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Granule cells born in the adult rat hippocampus can regulate the expression of GABAergic markers

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Abstract

The granule cells (GCs) of the dentate gyrus transiently express markers of the GABAergic phenotype early during development. However, GCs are generated throughout life, posing the question of whether the newborn neurons in the adult rodent recapitulate the development of the neurotransmitter phenotype of GCs generated during embryonic and early postnatal development. In this work we asked whether newborn GCs transiently express a GABAergic phenotype during their development in the adult rat. Using retroviral infection, we labeled dividing cells in the dorsal hippocampus with GFP, identified them as granule cells, and determined their expression of GABAergic markers at different developmental stages. We found that GFP-positive cells express Prox-1 and calbindin, identifying them as GCs. GABA or GAD₆₇ was expressed in 13% of GFP-positive cells at 7 dpi, in 16% at 10 dpi and in 20% at 15 dpi. At 30 dpi, however, no GFP-positive cell somata containing GABAergic markers were detected, but their mossy fiber boutons did contain GAD₆₇. Interestingly, developing GCs detected with doublecortin and PSA-NCAM in non-injected adult rats, did not express GABAergic markers, suggesting that retroviral injection/infection stimulates their transient expression. However, in non-injected rats, a number of mossy fiber boutons of newborn granule cells detected with PSA-NCAM did express GAD₆₇. Our findings reveal that developing GCs born in the adult are able to transiently up-regulate the expression of GABAergic markers to be detected in their soma in response to insults, while they constitutively express GAD₆₇ in their mossy fibers.

Keywords

Neurogenesis; Granule cells; Dentate gyrus; GAD; GABA; Prox-1; GFP; Doublecortin; PSA-NCAM

Introduction

The granule cells (GCs) of the dentate gyrus (DG), which constitute a glutamatergic projection to the hippocampus in the adult rodent, are known to transiently express markers

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of the GABAergic phenotype during their postnatal development (Gutiérrez et al., 2003; Safiulina et al., 2006). These markers disappear on completion of development but can be reexpressed by enhanced excitability (Gutiérrez, 2005). Therefore, besides expressing the markers of the glutamatergic phenotype (glutamate, VGlut-1), they express GABA, GAD₆₇, VGAT (Gómez-Lira et al., 2005; Gutiérrez et al., 2003; Lamas et al., 2001; Zander et al., 2010). Interestingly, during the first 4–6 days of life, their activation produces only GABA-R mediated responses in their post-synaptic target cells (Safiulina et al., 2006), after which time the same activation produces simultaneous glutamate-R- and GABA-R mediated responses until completion of development (Gutiérrez et al., 2003; Walker et al., 2001). Thus, in the GCs of adult rats, the GABAergic markers are down-regulated and GABAergic transmission disappears, and their stimulation produces exclusively glutamatergic responses (Gómez-Lira et al., 2005; Gutiérrez et al., 2003; Lamas et al., 2001; Safiulina et al., 2006).

Granule cells are generated throughout life, i.e., they are born in the adult rat and their physiological characteristics during their development are parallel to those described for GCs born in the embryonic period or early in the post-natal rat (Espósito et al., 2005). Although evidence has appeared suggesting that newborn GCs in the adult rat do not release GABA (Toni et al., 2008), there is no systematic analysis of their neurotransmitter phenotype to date. It is known that epileptic seizures up-regulate GAD₆₇ and GABA in the GCs and their mossy fibers (for a review see Gutiérrez, 2005), and that neurogenesis is also up-regulated (Parent et al., 1997). Interestingly, single seizures produce a strong up-regulation of GAD₆₇ in cells located in the infragranular layer of the DG, where most of GCs born in the adult are located (Ramírez and Gutiérrez, 2001), which suggested that GCs born in the adult can express a GABAergic phenotype.

The fact that many GCs express GABAergic markers during development, and that seizures up-regulate them in the adult, suggests that developing GCs born in the adult rat may express them as well. In this work we assessed the expression of GAD₆₇ and GABA in newborn GCs, identified by their expression of GFP, which was induced by retroviral infection. This analysis was carried out at 7, 10, and 15 days post-injection (dpi), as this is the critical period at which many GCs express the markers of the GABAergic phenotype (Gutiérrez et al., 2003) and at 30–35 dpi, when the newborn GCs are fully developed and integrated to the circuit (Ge et al., 2008).

Materials and methods

Viral vector production

We used a retroviral vector based on the Moloney murine leukemia virus to express enhanced GFP driven by a CAG promoter. Retroviral particles were assembled using three separate plasmids containing the capsid (CMV-vsvg), viral proteins (CMV-gag/pol) and transgene (CAG-GFP) (Tashiro et al., 2006). These were a gift from F.H. Gage (Salk Institute, La Jolla, CA). Plasmids were transfected to 293T cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The virus-containing supernatant was harvested 48 h after transfection, passed through a 0.22 µm filter and concentrated by two rounds of

ultracentrifugation (Tashiro et al., 2006). Expression titers were estimated on 293T cells by serial dilutions. Titters ranged from 5×10^7 cfu/ml to 3×10^8 cfu/ml.

Animals and stereotaxic surgery

For our studies we used 30 male Sprague Dawley rats (150–170 g) of 6 to 8 weeks of age; 23 of them were injected with the retrovirus, 3 were injected with PBS (at dpi 15) and 4 were used as control, non-injected rats. They were anesthetized (100 mg/kg ketamine and 5 mg/kg xylazine) and the virus was infused (1 μ l at 0.1 μ l/min) into the right dorsal DG (anteroposterior, -2.7 ; mediolateral, -1.7 ; dorsoventral, -3.4 ; Fig. 1A). A Hamilton syringe (26s-gauge needle with point style 4; Hamilton Co., Reno NV, USA) was used for all injections.

Immunohistochemistry

The animals were anesthetized and transcardially perfused with phosphate buffer (PB) 0.1 M, pH 7.4 followed by 4% paraformaldehyde (PFA). Rats injected with the retrovirus were prepared at 7, 10, 15 and 30 dpi. Brains were post-fixed overnight in 4% PFA and then transferred into 30% sucrose solution. Serial 30 μ m coronal sections of the brain were cut using a cryostat (Leica CM1510, Leica Microsystems, Wetzlar, Germany), and for our immunostaining analysis we selected one every 3 sectioned slices. Sections were incubated with BSA 5% in PBS Triton X-100 0.3%, for 2 h at room temperature and further incubated with primary antibodies at 4 °C overnight in PBS triton X-100 0.3% and BSA 1%. We used the following antibodies: chicken anti-GFP (1:1000, Abcam, Cambridge, USA), rabbit anti-Prox1 (1:1000, Chemicon, Temecula, CA), mouse anti-GAD₆₇ (1:1000, Chemicon), rabbit anti-GABA (1:1000, Sigma Aldrich, St Louis, MO, USA), rabbit anti-Calbindin D-28K (1:200, Chemicon), rabbit anti-doublecortin (DCX; 1:1000, Abcam) and mouse anti-PSA-NCAM (1:500, Chemicon). As negative controls, slices were simultaneously processed in the absence of the primary antibody. After rinsing 3 times in PBS Triton X-100 0.3%, the slices were incubated with secondary antibodies for 2 h at room temperature. They were: donkey anti-chicken FITC (1:100), goat anti-mouse TRITC (1:100), goat anti-rabbit TRITC (1:100), goat anti-rabbit Cy5 (1:100) and goat anti-mouse CY5 (1:100; all from Jackson ImmunoResearch, West Grove, PA). Finally, the slices were rinsed 3 times in PBS and mounted in slides using Vectashield with DAPI mounting medium (Vector, Burlingame, CA). All analyses were performed in double or triple-stained sections (GFP, Prox-1 and GAD₆₇/GABA; GFP, Calbindin D-28K and GAD₆₇/GABA; GFP and DCX; GFP, DCX and GAD₆₇; DCX and GAD₆₇; GFP and PSA-NCAM; PSA-NCAM and GAD₆₇) using an epifluorescent microscope (Axio Scope, Carl Zeiss) and analyzed with the software Axio Vision V4.8 (Carl Zeiss, Goettingen, Germany). Selected sections were analyzed with a confocal microscope (Leica TCS-SPE) and the software Leica LAS AF Lite (Leica Microsystems Wetzlar, Germany). Colocalization was assessed in three-dimensional reconstructions through the entire Z-axis of each cell GFP⁺, using consecutive optical slices at 0.5–1 μ m intervals. Cells were counted by eye in one every 3 slices from the ipsilateral side of the injection at 7, 10, 15 and 30 days post-retrovirus injection. Each experiment was performed at least in three different animals.

Electrophysiology

For electrophysiological recordings, 3 rats were injected with the virus in the dorsal hippocampus, as previously described. At 14 dpi, rats were decapitated under profound pentobarbital anesthesia and 300 μm thick slices were cut in a vibroslicer (Leica VT 1200, Wetzlar, Germany) submerged in sucrose-based cutting solution containing (in mM): 87 NaCl, 25 NaHCO₃, 25 glucose, 75 sucrose, 2.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂ and 4 MgCl₂ equilibrated with 95% O₂–5% CO₂, at 4 °C. Recordings were conducted with the patch clamp technique in the whole-cell mode, on the stage of a bright field/fluorescence microscope (Eclipse E600FN; Nikon, Tokyo). Putative fluorescent newborn GCs and adult GCs were recorded in the whole cell patch-clamp configuration in current clamp using borosilicate pipettes pulled to yield 4–6 M Ω (Flaming-Brown P-97 puller; Sutter Instrument Company, Novato, CA) and filled with K-gluconate intracellular solution (in mM: 133 K-gluconate, 7 KCl, 1.5 MgCl₂, 4 ATP-Mg, 0.5 GTP-Na, 0.5 EGTA, 10 HEPES, pH 7.2 with KOH). The artificial cerebrospinal fluid used contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 1 MgCl₂, 2 CaCl₂, and 25 glucose, and was constantly oxygenated. The whole-cell signals were recorded with a Multiclamp 700B amplifier (Molecular Devices, Palo Alto, CA), filtered at 5 kHz, digitized, acquired (Digidata 1440A; Molecular Devices), and off-line analyzed with the pClamp10.1 program (Molecular Devices).

Results

We determined the number of GFP-expressing (GFP⁺) newborn cells at 7, 10 and 15 dpi (Fig. 1B), which constitutes the critical period during which GABAergic markers are expressed in GCs of developing rats (Gutiérrez et al., 2003), and after 30–35 dpi, when newborn cells are completely developed. Therefore, as markers of the maturity of the newborn cells, DCX, calbindin and PSA-NCAM cells were determined in the GFP⁺ cells (Rao and Shetty, 2004; Snyder et al., 2009). Newborn, GFP⁺ cells were located in the subgranular and inner third of the granular layer of the DG (Fig. 1B), with morphological (Figs. 1C–F) and electrophysiological characteristics (Fig. 4I) that correspond to the developmental stages studied, as reported in Espósito et al. (2005).

We counted the number of GFP⁺ cells in one every 3 slices along the hippocampus (n=5 rats at each post-infection time) and found 54.7 ± 2.3 , 65.3 ± 2.1 , 52.4 ± 1.7 and 52.7 ± 1.7 cells per rat, at 7, 10, 15 and 30 dpi, respectively. We confirmed the expression of DCX/PSA-NCAM and calbindin in 70% and 30% of the GFP⁺ cells (at 10 dpi), respectively, identifying them as neurons (Figs. 2A–C). These results agree with previous reports (Espósito et al., 2005; Piatti et al., 2011; Snyder et al., 2009). Eight GFP⁺ cells at 12–14 dpi were randomly selected to electro-physiologically verify their neuronal phenotype. Contrary to mature, embryonically generated GCs, their high R_{input} , low C_m and the low amplitude and wide action potentials, evoked by depolarizing the membrane potential, identify the recorded cells as immature GCs (Fig. 4I; Espósito et al., 2005). The electrophysiological characteristics of the GFP⁺ and adult GCs are summarized in Table 1.

Because our goal was to assess the expression of GABAergic markers in developing newborn GCs, and because these GABAergic markers could be expressed in interneurons containing GFP, we first sought to identify the GFP⁺ cells as GCs by their expression of

prospero-related homeobox 1 gene (Prox1), a transcription factor specifically expressed in these cells and that controls its generation (Karalay et al., 2011; Pleasure et al., 2000). With this analysis, we determined that all GFP⁺ cells were Prox1-positive at the different post-infection times, identifying them as GCs (Figs. 3A–D). Therefore, we next determined the expression of the GABAergic markers GAD₆₇ and/or GABA in identified GFP⁺–Prox1⁺ GCs. By conducting a double fluorescent labeling of already-labeled GFP⁺ cells, against Prox1 and GAD₆₇ and/or GABA, we could confidently determine that from the total of identified GFP⁺ GCs analyzed from 5 rats at each post-infection time, 30 out of 219 (13%) at 7 dpi, 64 out of 392 (16%) at 10 dpi, and 54 out of 262 (20%) at 15 dpi, expressed GAD₆₇/GABA (Fig. 4H). Figs. 4(A–E) depict examples of GFP⁺–Prox1⁺ cells that express and that do not express the GABAergic markers at all post-infection times. To corroborate the correct determination of the GABAergic phenotype, we verified in 3 preparations that GFP⁺ cells that expressed GAD₆₇ also expressed GABA, which was the case (Fig. 4F). Interestingly, at 30 dpi no GFP⁺ granule cell (out of 265) was found to express either GAD₆₇ or GABA (Figs. 4G, H). However, because the mossy fibers normally express GAD₆₇ (for a review see Gutiérrez, 2005), we analyzed the expression of the enzyme in the mossy fibers of preparations at 15 and 30 dpi, when GFP⁺ cells already extend their axons to CA3 area. In agreement with previous reports, the stratum lucidum of CA3 was immunoreactive to GAD₆₇ (Fig. 5A1). We were able to find 7 GFP⁺ axons with giant boutons, identifying them as mossy fibers (Fig. 5A2), all of which had GAD₆₇ in their characteristic giant boutons (Fig. 5B).

The transient somatic expression of GAD₆₇/GABA in the aforementioned experiments could be due to either a developmental trait or to the insult that constitutes the injection/infection of the dividing cells with the retrovirus. Therefore, in 4 non-injected rats we conducted double labeling against GAD₆₇ or GABA, and DCX and PSA-NCAM, which are known to identify developing cells. Surprisingly, contrary to DCX⁺ cells from injected rats, where co-localization of DCX and GAD was evidenced (Fig. 6A), in non-injected rats no DCX⁺ or PSA-NCAM⁺ cell expressed GABAergic markers (Fig. 6B), suggesting that the injection of the retrovirus induced the expression of GABAergic markers in developing newborn cells. However, although the somata of GFP⁺ cells at 30 dpi did not express GABAergic markers, we confirmed that their giant mossy fiber boutons contained GAD₆₇ (Figs. 5C, D). Because the injection of the retrovirus implies a mechanical damage and possibly inflammation of the tissue (Breunig et al., 2008; Zhao, 2008), we investigated whether the injection of PBS alone was able to induce the expression of GABAergic markers. In rats injected with PBS, as with the retroviral infection, we found some PSA-NCAM⁺ positive cells expressing GAD₆₇ (Fig. 6C), suggesting that the injection itself is able to activate the expression of GABAergic markers. Further, because PSA-NCAM also stains mossy fibers from developing GCs, we could corroborate that some boutons also expressed GAD₆₇, as shown to happen in all the other experimental conditions (Fig. 6D).

Discussion

It has been described that the GCs of the DG transiently express a GABAergic phenotype during development, whereby the markers of this phenotype, GAD₆₇, GABA and the vesicular GABA transporter can be detected at varying degrees throughout the development

(Gómez-Lira et al., 2005; Gutiérrez et al., 2003; Zander et al., 2010). Moreover, during this period, stimulation of the mossy fibers produces monosynaptic GABA-R-mediated fast responses in their target cells (Gutiérrez et al., 2003; Kasyanov et al., 2004; Walker et al., 2001). Further, recent evidence demonstrates that some mossy fiber boutons co-release glutamate and GABA, while others only release glutamate or GABA. In the adult rat, however, boutons release exclusively glutamate (Beltran and Gutierrez, 2012). Considering this, we hypothesized that developing GCs born in the adult rat may recapitulate the transient expression of the GABAergic phenotype of GCs that occurs in the developing rat. This is important because the integration of GCs born in the adult rat impact on the modulation of hippocampal circuits, which participate in cognitive hippocampal-dependent tasks (reviewed by Marín-Burgin et al., 2012; Ming and Song, 2011).

In this work, we analyzed the expression of GAD₆₇ and GABA in newborn GCs identified by their expression of GFP after retroviral infection. The number of GFP⁺ cells that we detected is in agreement with the efficiency of the viral infection reported by several studies (Espósito et al., 2005; Piatti et al., 2011). The granule cell phenotype of the GFP⁺ cells was confirmed by their expression of Prox1, which is a transcription factor specifically expressed in GCs but not in interneurons (Pleasure et al., 2000); moreover, they had the morphological and electrophysiological characteristics of developing GCs (Espósito et al., 2005).

We determined that 15–20% of developing GFP⁺ GCs expressed GAD₆₇/GABA, while the adult, GFP⁺ GCs did not express these markers at 30 dpi. However, while these results refer to the somatic expression of the GABAergic markers, we observed that GFP⁺ mossy fiber boutons expressed GAD₆₇ at all ages. There may be several possible reasons why only this percentage of developing GFP⁺ cells expresses GAD₆₇, while adult GFP⁺ cells do not. First: a developmental trait. It has been shown that many, but not all GCs born perinatally express the GABAergic markers in their somata (Gutiérrez et al., 2003; Maqueda et al., 2003). Despite this, their stimulation provoke almost exclusively GABA-mediated responses in their post-synaptic target cells, during the first postnatal days (Safilina et al., 2006), and mixed glutamatergic–GABAergic responses until completion of development (Gutiérrez et al., 2003; Walker et al., 2001), when they become exclusively GABAergic (Gutiérrez, 2000; Gutiérrez et al., 2003). Second, GCs are born continuously but different cohorts of newborn can be found at a given time (Mathews et al., 2010; Piatti et al., 2011). It is possible that some cohorts do express GAD₆₇ (as found in developing rats; Gutiérrez et al., 2003) and they may be differentially integrated to circuits. Indeed, Mathews et al. (2010) proposed that although early and adult born cells are not different from each other, they might be recruited to different networks. We can hypothesize, that the GAD₆₇-expressing population may be distributed into such networks to serve a distinctive function, which is still to be determined. In this regard, the possible existence of reserve pools of neurons that can respecify their neurotransmitter phenotype (Dulcis and Spitzer, 2011), could apply to newborn GCs that can be recruited into dynamic, existing circuits.

Noteworthy, while GFP⁺ granule cell somata may not express GAD₆₇/GABA, we found that their mossy fibers did express GAD₆₇, as has been shown to occur normally in developing and adult mossy fibers (see also Gutiérrez et al., 2003; Ramírez and Gutiérrez, 2001; Sloviter et al., 1996). Third: injection/infection induced expression of GABAergic markers.

In our experiments using DCX and PSA-NCAM to identify developing GCs in non-injected rats, we found that none of these cells expressed GABAergic markers in their somata, while GAD was present in their mossy fiber boutons.

This may suggest that the injection constitutes an insult to which a number of GCs react by transiently expressing the GABAergic. This insult-induced expression, however, was not observed in the surrounding adult GCs, which did not express GAD₆₇ or GABA. Therefore, it can be hypothesized that developing GCs in the adult rat are more prone to express markers of the GABAergic phenotype after an insult. Indeed, it is recognized that the injection of retrovirus per se causes a lesion and inflammation (Breunig et al., 2008; Zhao, 2008). Moreover, it has been shown that newborn GCs overexpress GAD₆₇ after seizures (Jiang et al., 2004) and that newborn GCs in culture conditions can express a GABAergic phenotype in a manner dependent on activity, and by kainate or BDNF exposure (Babu et al., 2007), as previously described to occur in adult GCs (Gómez-Lira et al., 2005). Therefore, our data suggest that the retroviral infection is not the cause of the expression of GABAergic markers, as a number of newborn GCs from rats injected with PBS also expressed GAD₆₇ or GABA. On the other hand, newborn GCs in non-injected rats that were detected by DCX or PSA-NCAM did not express the GABAergic markers. Thus, provided that appropriate injection controls are considered, the use of the retroviral infection is well suited for the study of the neurotransmitter phenotype of the infected neurons.

Whether GCs containing GAD₆₇ in their mossy fibers are able to release GABA is still unknown. There is evidence showing that newborn GCs do not release GABA (Toni et al., 2008), but recent evidence has established that some identified mossy fiber boutons of developing GCs do co-release glutamate and GABA, while others release only glutamate (Beltran and Gutierrez, 2012), opening the possibility of having some cells that can release GABA. Different cohorts of cells participate in the integration of incoming inputs from the cortex in a differential manner (Marín-Burgin et al., 2012). Insults, like seizures, besides increasing the number of newborn GCs (Parent et al., 1997), can up-regulate the expression of a GABAergic phenotype in existing ones or turn on the GABA-releasing machinery (they constitutively express GAD₆₇ in their mossy fibers) converting them into GABA-releasing cells. In this way, they may contribute to dampen excitability in the microcircuit to which they belong, as it has been described that newborn GCs receive enhanced excitatory drive after seizures (Wood et al., 2011).

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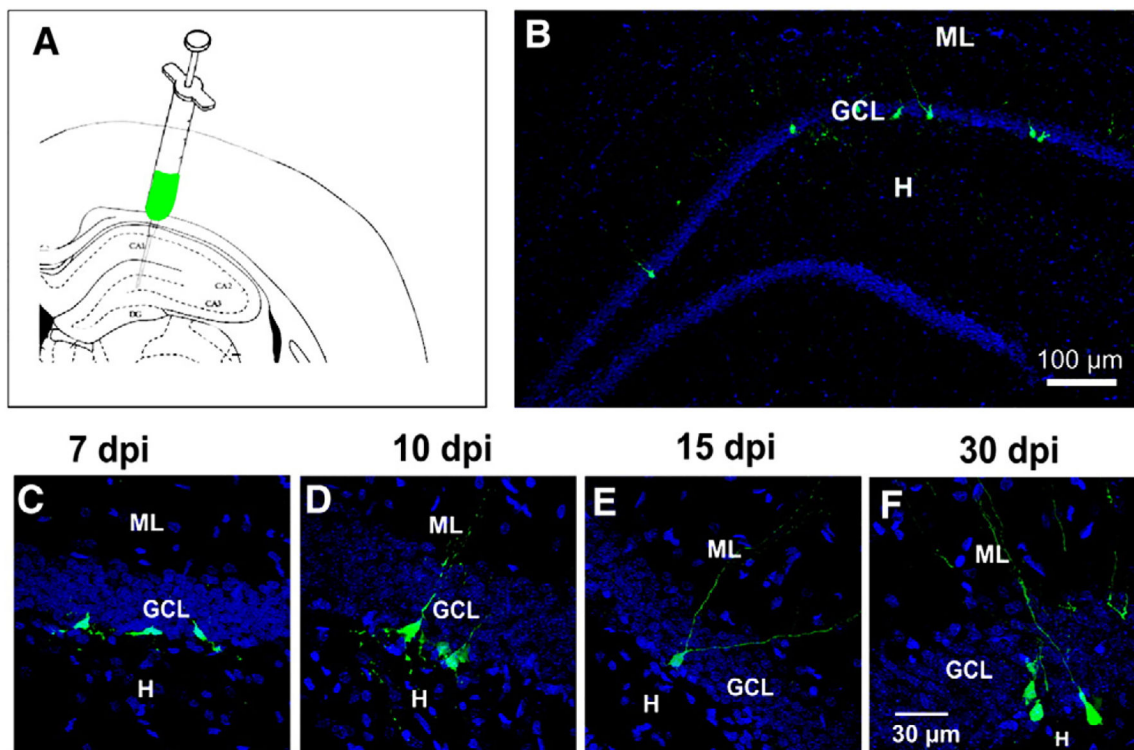


Fig. 1.

Newborn cells were detected in adult rats by retroviral infection. A) Schematic view of the hippocampus depicting the site of injection of the retrovirus. B) Panoramic view of a dentate gyrus with GFP⁺ cells in the inner granule cell layer at 15 dpi. Confocal images showing the morphology of GFP⁺ cells at the post-injection times analyzed: C) at 7, D) at 10, E) at 15 and F) at 30 dpi. DAPI is shown in blue. ML, molecular layer; GCL, granule cell layer; H, hilus. Calibration in F applies to panels C–E.

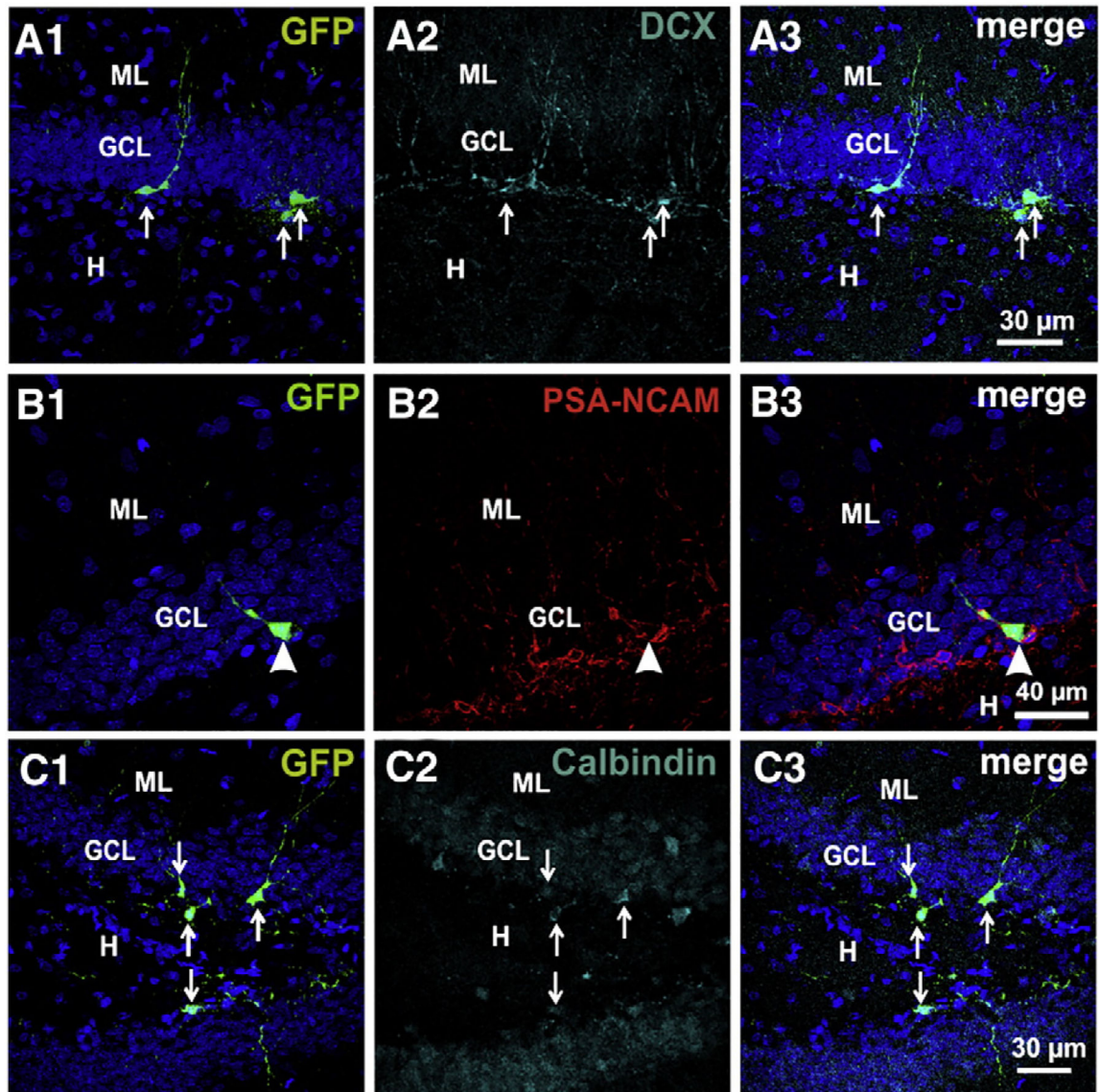


Fig. 2. GFP⁺ cells are newborn granule cells. Confocal images showing that developing GCs (all examples at 10 dpi) express DCX (A), PSA-NCAM (B) and calbindin (C).

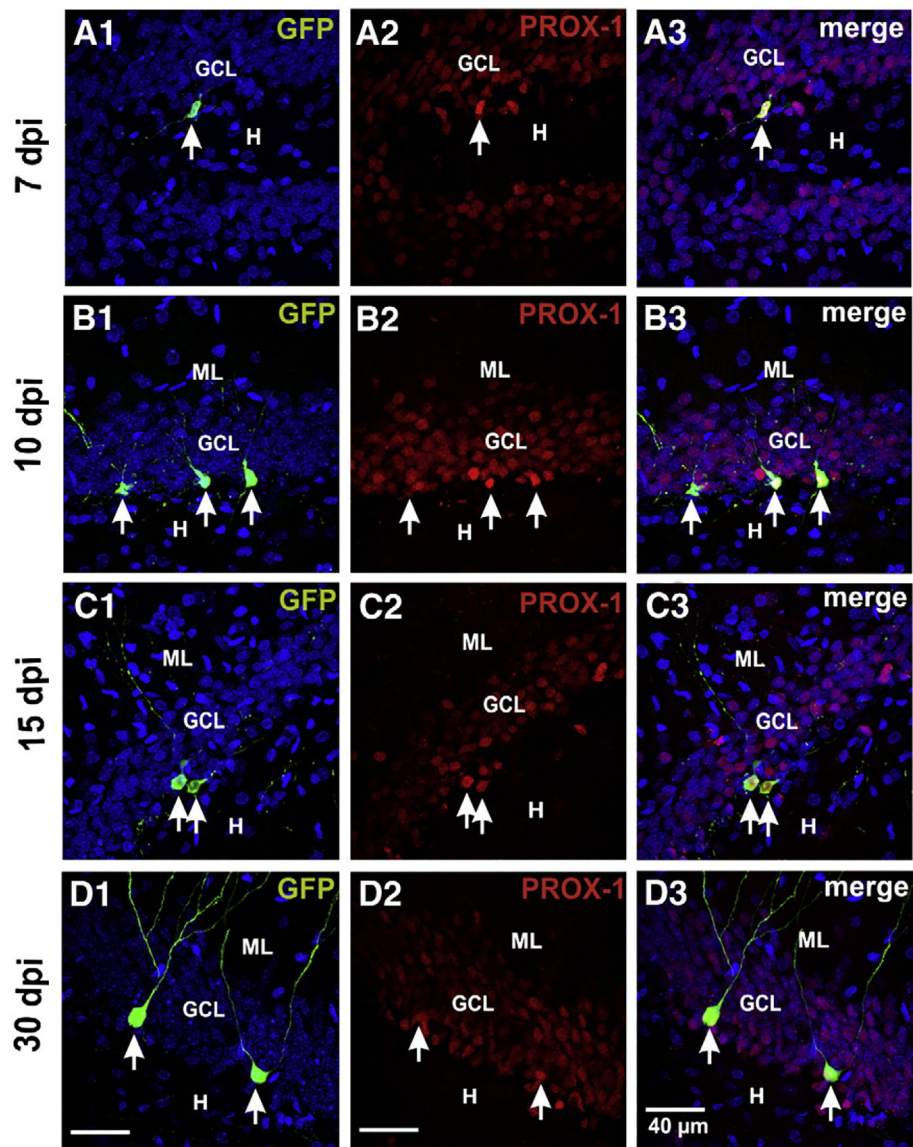


Fig. 3. Newborn cells were positively identified as GCs by their expression of Prox1, at 7 (C), 10 (D), 15 (E) and 30 dpi (F). ML, molecular layer; GCL, granule cell layer; H, hilus. Arrows signal the newborn GCs.

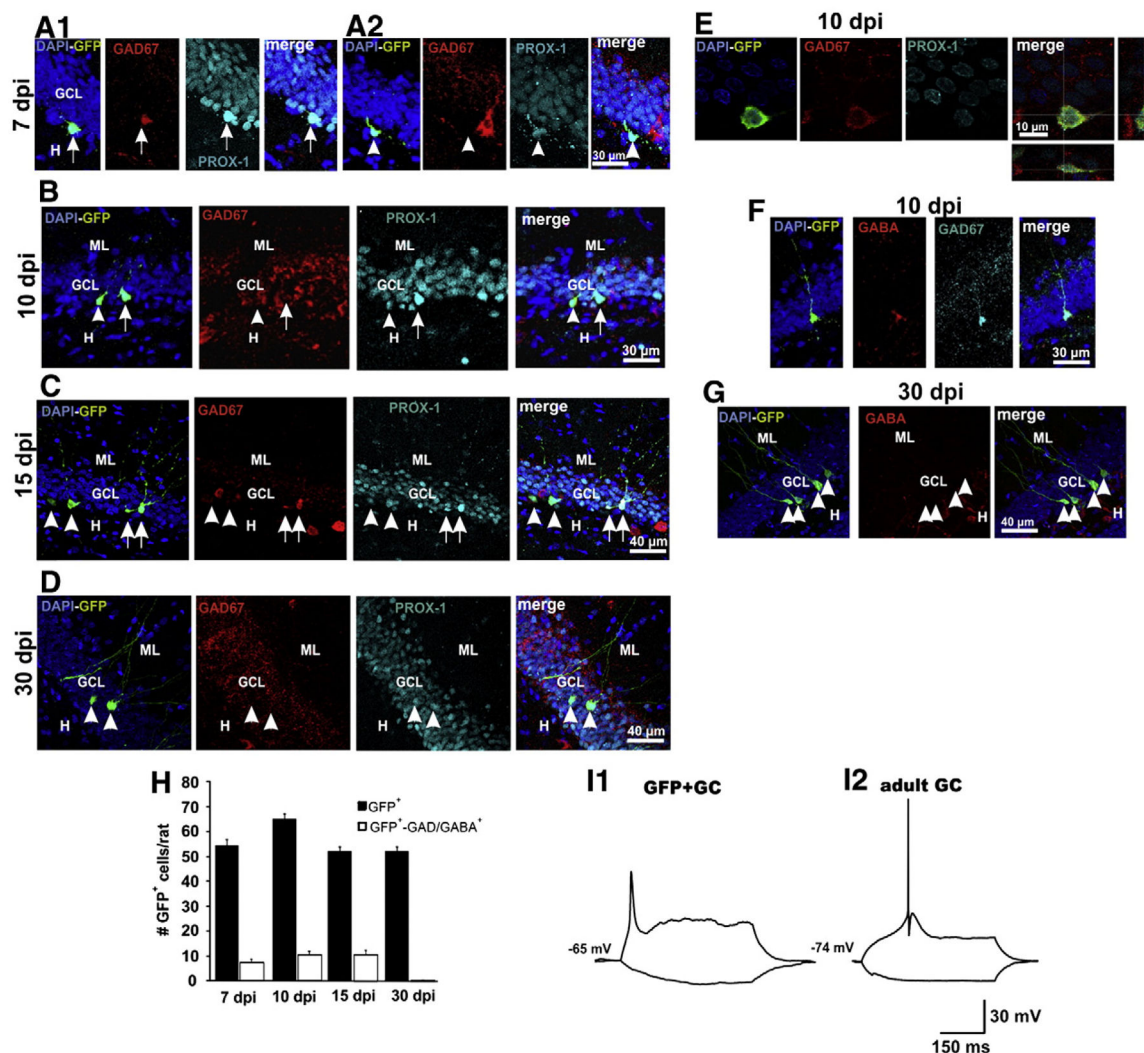


Fig. 4. Expression of GABAergic markers in GCs born in the adult rat. A1) Confocal images at 7 dpi showing a GFP⁺ granule cell expressing GAD₆₇ (arrow) and A2) a granule cell that does not express GAD₆₇ (arrowhead). A GAD₆₇⁺ interneuron can be observed beside the GFP⁺ granule cell. B and C) Images depicting newborn GCs at 10 and 15 dpi, respectively, which express (arrows) and do not express GAD₆₇⁺ (arrowheads). D) At 30 dpi, no GFP⁺ granule cell expressed GAD₆₇⁺. E) Three-dimensional reconstructions through the Z-axis of GFP⁺ cells (fifty optical slices at 0.5 μm intervals) were used to verify the co-localization of GFP, GAD₆₇ and Prox1. DAPI, blue; GAD₆₇ Red; Overlay with Prox1, in light blue, and orthogonal projections are shown on the right panel. F) Confocal images depicting the coexistence of GAD₆₇ and GABA in a GFP⁺ granule cell at 10 dpi. G) At 30 dpi, no GFP⁺ granule cell expressed GABA. Calibrations in the rightmost panels apply for all panels in the corresponding row. ML, molecular layer; GCL, granule cell layer; H, hilus. H) Mean number of GFP⁺ GCs per rat (black bars) and number of GFP⁺ GCs expressing GAD₆₇/GABA at the different postinjection times (n=5/bar). I) Electrophysiological recordings of a developing GFP⁺ granule cell at 14 dpi and of an adult granule cell.

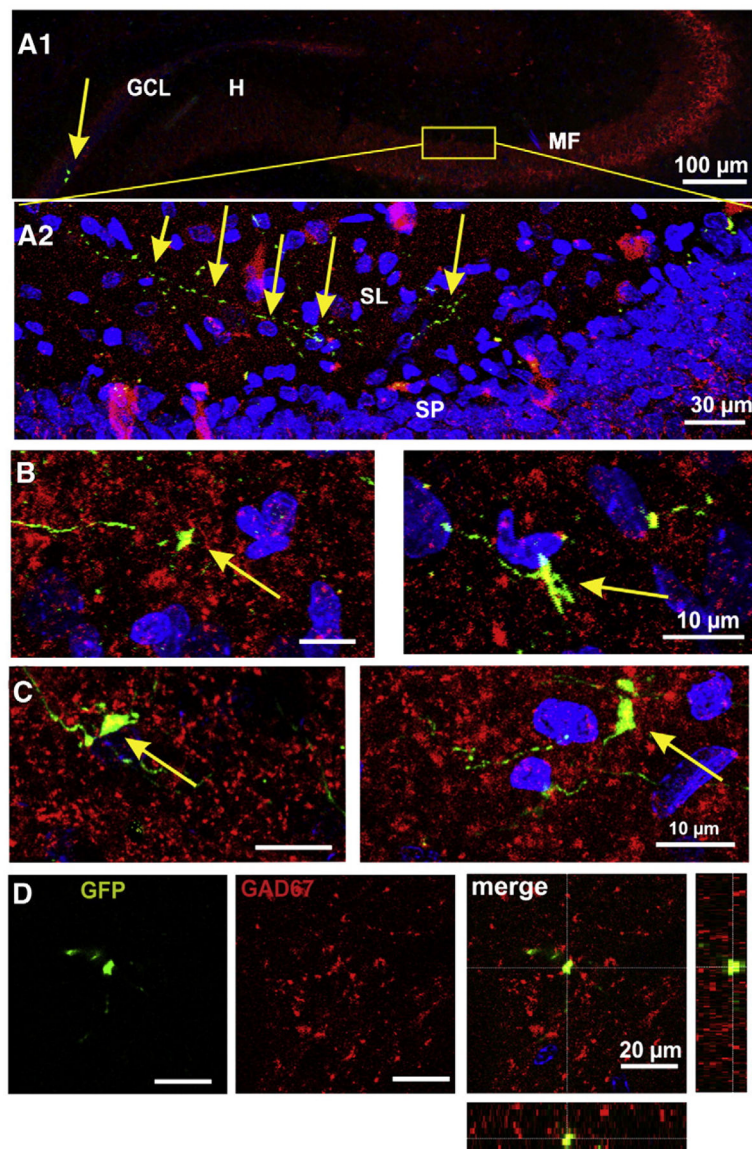


Fig. 5. The mossy fibers express GAD_{67} . A1) Panoramic view of the mossy fiber projection showing, on the left hand, two GFP^+ GCs (arrow) and GAD_{67}^+ mossy fibers. The area shown at high magnification in A2 is signaled. A2) Confocal image of an axon of a GFP^+ granule cell is shown along the stratum lucidum of CA3. B) Confocal images (50 optical slices of $0.5 \mu m$) of mossy fiber giant boutons expressing GAD_{67} at 15 dpi. C) Mossy fiber boutons of GFP^+ GCs at 30 dpi, did express GAD_{67} enzyme. D) Three-dimensional reconstructions through the Z-axis of GFP^+ boutons (fifty optical slices at $0.5 \mu m$ intervals) were used to verify the co-localization of GFP and GAD_{67} . Orthogonal projections are shown on the right most panel.

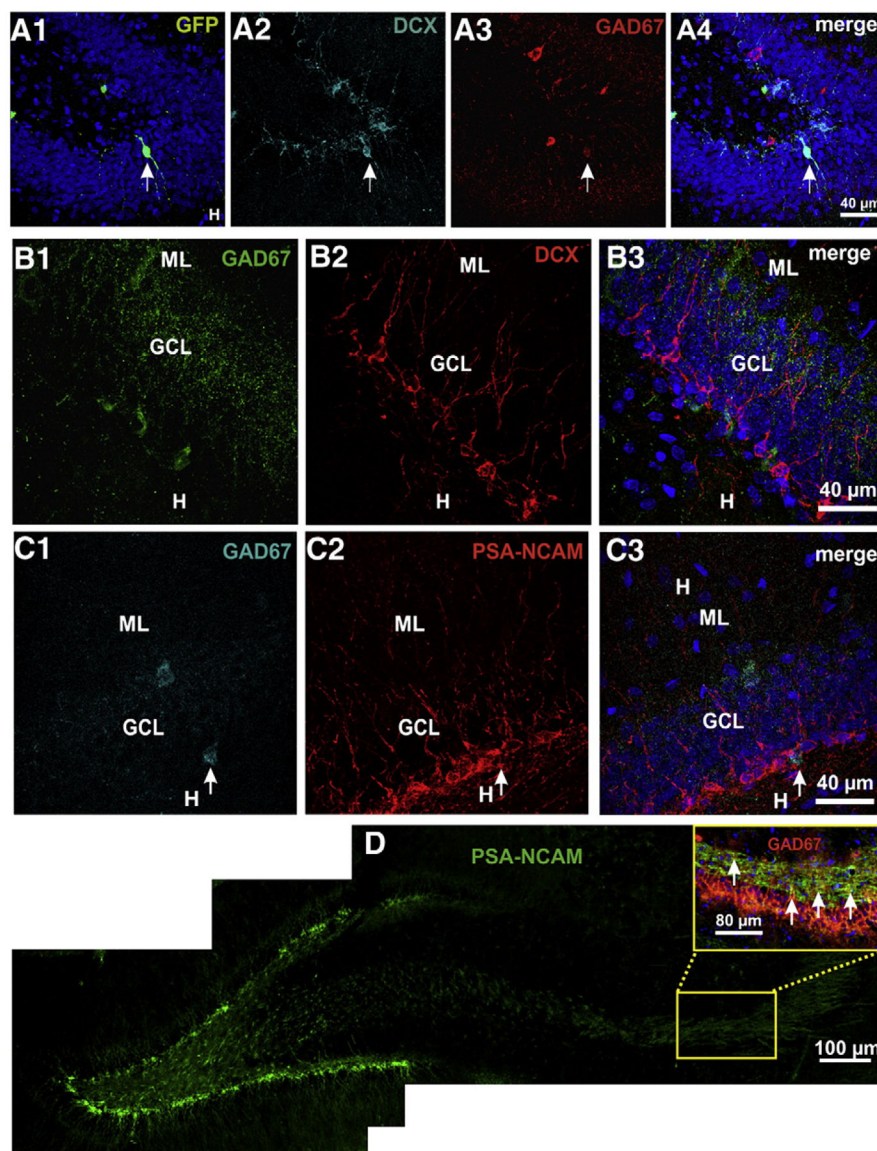


Fig. 6. Newborn GCs of injected rats, both with retrovirus and PBS, but not of control rats express GAD in their somata, while all express GAD₆₇ in their mossy fibers. A) GFP⁺ cell co-expressing DCX and GAD₆₇. B) Developing cells in non-injected adult rats, detected by their expression of doublecortin (DCX), did not express GAD₆₇. C) Some developing cells, detected by their expression of PSA-NCAM, expressed GAD₆₇ in PBS injected rats. D) In control, non injected rats, a number of PSA-NCAM⁺ mossy fibers expressed GAD₆₇.

Table 1

Electrophysiological properties of newborn and adult granule cells.

	GFP⁺	Adult
V rest (mV)	-64.7±1.0	-74.5±1.4
R input (GΩ)	2.8±0.1	0.43±0.01
C m (pF)	23.6±1.6	43.6±1.9
AP amp (mV)	40.4±1.6	80.4±2.9
AP slope (mV/ms)	18.4±4.2	171.8±36.8
AP half duration (ms)	5.5±1.3	1.32±0.1
Tau	74.7±4.4	31.1±3.5
Morphological class ^a	C	D
Position in the GCL ^a	1-2	1-3

Values are mean±s.e.m.; n=8 cells.

^aClassification of Espósito et al. (2005).

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