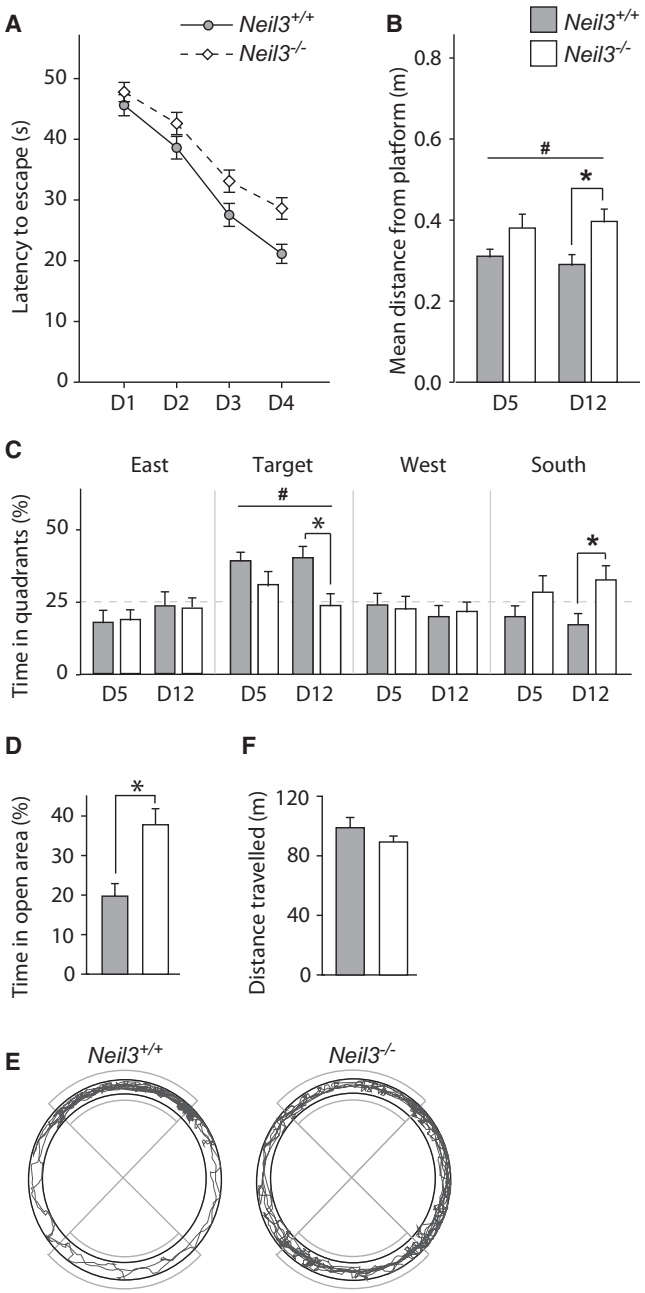

Figure 2. Learning and Memory Deficit and Reduced Anxiety in *Neil3*$^{-/-}$ Mice

(A–C) MWM trials revealed a slight learning and memory deficit in *Neil3*$^{-/-}$ mice compared with *Neil3*$^{+/+}$ mice. (A) Latency to locate the platform position and escape the water decreased across the learning trials (days 1–4). Repeated-measures analysis of variance (rmANOVA), main effect of test day, $p < 0.001$. (B) Mean distance from platform position during probe trials at days 5 and 12 (Gallagher's measure). #rmANOVA, main effect of genotype, $p = 0.02$; *t test, $p = 0.012$. (C) Percentage of time spent in the four quadrants of the tank during probe trials. #rmANOVA, main effect of genotype, $p = 0.001$; *Target quadrant, t test, $p = 0.008$; *South quadrant, t test, $p = 0.021$.

(D and E) *Neil3*$^{-/-}$ mice showed reduced anxiety in the elevated zero maze (*t test, $p = 0.002$). (E) Representative trace of position in the elevated zero maze. Closed and open quadrants are indicated.

(F) *Neil3*$^{-/-}$ mice showed no significant difference in activity level in the open field maze (t test, $p = 0.225$).

In (A)–(F), n = 16 *Neil3*$^{-/-}$ mice, and n = 14 *Neil3*$^{+/+}$ mice. Data are shown as the mean ± SEM. See also Figure S1.

neurospheres from aged *Neil3*$^{-/-}$ animals showed impaired capacity for repair of Gh lesions in ssDNA.

Anxiety-like Behavior and Impairment of Memory and Learning in *Neil3*$^{-/-}$ Mice

Reduced proliferation of NSPCs indicated defective neurogenesis in the adult *Neil3*$^{-/-}$ brain. Because adult neurogenesis in the hippocampus is important for learning and memory (Deng et al., 2010), we examined the behavioral phenotype of *Neil3*$^{-/-}$ mice. Aging of the animals revealed no significant changes in morbidity or mortality compared with *Neil3*$^{+/+}$ mice. The total body weight (*Neil3*$^{+/+}$ 34.0 ± 1.2 g, n = 9, versus *Neil3*$^{-/-}$ 35.5 ± 1.5 g, n = 13, mean ± standard error of the mean [SEM]) and organ weight (Figure S1) were normal at 18 months of age.

Hippocampal functions such as spatial learning and memory performance were examined in a Morris water maze (MWM), whereas anxiety and activity levels were examined in an elevated zero maze and open field test, respectively.

In the MWM, we found that mice of both genotypes swam well and learned the position of the hidden escape platform, as indicated by reduced latencies to find the platform (Figure 2A). During retention trials, the *Neil3*$^{-/-}$ mice searched significantly farther away from the platform position than the *Neil3*$^{+/+}$ mice (Figure 2B). The *Neil3*$^{-/-}$ mice also spent a significantly smaller percentage of time searching for the submerged escape platform within the target quadrant than the *Neil3*$^{+/+}$ mice (Figure 2C). Mice of both genotypes showed a preference for the target quadrant during the retention trial on day 5, but during the retention trial on day 12 the *Neil3*$^{-/-}$ mice spent significantly less time searching in the target quadrant and significantly more time searching in the opposite quadrant (Figure 2C).

When tested in the elevated zero maze, the *Neil3*$^{-/-}$ mice spent significantly more time in the open quadrants compared with the *Neil3*$^{+/+}$ mice (Figures 2D and 2E), indicating reduced anxiety-like behavior. This was not due to a difference in activity between the two genotypes, since they showed similar levels of activity in an open field test (Figure 2F).

Overall, the behavioral tests indicate that the *Neil3*$^{-/-}$ mice have a learning and memory deficit and a reduction in anxiety-like behavior.

Differential Expression of Excitatory and Inhibitory Receptor Subunits in the *Neil3*$^{-/-}$ Hippocampus

The MWM measures activities that strongly correlate with hippocampal plasticity, LTP, and NMDA receptor function (Vorhees and Williams, 2006), making it a key technique in the investigation of hippocampal circuitry. The behavioral differences between the *Neil3*$^{-/-}$ and *Neil3*$^{+/+}$ mice warranted an investigation of the expression of glutamatergic and GABAergic receptors in the hippocampal formation.

We examined the expression of receptor subunits in selected regions of the hippocampal formation (Figure 3A). To avoid biasing from the behavioral studies, we used unexposed

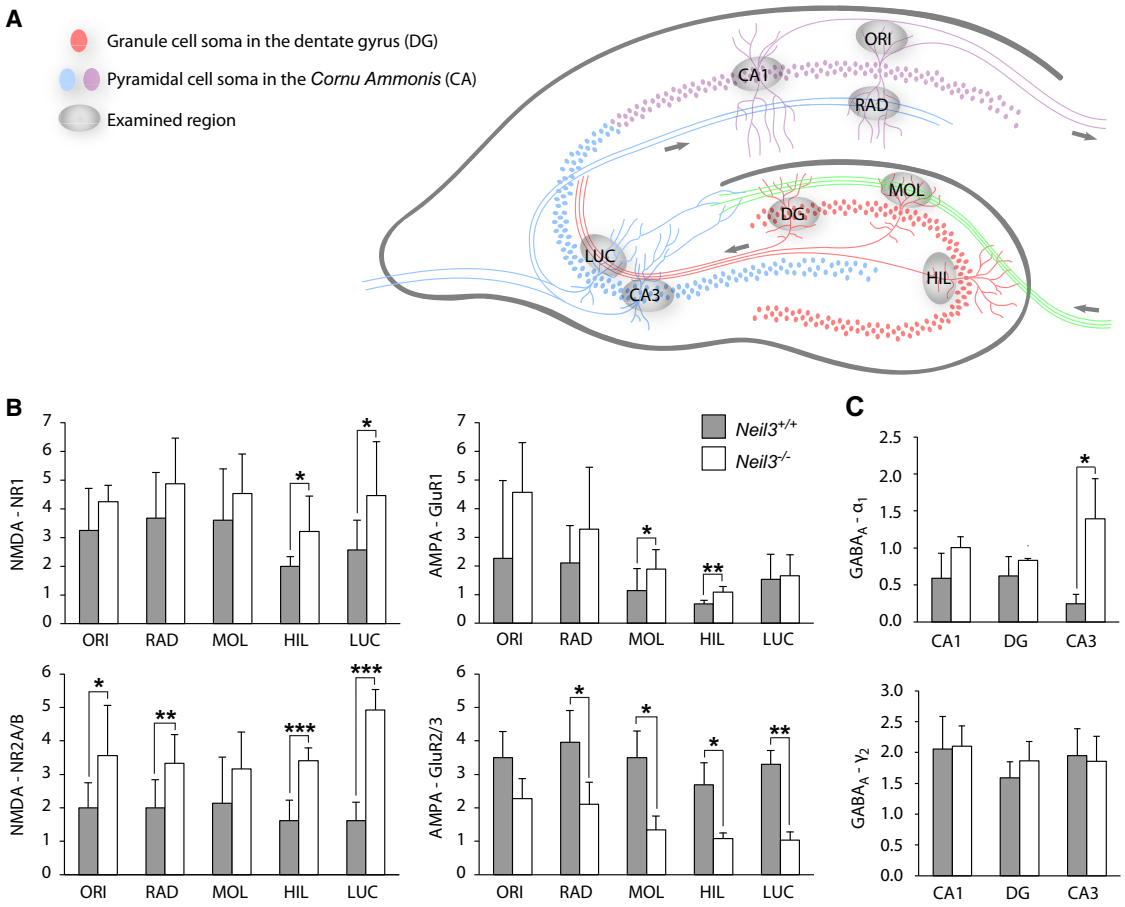

Figure 3. Differential Expression of Excitatory and Inhibitory Receptor Subunits in *Neil3*$^{-/-}$ and *Neil3*$^{+/+}$ Mice

Confocal microscopy of glutamatergic and GABAergic receptor subunits. Quantification was performed by measurement of the fluorescent intensity.

(A) Schematic illustration of a cross-section showing the hippocampal anatomy and neuronal circuitry. Arrows depict the traditional trisynaptic pathway: entorhinal cortex (EC) → dentate gyrus (DG) → CA3 → CA1 → EC. Excitatory synapses were sampled in the stratum oriens (ORI) and the stratum radiatum (RAD) within the CA1 region, the stratum moleculare (MOL) and the hilus (HIL) within the DG, and the stratum lucidum (LUC) within the CA3 region. Inhibitory synapses were sampled on CA1 pyramidal cells (CA1), DG granule cells (DG), and CA3 pyramidal cells (CA3).

(B) Immunoreactivity of excitatory NMDA and AMPA receptor subunits (p values by t test: NMDA NR1: HIL, .019; LUC, .020; NR2A/B: ORI, .016; RAD, .005; HIL and LUC, < .001. AMPA GluR1: MOL, .043; HIL, .001; GluR2/3: RAD, .049; MOL, .014, HIL, .015; LUC, .001).

(C) Immunoreactivity of inhibitory $GABA_A$ receptor subunits (*p = .023).

In (B) and (C), data are shown as the mean fluorescent intensity above background ± SD; three mice were analyzed per genotype. See also Figures S2 and S3.

animals. Confocal immunofluorescence z-stack analyses showed punctuate fluorophore labeling consistent with the localization of the NMDA and AMPA receptor subunits, and the $GABA_A$ receptor subunits in the hippocampal formation (Figure S2). Antibody specificity was confirmed by western blotting (Figure S3). Quantification revealed that expression of NR1 was significantly increased in the hilus of the DG and the stratum lucidum of the CA3 region, and NR2A/B was significantly increased in the stratum oriens and the stratum radiatum of the CA1 region, the hilus of the DG, and the stratum lucidum of the CA3 region in *Neil3*$^{-/-}$ mice compared with *Neil3*$^{+/+}$ mice (Figure 3B). GluR1 was significantly increased in the stratum moleculare and the hilus of the DG, whereas GluR2/3 showed a statistically significant decrease in labeling in the stratum radiatum of the CA1 region, the stratum moleculare and the hilus of the DG, and the stratum lucidum of the CA3 region. Expression of the $GABA_A$ receptor subunit α_1 was significantly increased at the CA3 pyramidal cell layer in *Neil3*$^{-/-}$ mice, whereas the γ_2 subunit did not display any significant differences in fluorescence labeling in any of the regions investigated (Figure 3C).

In sum, we observed a pattern of increased labeling of the excitatory NMDA receptor subunits NR1 and NR2A/B, the excitatory AMPA receptor subunit GluR1, and the inhibitory $GABA_A$ receptor subunit α_1 in *Neil3*$^{-/-}$ mice. The AMPA receptor subunit GluR2/3 showed a pattern of decreased labeling compared with *Neil3*$^{+/+}$ animals.

Decreased Postsynaptic Density Length and Increased Receptor Subunit Density in *Neil3*$^{-/-}$ Mice

Altered patterns of receptor subunit expression in *Neil3*$^{-/-}$ animals indicated a differential synaptic composition in the hippocampal formation. We employed quantitative immunogold

postembedding labeling and electron microscopy to examine the synapse morphology and the density of glutamatergic and GABAergic receptor subunits within the hippocampal formation. We examined NMDA receptor subunits NR1 and NR2A/B within excitatory synapses in the stratum radiatum of the CA1 region and the hilus of the DG, and the AMPA receptor subunit GluR1 within excitatory synapses in the stratum moleculare. In addition, the $GABA_A$ receptor subunit α_1 within inhibitory synapses in the CA3 region was examined.

NMDA and AMPA receptor subunit labeling was detected at the asymmetric, i.e., excitatory, synapses synapses on dendritic spines (Figure 4A). $GABA_A$ receptor subunit labeling at the symmetric inhibitory synapses of the CA3 pyramidal cells was also observed. The labeling was particularly strong at the postsynaptic membrane overlying the postsynaptic density (PSD). Most regions studied showed a pattern of increased gold particle density labeling for all receptor types and receptor subunits in *Neil3*$^{-/-}$ mice compared with *Neil3*$^{+/+}$ mice (Figure 4B). The *Neil3*$^{-/-}$ mice displayed a significant increase in gold particle density of the receptor subunits GluR1 in the stratum moleculare of the DG and $GABA_A$ α_1 in the CA3 pyramidal cells. Additionally, the PSD length in *Neil3*$^{-/-}$ mice was shorter than in *Neil3*$^{+/+}$ mice (Figure 4B).

Overall, changes were detected in all regions of the hippocampal formation in both excitatory and inhibitory synapses (Figure 4C).

DISCUSSION

Here, we demonstrate that proliferation of NSPCs derived from adult *Neil3*$^{-/-}$ mice is impaired, and that the capacity for repair of Gh in ssDNA is diminished. We reveal that mice lacking the Neil3 DNA glycosylase exhibit learning and memory deficits, as well as decreased anxiety-like behavior. Also, *Neil3*$^{-/-}$ mice show alterations in the expression of glutamatergic and GABAergic receptors, increased receptor density, and reduced PSD length. These data indicate that the composition of hippocampal synapses in vivo is changed as a consequence of Neil3 deficiency.

Maintenance of hippocampal memory and learning, as well as the associated normal behaviors, seem to be dependent on neurogenesis throughout adulthood (Imayoshi et al., 2008). The genesis of new DG granule cells begins in the SGZ, where NSPCs proliferate. While they are differentiating, cells destined for the neuronal lineage migrate into the inner layer of the DG, and during the second week after birth they start extending dendrites and axons (Zhao et al., 2006). During the third week of life, afferent and efferent connections are formed, and the adult-born granule cells are integrated in the neuronal network. At this age, granule cells are highly excitable and show strong synaptic plasticity, and network integration is influenced by local synaptic activity (Ge et al., 2006; Toni et al., 2007). By 8 weeks of age, the synaptic plasticity and physiological properties of adult-born granule cells are indistinguishable from those of mature granule cells. Hence, in an age window of 1–6 weeks, adult-born granule cells have the ability to influence memory and learning (Dupret et al., 2007; Trouche et al., 2009), suggesting that the behavioral deficits observed in *Neil3*$^{-/-}$ mice in this study were due to differential composition of the DG granule cells at the time of testing. Inhibition of adult neurogenesis halts the normal increase in DG granule cell number and impairs hippocampal learning and memory (Imayoshi et al., 2008). We have shown that the proliferation rate of NSPCs derived from the adult *Neil3*$^{-/-}$ brain was reduced, indicating that these animals were deficient in adult-born DG granule cells.

We also observed significant synaptic alterations in various regions of the hippocampal formation. The accumulation of oxidative damage in the CNS has been shown to result in decreased efficiency of LTP (Lynch, 2004; Serrano and Klann, 2004). The maintenance of LTP also involves the growth of the synaptic membrane of excitatory synapses (Bourne and Harris, 2012), suggesting that LTP may be impaired in *Neil3*$^{-/-}$ mice. Activation of $GABA_A$ receptors in the hippocampus was previously shown to have an anxiolytic effect (Engin and Treit, 2007). We speculate that the increase in $GABA_A$ receptors in *Neil3*$^{-/-}$ mice may contribute to the decreased anxiety-like behavior.

One of the major determinants of adult hippocampal neurogenesis is hippocampal-dependent learning (Epp et al., 2007; Gould et al., 1999). Receptor activation has been shown to increase hippocampal adult progenitor cell proliferation and differentiation (Deisseroth et al., 2004; Farrant and Nusser, 2005; Hardingham and Bading, 2010; Jacob et al., 2008; Krugers et al., 2010; Nacher and McEwen, 2006; Owens and Kriegstein, 2002), and as such, genesis of new neurons in the adult brain is influenced by an animal's experiences and synaptic activity. However, a major challenge has been to demonstrate the causal relationship between disrupted adult neurogenesis and deterioration of cognitive ability. Early approaches included antimitotic drug treatments and irradiation, which effectively blocked proliferation of progenitor cells during neurogenesis but had substantial side effects, such as general health deterioration and inflammation, that complicated the interpretation of behavioral studies. Recent studies using transgenic models involved the expression of suicide genes driven by various NSPC-specific promoters (Deng et al., 2010). The *Neil3*$^{-/-}$ mouse, which displays defective DNA damage repair (Sejersted et al., 2011), diminished NSPC proliferation, and aberrant behavior, represents an excellent model for further studies of the causal relationship among cognitive performance, oxidative damage, and neurogenesis.

The canonical function of Neil3 seems to be base excision with a preference for oxidative DNA base lesions such as spiroiminohydantoins and guanidinohydantoins (Liu et al., 2010), which are potent replication blocks in vivo (Henderson et al., 2003). Neil3 is the main DNA glycosylase for removal of these hydantoin lesions in ssDNA of NSPC (Sejersted et al., 2011). Our results suggest that *Neil3*$^{-/-}$ NSPCs in the SGZ fail to replicate and replenish DG granule cells due to age-associated accumulation of oxidative DNA damage. The sustenance of many mammalian tissues is dependent on replenishment of cells. *Neil3* is expressed in several proliferating tissues, and although no other phenotypic changes were noted in Neil3-deficient mice during the course of the current investigation, Neil3 likely has a broader function to remove DNA lesions and sustain proliferation. Further studies are required to determine the impact of Neil3 deficiency in other organ systems.

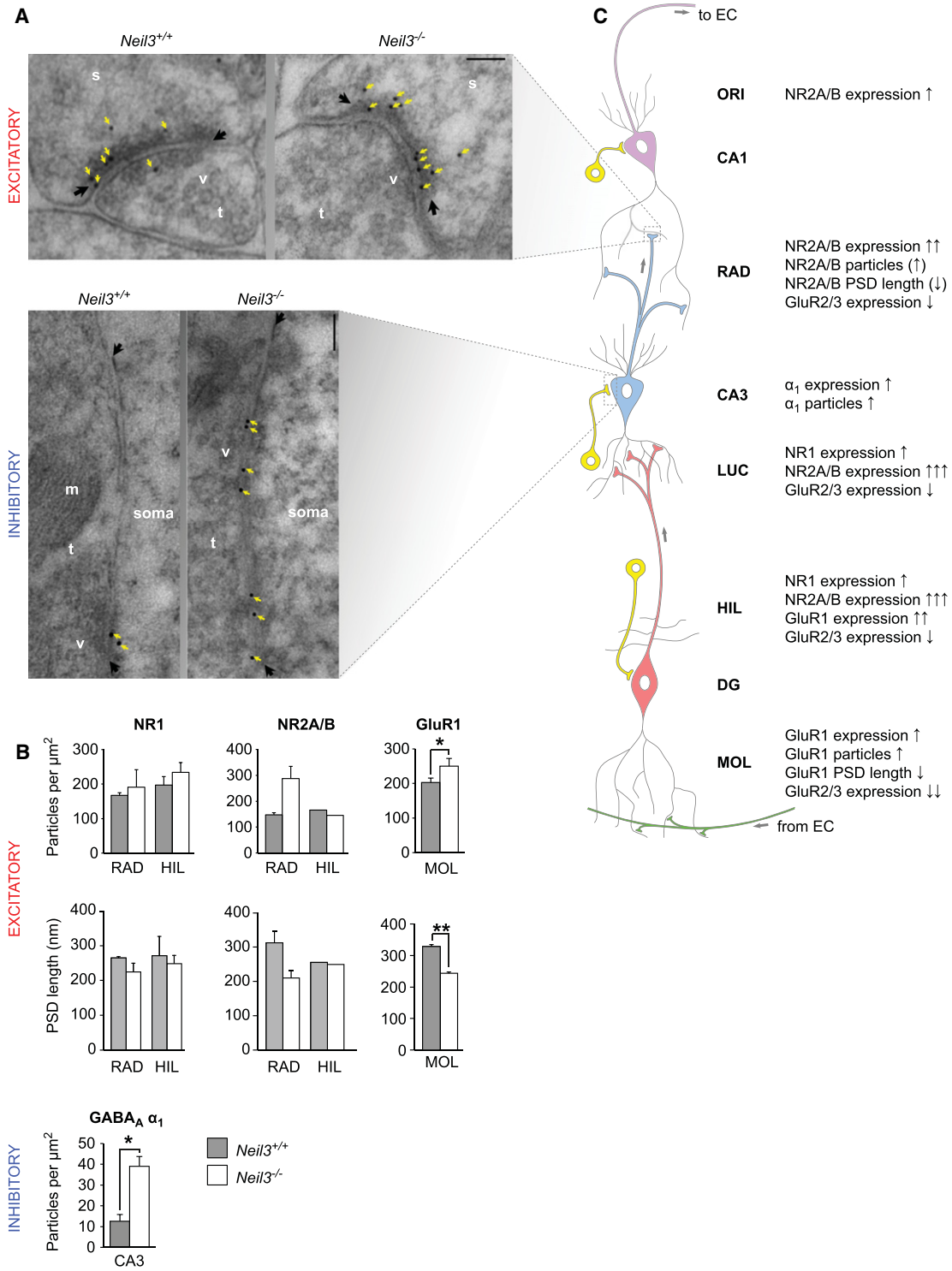

Figure 4. Altered Synapse Composition of the Hippocampal Formation in *Neil3*$^{-/-}$ Mice

Postembedding immunogold electron microscopy of glutamatergic and GABAergic synapses and receptor subunits.

(A) Micrographs illustrating representative excitatory synapses in the stratum radiatum and inhibitory synapses against the soma of CA3 pyramidal cells. Yellow arrows indicate the gold particles (black dots) representing labeled NR1 (Excitatory) and $GABA_A$ α_1 (Inhibitory) receptor subunits within the postsynaptic membranes (limited by black arrows). Scale bars: 100 nm. v, synaptic vesicles; t, presynaptic terminal; s, postsynaptic spine; m, mitochondria; soma, cell soma.

(B) Quantification of excitatory NMDA NR1 and NR2A/B, and AMPA GluR1 receptor subunits (*p = 0.046; **p = 0.005), and inhibitory $GABA_A$ α_1 receptor subunits (*p = 0.031). Gold particles within 25 nm of the postsynaptic membranes were counted and the membrane lengths were measured. Data are shown as mean

The free-radical theory of aging and accumulation of oxidative DNA damage has been put forward as a possible explanation for cognitive deficits (Barja, 2004; Harman, 1956, 1981). However, no direct evidence demonstrates that a defect in repair of oxidative DNA damage induces age-related neurodegeneration. Here, we present findings that support the role of oxidative DNA damage in cognitive decline. However, to our knowledge, no method is available for direct detection of hydantoin lesions in tissue. A comprehensive understanding of the mechanisms by which oxidative DNA damage and repair regulate adult neurogenesis remains to be achieved in further studies. Such insight will unveil novel therapeutic targets in neurodegenerative disease.

EXPERIMENTAL PROCEDURES

All methods and materials are described in detail in Extended Experimental Procedures.

Mice

All experiments were approved by the Norwegian Animal Research Authority and conducted in accordance with the laws and regulations controlling experimental procedures in live animals in Norway, and European Union Directive 86/609/EEC. *Neil3*$^{-/-}$ were generated as previously described (Sejersted et al., 2011).

In Vitro Expansion of NSPCs

The SVZ and the hippocampus were dissected from the brains of 18-month-old mice. Tissue was mechanically dissociated and filtered. Cells were resuspended in serum-free growth medium supplemented with glutamine, penicillin/streptomycin, growth factors, and heparin. Neurospheres were then passaged every 7 days. For quantification of sphere-forming cells, a colony-forming assay was set up at passage 3 and spheres exceeding 50 μm in diameter were counted after 10 days. DNA repair activity assays were performed as described previously (Sejersted et al., 2011).

MWM

The MWM (Vorhees and Williams, 2006) trials were carried out in a white circular pool. The mice were 8 months of age. An escape platform was located at a fixed position. During training trials the platform was kept 0.5–1 cm below the water surface, but during retention trials the escape platform was submerged to the bottom of the pool.

Elevated Zero Maze

The elevated zero maze task (Shepherd et al., 1994) was conducted in a custom-made white circular runway with alternating open and closed quadrants. The mice were 18 months of age. The mice were placed on the maze facing a closed quadrant and allowed 5 min for exploration of the apparatus.

Open Field Test

The open field test was conducted in square white arenas. The mice were allowed to explore freely for 45 min. The mice were 18 months of age.

Immunohistochemistry

Immunohistochemistry was performed following standard protocols. Fixed brains from 14-month-old mice were cryoprotected by incrementally permeating the brains in sucrose. Sagittal sections were incubated overnight with primary antibodies, rinsed in PBS, and incubated for 60 min with a secondary antibody. The sections were rinsed again and mounted with ProLong Gold Antifade Reagent. The fluorescent labeling was analyzed in z-stack scans obtained with a Zeiss Pascal laser scanning microscope. Three z-stacks were obtained with identical settings from each region of interest. The average fluorescence intensity above background was quantified with the use of a Zeiss LSM image browser.

Postembedding Immunogold Electron Microscopy

The procedure used for postembedding immunogold electron microscopy was previously described in detail (Bergersen et al., 2008). Tissue was collected from 15-month-old mice. Micrographs of clearly visible synapses were recorded randomly in selected subregions of the hippocampal formation with the use of a Tecnai 12 electron microscope. Excitatory synapses were identified as small axonal terminals forming asymmetric synapses onto dendritic spines. Inhibitory synapses were identified as small axonal terminals forming symmetrical synapses onto the cellular soma of pyramidal cells. The lengths of the postsynaptic membranes were measured, and the densities of gold particles associated with the postsynaptic membranes were calculated by taking a rectangular area of 50 nm and counting the number of gold particles located ±25 nm from the midline of the postsynaptic membranes, i.e., approximately the lateral resolution of the immunogold method (Bergersen et al., 2003, 2008).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.08.008.

LICENSING INFORMATION

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REFERENCES

Barja, G. (2004). Free radicals and aging. Trends Neurosci. *27*, 595–600.

Bergersen, L., Ruiz, A., Bjaalie, J.G., Kullmann, D.M., and Gundersen, V. (2003). GABA and GABAA receptors at hippocampal mossy fibre synapses. Eur. J. Neurosci. *18*, 931–941.

Bergersen, L.H., Storm-Mathisen, J., and Gundersen, V. (2008). Immunogold quantification of amino acids and proteins in complex subcellular compartments. Nat. Protoc. *3*, 144–152.

Bourne, J.N., and Harris, K.M. (2012). Nanoscale analysis of structural synaptic plasticity. Curr. Opin. Neurobiol. *22*, 372–382.

Dalhus, B., Laerdahl, J.K., Backe, P.H., and Bjørås, M. (2009). DNA base repair—recognition and initiation of catalysis. FEMS Microbiol. Rev. *33*, 1044–1078.

subunit-representing particle number per μm^2 ± SD, and as mean PSD length ± SD. Two mice were used per genotype, except for NR2A/B HIL, where the data are based on one mouse.

(C) A summary of significant changes in expression (Figure 3), particle density, and PSD length in *Neil3*$^{-/-}$ animals as compared with *Neil3*$^{+/+}$ animals. ↑, $p < 0.05$; ↑↑, $p < 0.01$; ↑↑↑, $p < 0.001$; (↑), near significant. The excitatory trisynaptic pathway is illustrated with inhibitory interneuron (yellow) input. See also Figure S3.

Deisseroth, K., Singla, S., Toda, H., Monje, M., Palmer, T.D., and Malenka, R.C. (2004). Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron *42*, 535–552.

Deng, W., Aimone, J.B., and Gage, F.H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat. Rev. Neurosci. *11*, 339–350.

Dupret, D., Fabre, A., Döbrössy, M.D., Panatier, A., Rodríguez, J.J., Lamarque, S., Lemaire, V., Oliet, S.H., Piazza, P.V., and Abrous, D.N. (2007). Spatial learning depends on both the addition and removal of new hippocampal neurons. PLoS Biol. *5*, e214.

Engin, E., and Treit, D. (2007). The role of hippocampus in anxiety: intracerebral infusion studies. Behav. Pharmacol. *18*, 365–374.

Englander, E.W. (2008). Brain capacity for repair of oxidatively damaged DNA and preservation of neuronal function. Mech. Ageing Dev. *129*, 475–482.

Epp, J.R., Spritzer, M.D., and Galea, L.A. (2007). Hippocampus-dependent learning promotes survival of new neurons in the dentate gyrus at a specific time during cell maturation. Neuroscience *149*, 273–285.

Farrant, M., and Nusser, Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat. Rev. Neurosci. *6*, 215–229.

Ge, S., Goh, E.L., Sailor, K.A., Kitabatake, Y., Ming, G.L., and Song, H. (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature *439*, 589–593.

Gould, E., Beylin, A., Tanapat, P., Reeves, A., and Shors, T.J. (1999). Learning enhances adult neurogenesis in the hippocampal formation. Nat. Neurosci. *2*, 260–265.

Hardingham, G.E., and Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat. Rev. Neurosci. *11*, 682–696.

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. J. Gerontol. *11*, 298–300.

Harman, D. (1981). The aging process. Proc. Natl. Acad. Sci. USA *78*, 7124–7128.

Hegde, M.L., Hazra, T.K., and Mitra, S. (2008). Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. *18*, 27–47.

Henderson, P.T., Delaney, J.C., Muller, J.G., Neeley, W.L., Tannenbaum, S.R., Burrows, C.J., and Essigmann, J.M. (2003). The hydantoin lesions formed from oxidation of 7,8-dihydro-8-oxoguanine are potent sources of replication errors in vivo. Biochemistry *42*, 9257–9262.

Hildrestrand, G.A., Neurauter, C.G., Diep, D.B., Castellanos, C.G., Krauss, S., Bjørås, M., and Luna, L. (2009). Expression patterns of Neil3 during embryonic brain development and neoplasia. BMC Neurosci. *10*, 45.

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., Mori, K., Ikeda, T., Itohara, S., and Kageyama, R. (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat. Neurosci. *11*, 1153–1161.

Jacob, T.C., Moss, S.J., and Jurd, R. (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat. Rev. Neurosci. *9*, 331–343.

Jeppesen, D.K., Bohr, V.A., and Stevnsner, T. (2011). DNA repair deficiency in neurodegeneration. Prog. Neurobiol. *94*, 166–200.

Krugers, H.J., Hoogenraad, C.C., and Groc, L. (2010). Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. Nat. Rev. Neurosci. *11*, 675–681.

Landgren, H., and Curtis, M.A. (2011). Locating and labeling neural stem cells in the brain. J. Cell. Physiol. *226*, 1–7.

Liu, M., Bandaru, V., Bond, J.P., Jaruga, P., Zhao, X., Christov, P.P., Burrows, C.J., Rizzo, C.J., Dizdaroglu, M., and Wallace, S.S. (2010). The mouse ortholog of NEIL3 is a functional DNA glycosylase in vitro and in vivo. Proc. Natl. Acad. Sci. USA *107*, 4925–4930.

Lynch, M.A. (2004). Long-term potentiation and memory. Physiol. Rev. *84*, 87–136.

Morland, I., Rolseth, V., Luna, L., Rognes, T., Bjørås, M., and Seeberg, E. (2002). Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. Nucleic Acids Res. *30*, 4926–4936.

Nacher, J., and McEwen, B.S. (2006). The role of N-methyl-D-asparate receptors in neurogenesis. Hippocampus *16*, 267–270.

Owens, D.F., and Kriegstein, A.R. (2002). Is there more to GABA than synaptic inhibition? Nat. Rev. Neurosci. *3*, 715–727.

Rolseth, V., Rundén-Pran, E., Luna, L., McMurray, C., Bjørås, M., and Ottersen, O.P. (2008). Widespread distribution of DNA glycosylases removing oxidative DNA lesions in human and rodent brains. DNA Repair (Amst.) *7*, 1578–1588.

Sejersted, Y., Hildrestrand, G.A., Kunke, D., Rolseth, V., Krokeide, S.Z., Neurauter, C.G., Suganthan, R., Atneosen-Åsegg, M., Fleming, A.M., Saugstad, O.D., et al. (2011). Endonuclease VIII-like 3 (Neil3) DNA glycosylase promotes neurogenesis induced by hypoxia-ischemia. Proc. Natl. Acad. Sci. USA *108*, 18802–18807.

Serrano, F., and Klann, E. (2004). Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Res. Rev. *3*, 431–443.

Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J., and Dourish, C.T. (1994). Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology (Berl.) *116*, 56–64.

Takao, M., Oohata, Y., Kitadokoro, K., Kobayashi, K., Iwai, S., Yasui, A., Yonei, S., and Zhang, Q.M. (2009). Human Nei-like protein NEIL3 has AP lyase activity specific for single-stranded DNA and confers oxidative stress resistance in *Escherichia coli* mutant. Genes Cells *14*, 261–270.

Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H., and Gage, F.H. (2007). Synapse formation on neurons born in the adult hippocampus. Nat. Neurosci. *10*, 727–734.

Torisu, K., Tsuchimoto, D., Ohnishi, Y., and Nakabeppu, Y. (2005). Hematopoietic tissue-specific expression of mouse Neil3 for endonuclease VIII-like protein. J. Biochem. *138*, 763–772.

Trouche, S., Bontempi, B., Roullet, P., and Rampon, C. (2009). Recruitment of adult-generated neurons into functional hippocampal networks contributes to updating and strengthening of spatial memory. Proc. Natl. Acad. Sci. USA *106*, 5919–5924.

Vorhees, C.V., and Williams, M.T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. *1*, 848–858.

Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., and Gage, F.H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J. Neurosci. *26*, 3–11.