

Synaptic Integration of Adult-Born Hippocampal Neurons Is Locally Controlled by Astrocytes

Highlights

- Astrocytes control the dendritic maturation and survival of adult-born neurons
- Astrocytes locally regulate the formation of dendritic spines on adult-born neurons
- Astrocytes regulate the functional synaptic integration of adult-born neurons
- These effects are mediated by the astrocytic vesicular release of D-serine

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In Brief

Adult neurogenesis plays a crucial role in learning and memory. Sultan et al. show that by releasing D-serine, astrocytes support the maturation of new neurons that cross their territories. This finding reveals a critical role for glia in brain plasticity.



Synaptic Integration of Adult-Born Hippocampal Neurons Is Locally Controlled by Astrocytes

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SUMMARY

Adult neurogenesis is regulated by the neurogenic niche, through mechanisms that remain poorly defined. Here, we investigated whether niche-constituting astrocytes influence the maturation of adult-born hippocampal neurons using two independent transgenic approaches to block vesicular release from astrocytes. In these models, adult-born neurons but not mature neurons showed reduced glutamatergic synaptic input and dendritic spine density that was accompanied with lower functional integration and cell survival. By taking advantage of the mosaic expression of transgenes in astrocytes, we found that spine density was reduced exclusively in segments intersecting blocked astrocytes, revealing an extrinsic, local control of spine formation. Defects in NMDA receptor (NMDAR)-mediated synaptic transmission and dendrite maturation were partially restored by exogenous D-serine, whose extracellular level was decreased in transgenic models. Together, these results reveal a critical role for adult astrocytes in local dendritic spine maturation, which is necessary for the NMDAR-dependent functional integration of newborn neurons.

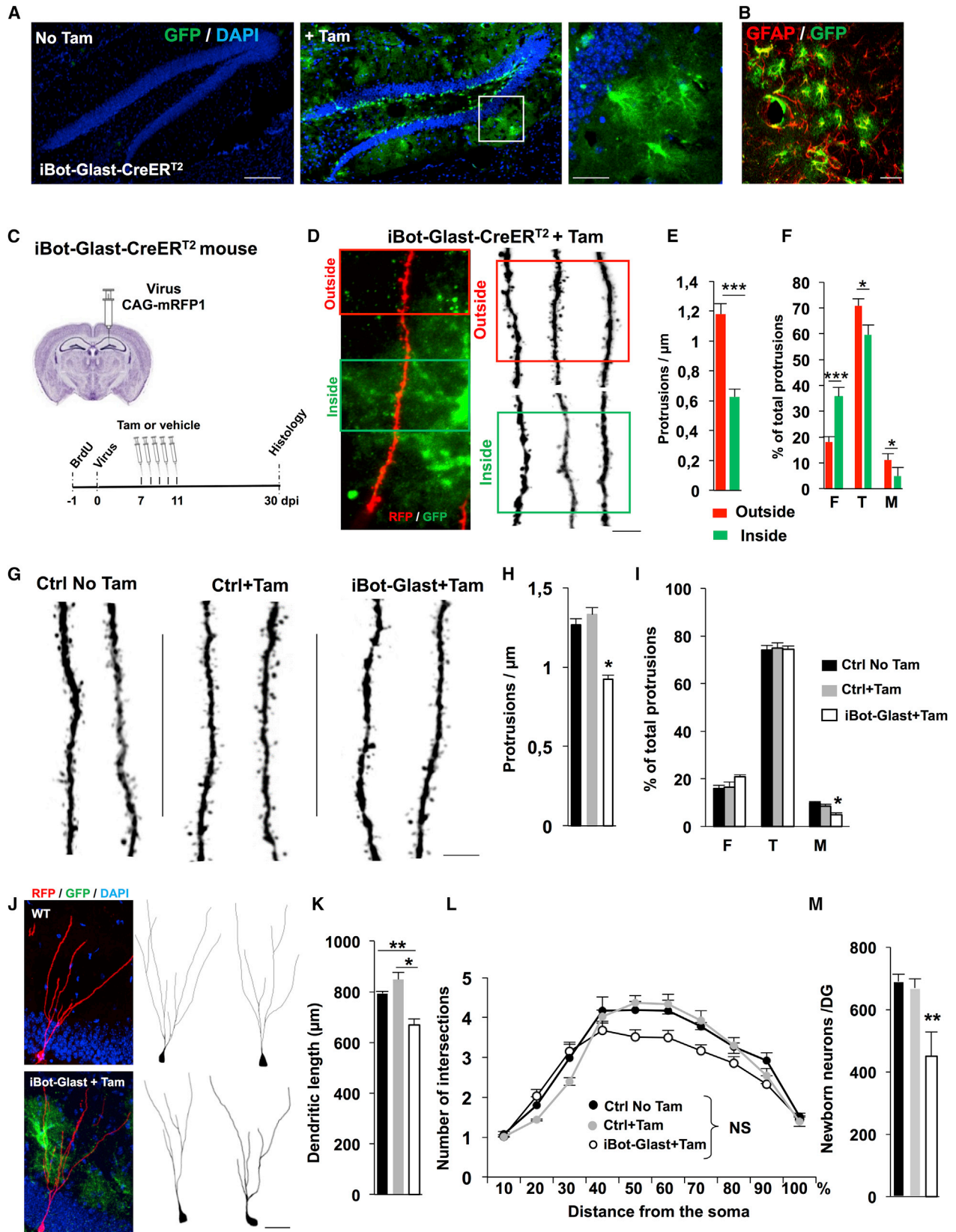
INTRODUCTION

Adult neurogenesis occurs in the brains of most mammals (Altman and Das, 1965), including humans (Eriksson et al., 1998), and contributes to learning and memory (Aimone and Gage, 2011; Zhao et al., 2008). The survival and synaptic integration of adult-born hippocampal neurons is enhanced by neuronal network activity or the induction of long-term potentiation (LTP), a physiological mechanism of synaptic plasticity (Bruehl-Jungerman et al., 2006). In turn, the incorporation of adult-born neurons with increased synaptic plasticity (Ge et al., 2007; Schmidt-Hieber et al., 2004) enhances LTP and hippocampal-dependent learning performances (Kempermann and Gage, 1999). Adult neurogenesis is highly regulated and a growing number of studies indicate that the direct cellular environment

of adult neural stem cells, the neurogenic niche, provides pro-neurogenic cues (Song et al., 2002). A major component of the neurogenic niche is the astroglia, which releases soluble molecules that control distinct steps of adult neurogenesis, including the proliferation of the stem cells (Cao et al., 2013), their differentiation into neuronal lineage (Song et al., 2002), and their survival (Platel et al., 2010). Here, we examined whether astrocytes regulate the synaptic integration of adult-born neurons, a major step of adult neurogenesis.

Previous studies have shown that during development, astrocytes promote the formation of synapses in distinct neuronal populations (Allen, 2013). Indeed, soluble molecules contained in astrocyte-conditioned medium induce the formation of synapses on early postnatal retinal ganglion cells (Banker, 1980; Pfrieger and Barres, 1997). These molecules display a great diversity of class, ranging from protein components of the extracellular matrix, such as thrombospondin to membrane lipids such as cholesterol (Christopherson et al., 2005; Mauch et al., 2001). They also express a variety of properties, since some astrocyte-secreted molecules such as thrombospondins induce the formation of structurally mature but functionally silent synapses because of a lack of AMPA receptors (AMPA receptors) (Christopherson et al., 2005), whereas others molecules such as Glypicans are involved in the functional maturation of synapses by facilitating the insertion of AMPA receptors in the postsynaptic membrane (Allen et al., 2012).

However, the formation of synapses on adult-born neurons differs from developmental synaptogenesis on two major aspects: first, during perinatal development, pre- and postsynaptic neurons mature concomitantly and newly formed dendritic spines contact immature axon terminals. In the adult hippocampus, in contrast, new spines preferentially contact pre-existing, functionally mature axon terminals that already synapse with other dendritic spines, thereby forming multiple-synapse boutons, where two or more postsynaptic neurons contact the same axonal terminal (Toni et al., 2007) (Figure S1A). Over time, multiple-synapse boutons transform into single-synapse boutons (Toni et al., 2007), suggesting that new spines replace more mature spines through a competitive process occurring between immature and mature neurons (Toni and Sultan, 2011). This process may play a role in the integration of new neurons into the adult neuronal network and regulate the functional implication of new neurons on the hippocampus (Bergami and Berninger, 2012; Tashiro et al., 2006). Furthermore, the



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synaptic integration of new neurons is strongly activity dependent. Indeed, during an early developmental stage, new neurons express NMDA receptor (NMDAR)- but not AMPAR-mediated glutamatergic synaptic transmission and are therefore functionally silent. At this stage, GABA_A receptor-mediated depolarization enables NMDAR activation, the expression of AMPA receptors, and synapse unsilencing, a mechanism that is also induced by exposure to an enriched environment (Chancey et al., 2013). Interfering with GABA_A-mediated depolarization results in impaired dendritic development and synaptic integration (Ge et al., 2006). Similarly, the cell-specific knockout of the essential NR1 subunit of the NMDA receptor drastically reduces the synaptic integration and survival of new neurons (Tashiro et al., 2006), which can be partially rescued by reducing overall NMDA receptor activity with injections of the specific antagonist CPP during a critical phase of maturation (Tashiro et al., 2006, 2007). Together, these studies indicate that the integration of new neurons depends on neuronal network activity and an activity-dependent competitive process between immature and mature neurons. Second, developmental synaptogenesis occurs in the presence of highly proliferative, immature astrocytes, which release pro-synaptogenic molecules (Allen, 2013; Allen et al., 2012; Christopherson et al., 2005; Pfrieger and Barres, 1997). In contrast, in the adult brain, the generation of glia is drastically reduced (Ge et al., 2012) and as a consequence, newborn neurons are principally in contact with mature, pre-existing astrocytes (Krzisch et al., 2015). Furthermore, the reduction in astrocytic signaling with age (Okamoto et al., 2011; Sun et al., 2013) suggests that mature astrocytes may be less amenable to synaptogenesis than immature astrocytes. It is therefore unclear to which extent adult astrocytes may contribute to synaptogenesis and play a role in this late phase of adult neurogenesis.

In the current study, we examined the role of exocytosis from astrocytes on the synaptic integration of adult-born hippocam-

pal neurons. We used two distinct conditional transgenic mice to manipulate exocytosis from astrocytes during the maturation stage of new neurons identified and birth dated by viral-mediated gene transfer (Figure S1B). We found that blocking vesicular release from astrocytes resulted in impaired dendritic maturation of new neurons: dendrites were shorter and less branched and bore fewer dendritic protrusions with an immature morphology. This effect was specific for adult-born neurons and restricted to the territories of impaired astrocytes, revealing a local regulation of synapse formation within specific dendritic segments newborn neurons. These impairments were accompanied by a reduced extracellular D-serine concentration and reduced NMDAR currents, which were alleviated by application of the co-agonist D-serine. Finally, we found that the chronic administration of D-serine restored the dendritic development of new neurons in the absence of astrocytic vesicular release. Together, these results indicate that D-serine released by astrocytes is required for the NMDAR-dependent maturation, synaptic integration, and survival of adult-born hippocampal neurons. These results underline the fundamental role of astrocytes in the regulation of adult brain plasticity.

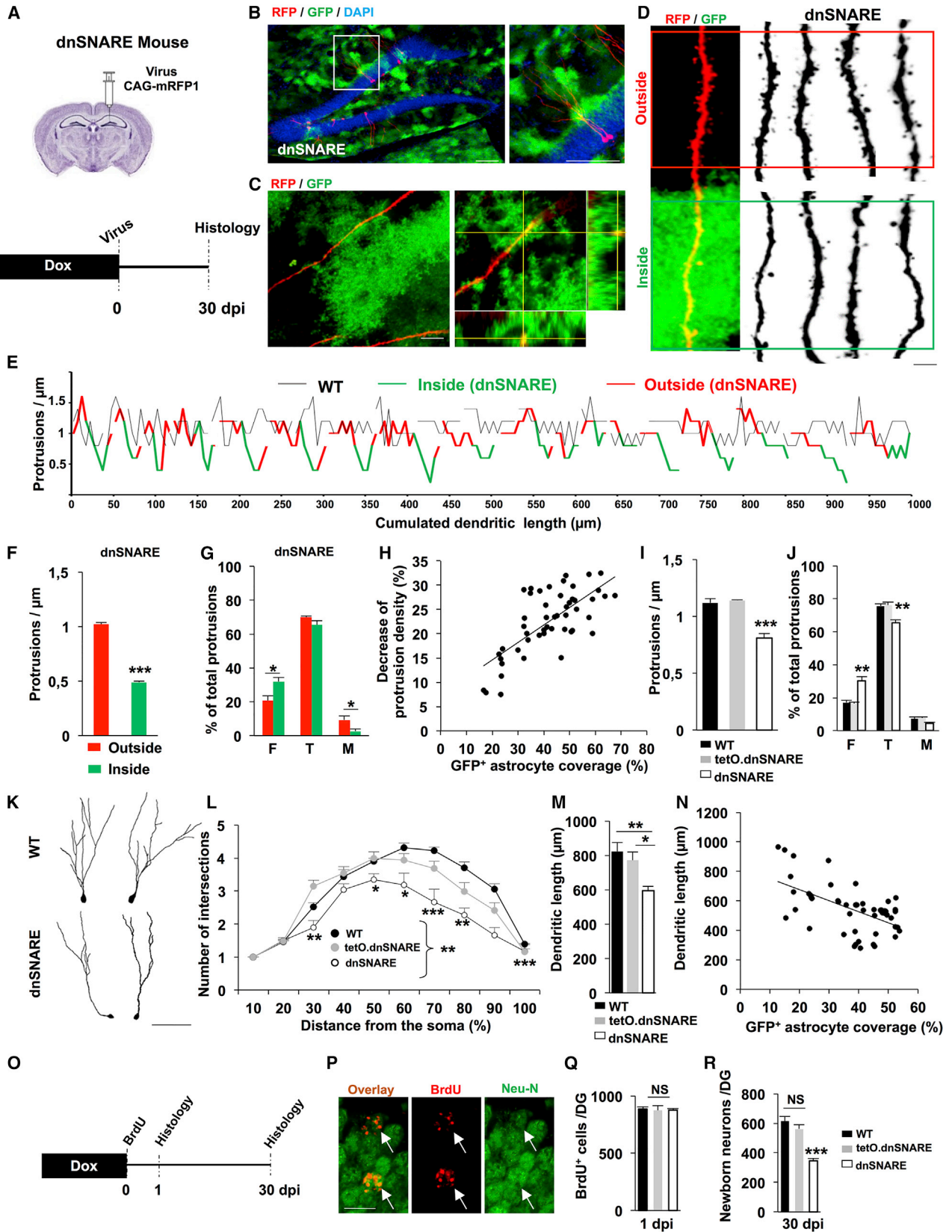
RESULTS

Inducible Inhibition of Vesicular Release in Astrocytes

To investigate the role of astrocytes on the maturation of adult-born hippocampal neurons, we used two complementary mouse models enabling the control of SNARE-dependent exocytosis in astrocytes. The first model, the iBot-Glast-CreER^{T2} mouse line, enables the tamoxifen-inducible, stable expression of the *Clostridium botulinum* toxin serotype B light chain (boNT/B) and the enhanced GFP in astrocytes (Figures 1A–1B; Figures S2A–S2C). boNT/B cleaves the vesicle-associated membrane synaptobrevin-2, a component of the SNARE complex, resulting in

Figure 1. BoNT Expression in Astrocytes Impairs the Maturation and Survival of New Neurons as Well as Dendritic Spine Formation

- (A) Confocal micrographs of the dentate gyrus of iBot-Glast-CreER^{T2} mice, before and 3 weeks after the induction of transgene expression with tamoxifen (Tam) injections showing the mosaic pattern of transgene expression (scale bars: left and middle, 100 μ m; right, 50 μ m).
- (B) Confocal micrograph of GFAP immunostaining (red) on an iBot-Glast-CreER^{T2} Tam-injected mouse hippocampal section showing the mosaic pattern of transgene expression. Scale bar: 50 μ m.
- (C) Experimental timeline: iBot-Glast-CreER^{T2} (n = 5 mice) were injected with BrdU (3 injections IP, 100 mg/kg every 2 hr) followed 1 day later by an intra-hippocampal injection with a RFP-encoding moloney virus (day 0). Transgene expression was induced by daily injections of Tam from 7 to 11 days post virus injection (dpi) and hippocampal slices were analyzed at 30 dpi. Control animals consisted of tamoxifen-injected animals (Ctrl+Tam: n = 3 monogenic iBot + 2 WT littermate mice) and animals injected with vehicle (Ctrl No Tam: n = 2 iBot-Glast-CreER^{T2} + 1 WT mice).
- (D) Confocal micrographs of spiny dendrites from new neurons in iBot-Glast-CreER^{T2} injected with Tam intersecting with (inside) and exiting (outside) transgene-expressing astrocytes. Scale bar: 5 μ m.
- (E) Dendritic protrusion density on adult-born neurons in dendritic segments inside and outside of transgene-expressing astrocytes (bilateral Student's t test, ***p < 0.001).
- (F) Proportion of protrusions with filopodia (F), thin (T), and mushroom (M, see Experimental Procedures section) morphologies. The proportion of filopodia was greater (bilateral Student's t test, ***p < 0.001) and the proportion of mushroom spines was smaller (Mann-Whitney test, *p < 0.05) in dendritic segments intersecting transgene-expressing astrocytes in iBot-Glast-CreER^{T2} injected with Tam.
- (G) Confocal micrographs of dendrites of newborn neurons from iBot-Glast-CreER^{T2} injected with tamoxifen (iBot-Glast + Tam) and control mice. Scale bar: 5 μ m.
- (H) The global dendritic protrusion density on new neurons was smaller in iBot-Glast + Tam than control mice (one-way ANOVA, $F_{2,12} = 22,2036$, p = 0,0002; post hoc Tukey's HSD test, *p < 0.05).
- (I) The proportion of mushroom spines on adult-born neurons was smaller in iBot-Glast + Tam than in control mice (filopodia [F] one-way ANOVA $F_{2,12} = 1.9260$, p = 0.1961, thin [T] one-way ANOVA $F_{2,12} = 0.042$, p = 0.9590, and mushroom [M], one-way ANOVA $F_{2,12} = 7.47$, p = 0.0103; post hoc Tukey's HSD test, *p < 0.05).
- (J) Confocal micrographs (left) and 3D reconstructions (right) of RFP⁺ new neurons at 30 dpi. Scale bar: 20 μ m.
- (K) Newborn neurons dendritic length (one-way ANOVA; post hoc Tukey's HSD test, *p < 0.05; **p < 0.01).
- (L) Scholl analysis of dendritic complexity (two-way ANOVA with repeated-measures $F_{2,10} = 1.18$, p = 0.34).
- (M) Number of new neurons (co-expressing BrdU and Neu-N) in the DG (one-way ANOVA $F_{2,12} = 12.55$, p = 0.0019; post hoc Tukey's HSD test, **p < 0.01). Values represent the mean \pm SEM.



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impaired vesicular release (Schiavo et al., 1992; Slezak et al., 2012). The second mouse model, the dnSNARE mouse line, expresses the dominant-negative SNARE domain of the synaptobrevin-2 protein and GFP in astrocytes under the control of a tet-off tetracycline transactivator system controlled by doxycycline (Dox; Figures S2D–S2G) (Pascual et al., 2005). To directly assess the effect of this construct on vesicular release, we examined exocytosis in vitro on hippocampal astrocytes using total internal reflection fluorescence (TIRF) microscopy. When compared to wild-type (WT), astrocytes from dnSNARE mice showed a 91% reduction in the number of fusion events (Figures S2H–S2J). Thus, interfering with synaptobrevin-2 function in astrocytes resulted in a drastic reduction of vesicular fusion.

Inhibition of Astrocytic SNARE-Mediated Release Locally Impairs the Dendritic Maturation and Survival of Adult-Born Neurons

We then assessed the maturation of adult-born neurons in mice expressing boNT/B or dnSNARE in astrocytes. Newly formed cells were identified using injections of either the cell proliferation tracer 5-Bromo-2-deoxyuridine (BrdU) or an engineered Moloney murine leukemia virus encoding for the red fluorescent protein (RFP) in dividing cells (see Supplemental Experimental Procedures). In each mouse line, we labeled newly formed cells before the induction of transgene expression in astrocytes: for the iBot mouse line, new neurons were labeled 7–8 days before tamoxifen injections (Figure 1C) and for the dnSNARE mouse line, Dox was withdrawn immediately after the labeling of new neurons (Figure 2A). The neuronal progenies were analyzed 30 days post-injection (dpi). Therefore, the labeled neural progenitors could proliferate in absence of transgene expression, whereas their progenies, newly formed neurons, matured in the presence of astrocytes expressing the respective transgenes. Also, since adult neural stem cells express GLAST and GFAP (Kronenberg et al., 2003), this specific experimental design

was important to avoid transfer of transgene expression in the labeled new neurons. Consistently, immunostaining against GFP followed by both confocal microscopy and electron microscopy (EM) in the molecular layer and the granule cell layer of the dentate gyrus (DG) revealed a strong expression of GFP in astrocytes but not in neurons or other cell types (Figures S2B, S2C, and S3). Notably, $35\% \pm 12\%$ ($n = 5$ mice) of GFAP-immunostained astrocytes co-expressed GFP in iBot-Glast-CreER^{T2} Tam-injected mice (Figures 1A and 1B) and $49.9\% \pm 1.3\%$ in dnSNARE mice ($n = 5$ mice) (Figures S7A–S7D), indicating a mosaic expression of transgenes. Therefore, astrocytes not expressing the transgenes could be used as internal controls. Since astrocytes occupy distinct, non-overlapping territories (Bushong et al., 2002, 2004) and the dendrites of any given adult-born neuron crossed the territories of both transgene-expressing astrocytes and normal astrocytes, we assessed whether the locally confined presence of the transgene affected protrusions along a given dendrite. Remarkably, dendritic protrusion density and protrusion head diameter were significantly reduced in the dendritic segments that crossed transgene-expressing astrocytes in both iBot-Glast-CreER^{T2} Tam-injected mice (Figures 1D–1F) and dnSNARE mice (Figures 2B–2G). In contrast, segments of the same neurons that extended outside of transgene-expressing astrocytic territories displayed comparable spine density to new neurons generated in control littermate mice (bilateral t test $p > 0.05$ for iBot-Glast-CreER^{T2} Tam-injected mice, ANOVA $F(2, 14) = 3.34$; $p > 0.05$ for dnSNARE animals). The reduction of protrusion density and maturation observed in both mice was not due to GFP expression in astrocytes, since new neurons formed in GFAP-GFP transgenic mice, which lack dnSNARE or boNT/B expression, showed homogeneous protrusion density along their dendrites (Figure S4). Therefore, the dendrites of new neurons displayed reduced spine density and spine maturation exclusively in segments that crossed transgene-expressing astrocytes and this reduction

Figure 2. dnSNARE Expression in Astrocytes Impairs the Maturation and Survival of New Neurons as Well as Dendritic Spine Formation

- (A) Experimental timeline: Dox was withdrawn immediately after virus injection and mice were analyzed 30 days later.
- (B and C) Confocal micrographs of dendritic segments of new neurons (red) extending inside and outside of transgene-expressing astrocytes (green), with high- (B) and low- (C) magnification views.
- (D) Confocal micrographs of dendrites of new neurons in dnSNARE mice. Right: dendrites that extend into transgene-expressing astrocytes (inside) as well as into astrocytes not expressing the transgenes (outside). Note the thinner dendritic diameter and lower spine density of the dendrites upon entering transgene-expressing astrocyte territories. Scale bars: (B) 100 μm , (C) 10 μm , and (D) 5 μm .
- (E) Line plot of the dendritic protrusion density along several dendritic segments for a total cumulated length of 1,000 μm . Individual dendritic segments are separated by a blank space. Note the heterogeneity in spine density along dendrites in dnSNARE mice.
- (F and G) Summaries of protrusions density (F; bilateral Student's t test) and proportion of protrusions with filopodia (F), thin (T), and mushroom (M) morphologies in the different segments (G; bilateral Student's t test for F and M and Welch's t test for T, * $p < 0.05$).
- (H) Correlation between the proportion of dendritic length of new neurons that intersects transgene-expressing astrocytes and the decrease in protrusion density (Spearman's $\rho = 0.6304$; $p < 0.0001$).
- (I and J) Histogram of the global protrusion density (I; one-way ANOVA: $F_{2,14} = 19.22$, $p < 0.0001$ followed by post hoc Tukey's HSD) and morphology (J; one-way ANOVA followed by post hoc Tukey's HSD: filopodia [F] $F_{2,14} = 14.67$, $p = 0.0006$; thin [T] $F_{2,14} = 11.64$, $p = 0.0015$; and mushroom [M] $F_{2,14} = 3.71$, $p = 0.0555$) of newborn neurons in WT, single transgenic, and dnSNARE mice.
- (K) 3D reconstructions of new neurons from WT and dnSNARE mice. Scale bar: 50 μm .
- (L) Scholl analysis of dendritic branching (two-way ANOVA $F_{2,12} = 12.25$, $p = 0.0013$ followed by post hoc Tukey's HSD).
- (M) Dendritic length of new neurons (one-way ANOVA $F_{2,14} = 6.5917$, $p = 0.0117$ followed by post hoc Tukey's HSD).
- (N) Correlation between dendritic length and coverage by transgene-expressing astrocytes (Spearman's $\rho = -0.4734$; $p = 0.0005$).
- (O) Experimental timeline: Dox was withdrawn immediately after BrdU injections and mice were analyzed 1 or 30 days later.
- (P) Confocal micrographs of BrdU-Neu-N labeling of new neurons at 30 dpi; scale bars: 10 μm .
- (Q) Number of BrdU⁺ cells at 1 dpi (one-way ANOVA $F_{2,12} = 0.04$, $p = 0.9517$).
- (R) Number of BrdU⁺-NeuN⁺ surviving neurons at 30 dpi (one-way ANOVA $F_{2,12} = 31.10$, $p < 0.0001$ followed by post hoc Tukey's HSD). For all, values represent the means \pm SEM. NS: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

followed the shape of transgene-expressing astrocytes' territories (Figure 2E). This observation indicates that astrocytes locally control spine density in specific segments of the growing dendritic tree.

We next examined whether the sum of local reductions in dendritic spine density resulted in reduction of global spine density on new neurons. In dnSNARE animals, individual neurons intersected with the territories of several transgene-expressing astrocytes, averaging $38.3\% \pm 1.7\%$ of the total length of their dendritic arbor ($n = 50$ neurons, range: 17% to 67%). Since spine density was 0.49 spines/ μm in these territories (Figure 2F) and spine density on adult-born neurons in WT mice averaged 1.12 spines/ μm (Figure 2I), the predicted total spine density on entire newborn neurons in dnSNARE mice was expected to drop to 0.87 spines/ μm , which is remarkably similar to the measured total spine density (0.81 ± 0.1 spines/ μm ; Figure 2I). Similarly, global spine morphological maturation was impaired on new neurons in dnSNARE mice (Figure 2J). Furthermore, the reduction in total dendritic protrusion density on individual neurons was correlated with the proportion of their dendritic arbor covered by transgene-expressing astrocytes (Figure 2H). Similar observations were done in iBot-Glast-CreER^{T2} Tam-injected mice: the global density of dendritic protrusions and the proportion of protrusions with a mature morphology were reduced compared to control, tam-injected animals, and control animals injected with vehicle (No Tam; Figures 1G–1I). Together, these results indicate that blocking astrocyte exocytosis induced local reductions of spine density and maturation, which resulted in reduction of total spine density on new neurons.

Next, we examined dendritic development of new neurons using Scholl analysis. In dnSNARE mice, the dendritic arborization of individual neurons was decreased as compared to wild-type or single transgenic mice (Figures 2K and 2L). Dendritic length was also decreased and was inversely correlated with the proportion of their arbor that intersected transgene-expressing astrocytes (Figures 2M and 2N), consistently with the observation that the sum of local impairments of dendritic maturation reduced global maturation of new neurons. In iBot-Glast-CreER^{T2} Tam-injected mice, the dendritic length of newborn neurons but not their arborization was also reduced (Figures 1J–1L). Thus, blocking vesicular release from astrocytes reduced the dendritic maturation of new neurons.

The survival of adult-born neurons depends on their synaptic integration during a critical period (Tashiro et al., 2006, 2007). We therefore examined whether the reduction of dendritic maturation on newborn neurons in dnSNARE mice was accompanied by alterations in their survival. Mice were injected with BrdU and Dox was withdrawn shortly thereafter. The number of newly divided cells was analyzed by immunohistochemistry 1 day later (Figure 2O). The number of BrdU⁺ cells was not different between dnSNARE and control mice (Figures 2P and 2Q), indicating that cell proliferation was not altered shortly after Dox withdrawal, consistently with a latency in transgene expression after Dox withdrawal (Figures S2F and S2G). When mice were analyzed two weeks after BrdU injections and Dox removal, no difference in BrdU⁺ cells density was observed between dnSNARE and WT mice (data not shown, bilateral *t* test $p > 0.05$). In contrast, when BrdU immunostaining was assessed

1 month after injection and doxycycline removal, cells that divided before Dox removal but matured after transgene expression showed a 40% decrease in survival in dnSNARE compared to WT mice (Figure 2R). Similar observations were found with the iBot mouse line (Figure 1M). Thus, the impaired dendritic maturation was accompanied with reduced survival of new neurons.

dnSNARE Expression in Astrocytes Reduces the Synaptic Integration of New Neurons

To assess whether dendritic protrusions from adult-born neurons formed mature synapses with presynaptic boutons, new neurons were identified with a GFP-encoding virus, followed by serial section immuno-EM. Within the territories of dnSNARE transgene-expressing astrocytes as well as in territories of astrocytes not expressing the transgenes, all the observed dendritic spines from new neurons formed synapses, defined by the presence of presynaptic vesicles accumulation, a widening of the synaptic cleft, cleft material, and a postsynaptic density (Figures 3A–3C). Furthermore, the proportion of spines forming multiple synapse boutons (MSBs) was similar between territories in mature neurons (GFP⁻ spines; 42.3% in territories of transgene-expressing astrocytes, 46.1% outside transgene-expressing territories), as well as in newborn neurons (73% versus 71%, respectively, data not shown), consistent with previous observations (Toni et al., 2007). However, consistent with confocal microscopy observations, the protrusion head volume on newborn neurons was significantly smaller in dendritic segment within the territory of transgene-expressing astrocyte than in dendritic segments outside these territories or in WT mice (nonparametric Wilcoxon test, $p = 0.003$, data not shown).

Direct contact with astrocytes regulates the stabilization and maturation of dendritic spines (Nishida and Okabe, 2007). To examine whether dnSNARE expression in astrocytes may interfere with the fine structure of their processes and the contact they establish with new neurons, we examined the proportion of surface area of synapses that was ensheathed by astrocytes. On both GFP⁺ newborn neurons and GFP⁻ neurons, transgene-expressing astrocytes ensheathed the same proportion of synaptic surface area as astrocytes not expressing the transgene and wild-type astrocytes (Figures 3D and 3E). This indicates that the effect of dnSNARE-expressing astrocytes on new neurons' maturation was due to impaired astrocytic function and not to impaired structural integrity.

We next assessed the functional integration of newborn neurons in dnSNARE mice. Impaired dendritic maturation may interfere with the functional integration of adult-born neurons and result in functional deficits of excitatory synaptic transmission. Since AMPA receptors are absent from filopodia and their density correlates with dendritic protrusion head diameter (Matsuzaki et al., 2001; Takumi et al., 1999), the combination of decreased dendritic protrusion head diameter (15%, data not shown), decreased dendritic protrusion density (28%, Figure 2I), and decreased dendritic length (28%, Figure 2M) observed on adult-born neurons in dnSNARE mice may result in an overall reduction of glutamatergic synaptic input. To assess synaptic transmission in newborn neurons, we performed whole-cell patch-clamp recordings in acute brain slices from adult WT or dnSNARE mice and measured synaptic currents in response to

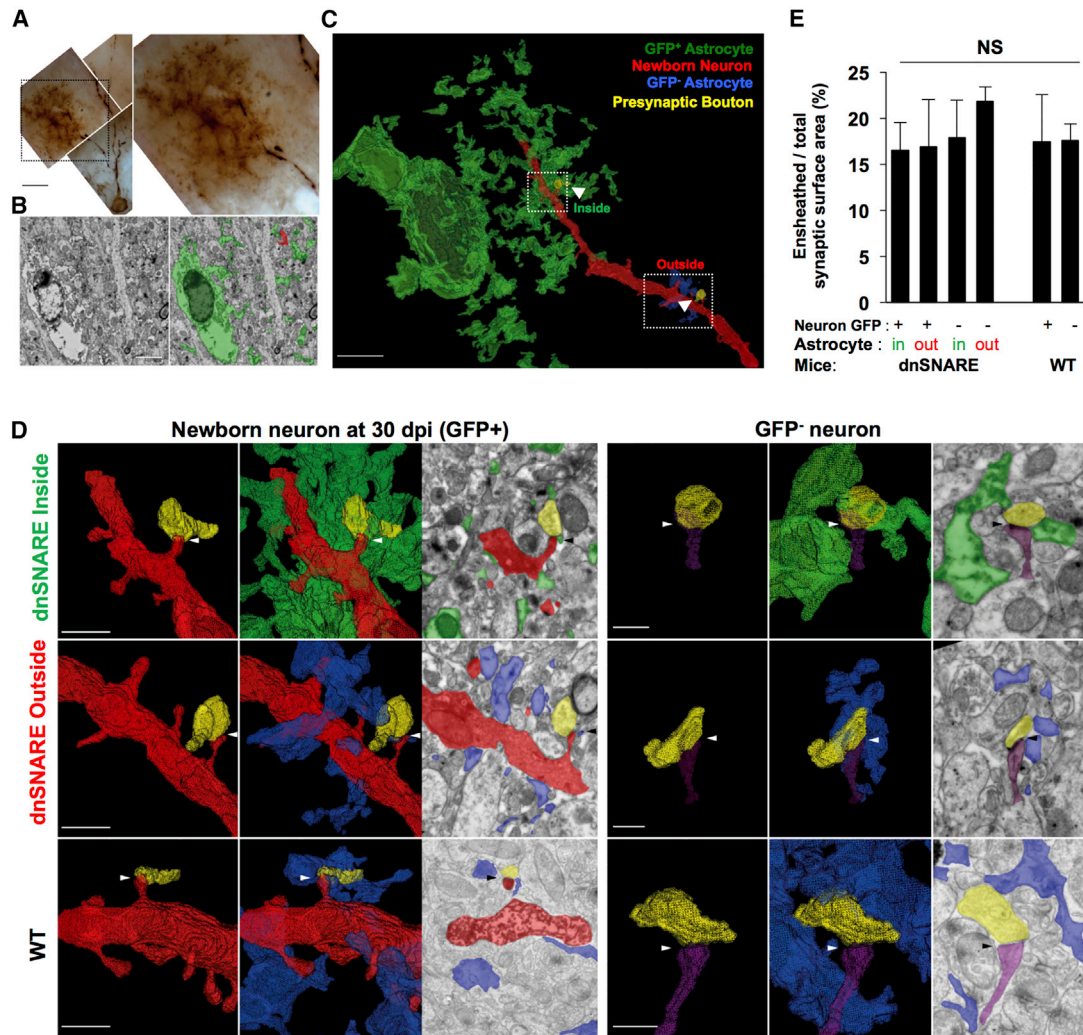


Figure 3. The Fine Structure of Perisynaptic Processes of dnSNARE-Expressing Astrocytes Is Similar to WT

(A) Peroxidase-labeled new neuron dendrite (30 dpi) intersecting the territory of an immunoperoxidase-labeled transgene-expressing astrocyte that was further examined with electron microscopy.

(B) Electron micrographs of the same region, showing the labeled dendrite (red) and astrocyte (green).

(C) This astrocyte and dendrite were serially sectioned and 3D reconstructed. Two axons (yellow) forming synaptic contacts (arrowheads) with adult-born neuron dendritic spines (red), inside and outside of the transgene-expressing astrocyte (green). Processes of an astrocyte not expressing the transgene (blue) have been reconstructed for comparison. Scale bars: (A) 20 μm , (B) 10 μm , and (C) 5 μm .

(D) 3D reconstructions from electron micrographs of dendritic spines and their perisynaptic processes. In dnSNARE and WT mice, newborn neurons (GFP⁺) are shown in red and random neurons (GFP⁻) in purple. Also shown are some axon terminals (yellow) and all astrocytic perisynaptic processes (green in GFP⁺ astrocytes, blue outside GFP⁺ astrocytes and in WT mice). Scale bars: 0.5 μm .

(E) Histogram showing the proportion of synaptic surface area (from new neuron or from random neurons) that is ensheathed by dnSNARE-expressing astrocyte, astrocyte not expressing dnSNARE, or WT astrocyte (Kruskal-Wallis, $p = 0.6185$). Each value represents the mean \pm SEM.

extracellular perforant-path stimulation. Adult-born neurons in dnSNARE mice, as compared to WT mice, displayed a significant reduction of AMPA receptor-mediated excitatory postsynaptic currents (EPSCs) by about 70% (Figures 4A and 4B). NMDA receptor-mediated EPSCs were also significantly reduced, but less so than AMPAR EPSCs, resulting in a two-times larger NMDA EPSC/AMPA EPSC ratio (Figures 4C and 4D). The reduced amplitude of evoked synaptic currents suggests that the young neurons might have formed fewer excitatory synapses. To assess the number of functional synaptic release sites,

we recorded miniature EPSCs (mEPSCs) in the presence of TTX. Newborn neurons in dnSNARE mice showed reduced frequency and amplitude of mEPSCs, without changes in mEPSC decay time course, consistent with a lower dendritic spine density and a higher proportion of immature synapses (Chancey et al., 2014; Schmidt-Salzmann et al., 2014) (Figures 4E–4J). Altogether, morphological and electrophysiological data converge to the conclusion that inhibition of exocytosis in astrocytes reduces the number and size of functionally mature synapses in newborn neurons.

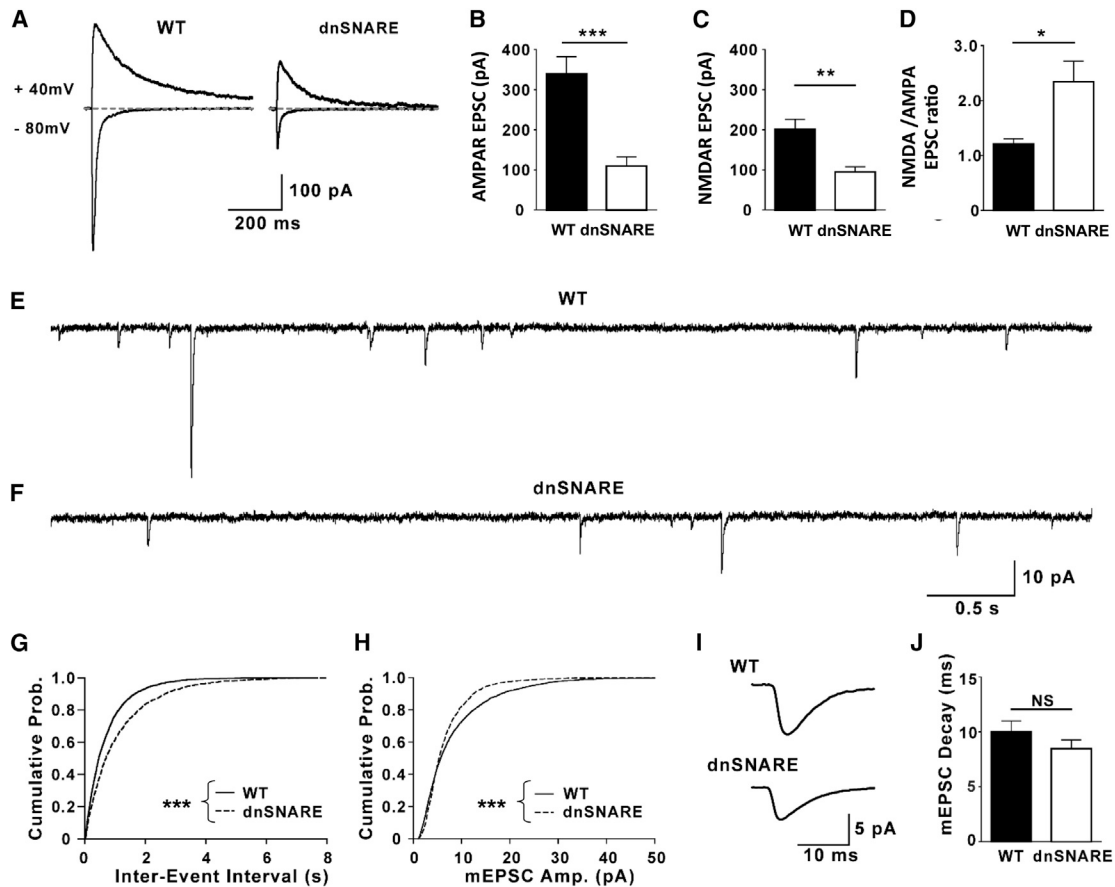


Figure 4. Impaired NMDA- and AMPA-Mediated Synaptic Transmission in Newborn Neurons in dnSNARE Mice

(A) AMPAR- and NMDAR-mediated excitatory postsynaptic currents (EPSCs; recorded at -80 mV and $+40$ mV, respectively) in adult-born neurons from WT and dnSNARE mice at 30 dpi. Dotted line indicates 0 pA.

(B) Histogram of the AMPAR-EPSCs in new neurons from dnSNARE and WT mice (WT: 340.5 ± 42.0 pA, dnSNARE: 109.5 ± 23.0 pA; $p = 0.0005$, non-parametric Mann Whitney test; $n = 9$ and 11 , respectively).

(C) Histogram of the NMDAR-EPSC currents in adult-born neurons from WT and dnSNARE mice at 30 dpi (WT: 201.3 ± 24.9 pA, dnSNARE: 95.0 ± 12.8 pA; $p = 0.005$, nonparametric Mann Whitney test; $n = 9$ and 11 for WT and dnSNARE, respectively).

(D) NMDA EPSC/AMPA EPSC ratio in adult-born neurons from WT and dnSNARE mice at 30 dpi (WT: 1.21 ± 0.10 , dnSNARE: 2.35 ± 0.37 ; $p = 0.01$, nonparametric Mann Whitney test; $n = 9$ and 11 for WT and dnSNARE, respectively).

(E and F) Example traces of 6 s recordings of miniature EPSCs (mEPSCs) from newly generated neurons in WT (E) and dnSNARE (F) mice, as indicated.

(G) Cumulative probability plot of inter-event intervals (IEIs) of all mEPSCs recorded from newborn neurons in WT and dnSNARE mice, respectively. Longer average mEPSC interval was detected in dnSNARE mice (WT: 0.71 ± 0.01 s, total 3,460 events from 8 cells, dnSNARE: 1.10 ± 0.03 s, total 1,606 events from 6 cells. $p < 0.001$, nonparametric Kolmogorov-Smirnov test).

(H) Cumulative probability plot of mEPSCs amplitude from newborn neurons in WT and dnSNARE mice, respectively (WT: 8.37 ± 0.13 pA, total 3,468 events from 8 cells, dnSNARE: 6.87 ± 0.15 pA, total 1,612 events from 6 cells. $p < 0.001$, nonparametric Kolmogorov-Smirnov test).

(I) Typical average mEPSCs obtained from single newborn neurons in a WT or dnSNARE mouse.

(J) Histogram of the mEPSC decay time constant in new neurons of WT and dnSNARE mice (WT: 8.48 ± 0.79 ms, dnSNARE: 10.03 ± 0.96 ms. $p = 0.23$, nonparametric Mann Whitney test. $n = 6$ and 8 for WT and dnSNARE mice, respectively). Each value represents the mean \pm SEM.

dnSNARE Expression in Astrocytes Does Not Interfere with the Stability of Mature Synapses or with Synapses on Mature Neurons

To examine whether the reduced dendritic maturation of newborn neurons was transient, we examined them at 21 and 90 dpi (Figures S5 and S6, respectively). Similar to 30 dpi, dendritic protrusion density and diameter in segments intersecting transgene-expressing astrocytes was reduced at 21 and 90

dpi in dnSNARE as compared to WT mice, indicating that astrocytic impairment resulted in early-onset and long-lasting maturation defects in newborn neurons. Furthermore, the survival of BrdU⁺/NeuN⁺ new neurons in dnSNARE mice was reduced by 20% at 21 dpi, 43% at 30 dpi (Figure 2R), and 45% at 90 dpi, indicating that astrocytic vesicular release was particularly important for the survival of new neurons during the entire period of excitatory synapse formation.

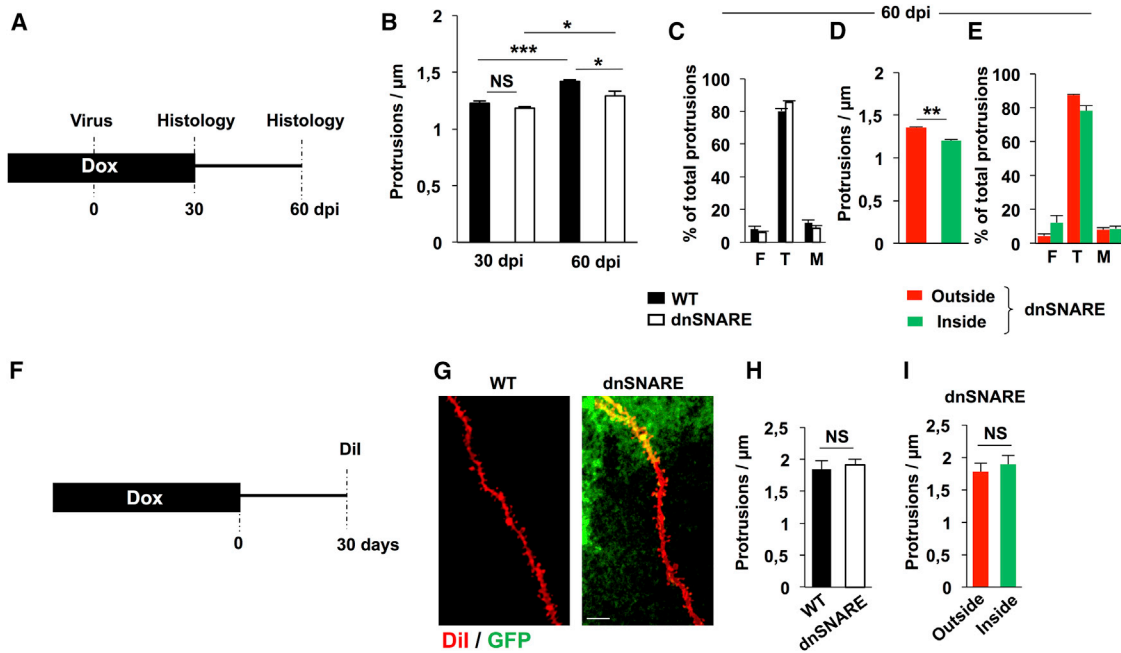


Figure 5. Pre-established Spines Are Not Destabilized by dnSNARE Expression in Astrocytes

(A) Experimental timeline: Moloney virus was injected 30 days before Dox removal. Mice were sacrificed and examined at 30 or 60 days post injection (dpi). (B) Dendritic protrusion density at 30 and 60 dpi (one-way ANOVA $F_{3,15} = 18.61$, $p < 0.0001$ followed by post hoc Tukey's HSD). (C) Dendritic protrusion morphology on new neurons in dnSNARE or WT mice at 60 dpi. (D) Dendritic protrusion density at 60 dpi in dendritic segments inside or outside of transgene-expressing astrocytes. (E) Dendritic protrusion morphology at 60 dpi in dendritic segments inside or outside of transgene-expressing astrocytes. (F) Experimental timeline: Dox was removed and 30 days later, mice were sacrificed and random dendrites were labeled with the lipophilic dye Dil. (G) Confocal micrographs of Dil-labeled dendrites (red). Scale bar: 5 μm . (H and I) Dendritic protrusion density on entire Dil-labeled dendrites (H) and on dendritic segments inside or outside of transgene-expressing astrocytes (I). For all, each value represents the mean \pm SEM. Bilateral Student's t test for all. NS: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

The observed reduction in spine density may be due to impaired dendritic spine formation, impaired stabilization, or both. To test these possibilities, we first assessed whether spines formed prior to transgene expression may be destabilized upon impaired astrocytic function. To this aim, we induced dnSNARE expression by removing Dox 30 days after viral infection, a time point when new neurons have already formed most dendritic protrusions (Toni et al., 2007) and neuronal morphology was analyzed immediately (i.e., at 30 dpi) and 30 days later (60 dpi). At 30 dpi, spine density was similar between WT and dnSNARE mice (Figures 5A and 5B), indicating that spine formation was not altered upon transgene repression by Dox. At 60 dpi, however, the density and maturation of dendritic protrusions of adult-born neurons was slightly but significantly decreased in dnSNARE compared to WT mice and this reduction was observed only in dendritic segments intersecting transgene-expressing astrocytes (Figures 5B–5E). However, in both mice, protrusion density at 60 dpi was greater than at 30 dpi (bilateral t test $p < 0.05$), suggesting that blocking vesicular release from astrocytes did not prune dendritic protrusions formed prior to the induction but reduced the formation of new protrusions.

Next, we tested whether dendritic protrusions from mature neurons may be affected by impaired astrocytic function. Thirty

days after Dox removal, random dendrites (i.e., principally from mature granule neurons) of dnSNARE and WT mice were fluorescently labeled using the application of the lipophilic dye Dil in the molecular layer. In contrast to newly-generated adult-born neurons, we found no difference in dendritic protrusion density in mature neurons between groups of mice and between dendritic segments inside and outside territories of transgene-expressing astrocytes (Figures 5F–5I).

Similarly, GFP⁻ synapse density (i.e., synapses from random neurons) measured by serial-section EM and unbiased stereology was similar between territories of transgene-expressing astrocytes and the territories of astrocytes not expressing the transgene (total of synapses analyzed: 696; t test, $p > 0.05$, data not shown). Furthermore, serial-section EM and 3D reconstructions of GFP⁻ spines inside and outside the territories of transgene-expressing astrocytes had similar spine head volume as in WT mice (total number of spines analyzed: 46; Kruskal Wallis, $p = 0.1134$, data not shown).

These results indicate that dnSNARE expression in astrocytes did not interfere with the stability or the morphology of pre-established synapses from both mature and adult-born neurons. Thus, the reduction in dendritic protrusion density in the territories of transgene-expressing astrocytes was specific to new neurons.

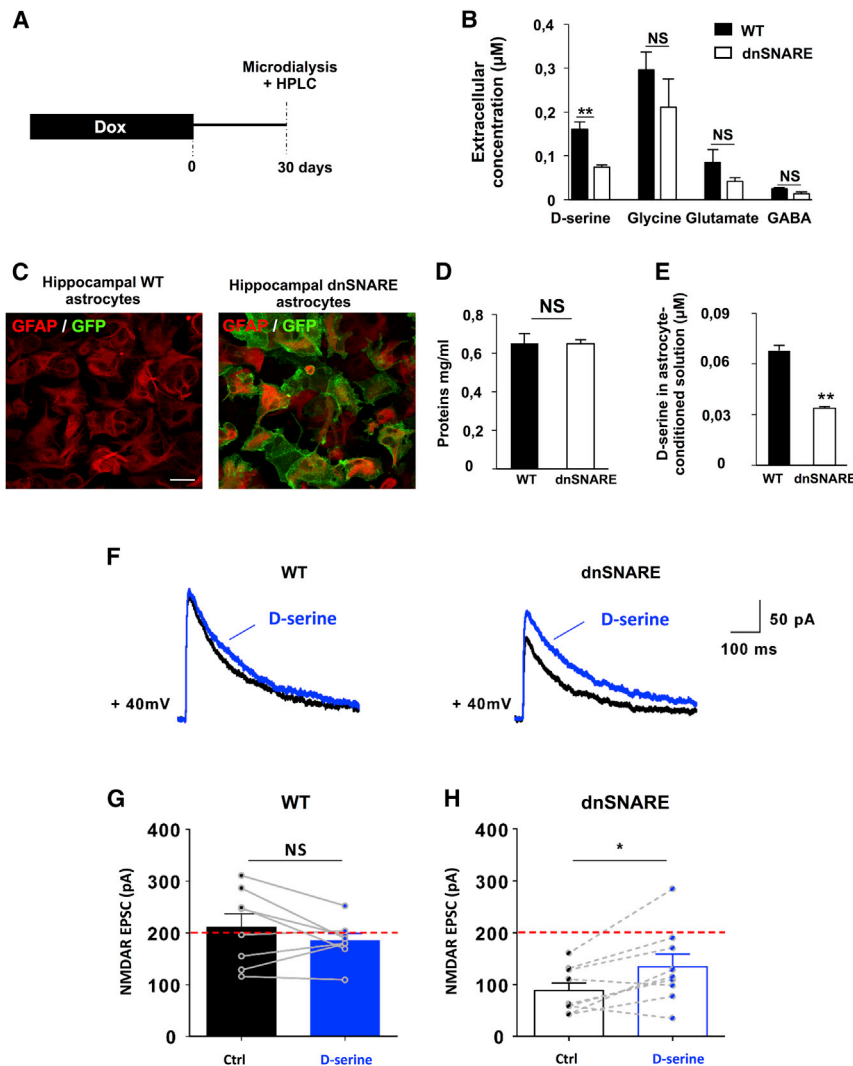


Figure 6. In dnSNARE Mice, Extracellular D-Serine Is Reduced and Its Exogenous Application Partially Rescues NMDAR EPSCs in Newborn Neurons

(A) Experimental timeline: microdialysis was performed in the DG 30 days after Dox withdrawal.

(B) Extracellular concentration of D-serine, glycine, glutamate, and GABA in the DG of dnSNARE and WT mice analyzed by HPLC ($n = 5-6$ mice per group, nonparametric Wilcoxon test for each molecule).

(C) Sample confocal images of GFAP immunostained hippocampal astrocytic culture from WT and dnSNARE mice. In dnSNARE astrocytic culture 60% ($\pm 8.5\%$) of astrocytes expressed GFP. Scale bar: 20 μm .

(D) Concentration of proteins (mg/ml) measured in astrocytic culture used to condition the medium analyzed by HPLC (3 samples per group, nonparametric Wilcoxon test).

(E) HPLC analysis of D-serine concentration in medium conditioned by dnSNARE and WT astrocytes (3 samples per group, nonparametric Wilcoxon test).

(F) Example traces of NMDAR-EPSCs (recorded at +40 mV) prior to (in black) and during 50 μM D-serine application (in blue) in newborn neurons from WT and dnSNARE mice at 30 dpi.

(G) NMDAR-EPSC amplitude prior to and during D-serine application in newborn neurons from WT mice at 30 dpi (211.1 ± 25.9 pA and 184.8 ± 14.0 pA, $p = 0.06$, non-parametric Wilcoxon matched pairs test, $n = 8$ pairs).

(H) NMDAR-EPSC amplitude prior to and during D-serine application in newborn neurons from dnSNARE mice at 30 dpi (88.4 ± 14.8 pA and 134.1 ± 24.2 pA, nonparametric Wilcoxon matched pairs test, $n = 9$ pairs). The dashed red line in (G) and (H) indicates the mean NMDAR-EPSC amplitude in newborn neurons from WT mice, as shown in Figure 4C. Values represent the mean \pm SEM. NS, not significant; * $p < 0.05$, ** $p < 0.01$.

D-serine Mediates the Astrocyte-Dependent Synaptic Integration of Adult-Born Neurons

Finally, we assessed the mechanisms involved in the regulation of astrocyte-dependent synaptic integration of adult-born neurons. During their development, adult-born neurons transition through a silent stage, during which glutamatergic transmission is mediated by NMDA receptors only. During this period, the pairing of GABA-mediated depolarization and synaptic stimulation enables the activity-dependent expression of AMPA receptors and synapse unsilencing (Chancey et al., 2014), whereas the knockdown of the essential NR1 subunit of the NMDA receptor results in the drastic elimination of new neurons (Tashiro et al., 2006). Astrocytes release several molecules known to target neurotransmitter receptors (Araque et al., 2014). We therefore examined whether dnSNARE expression in astrocytes affected the extracellular level of agonists of glutamatergic and GABAergic receptors, namely D-serine, Glycine, Glutamate and GABA, using microdialysis in the DG followed by HPLC. One month after Dox removal, no

difference was observed in the extracellular concentrations of Glutamate, Glycine, and GABA in the DG of dnSNARE mice as compared to WT animals. In contrast, the extracellular concentration of D-serine was significantly decreased in dnSNARE mice (Figures 6A and 6B). D-serine is an NMDA receptor co-agonist, known to be released by astrocytes in a SNARE-dependent manner (Fellin et al., 2009; Henneberger et al., 2010; Martineau et al., 2013). However, D-serine can also be synthesized and released by neurons (Kartvelishvily et al., 2006). To directly assess the astrocytic release of D-serine, we analyzed the concentration of D-serine in a buffered saline solution (HEPES Tyrode's solution) conditioned by purified hippocampal astrocytes. Consistent with our in vivo observations, D-serine concentration was significantly reduced in medium conditioned by dnSNARE-expressing astrocytes as compared to medium conditioned by WT astrocytes (Figures 6C–6E). This effect was not due to difference in culture conditions, since protein content was similar between groups (Figure 6D). These results indicate that blocking astrocytic

exocytosis was accompanied by a reduction of extracellular D-serine in the DG of dnSNARE mice.

The reduced extracellular D-serine concentration in dnSNARE mice may underlie the reduced NMDAR EPSCs observed in new neurons. To test this possibility, we measured the effect of exogenous D-serine application on synaptic NMDAR EPSCs in newborn neurons in acute brain slices from adult mice at 30 dpi. D-serine (50 μ M) significantly increased NMDAR EPSCs in newborn neurons in dnSNARE mice, but not in WT mice (Figures 6F–6H).

Finally, to test the possibility that neuronal impairment in dnSNARE mice resulted from a reduction in D-serine release, we assessed the effect of a chronic administration of D-serine on the dendritic maturation of adult-born neurons in dnSNARE mice. Mice were injected with an RFP-expressing virus and withdrawn from Dox. Twenty-two days later, D-serine was injected daily for 8 days (50 mg/kg, i.p. from 22 to 29 dpi) and neuronal maturation was examined at 30 dpi (Figure 7A). Consistent with blood-brain barrier permeability to D-serine (Pernot et al., 2012), intraperitoneal D-serine injections cancelled the difference in extracellular brain concentration between dnSNARE and WT mice (Figure 7B). In dnSNARE mice, D-serine administration restored dendritic length and arborization complexity of adult-born neurons to WT levels (Figures 7C and 7D) as well as the density and the size of dendritic protrusions (Figures 7E–7G). D-serine also increased dendritic protrusion density on new neurons in WT mice (Figure 7E) but consistently with the observation that NMDAR co-agonists sites were depleted in dnSNARE mice, the effect of D-serine on spine density was greater in dnSNARE mice (56% increase) than in WT mice (33% increase). Similarly, the effect of D-serine on protrusion density was greater in dendritic segments crossing the territories of transgene-expressing astrocytes (inside; 110% increase) than outside (41%, Figures 7H–7J).

The percentage of GFP⁺ astrocytes and the density of GFAP⁺ cells were similar between treated and untreated mice (Figure S7), indicating that D-serine did not modify the number of astrocytes expressing the transgene or the total number of astrocytes.

Thus, the exogenous administration of D-serine restored defective dendritic maturation and formation of dendritic protrusions on newborn neurons in dnSNARE mice. Taken together, these results suggest an important role of astrocytic D-serine release in locally controlling synaptic integration of newly generated granule cells into the adult hippocampal circuitry.

DISCUSSION

This study provides direct *in vivo* evidence that astrocytes regulate the synaptic integration of new neurons in the adult hippocampus. By blocking exocytosis from a subset of astrocytes after the labeling and birth dating of a cohort of newborn cells, we examined the role of astrocytes in the maturation of new hippocampal neurons. As schematized in Figure S8, we found that within their territories, individual astrocytes regulate synapse formation on adult-born neurons in a D-serine-dependent manner. This effect is crucial for the dendritic maturation and functional integration of new neurons, as well as for the survival of these

cells during the critical period of their maturation. These results show that the maturation and synaptogenesis on adult-born neurons depends on cues provided by their direct cellular environment, namely astrocytes. These results have broad implications for the role of astrocytes in the adult brain and demonstrate that a molecule known to play a role in adult brain plasticity can also regulate the development of neurons generated in the adult brain.

Vesicular release from astrocytes and its role in neuronal function is a highly debated topic, principally as a result from the intricate communication and interdependence between these two cell types. Recently, a study from Fujita et al. (2014), echoed by an expert opinion review (Sloan and Barres, 2014), has cast doubt on the role of astrocytic vesicular release in neuronal function. Indeed, Fujita et al. suggested that in one of the best transgenic mouse models to study astrocytic vesicular release so far, the dnSNARE mouse, which we also examined in our study, neurons may also express small quantities of the transgenes and implied that results obtained with this mouse line may account for neuron-specific (rather than astrocyte-specific) effects. However, this study and the functional relevance of these measurements have been criticized (<http://www.ncbi.nlm.nih.gov/pubmed/25505312/#comments>). Furthermore, the results we present here do not support these conclusions for two reasons: first, the impairment in dendritic spine formation that we observed here was only visible on new neurons and not on mature neurons, only upon withdrawal of Dox, and, most importantly, were exclusively found on the dendritic portions that crossed the territories of transgene-expressing astrocytes. The reduction in dendritic spine density and maturation on the dendrites of newborn neurons followed exactly the borders of transgene-expressing astrocytes and returned to normal (i.e., similar to neurons newly generated in wild-type mice) as soon as the same dendrites exited the territories of blocked astrocytes (Figure 2E). Second, these observations have been confirmed using iBot mice, expressing a different transgene inhibiting synaptobrevin-2 function (botulinum toxin instead of dnSNARE) using a different promoter (GLAST instead of GFAP) and a different induction system (tamoxifen-induced Cre recombinase instead of Dox) than the dnSNARE mouse. Together, these results indicate that the impaired development of new neurons cannot be explained by a—yet to be confirmed—neuronal expression of dnSNARE. Furthermore, they underline the necessity of fine morphological inspection to discriminate between astrocytic and neuronal protein localization. They also demonstrate that a careful morphological analysis, combined with the use of several controls, including internal controls, can unambiguously reveal astrocyte-specific effects on neurons.

D-serine is a co-agonist of the NMDA receptor (Schell et al., 1995) that regulates synaptic plasticity in the mature brain. By activating the glycine site of the NMDA receptor, D-serine enhances LTP induction (Bashir et al., 1990), whereas impaired exocytosis of D-serine from astrocytes reduces LTP expression in the hippocampus (Henneberger et al., 2010; Panatier et al., 2006). Glutamate release from astrocytes has been shown to play a role in neuroblast migration from the subventricular zone to the olfactory bulb (Platel et al., 2010), but a role of D-serine in the synaptic integration of adult-born neurons was never

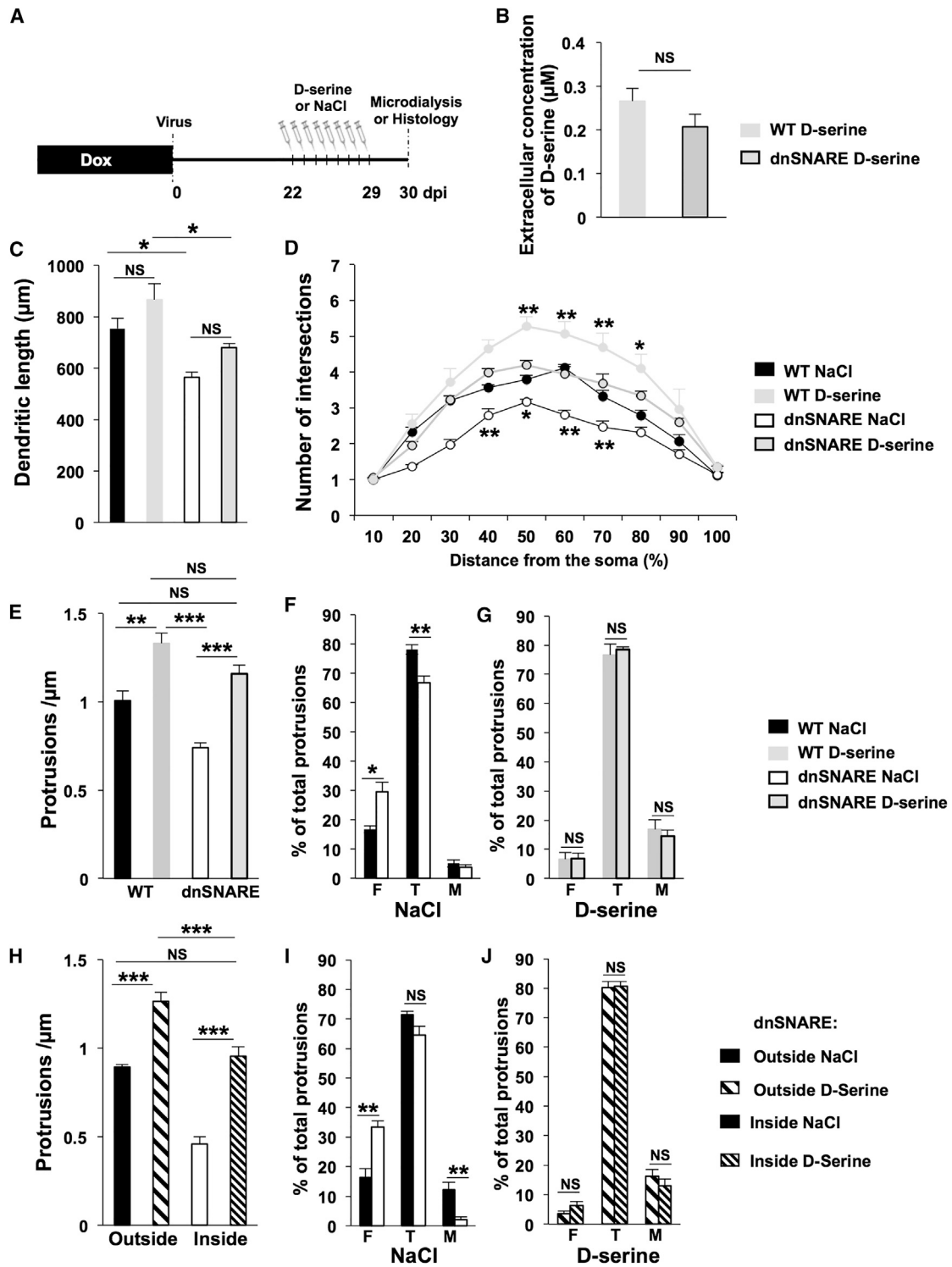


Figure 7. D-serine Administration Restores the Maturation of New Neurons in dnSNARE Mice

(A) Experimental timeline: Dox was withdrawn immediately after viral injection and D-serine (50 mg/kg, i.p.) or vehicle (NaCl 0.9%) was injected daily, from 22 to 29 dpi. Mice were analyzed at 30 dpi.

(B) Extracellular concentration of D-serine in the DG of dnSNARE and WT mice treated with D-serine ($n = 5-6$ mice per group, nonparametric Wilcoxon test).

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reported. During their maturation, new granule neurons first receive GABAergic innervation (Li et al., 2012; Tozuka et al., 2005), which is excitatory, due to high intracellular chloride concentration (Chancey et al., 2013; Ge et al., 2008). Then, the first glutamatergic synaptic inputs on these cells express NMDA receptors and lack AMPA receptors thus forming silent synapses (Chancey et al., 2013; Schmidt-Salzmann et al., 2014). At this stage, GABA-mediated depolarization relieves the voltage-dependent Mg^{2+} block from the NMDA receptors and enables their activation by glutamate. Such concomitant action of GABAergic and glutamatergic inputs enables the expression of AMPA receptors at the postsynaptic membrane and the unsilencing of newborn neurons (Chancey et al., 2013). In this context, the release of the NMDAR co-agonist D-serine by astrocytes represents an ideal way to promote this process and to contribute to the activity-dependent synaptic integration of new neurons. Consistent with this possibility, we observed that blocking astrocytic vesicular release resulted in impaired AMPAR currents, reduced maturation of dendritic spines, and decreased survival of new neurons. These observations are in line with previous observations on the role of the NR1 subunit of the NMDA receptor on new neuron survival (Tashiro et al., 2006, 2007). They extend on these previous observations in showing that the impairment in survival is not detectable before glutamatergic synapse formation (at about 14 dpi) and extend until the end of synaptogenesis (between 30 and 90 dpi), suggesting that the formation of excitatory synaptic inputs is crucial for the long-term survival of adult-born neurons. Our observations of reduced dendritic length and branching of new neurons in dnSNARE and iBot-Glast-CreER^{T2} mice also support previous reports of a role for NMDAR in dendritic development. Indeed, recent studies showed that the ablation of the NR2B subunit from newborn neurons reduces their dendritic complexity (Kheirbek et al., 2012), whereas learning experience enhances the dendritic maturation of new neurons in an NMDAR-dependent manner (Tronel et al., 2010).

Although our results clearly show an important role of D-serine in neuronal maturation in the adult brain, they do however not exclude the involvement of additional astrocyte-secreted molecules. Several other molecules are known to be released by astrocytes and to regulate synaptogenesis during development (Allen, 2013), such as Thrombospondin, Glypicans, TGF β , TNF α , GABA, or adenosine. In particular, adenosine is released in a synaptobrevin-2-dependent manner and its extracellular concentration may be reduced in dnSNARE mice (Schmitt et al., 2012). In the somato-sensory cortex, it was reported that a reduction of extracellular adenosine concentration in dnSNARE

mice decrease the amplitude of NMDAR EPSCs and surface expression of NR2B subunits without affecting synaptic AMPA receptors (Deng et al., 2011). By contrast, in the adult hippocampus, we have observed a strong reduction of synaptic AMPA EPSCs, leading to an increase in the NMDA/AMPA ratio (Figure 4D). Furthermore, bath application of D-serine in slices largely rescued the NMDAR deficit in newborn neurons in dnSNARE mice. Taken together, although we cannot exclude a further contribution of additional astrocyte-released factors, D-serine appears to make a major contribution to the local control of spine formation on newborn hippocampal granule neurons.

Dendritic protrusions express a constant number of NMDAR and a number of AMPAR that is correlated with the diameter of their tip (Takumi et al., 1999). Therefore, the structural correlate of silent synapses are dendritic protrusions with little or no enlargement of their tip, such as filopodia. Filopodia point toward and touch pre-existing axon terminals that are already involved in synapses with dendritic spines of other neurons. Upon conversion into dendritic spines, they form multiple synapse boutons, on which they compete with more mature neurons for stability on the axon terminal (Toni et al., 2007). In a recent study, we found that pre-existing, mature astrocytes ensheath synapses on adult-born neurons independently of their age or the size of the dendritic protrusions (Krzisch et al., 2015). Thus, by contacting growing filopodia or immature dendritic spines, astrocytes are ideally positioned to supply D-serine to immature, silent dendritic protrusions, thereby enabling their maturation.

Astrocytes occupy distinct, non-overlapping territories (Bushong et al., 2002). The observation that individual astrocytes regulate the maturation of dendritic segments that intersect with their territories provides a clear demonstration that the structural boundaries of astrocytes delineate their functional impact on neurons. Since astrocytic vesicular release is regulated by neuronal activity (Bezzi and Volterra, 2001), individual astrocytes may serve as intermediates, coupling neuronal network activity to the local synaptic recruitment of adult-born neurons into the hippocampal synaptic circuitry. In turn, new neurons enhance the plasticity of neuronal networks into which they integrate (Schmidt-Hieber et al., 2004). Such “on demand” integration may serve to recruit new neurons in particularly active computational hotspots, thereby contributing to memory and the discrimination of learned events (Aimone and Gage, 2011). Conversely, astrocytic deficiency may result in impaired adult neurogenesis and cognitive deficits, as illustrated in Alexander’s disease, which is characterized by a mutated form of GFAP in astrocytes and results in impaired adult neurogenesis and learning performance (Hagemann et al., 2013).

(C and D) Histogram of the total dendritic length (C; one-way ANOVA $F_{3,19} = 8.5882$, $p = 0.0013$ followed by post hoc Tukey’s HSD) and dendritic arborization complexity (D; two-way ANOVA with repeated-measures $F_{3,16} = 18.55$, $p < 0.0001$ followed by post hoc Tukey’s HSD) of newborn in dnSNARE and WT mice after D-serine or vehicle injections.

(E–G) Histogram of the dendritic protrusion density (E), one-way ANOVA $F_{2,17} = 23.33$, $p < 0.0001$ followed by post hoc Tukey’s HSD) and their morphology in newborn neurons of dnSNARE and WT mice after vehicle (F) or D-serine (G) treatment (bilateral Student’s t test between categories filopodia [F], thin [T], or mushroom [M]).

(H–J) Histogram of the protrusion density (H; one-way ANOVA $F_{3,19} = 60.60$, $p < 0.0001$ followed by post hoc Tukey’s HSD) and their morphology in newborn neurons of dnSNARE inside (thin dashed lines) and outside (thick dashed lines) transgene-expressing astrocytes after vehicle (I) or D-serine (J) injections (bilateral Student’s t test between categories filopodia [F], thin [T], or mushroom [M]). For all, each value represents the mean \pm SEM. NS, not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Astrocytes are key constituents of the neurogenic niche and play a fundamental role in the regulation of neural progenitor proliferation (Song et al., 2002). We show here that, by providing the necessary cues, mature astrocytes enable the dendritic maturation, synaptic integration, and survival of new neurons in the adult hippocampus. This regulation demonstrates a crucial role of the neurogenic niche in locally regulating and adjusting the late stages of adult neurogenesis, to fine tune network plasticity and hippocampal function.

EXPERIMENTAL PROCEDURES

Experimental Animals

dnSNARE mice were generated by crossing (1) the GFAP-tTA line expressing the tet-off tetracycline transactivator under the control of the GFAP promoter and (2) the tetO.dnSNARE line containing a tet-operator-regulated dominant-negative soluble N-ethylmaleimide-sensitive factor attachment protein receptor (dnSNARE) domain of synaptobrevin 2 as well as the reporter genes lacZ and GFP (Pascual et al., 2005).

iBot-Glast-CreER^{T2} mice were obtained by crossing two lines of transgenic mice (Slezak et al., 2012): (1) the iBot line, that contains a CAG (cytomegalovirus early enhancer and chicken beta-actin) promoter, a floxed-STOP cassette upstream of the gene encoding the *Clostridium botulinum* neurotoxin serotype B light chain (BoNT/B) followed by an GFP, and (2) the Glast-CreER^{T2} T45-72 line in which the expression of tamoxifen-inducible Cre recombinase (CreER^{T2}) is under the control of the astrocyte-specific promoter GLAST.

Stereotaxic Viral Injections

Dividing neuronal progenitor cells and their progenies, we identified and stably labeled using a MoMuLv encoding RFP or GFP under the control of the CAG promoter (Zhao et al., 2006). Viral injection was performed as previously described (Sultan et al., 2013). 1.5 μ l of retroviral vector (final titer 10E8 pfu/ml) was injected in the DG (Bregma: -2 mm anteroposterior, 1.75 mm lateral and -2.00 mm dorsoventral) using a calibrated 5 μ l Hamilton syringe fitted with a 33G needle.

Statistical Analysis

Hypothesis testing was two tailed. All analyses were performed using JMP 10. First, Shapiro-Wilk tests were performed on each group of data to test for distribution normality. When the distribution was not normal, a non-parametric Kruskal-Wallis test was used. Otherwise, analysis was performed using parametric tests. Homoscedasticity of variances was tested by Bartlett's or Levene's tests and adequate analysis of variance (ANOVA or Welch's ANOVA) was performed, followed by a post hoc multiple comparisons procedure Tukey's HSD test or t test with Bonferroni correction. For two sample comparisons, when the distribution was normal, the equality of variance of the groups was tested by a bilateral F-test and the adequate unpaired t test was used. When the distribution was not normal, a Mann-Whitney test was used. For the Sholl analysis, parametric tests were used: two-way ANOVA with repeated-measures was performed, followed by a post hoc Tukey's HSD test. All data are presented as mean \pm SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and seven figures and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2015.10.037>.

A video abstract is available at <http://dx.doi.org/10.1016/j.neuron.2015.10.037#mmc3>.

AUTHOR CONTRIBUTIONS

N.T., J.B., P.B., and S.S. conceived and designed the experiments. S.S., L.L., J.M., F.P., E.G., J.L., and P.B. performed the experiments. S.S., L.L., J.M., F.C., P.B., J.B., and N.T. analyzed and discussed the data. S.S., F.W.P.,

J.B., and N.T. wrote the manuscript. N.T., F.W.P., J.B., and P.B. provided financial support.

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