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Inducible gene deletion in astroglia and radial glia – a valuable tool for functional and lineage analysis

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ABSTRACT

Astrocytes are thought to play a variety of key roles in the adult brain, such as their participation in synaptic transmission, in wound healing upon brain injury and adult neurogenesis. However, to elucidate these functions in vivo has been difficult due to the lack of astrocyte-specific gene targeting. Here we show that the inducible form of Cre (CreERT2) expressed in the locus of the astrocyte-specific glutamate transporter (GLAST) allows precisely timed gene deletion in adult astrocytes as well as radial glial cells at earlier developmental stages. Moreover, postnatal and adult neurogenesis can be targeted at different stages with high efficiency as it originates from astroglial cells. Taken together, this mouse line will allow dissecting the molecular pathways regulating the diverse functions of astrocytes as precursors, support cells, repair cells and cells involved in neuronal information processing.

INTRODUCTION

Astrocytes are not only the most frequent cell type in the adult brain, but also thought to perform many diverse functions. For example, astrocytes play crucial roles in ion and glutamate homeostasis, the formation of the blood-brain-barrier (BBB), the modulation of cerebral blood flow as well as in synaptogenesis and synaptic transmission (Christopherson et al. 2005; Goritz et al. 2005; Hoke and Silver 1994; Mauch et al. 2001). In fact, the term 'tripartite synapse' describes the intricate association of astrocytes at the synapses and several studies highlight their functional contribution to the modulation of synaptic transmission (Araque et al. 1999). Moreover, some cells with astroglial features in the lateral wall of the lateral ventricle, the subependymal zone (SEZ), as well as in the subgranular layer of the dentate gyrus (SGL), act as adult neural stem cells at the source of ongoing adult neurogenesis (Belluzzi et al. 2003; Carleton et al. 2003; Doetsch et al. 1999a; Liu et al. 2003; Seri et al. 2001). However, despite these important functions of astrocytes, it has been difficult to elucidate the molecular mechanisms by which astrocytes exert such diverse roles due to the lack of astrocyte-specific tools to manipulate gene expression.

A very valuable tool has so far been the glial fibrillary acidic protein (GFAP)-promoter that allows examining some astrocyte populations in living slices, e.g. by their GFP expression (Matthias et al. 2003; Nolte et al. 2001). However, the GFAP-promoter is expressed only in small subpopulations of astrocytes in the adult brain, leaving many astroglial cells 'in the dark'. Moreover, other transgenic mice, such as the ones utilizing parts of the S100β-promoter, exhibit expression in a large proportion of other cell types, such as oligodendroglia (Vives et al. 2003; Zuo et al. 2004). Notably, all these approaches employed transgenic mice and no attempt has so far been used to directly target of astroglia-specific genes.

Moreover, there is another hurdle to manipulate gene expression specifically in astrocytes, namely that astrocytes share most, if not all, characteristic expression of genes with the ubiquitous glial cell type in the developing brain, the radial glial cells (for review, see Mori et al. 2005). For example, the astrocyte specific L-glutamate/L-aspartate transporter (GLAST), GFAP, vimentin, S100β, glutamine synthase (GS), the extracellular matrix molecule Tenascin-C, the brain lipid binding protein (BLBP) and nestin are expressed in astrocytes in the adult brain as well as in radial glial cells during development (Mori et al. 2005). This similarity between radial glial cells and astrocytes in regard to gene expression has hampered so far the construction of astrocyte-specific Cre lines that would not also mediate recombination in radial glia. Indeed, recombination mediated by the human (h)GFAP-promoter is inherited to all the neurons generated from radial glial cells (Malatesta et al. 2003), as is the case for a BLBP-Cre line (Anthony et al. 2004). The mouse (m)GFAP-promoter element apparently mediates later recombination starting around E18

(Garcia et al. 2004; Imura et al. 2003) and thereby affects radial glial cells at later developmental stages (E18) as well as astrocyte precursors. Thus, targeted mutagenesis in adult astrocytes has so far not been feasible, impairing our understanding of the molecular mechanisms regulating the diverse functions of astroglial cells in the adult brain.

To overcome these limitations we have chosen to target the inducible form of the Cre recombinase (CreERT2) that also proved to work efficiently in cells of the oligodendrocyte lineage (Doerflinger et al. 2003; Leone et al. 2003) to the GLAST locus (Hagiwara et al. 1996; Watase et al. 1998). The fusion of Cre to the ligand binding domain of the modified estrogen receptor (ERT2) is restricted in the cytoplasm and translocates only upon tamoxifen stimulation into the nucleus where it can then mediate recombination (Feil et al. 1996; Feil et al. 1997; Metzger and Chambon 2001; Metzger et al. 1995). CreERT2 was targeted to the locus of GLAST as GLAST is one of the best markers of adult astrocytes and radial glial cells (Barry and McDermott 2005; Hartfuss et al. 2001; Shibata et al. 1997; Williams et al. 2005). This allows mediating recombination at specific time points either in radial glial cells or adult astrocytes, including adult neural stem cells.

MATERIALS AND METHODS

Generation of mice

GLAST genomic DNA was obtained by screening the genomic DNA library (Hagiwara et al. 1996). Cre-ERT2-poly-A cassette or Cre-IRES-hrGFP-poly-A cassette (Stratagene) was inserted into the EcoRI site of the exon2 encoding the translation initiation site. The neomycin resistance gene cassette (PGK-neo-poly-A) flanked by FRT sequences was used for positive selection of targeted ES cells. A DTA cassette without poly-A signal was added at the 3' end of each targeting vectors for negative selection. The constructs were electroporated into the 129SVJ derived TBV2 ES cell line and screened for homologous recombination according to standard procedures. Recombined ES cells were injected into C57bl/6 blastcysts to generate chimeras. Later, heterozygous mice were crossed with hACTB::FLPe transgenic mice (mutated FLP recombinase driven by human β-actin regulatory sequences) to delete the neomycin cassette (Rodriguez et al. 2000).

Genotyping

Southern blotting

20μg ES cell DNA was digested with HindIII or EcoRI to check the recombination with the 5' and 3' probes as depicted in Fig. 1 Radio labeled probes were hybridized with the membrane as described previously (Mori et al. 2004).

PCR genotyping

Mouse tail DNA was used for PCR genotyping. The following primers were used for GLAST::CreERT2 genotyping (GLAST F8 (5'-GAGGCACTTGGCTAGGCTCTGAGGA-3'), GLAST R3 (5'-GAGGAGATCCTGACCGATCAGTTGG-3'), and CER1 (5'-GGTGTACGGTCAGTAAATTGGACAT-3')), for GLAST::Cre-IRES-hrGFP genotyping (GLAST F8, GLAST R3, and CreR1 (5'-ACACCTTCCTCTTCTTCTTGGGCAT-3')), for the deletion of the neomycin cassette (PGKF3 (5'-ACTTGTGTAGCGCCAAGTGCCAGCG-3') and FRTR2 (5'-GATAACACCCCCCAGGCATTCATGC-3')) are indicated in Fig. 1 and Supplemental Fig. 1, for hACTB::FLPe genotyping (sense primer (5'-CTAATGTTGTGGGAAATTGGAGC-3') and antisense primer: (5'-CTCGAGGATAACTTGTTTATTGC-3')).

Tamoxifen administration

Tamoxifen (SIGMA, T-5648) was dissolved in corn oil (SIGMA, C-8267) at 37^{0}C for several hours to prepare the 20mg/ml solution (Zervas et al. 2004). To optimize Cre activity induction in the adult brain, we injected Tamoxifen (Tx) intraperioneally according to four different protocols: protocol1: 1mg/day for 10 days by intraperioneal injection (IP) (Leone et al. 2003), protocol 2: twice 1mg/day for 5 days by IP (Leone et al. 2003), protocol 3 : 5mg/day for 2 days by oral gavage (Ahn and Joyner 2005), protocol 4: twice 2mg/day for 5 days by IP. Protocol 2 was the most efficient to induce Cre activity in the adult brain. In control mice, corn oil was injected. Adult mice that received Tx were analyzed 7, 10 days or 4 months after the last Tx injection. For embryonic induction of Cre activity, 3mg Tx was administrated per mouse by oral gavage of timed-pregnant mothers (Zervas et al. 2004).

Immunostaining

Adult mice were transcardinally perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) and brains were post-fixed in the same fixative over night followed by cryoprotection by 30% sucrose in PBS at 4^{0}C. 12 μm frontal sections were cut at the cryostat and processed for immunohistochemistry as described previously (Hartfuss et al. 2001). Following antibodies were used in PBS containing 10% normal goat serum and 0.5% TritonX: anti-β galactosidase (β–gal) (Cappel, rabbit, 1:2000), anti-S100β (SIGMA, IgG1, 1:1000), anti-NeuN (Chemicon, IgG1, 1:50), anti-Cre (Covance Research Product, rabbit, 1:5000), anti-CC1 (Calbiochem, IgG2b, 1:200), anti-glutamine synthase (GS, DB transduction lab, IgG2a, 1:250), anti-GFAP (SIGMA, IgG1, 1:100), anti-Double cortin (DCX, Chemicon, Guinea pig, 1:2000), anti-Calbindin (Swant, mouse IgG, 1: 5000), anti-Parvalbumin (Swant, mouse IgG1, 1:5000).

X-gal histochemistry

For whole mount staining, embryos were fixed with 2%PFA/0.2.% glutaraldehyde in PBS on

ice for 20 minutes. For X-gal staining on sections, embryonic brains were fixed with 2% PFA in PBS for 2 hours and cryoprotected with 30% sucrose in PBS. The embryos or sections were with 2mM MgCl, 5mM EGTA/PBS for 30 minutes and 2mMMgCl, 0.02% Nonidet-P40, 0.01% sodium deoxycholate in PBS for twice 5 minutes at room temperature. Then they were incubated in 5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4[Fe(CN)_6]$, 2 mM $MgCl_2$, 0.02% Nonidet-P40, 0.01% sodium deoxycholate, 1 mg/ml X-Gal in PBS at room temperature in the dark for several hours to visualize β-gal activity as a blue reaction product, washed with PBS and postfixed over night in 4% PFA in PBS.

RESULTS

Generation of mice

As described above, we targeted exon 2 of the GLAST locus, 66 base pairs upstream of the translation initiation site with two constructs: one containing CreERT2 (Fig. 1a) and one containing the normal form of Cre (Cre-IRES-hrGFP, Supplemental Fig. 1) in order to allow immunocytochemical localization of Cre-expression from the GLAST-locus. Both targeting constructs also contained the neomycin resistance gene flanked by FRT sequences, and a DTA cassette without poly-A signal (Fig. 1a, Supplemental Fig. 1a). After linearization of the targeting vectors with NotI, they were electroporated into the TBV2 ES cells and recombined ES cells were selected by neomycin. Recombination was confirmed by Southern blot with both 5' and 3' probes (Fig. 1b,c, Supplemental Fig. 1b,c). Neomycin cassettes were then deleted by crossing with hACTB:FLPe mice, in which the expression of the mutated FLP recombinase (FLPe) is driven by the human β-actin regulatory sequences (Rodriguez et al. 2000), Fig. 1d-f, Supplemental Fig.1d-f). Only heterozygous mice with a targeting construct lacking the neomycin cassette were used for further experiments.

Specificity of Cre expression in astrocytes and radial glia

In order to examine whether Cre expressed in the GLAST locus is targeted to the correct cell types, we first examined the localization of Cre in adult and embryonic GLAST::Cre-IRES-hrGFP mice, as the inducible form of Cre (CreERT2) can not be localized by immunocytochemistry (Casanova et al. 2002). As expected, the vast majority of Cre-immunoreactive (IR) cells in the adult brain were S100β-IR or GS-IR astrocytes (Supplemental Fig. 2a-c, Table 1a). Only few Cre-IR cells contained NeuN-immunoreactivity (1.6%) (Table 1a) and we found virtually no colocalization with NG2, a marker for glial precursor cells with diverse properties (Nishiyama et al. 1996)Matthias et al., 2003) (data not shown). However, we noted a small proportion of Cre-IR cells that were double-labelled with CC1, an antigen contained in oligodendrocytes (adenomatous polyposis coli, APC) (Bhat et al. 1996) (Table 1a). However, as previously reported (Leroy et al. 2001), CC1-immunoreactivity is also contained in astrocytes (in our hands 58% of CC1-IR cells were also

S100β-immunoreactive, Supplemental Fig. 2g-i, Table 1b). The S100β/CC1-double-IR cells can be discriminated from the cells that contain only CC1 by their nuclear size (see e.g. Buffo et al. 2005). Oligodendrocytes and CC1-only IR cells have small nuclei while the S100β/CC1-double-IR cells have significantly larger nuclei (Buffo et al. 2005). Indeed, most Cre/CC1-double IR cells were also S100β-positive and had large nuclei, suggesting that they are not mature oligodendrocytes. However, some of these cells may also represent precursors with lineage properties shared by the astrocyte and oligodendrocyte lineage. Taken together, we conclude that Cre expressed from the GLAST locus is mostly expressed in astroglial cells.

While virtually all Cre-IR cells are astrocytes, these may be a subset of the total astrocyte population. Astrocytes are a very heterogeneous population and also GLAST does not fully overlap with the other antigens typical for astrocytes, such as S100β or GS. Indeed, only 76% of S100β-IR cells are also GLAST-IR and accordingly only about 60% of all S100β-IR cells were Cre-IR cells (Table 1b). The smaller proportion of Cre-IR cells compared to GLAST-IR cells may be due to the affinity of the different antisera or to slight differences in gene expression levels. It is not possible to quantify reliably the number of Cre-IR cells that are GLAST-IR, as GLAST-immunoreactivity forms such a dense network of processes that virtually all Cre-IR nuclei are surrounded by GLAST-immunoreactivity. However, from this careful and extensive co-localization analysis with several astroglial markers (S100β, GS), we conclude that Cre expressed from the GLAST-locus is targeted to a large subset (60-80%) of astrocytes, corresponding to their frequency to those that endogenously express GLAST. Cre expressed from the GLAST locus was also expressed in the Müller Glia in the retina and the Bergmann Glia in the cerebellum (Supplemental Fig. 3 and data not shown), two special populations of adult astrocytes.

During development, Cre was observed in GLAST-IR radial glial cells in the telencephalon of GLAST::Cre-IRES-hrGFP mice at embryonic day (E) 12 and 16 (data not shown) consistent with our previous reports on GLAST in cortical radial glial cells (Hartfuss et al. 2001; Malatesta et al. 2003; Malatesta et al. 2000). In contrast to the strong expression of Cre from the GLAST locus, the GFP levels directed by the IRES sequence were virtually undetectable even after removal of the neomycin cassette (data not shown) with the exception of a much better signal in the Bergmann glia. Taken together, within the nervous system Cre expressed from the GLAST locus is specifically targeted to virtually all radial glia and a large subset of astrocytes.

Inducible Cre-mediated recombination in the adult brain

Cell-type specificity of inducible Cre-mediated recombination in the adult brain

Upon Tamoxifen (Tx) application by intraperitoneal injection into adult mice heterozygous for GLAST::CreERT2 and the Cre-reporter R26R (Soriano 1999), we observed a large number of

cells throughout the brain in which the reporter β-galactosidase was activated (Fig. 2a). Virtually all of the β-galactosidase-positive cells in the brain parenchyma had the morphology of protoplasmic astrocytes, while cells in the White Matter (WM) were also of different morphology (Fig. 2a). Indeed, co-localization of β-galactosidase-immunostaining with astroglial markers (S100β, GS or GFAP) showed that the vast majority of recombined β-galactosidase-IR cells (84.9%) were also immunoreactive for the respective astroglial marker (Fig. 2b-d, Supplemental Fig. 4a-f, and Table 2). Very few Cre-IR cells were weakly NeuN-immunoreactive (Fig. 2e and Table 2) in the cerebral cortex, while we observed a higher number of β-galactosidase-IR neurons in regions where adult neurogenesis continues. Consistent with the expression of Cre from the GLAST-locus in some CC1-positive cells, we also observed only some β-galactosidase-IR cells that were also CC1-IR (6%, Table 2, Fig. 2f). Similarly, we observed a minor population of β-galactosidase-IR cells that were NG2- or Sox10-positive (data not shown). Taken together, the vast majority of inducibly recombined β-galactosidase-IR cells were S100β-positive astrocytes demonstrating that this mouse line allows targeting selectively astroglial cells in the adult mammalian brain in vivo.

Efficiency of inducible recombination in the adult brain

Efficiency of inducible recombination was examined in two reporter lines, the R26R reporter as a ubiquitous reporter line (Soriano 1999), and the Cx43::LacZ mice (Theis et al. 2003) as specific reporter line for astrocytes containing connexin 43. In both lines, very few β-gal-IR cells could be detected in animals treated with oil without containing Tx (compare Fig. 3a and b), supporting a low degree of leakiness of this construct consistent with previous work (Guo et al. 2002; Indra et al. 1999; Leone et al. 2003). Next we varied the protocol of Tx application as described in the Methods (Table 2, 3a,b). In both reporter lines we found the highest number of cells expressing reporter (β-gal in both lines) with the protocol 2 administering twice 1mg Tx daily for 5 days (cortex: 29.5% of S100β-IR cells in R26R; 15.5% in Cx43::LaxZ; Fig. 2b-d, and Table 3a, b). Further increase in the Tx dose (twice 2mg/day for 5 days; protocol 4) did not notably increase the recombination efficiency (72.9% and 93.8% of protocol 2 in the cortical grey matter and S100β-IR Bergmann glia respectively) (Table 3a). However, shortening of Tx-application as in protocol 3 can give comparable recombination efficiency in the cortical grey matter (117.4% of protocol 2 in the cortical grey matter using the Cx43::LacZ reporter line; Table 3b), but allows fate mapping or conditional gene deletion in a shorter time window (2 days) rather than the 5-10 days of Tx application in the other protocols. Taken together, about a third of all astrocytes can be induced to recombine in the adult brain.

Notably, the proportion of cells exhibiting inducible recombination was consistently lower in the brain of Cx43::LacZ as reporter line (compare Table 3a and b). This prompted us to examine the total reporter activity in these mice by crossing them with nestin-Cre (Graus-Porta et al. 2001) or hGFAP-Cre (Zhuo et al. 2001) mouse lines that induce

recombination already at embryonic day 12 and 13 respectively (Malatesta et al. 2003). Despite this early recombination, only half of all S100β-IR cells expressed the β-gal reporter gene in adult offspring of these crosses (nestin-Cre x Cx43::LacZ: 53.3% of S100β, n=169; hGFAP-Cre x Cx43::LacZ: 47.1% of S100β, n=223). These data suggest to us, that only half of the S100β-IR astrocytes actually have high levels of connexin 43, consistent with only 60-70% of all S100β-positive cells containing high levels of GLAST. Again, these data support the view that astrocytes are heterogeneous.

We also compared the efficiency of inducible Cre-recombination in different brain regions. This was generally comparable within the parenchyma, such as in the cortex and the striatum where about 30% of all astrocytes had turned on β-gal (Table 3a). Notably, the highest recombination efficiency occurred in S100β-IR Bergmann glia of the cerebellum where almost all Bergmann glia were also β-galactosidase-IR in R26R mice (85.7%, Table 3a; 80.6% in Cx43::lacZ mice, Table 3b). A similarly high frequency of adult-induced recombination was observed in the retina (Supplemental Fig. 4) where many GS-IR Müller Glia (and some other astrocytes) were β–gal-positive in mice heterozygous for GLAST::CreERT2 and R26R. Taken together, this mouse line allows targeting a reasonable proportion of astrocytes throughout the adult brain and virtually all Bergmann glia.

Inducible recombination in adult neural stem cells with high efficiency

A region with relatively high recombination efficiency in the adult brain was the adult SEZ lining the lateral wall of the lateral ventricle (Fig. 3c, g-i), where neurogenesis continues life-long from astrocyte-like stem cells (Doetsch et al. 1999a). Interestingly, the majority of astrocytes in this region initiated recombination upon Tx treatment in adult mice heterozygous for GLAST::CreERT2 and Cx43::LacZ (65.9% of GFAP-IR cells were also β-gal-IR; Table 3b). These astrocytes were indeed at the source of adult neurogenesis as many neuroblasts in the rostral migrating stream and neurons in the olfactory bulb expressing the reporter gene were observed in mice heterozygous for GLAST::CreERT2 and R26R some time after Cre induction. 10 days after the last induction, many recombined cells were in the rostral migrating stream and few in the olfactory bulb (below 10% of all OB neurons were β-gal-IR; Fig. 2g, Fig. 4a-c), but after 4 months half of all interneurons in the olfactory bulb were labeled (Fig. 4d-f). Thus, this mouse line allows targeting adult neural stem cells specifically in vivo and thereby also manipulating all newly generated neurons.

Induction of Cre-activity in radial glial cells during development

As Cre in the GLAST locus is also targeted to radial glial cells in the developing CNS, the GLAST::CreERT2 mice should also allow to induce recombination at specific times during development in radial glial cells. Indeed, induction is already possible at E12 by administration of single dose of 3mg Tx into pregnant GLAST::CreERT2 x R26R mice

(Fig.5a-c), thereby demonstrating an excellent developmental resolution of recombination mediated in these mice. However, at early embryonic stage we also detected recombination outside the nervous system in the oral mesenchyme and some bones. Because we could also detect Cre expression in the oral mesenchyme in the developing GLAST::Cre-IRES-hrGFP mice, GLAST is expressed not only in the nervous system during embryonic stages (data not shown) (Gray et al. 2001; Huggett et al. 2002). As the X-gal staining in some bones was also observed in the oil control of GLAST::CreERT2 x R26R mice, but not in WT mice (Fig. 5c), this signal is due to some degree of leakiness of Cre in bone tissue.

Reporter gene activity within the nervous system after induction at E12 was mostly detected in the anterior forebrain, in the lateral ganglionic eminence (LGE) and at the border to the cortex (Fig. 5d-e) consistent with the onset of GLAST-expression in this region (insert in Fig. 5b and (Mori et al. 2005). The levels of Cre-expression seem still to be too low at E12 to induce recombination in the developing spinal cord consistent with the late upregulation of GLAST-immunostaining in this region (data not shown). Indeed, when Tx was administrated at E14 when also the endogenous expression levels of GLAST were better, many radial glial cells expressed the reporter gene in both the ventral and dorsal telencephalon and the spinal cord (Fig.5d-e, and data not shown). When animals were allowed to survive for longer times after embryonic Tx-administration (until postnatal day (P) 2 or 21), the progeny of early and late radial glial cells could nicely be monitored. Tx-administration at E12 or 14 resulted in reporter-gene-positive neurons in the cortex in intermediate or upper layers respectively (Supplemental Fig. 5a-c and data not shown), further supporting the neurogenic role of radial glial cells (Anthony et al. 2004; Malatesta et al. 2003; Malatesta et al. 2000; Miyata et al. 2001; Miyata et al. 2004; Noctor et al. 2001; Noctor et al. 2004). Notably, this mouse line now also allowed to look at the progeny of cerebellar radial glial cells which was not possible in the previous lines that also express Cre in external granule cells (Malatesta et al. 2003; Marino et al. 2000; Zhuo et al. 2001). As Purkinje neurons are generated at E12 (Goldowitz and Hamre 1998), we applied Tx at E12. Indeed, GLAST expressing radial glial cells at E12 generated Purkinje neurons (Fig. 6a-c). Upon Cre-induction at E14, recombination resulted in many labeled granule (Fig. 6 j-l), basket and stellate (Fig. 6 g-i) neurons as well as in Bergmann glia (Fig. 6 m-o) of the cerebellum at 3 weeks postnatal. These data suggest that also cerebellar radial glial cells generate neurons, including Purkinje cells, and glial cells, including Bergmann glia.

For some experiments, however, it may be desirable not to target neurons, but only glial cells including their precursors. Consistent with the loss of the neurogenic potential in late embryonic radial glia, application of Tx at E18 hardly resulted in β-gal IR neurons (Fig. 7d-f, Table 4) except DCX-IR migrating neuroblasts in the anterior SEZ (Fig. 7m-o), SGL in the hippocampus (Fig. 7p-r), and mature neurons in the olfactory bulb (Fig. 7j-l) (22.5$\pm$10.0%,

n=1860 and 16.6±5.1%, n=438 of NeuN-IR neurons were β-gal-IR cells in the granular cell layer and glomerular cell layer respectively). Thus, induction of Cre-activity at late embryonic stages allows targeting specifically neurons that are generated postnatally. This should allow determining the function of ongoing neurogenesis in the adult. Interestingly, only about half of all astroglial cells (41.9%) in the cerebral cortex exhibited reporter gene activity in R26R crosses (Table 4), suggesting that late radial glial cells only contribute to a subset of adult astrocytes. Moreover, recombination initiated in late embryonic radial glial cells also resulted in reporter gene activity in oligodendrocytes (Table 4), suggesting that late radial glial cells still contribute to the oligodendrocyte lineage, as previously suggested (Fogarty et al. 2005; LeVine and Goldman 1988). The proportion of oligodendrocytes derived from E18 radial glial cells was considerably higher in the striatum (41.9%, Table 4) than in the cortical gray matter (26.4%, Table 4), consistent with the continuation of oligodendrogliogenesis from lateral wall precursors throughout adulthood (Hack et al. 2005). We also observed oligodendrocytes labeled after Tx application at E18 in other regions of the brain, such as the olfactory bulb. Finally, we could also observe ependymal cells as a progeny of late embryonic radial glial cells (Fig. 7 g-i), consistent with previous data using Cre-adenovirus injections to fate map early postnatal radial glial cells (Merkle et al. 2004; Tramontin et al. 2003). Taken together, the GLAST::CreERT2 mice prove as a valuable tool not only to initiate recombination specifically in astrocytes of the adult brain, but also to examine fate changes of radial glial cells during development by inducing recombination in early or late radial glia respectively.

DISCUSSION

Here we described the recombination mediated by CreERT2 under control of the GLAST locus. This new mouse line allows efficient targeting of radial glial cells at different developmental stages, a versatile tool to examine the molecular determinants of neurogenic versus gliogenic radial glial cells. Importantly, recombination can be mediated also in the adult brain specifically in astrocytes, both in neurogenic astroglial-like cells located in the adult SEZ and SGL as well as in other astrocyte populations throughout the brain, including the Bergmann Glia of the cerebellum and the Müller Glia in the retina. This allows for the first time to address the function of specific genes exclusively in astroglial cells in vivo – experiments that could not be done previously.

Induction of Cre-mediated recombination – specific, fast and efficient

In order to delete genes of interest in astroglial cells, the foremost requirement is the specificity of recombination. So far, Cre-mediated recombination was possible only in astrocytes along with other cell types and throughout development (Anthony et al. 2004; Garcia et al. 2004; Malatesta et al. 2003; Zhuo et al. 2001), as mediated by the human or the mouse GFAP promoter elements. In GLAST::CreERT2 mice treated with tamoxifen about

90% of all cells expressing reporter gene induced in the adult brain were S100β-IR (Table 2). Most crucially, very little reporter gene activity was detectable in neurons or oligodendrocytes, further supporting the astrocyte-specific recombination mediated by adult induction in these mice. It is also important to note that specific populations of astrocytes in the adult brain, such as Bergmann glia or Müller glia, can be targeted using this mouse line. This line therefore complements mouse lines available for specific gene deletion in neurons (Beggs et al. 2003) and oligodendrocytes (Doerflinger et al. 2003; Leone et al. 2003) and closes the lack of tools for astroglia-specific gene targeting.

Interestingly, closer analysis of expression from the GLAST locus also revealed an important heterogeneity of astroglial cells, in line with many previous reports (for review see : Hoke and Silver 1994; Miller et al. 1994; Mori et al. 2005; Walz 2000). The 'classical' astrocytes are characterized by passive conductance and gap junctional coupling, the latter mediated largely by Connexin 43 (Nagy et al. 2003; Nagy and Rash 2000). However, there are other astrocyte subtypes that exhibit more active conductance and are not coupled by gap junctions (Matthias et al. 2003; Wallraff et al. 2004). To address this diversity we choose two reporter lines – the ubiquitous R26R line as well as the Cx43::LacZ mice that monitor recombination in astrocytes expressing high levels of Cx43. Interestingly, even upon recombination initiated at E13 by nestin-Cre (Betz et al. 1996; Graus-Porta et al. 2001; Tronche et al. 1999) Cx43-positive astrocytes constituted only about 50% of all S100β-IR cells in the adult cortex, suggesting that Cx43::LacZ labels only a subtype of astrocytes, namely those that are supposedly coupled by gap-junctions. Similarly, only in 60-70% of S100β-positive cells were also GLAST-IR (detected both by double-staining of S100β and GLAST as well as S100β and Cre in the GLAST::Cre-IRES-hrGFP mice), consistent with the expression of S100β also in other glial cell types such as oligodendrocytes and oligodendrocyte precursors (Buffo et al. 2005; Deloulme et al. 2004). Thus, the subpopulation of S100β–IR cells that expresses high levels of GLAST and is functionally coupled by gap-junctions, both criteria of classical astrocytes (Chaudhry et al. 1995; Shibata et al. 1997; Wallraff et al. 2004), is targeted in the GLAST::CreERT2 mouse line. Interestingly, astroglial-like cells in the adult SEZ and SGL that are at the source of neurogenesesis also belong to this group of astrocytes with high levels of GLAST and Connexin 43 expression (see below).

For astro-/radial glia-specific recombination, the induction of Cre activity can be optimized in regard to speed or efficiency. Cre-mediated recombination can be observed already 1 day after tamoxifen application at least at embryonic ages. Also in the adult brain, similar efficiency in recombination was achieved by Tx application for 2 or 5 days, demonstrating that induction of Cre-mediated recombination is feasible within a reasonable time frame and with cell type specificity. However, the efficiency of recombination varies in different regions of the adult brain, possibly due to differences in Tx accessibility modified by the blood-brain barrier. The

highest efficiency of recombination occurred in Bergmann glia of the cerebellum, where CreERT2-mediated recombination was observed in virtually each cell. In the cerebral cortex or basal ganglia about 30% of all S100β-IR cells, i.e. about 60% of the 'classical' GLAST-positive astrocytes can be targeted in this mouse line. Since the recombination in a subset of astrocytes can be traced by reporter gene activity, this allows comparing the phenotype of the targeted astrocytes with neighboring WT astrocytes in an otherwise unaffected environment. The phenotype and behaviour of the astrocytes genetically targeted by GLAST::CreERT2-mediated recombination therefore provides an ideal tool to examine cell-autonomous gene function in astrocytes.

Applications of GLAST::CreERT2 mediated gene deletion during development

Only recently has a novel role of radial glial cells in neurogenesis been discovered (Anthony et al. 2004; Malatesta et al. 2003; Malatesta et al. 2000; Miyata et al. 2001; Miyata et al. 2004; Noctor et al. 2001; Noctor et al. 2004). The GLAST::CreERT2 mice now allow examining neurogenesis from radial glial cells at different stages of development. Indeed, analysis of the progeny of radial glial cells in the cerebellum was problematic before as GFAP-promoter driven constructs also express in external granule neuron precursors (Marino et al. 2000). Now using induction of Cre-mediated recombination at E12 or E14 in GLAST::CreERT2 mice we could show that radial glial cells generate Purkinje neurons and basket, stellate, and granule neurons at E12, while recombination induced at E14 resulted exclusively in labelling of granule, basket and satellite neurons as the progeny of E14 cerebellar radial glia. Moreover, the inducible form of Cre targeted to the GLAST locus now allows to examine the glial progeny of radial glial cells which was not feasible before as all astroglial cells also expressed Cre under mGFAP, hGFAP or BLBP-promoter elements (Anthony et al. 2004; Garcia et al. 2004; Malatesta et al. 2003). Indeed, Cre-mediated recombination at embryonic stages in the cerebellum revealed Bergmann Glia as a derivative of earlier radial glial cells (Yamada et al. 2000).

While radial glial cells contribute to neurogenesis at earlier stages, at later stages they generate exclusively astrocytes and oligodendrocytes. Interestingly, when recombination was induced at E18, astrocytes and oligodendrocytes, but no neurons were derived from this late population of radial glial cells with the exception of the olfactory bulb and dentate gyrus, the only regions where neurogenesis continues life-long. These data thus confirm previous lineage tracing experiments with retroviral vectors demonstrating cells generating astrocytes and oligodendrocytes in the postnatal telencephalon (Levison and Goldman 1993; Levison and Goldman 1997; Luskin et al. 1988; Zerlin et al. 1995). Thus, late radial glial cells may correspond to an in vivo equivalent of an O2A precursor with the potential to generate either astrocytes or oligodendrocytes, but further clonal analysis is needed to determine the proportion of bi- or unipotent radial glial cells at this stage. Moreover, Cre-mediated

recombination at the end of neurogenesis in the mammalian brain may allow dissecting the molecular signals responsible for the end of neurogenesis from radial glial cells in most regions of the mammalian brain, but not in other vertebrate classes (Birse et al. 1980; Garcia-Verdugo et al. 2002; Goldman and Nottebohm 1983; Lopez-Garcia et al. 1988).

Potential applications of GLAST::CreERT2 mediated gene deletion in the adult brain

As mentioned above, recombination induced in GLAST-positive cells at E18 labels exclusively glial cells in most regions, except those where neurogenesis continues. This allows targeting postnatally generated neurons and elucidating their role in the olfactory bulb or dentate gyrus. In fact, our lineage analysis has shown that shortly after induction of Cre only a few interneurons in the olfactory bulb (OB) were labelled due to recombination in the adult neural stem cells, while the proportion of labelled interneurons in the OB rises to 30% of all interneurons 4 months after recombination was induced (Fig. 4). These data show for the first time the contribution of postnatal neurogenesis to the neuronal pool in the respective adult brain region, a crucial information for animal models of depression that have been linked to modifications in adult neurogenesis (Santarelli et al. 2003). Besides targeting all postnatally or adult generated neurons, this mouse line allows for the first time examining gene deletion in adult neural stem cells themselves. A recent publication using the inducible form of Cre in the Gli1 locus also allows the induction of recombination in adult neurogenesis (Ahn and Joyner 2005). However, Cre expressed from the Gli1-locus is also contained in neuroblasts, transit-amplifying cells as well as stem cells and only if fast-dividing precursors are killed by AraC infusion (Doetsch et al. 1999a; Doetsch et al. 1999b), recombination occurs in the only surviving cells, namely the slow dividing stem cells (Ahn and Joyner 2005). However, induction of Cre activity by tamoxifen in the GLAST::CreERT2 mice allows targeting exclusively astrocytes in the adult SEZ and SGL without the need of AraC infusion and hence direct genetic manipulations to the stem cells first. Therefore this mouse line allows for the first time to perform functional genetics on adult neural stem cells in vivo.

Not only adult neural stem cells, but also normal astrocytes in the brain parenchyma and white matter can now be manipulated in the adult brain in vivo. This will be particularly relevant for two areas of research addressing the role of astrocytes in brain lesion and neuronal plasticity. Astrocytes react to brain injury and migrate to the site of the lesion. Their reaction includes up-regulation of certain intermediate filament proteins (GFAP, vimentin), extracellular matrix molecules, such as Tenascin C, and hypertrophy of the cell somata and their processes (Pekny and Nilsson 2005). However, the signals mediating astroglial responses in injury are not yet well understood, given that reactive gliosis occurs in lesions with inflammation and disruption of the blood brain barrier (like stab wound or ischemia) as well as in neurodegenerative models where the blood brain barrier is intact (see Buffo et al., 2005).

A similar issue of contention has been the contribution of astrocytes to synaptogenesis and synaptic plasticity. Astrocytes closely enwrap all CNS synapses and evidence is mounting for their crucial role in synaptogenesis (Christopherson et al. 2005; Ullian et al. 2004). However, this analysis has so far relied on in vitro models. The GLAST::CreERT2 mice now allows to delete genes implicated in a crucial role for astrocyte-mediated synaptic processing (Araque et al. 1999) in vivo to test their function. In this context it is important to consider the role of GLAST in synaptic transmission as it contributes to remove glutamate from the synaptic cleft. Indeed, the absence of GLAST affects synaptic transmission in the cerebellum, retina and by a metabolic crosstalk also in the developing cortex (Harada et al., 1998; Takayasu et al., 2005; Voutsinos-Porche et al., 2003; Watase et al., 1998). Notably, some aspects of glutamate clearance from the synaptic cleft can be compensated by GLT1, the other major glutamate transporter in astrocytes (Stoffel et al. 2004), but it is important to use heterozygous GLAST::CreERT2 mice (as we have done here) for all experiments to exclude functional defects due to lack of GLAST function. No defects were so far observed in mice with only one functional allele for GLAST (Harada et al. 1998; Takayasu et al. 2005; Voutsinos-Porche et al. 2003; Watase et al. 1998). Taken together, these few examples highlight the great potential of this new mouse line for both fate mapping as well as functional analysis of astroglial cells.

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Average % / section	Cortex (GM)	striatum
% S100β / Cre	84.9±11.3% (n=340)	74.6±4.3% (n=142)
% GS / Cre	81.6±4.5% (n=247)	82.5±6.4% (n=191)
% CC1 / Cre	8.1±7.1% (n=89)	9.2±5.9% (n=121)
% NeuN / Cre	1.6±2.7% (n=118)	2.8±3.9% (n=38)

Table 1a

Specificity of Cre-expression in adult GLAST::Cre-IRES-hrGFP mice. (n= number of cells)

Average % / section	Cortex (GM)	Striatum
% Cre / S100β	58.8±5.7% (n=425)	51.9±4.5% (n=205)
% Cre / GS	52.8±7.2% (n=386)	49.3±15.5% (n=308)
% GS / S100β	89.1±2.2% (n=159)	66.1±7.8% (n=124)
% S100β / GS	89.6±4.2% (n=158)	85.5±15.0% (n=98)
% Cre / CC1	19.0±18.0% (n=34)	12.9±9.8% (n=94)
% CC1 / S100β	25.7±12.2% (n=303)	45.0±5.1% (n=128)
% S100β / CC1	58.0±10.7% (n=120)	76.3±10.7% (n=76)
% Cre / NeuN	0.3±0.5% (n=675)	0.7±0.9% (n=213)

Table 1b

Efficiency of Cre-expression/Astrocyte heterogeneity in adult GLAST::Cre-IRES-hrGFP mice. (n= number of cells)

	Protocol 2			Protocol 4	
Average % /section	Cortex (GM)	striatum	Bergmann glia	Cortex (GM)	Bergmann glia
% S100β/β-gal	84.9±7.5% (n=7)	82.7±14.9% (n=4)	97.7±2.3% (n=4)	89.0±9.8% (n=3)	96.6±4.0% (n=3)
% NeuN/β-gal	2.1±3.1% (n=6)	0.7±2.0% (n=3)		6.0±5.7% (n=3)	
% CC1/β-gal	6.2±5.3% (n=3)				

Table 2

Specificity of inducible Cre-mediatad recombination in adult mice heterozygous for GLAST::CreERT2 and R26R.

(n=number of animals)

	Protocol 2			Protocol 4	
Average % /section	Cortex (GM)	striatum	Bergmann glia	Cortex (GM)	Bergmann glia
% β-gal/S100ß	29.5±10.7% (n=7)	29.0±4.4% (n=4)	85.7±10.3% (n=4)	21.5±6.0% (n=3)	80.4±8.9% (n=3)
% β-gal/NeuN	0.1±0.2% (n=4)	0.04±0.1% (n=3)		0.3±0.4% (n=3)	
% β-gal/CC1	5.8±5.6% (n=3)				

Table 3a

Efficiency of inducible Cre-mediated recombination in adult mice heterozygous for GLAST::CreERT2 and R26R.

(n=number of animals)

Average % / section	Protocol 1(n=3)	Protocol 2(n=3)	Protocol 3(n=3)
cortex % β-gal / S100β	6.6±2.7%	15.5±6.1%	18.2±7.9%
Bergmann glia % β-gal / S100β	43.6 ±20.0%	79.7±12.1%	80.6±18.2%
SEZ % β-gal / GFAP	41.6±22.6%	65.9±15.6%	49.4±14.6%

Table 3b

Efficiency of inducible Cre-mediated recombination in adult mice heterozygous for GLAST::CreERT2 and Cx43::LacZ.

(n=number of animals)

Average % / section	Cortex (GM)	Cortex (WM)	striatum
% β-gal / S100β	41.9±8.1%	30.6±7.5%	43.0±7.6%
% S100β / β-gal	93.3±1.5%	25.9±9.5%	64.3±14.8%
% β-gal / NueN	0.3±0.3%		0.3±0.4%
% NeuN / β-gal	3.7±3.9%		2.1±2.7%
% β-gal / CC1	26.4±15.7%	36.8±10.8%	41.9±13.2%
% CC1 / β-gal	12.2±6.0%	61.7±24.0%	46.5±13.0%

Table 4

Lineage analysis of radial glial cells at E18 embryo heterozygous for GLAST::CreERT2 and R26R.

(n=number of animals)

Figure legends

Figure 1

Targeting strategy and genotyping

The panel in (a) depicts the targeting strategy of the GLAST locus. Sites for southern blotting are indicated in green, and PCR genotyping primer sites are indicated by red arrows. Panels (b, c) depict examples of southern blots of WT and heterozygous embryonic stem (ES) cells and panels (d-f) depict examples of PCR genotyping of GLAST::CreERT2 mice with the genotype indicated on top of the panels. Lines 3, 8 and 13 depict the PCR results after deletion of the neomycin cassette by crossing GLAST::CreERT2 heterozygous mice with hACTB::FLPe. The positive control in lane 4, 9 is DNA from recombined ES cells and lane 14 is the positive control for FLPe PCR genotyping (DNA from hACTB:FLPe mouse tail).

Figure 2

Induction of Cre activity in the adult brain monitored in R26R-reporter mice.

Cre activity was induced in the adult GLAST::CreERT2 x R26R by injection of 1mg Tamoxifen twice a day for 5 days (protocol 2) and analyzed at 10 days after induction. Successful recombination was monitored by immunostaining for β-galactosidase (green in a; red in b-g). Note the large number of protoplasmic astrocytes immunoreactive for β-galactosidase in the grey matter (GM) of the cerebral cortex (a), and astrocytes with a notably different morphology in the cortical white matter (WM). The astrocyte identity of the recombined cells was confirmed by double-staining for S100β (b-d), and hardly any colocalization of β-galactosidase-positive cells with the neuronal marker NeuN (e) or CC1 (f), an antigen detected in oligodendrocytes was observed. As Cre activity was also induced in the GFAP-IR adult neural stem cells in the SEZ lining the lateral ventricle (LV, g), their descendants, the doublecortin (DCX)-positive neuroblasts inherited the β-galactosidase expression (g). Scale bars: 200μm (a), 100 μm (b-g).

Figure 3

Induction of Cre activity in the adult brain monitored in the astroglia-specific Cx43::LacZ-reporter mice.

Cre activity was induced by injection of Tamoxifen as described in Fig. 2 and examined at 7 days after induction in GLAST::CreERT2 and Cx43::LacZ heterozygous mice by β-galactosidase-immunostaining (white in a-c, red in d,f,g,i). Note the nuclear localization of β-galactosidase-IR in the Cx43::LacZ mice. A dense band of β-galactosidase-IR Bergmann glia (identified by S100β-IR, not shown) is visible in the cerebellum after induction with Tamoxifen, while virtually no β-galactosidase-IR cells can be detected after oil administration (b). Note the dense band of β-galactosidase-IR cells lining the lateral ventricle (LV) in the subependymal zone (SEZ) next to the striatum (stri) where astrocyte-like adult neural stem

cells are located. Co-localization of β-galactosidase-IR (red) with GFAP-IR (green) confirmed the astroglial identity in the adult neurogenic regions (d-f; SGL, g-i; SEZ). Scale bars : 200 μm (a-c). 100 μm (d-e). CTX: cerebral cortex; CC: corpus callosum; Stri: striatum; LV: lateral ventricle; SEZ: subependymal zone; SGL: subgranular layer of the hippocampus.

Figure 4

Monitoring neuronal turn-over in adult neurogenesis by induction of Cre-based mediated fate mapping.

Cre-mediated recombination was induced in adult GLAST::CreERT2 and R26R heterozygous mice by injection of 1mg Tamoxifen twice a day for 5 days (protocol 2) and monitored by β-galactosidase-IR 10 days (a-c) or 4 months (d-f) after the last injection. At 10 days after the last Tamoxifen injection, only few NeuN-positive neurons were also β-galactosidase-positive in the granule cell layer (GCL) of the olfactory bulb (arrows a-c) indicating that they were derived from GLAST-positive adult neural stem cells in which recombination occurred. Neurons that are not β-galactosidase-positive are indicated by arrowheads. Notably, 4 month after recombination in adult neural stem cells half of all neurons in the GCL were also β-galactosidase-positive (arrows in d-f) suggesting that they have been generated from stem cells within this time. Scale bar: 100μm.

Figure 5

Induction of Cre activity in the embryonic brain monitored in R26R-reporter mice.

A single dose of 3mg tamoxifen was administrated to pregnant GLAST::CreERT2 and R26R heterozygous mice that were sacrificed one day or two days later. Whole embryos (a-c) and 12μm-thick sections (d-h) were processed for X-gal staining (a-e) or β-galactosidase immunostaining (f-h). Lateral view (a), ventral view (b), and dorsal view (c) of E13 embryos 1 day after Tamoxifen treatment. While few cells expressed β-galactosidase after induction at E12, when GLAST is still weak and mostly expressed at the cortico-striatal boundary (inset in (b) indicates GLAST-immunoreactivity in the telencephalon at E12), many cells in the telencephalic ventricular zone were β-galactosidase-IR after induction at E14 (d: anterior telencephalon, e: posterior telencephalon). Notably, many GLAST-IR radial glial cells (green in g, h) could be induced to recombine and express β-galactosidase (red in f,h; examples indicated by arrows) after a single Tamoxifen application at E14 (f-h). Scale bars: 0.5cm (a-c), 200μm (d-e), 50μm (f-h). CTX: cerebral cortex; LGE: lateral ganglionic eminence; MGE: medial ganglionic eminence; BG: basal ganglia.

Figure 6

Progeny of radial glia in the cerebellum at different developmental stages.

Tamoxifen was administrated to GLAST::CreERT2 and R26R heterozygous mice at E12 (a-c) or E14 (d-o) and the progeny of recombined radial glia was analyzed by β-galactosidase-IR in

the cerebellum at P21/22. Note that Purkinje cells (detected by Parvalbumin-IR (b,c) or by Calbinin-IR (e,f)) are β-galactosidase-labeled only if recombination is initiated at E12 (a-c, white arrows), but not at E14 (arrowheads in d-i indicate Calbindin- or Parvalbumin-IR β-galactosidase-negative Purkinje cells). Parvalbumin-IR stellate and basket neurons (grey arrows in a-c and arrows in g-i), NeuN-IR granule neurons (j-l), and S100β-IR Bergmann glia (m-o) were generated by radial glial cells induced to recombine at E12 or E14 respectively. Scale bars: 100 μm (a-i, m-o); 50 μm (j-l). ML: Molecular Layer; PCL: Purkinje Cell Layer; GCL: Granule Cell Layer.

Figure 7

Progeny of radial glia induced to recombine at embryonic day 18.

Tamoxifen was administrated to GLAST::CreERT2 and R26R at E18 and analyzed by β-galactosidase-IR at P21. Recombination (β-gal expression, red) was detected in S100β-IR astrocytes in the cortical gray matter (arrows in a-c) and CC1-IR oligodendrocytes (data not shown, but see Table 4). Virtually no NeuN-IR neurons (green in e) were also β-galactosidase-IR (red in d, f; arrowheads), indicating that cortical radial glial cells do no longer generate neurons at this stage. Note, however, the S100β-IR epemdymal cells (arrows in g-i) surrounding the lateral ventricle. In contrast to the cerebral cortex where radial glia are no longer neurogenic at E18, E18 radial glial cells in the ventral telencephalon generated adult neural stem cells in the SEZ and SGL and their descendants (β-galactosidase-IR cells) differentiated into DCX-IR neuroblasts (arrows in m-r) and olfactory bulb neurons (arrows in j-k). Scale bars: 50μm (a-l), 50 μm (m-r).

Supplemental Figure Legends

Supplemental Figure 1

Targeting strategy and genotyping of GLAST::Cre-IRES-hrGFP mice

The targeting construct Cre-IRES-hrGFP and the GLAST locus are depicted in panel a. Sites for southern blotting are indicated in green, and PCR genotyping primer sites are indicated by red arrows. Panels (b, c) depict examples of southern blots of WT and heterozygous embryonic stem (ES) cells and panels (d-f) depict examples of PCR genotyping of GLAST::CreIREShrGFP mice with the genotype indicated on top of the panels. Lane 16, 22, 27: wild type C57black/6; lane 20, 26: positive control for PCR genotyping (recombined ES cell DNA); lane 17, 23, 29: GLAST::Cre-IRES-hrGFP heterozygous mice; lane 18, 19, 24, 25, 30, 31: GLAST::Cre-IRES-hrGFP heterozygous mice crossed with hACTB::FLPe to delete the neomycine cassette; lane 14 and 32: positive control for FLPe PCR genotyping (DNA from hACTB:FLPe mouse tail).

Supplemental Figure 2

Specificity of Cre expression in the adult cortex of GLAST::Cre-IRES-hrGFP mice.

Fluorescent micrographs of frontal sections of the cerebral cortex of GLAST::Cre-IRES-hrGFP mice immunostained for Cre (green in a, d, g) and the cell-type specific antigens for S100β label astrocytes (b, c), NeuN to label neurons (e, f) and CC1 to label oligodendrocytes (h, i). Note that virtually all Cre-IR cells were S100β-positive astrocytes, but did not co-localize with NeuN. Very few Cre-IR cells were also CC1-IR (arrows in the lower right insets in g-h), but most of these had big somata and were often S100β and CC1-double positive (arrows in the upper right insets in g-h). CC1-IR cells with small somata without S100β– or Cre-IR are indicated by arrowheads in insets in g-h. Scale bar: 50 μm.

Supplemental Figure 3

Cre expression in the glial cells of the retina in GLAST::Cre-IRES-hrGFP mice.

Fluorescent micrographs of frontal sections of the retina stained for Cre (a, d) and glutamine-synthase (GS) to label Müller glia (b, c) and glia fibrillary acidic protein (GFAP) to label astrocytes (e, f). Note that Cre is expressed in most Müller cells, but only few astrocytes. Scale bar: 50 μm. RGC: retinal ganglion cell layer; INL: inner nuclear layer.

Supplemental Figure 4

Induction of Cre activity in Müller Glia and astrocytes of the retina in adult GLAST::CreERT2 mice.

Cre-mediated recombination was induced in adult GLAST::CreERT2 and R26R heterozygous mice by injection of 1mg Tamoxifen twice a day for 5 days (protocol 2) and monitored by β-galactosidase-IR 10 days later. Note that virtually all β-galactosidase-IR cells (a, d, g) were

glutamine synthase (GS)-IR Müller Glia (arrows in a-c) and GFAP-IR astrocytes (arrows in d-f), but not NeuN-IR neurons (arrowheads in g-i). Scale bar: 50μm.

Supplemental Fig. 5

Progeny of radial glia induced to recombine at embryonic day 12.

Cre activity was induced in GLAST::CreERT2 and R26R heterozygous mice at E12 and β-galactosidase-IR was analyzed at P22. In the cortex, β-galactosidase-IR (red in a, c, d, f, g, i) was detected in S100β-positive astrocytes (a-c), NeuN-positive neurons (d-f) and CC1-positive oligodendrocytes (g-i). Arrows indicate examples of double-labelled cells. Scale bar: 100 μm. CTX(GM): cortical gray matter; CTX(WM): cortical white matter; Stri: Striatum.

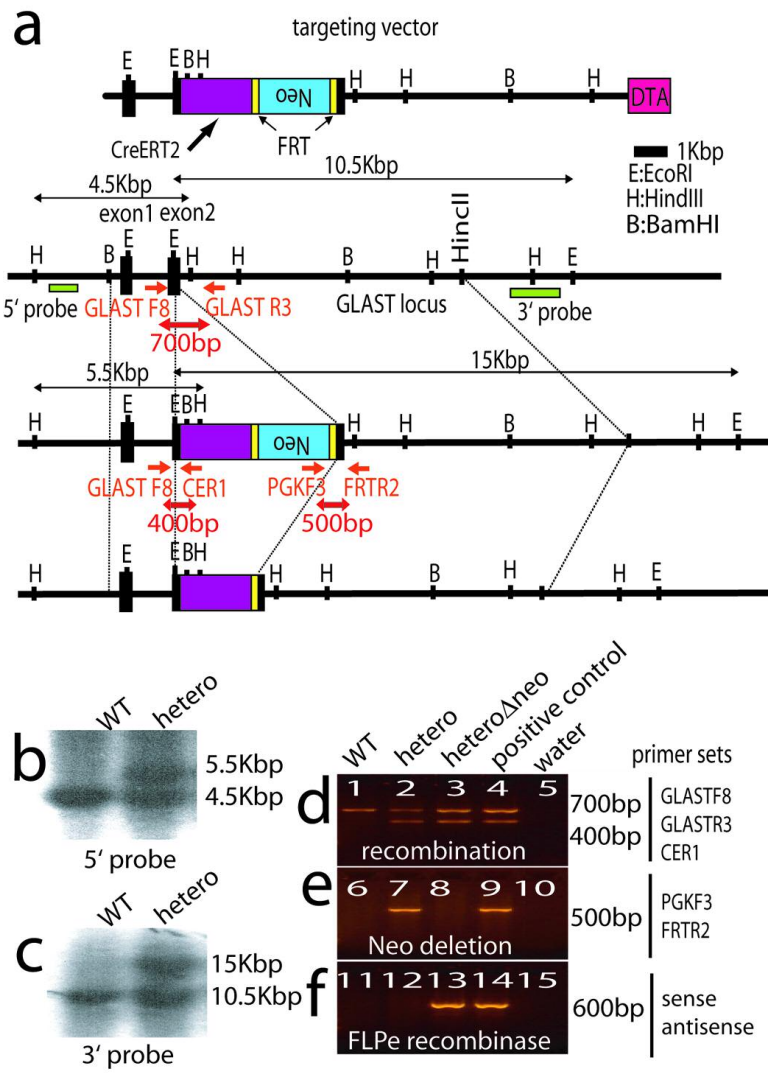


Figure 1 Mori et al.

Targeting strategy and genotyping

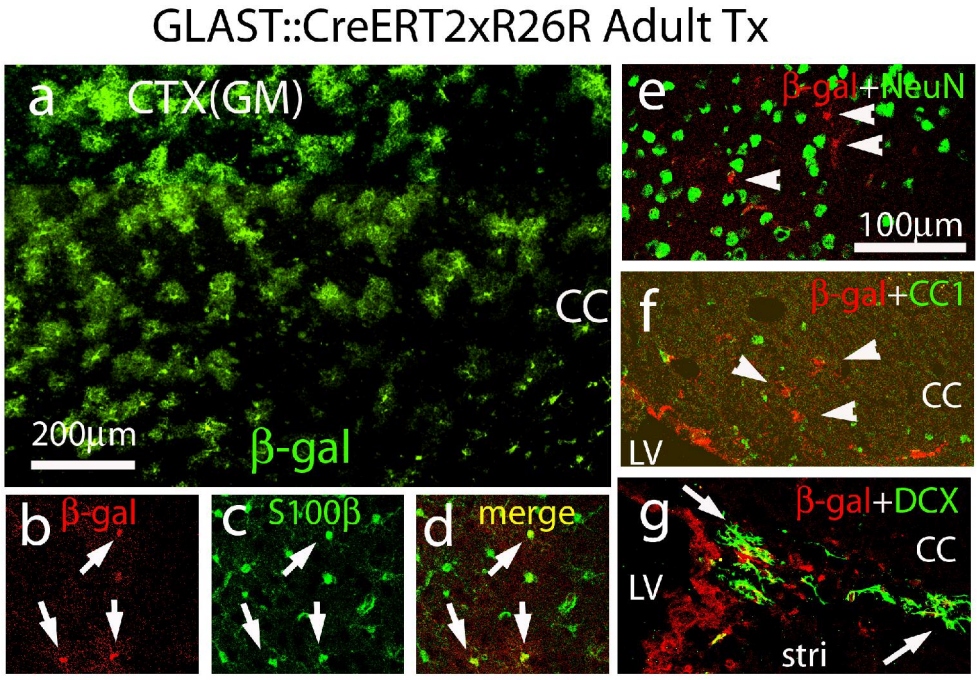


Figure 2 Mori et al.

Induction of Cre activity in the adult brain monitored in R26R-reporter mice.

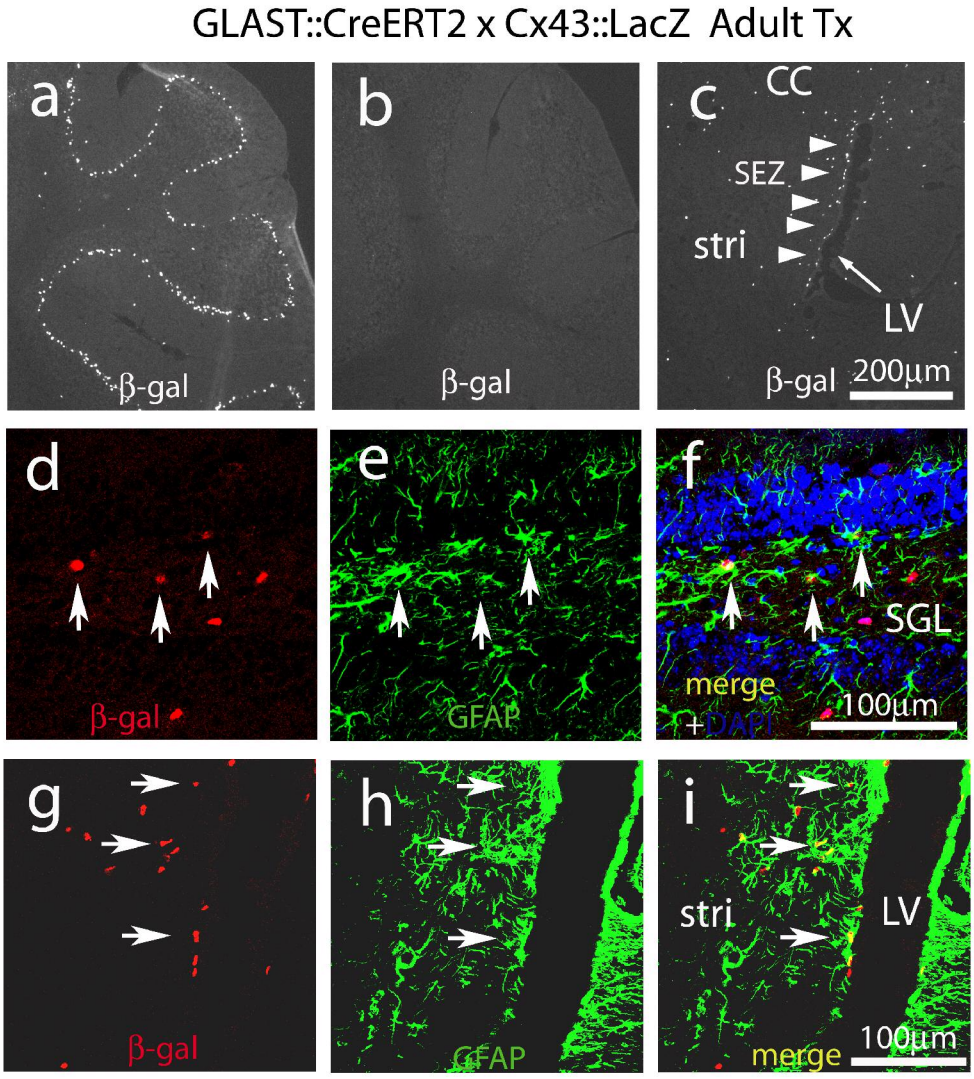


Figure 3 Mori et al.

Induction of Cre activity in the adult brain monitored in the astroglia-specific Cx43::LacZ-reporter mice

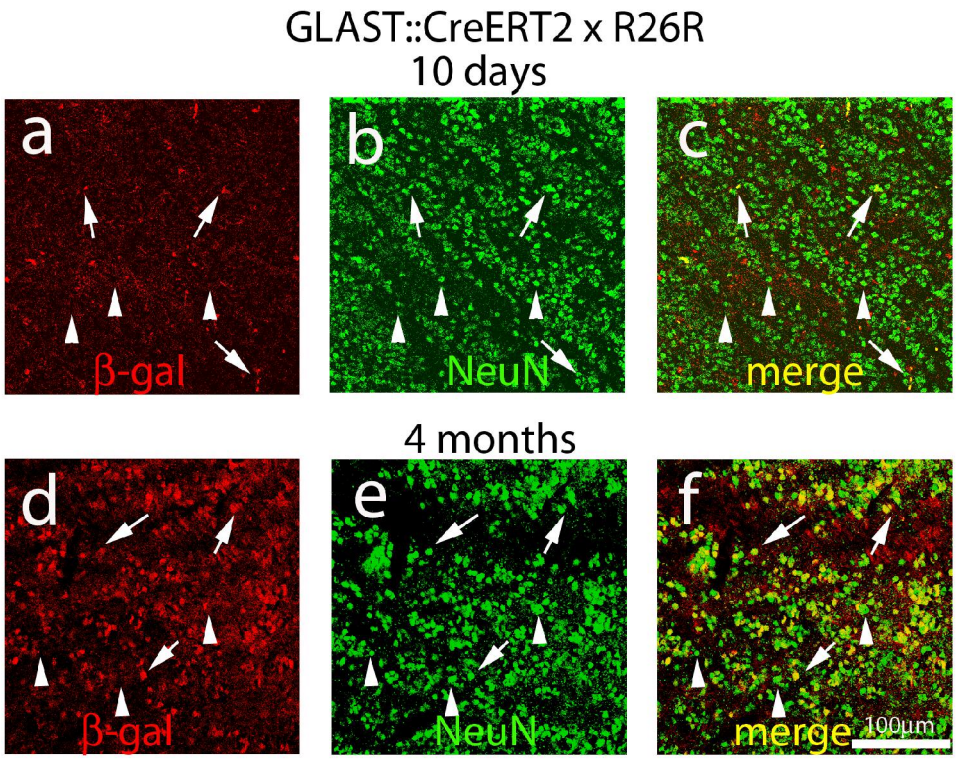


Figure 4 Mori et al.

Monitoring neuronal turn-over in adult neurogenesis by induction of Cre-based mediated fate mapping

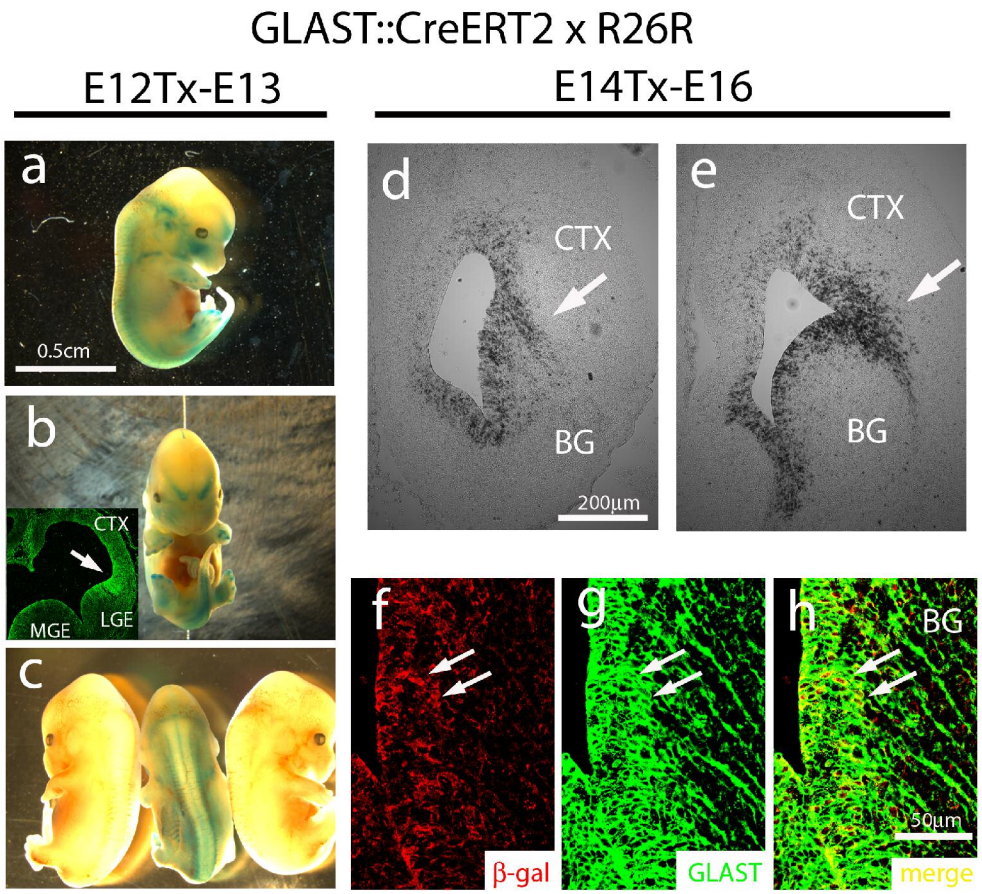


Figure 5 Mori et al.

Induction of Cre activity in the embryonic brain monitored in R26R-reporter mice

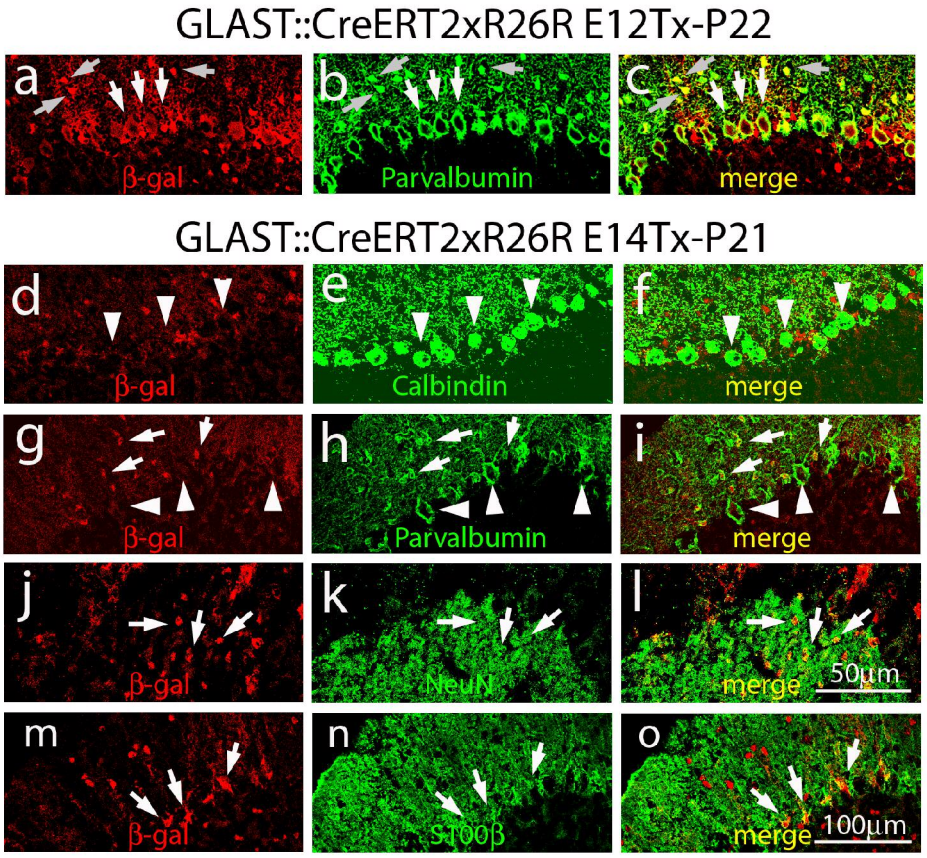


Figure 6 Mori et al.

Progeny of radial glia in the cerebellum at different developmental stages

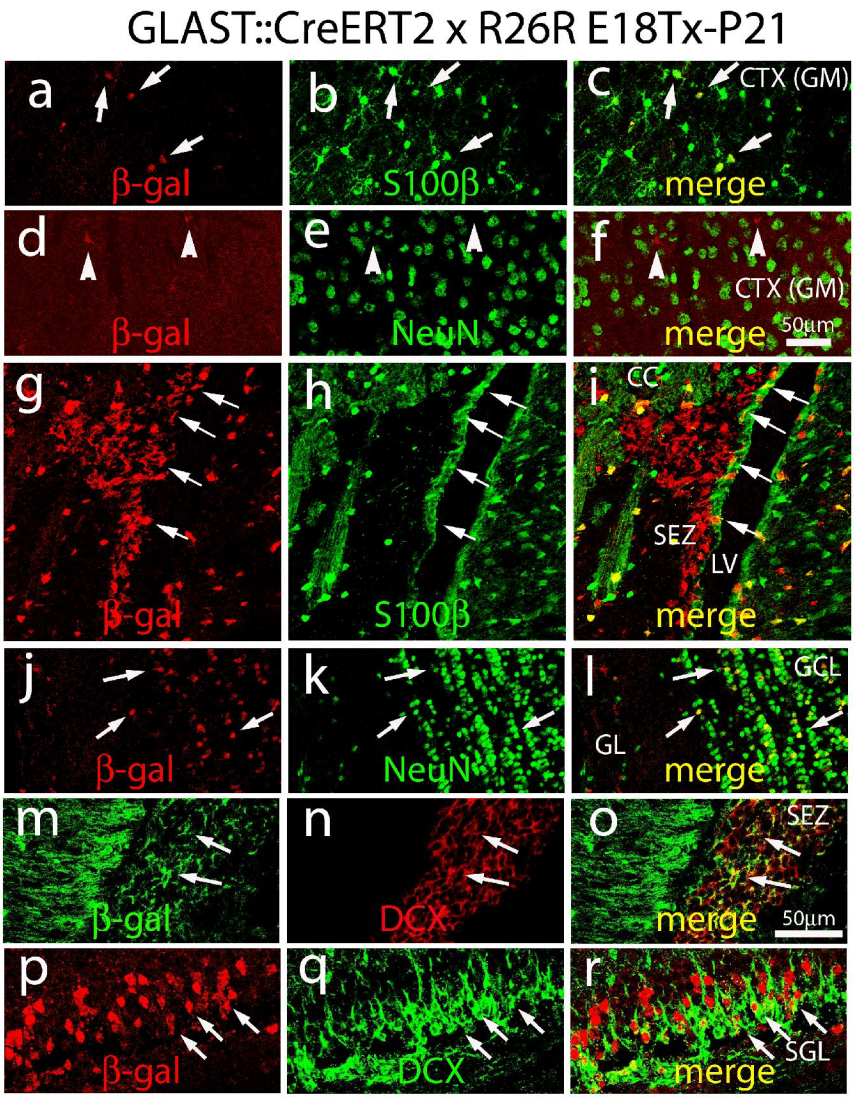
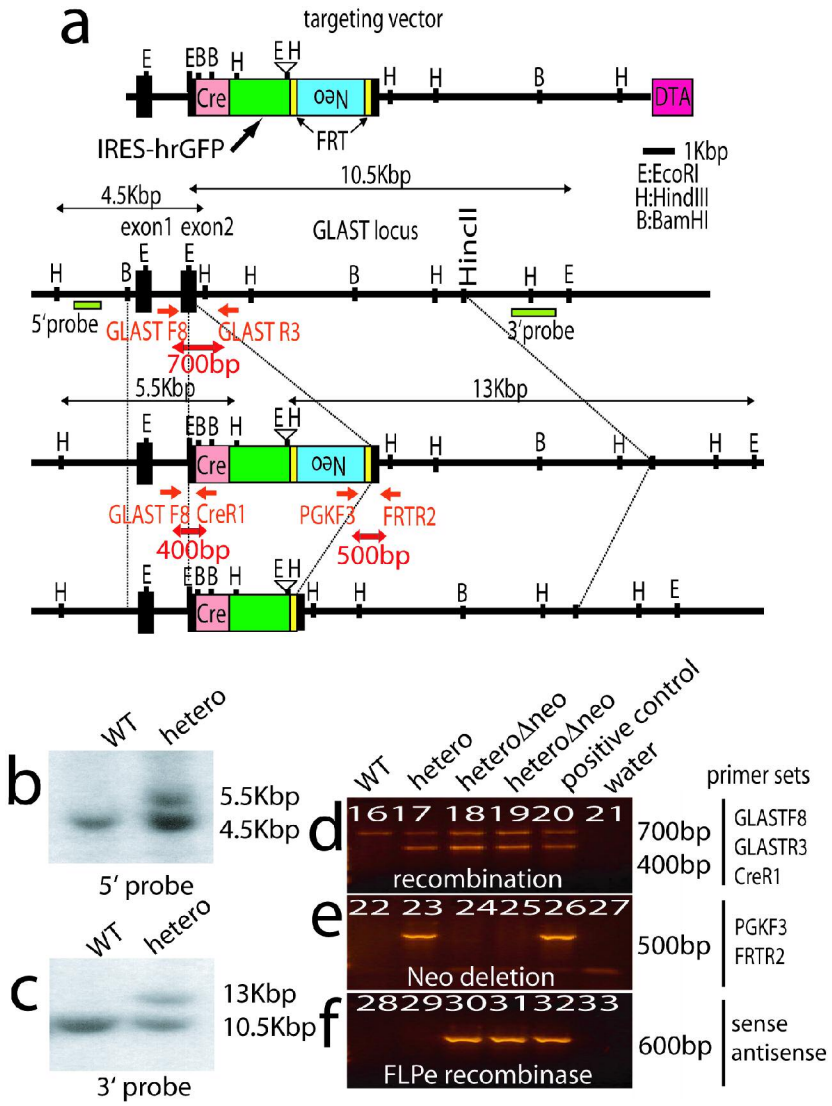


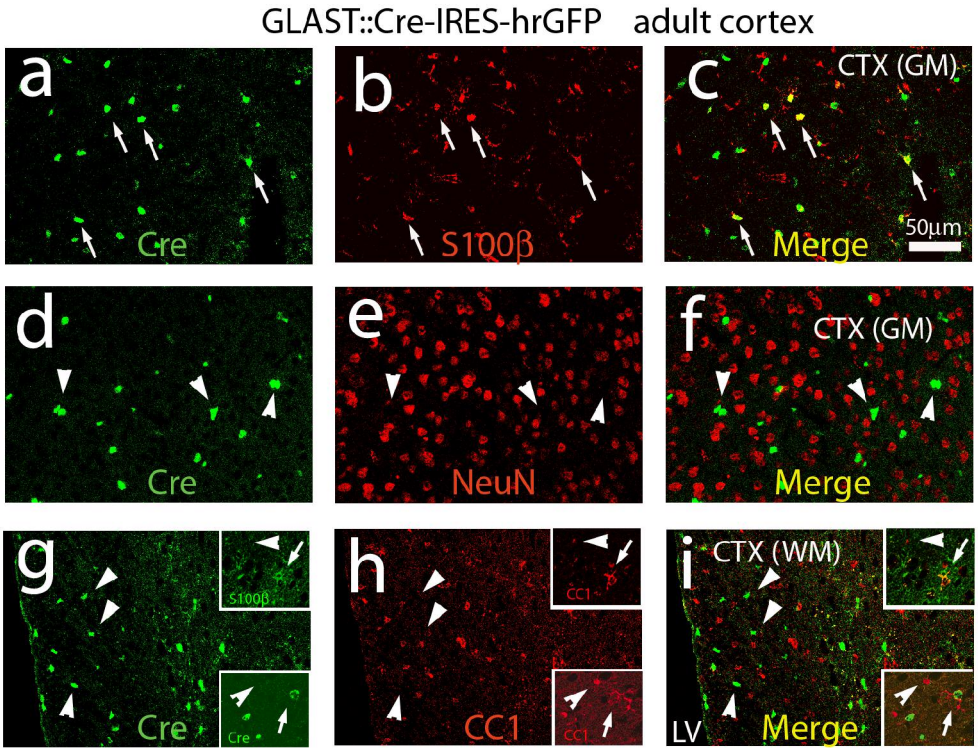
Figure 7 Mori et al.

Progeny of radial glia induced to recombine at embryonic day 18



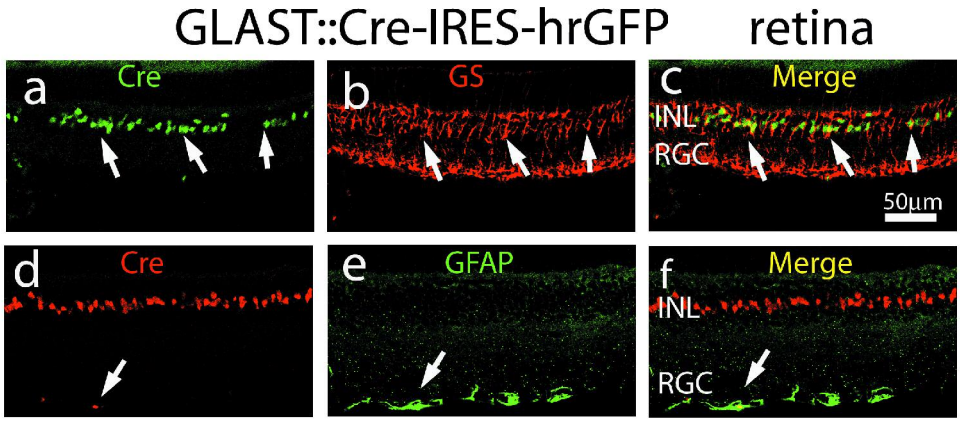
Supplemental Figure 1 Mori et al.

Targeting strategy and genotyping of GLAST::Cre-IRES-hrGFP mice



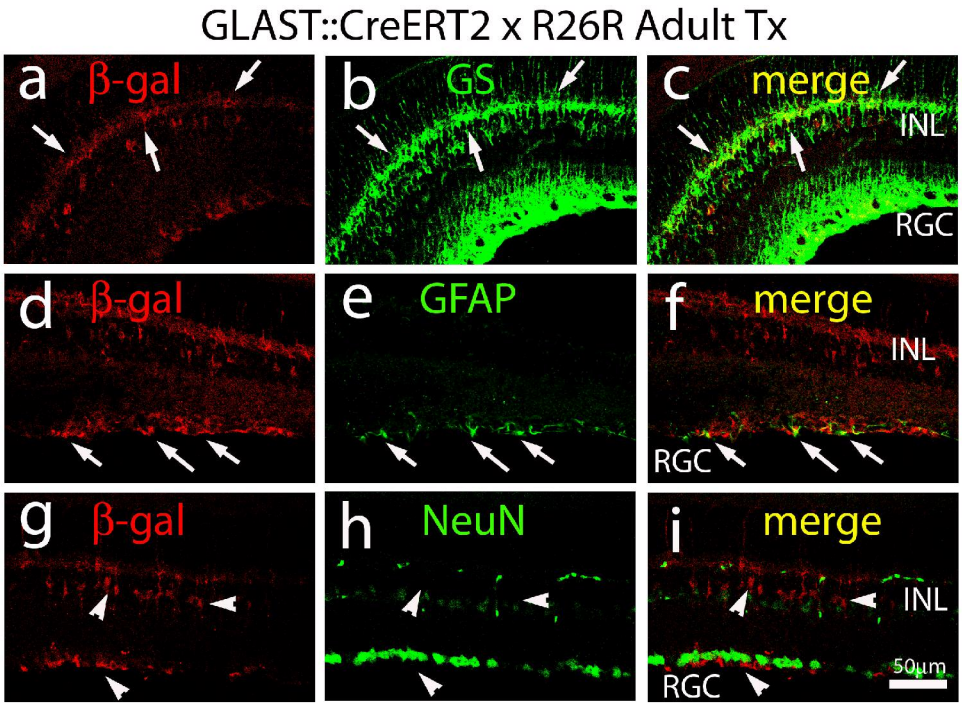
Supplemental Figure 2 Mori et al.

Specificity of Cre expression in the adult cortex of GLAST::Cre-IRES-hrGFP mice



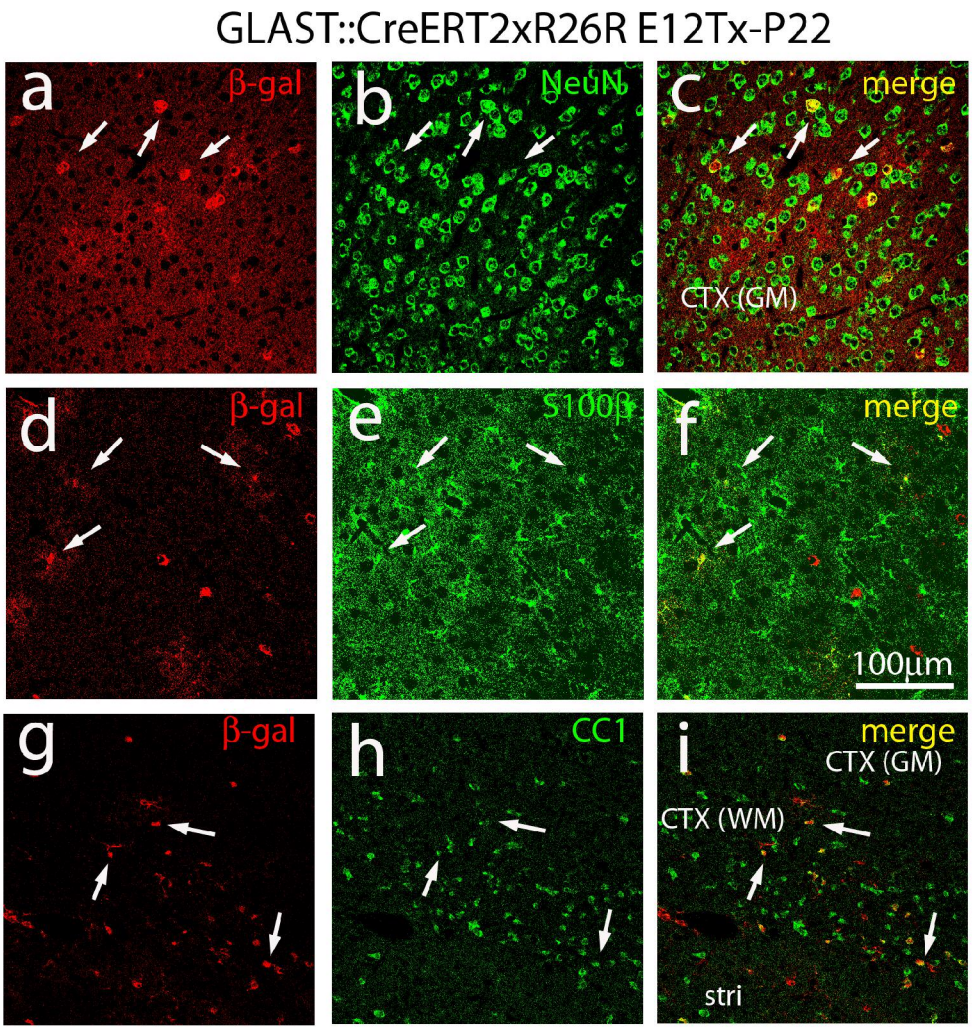
Supplemental Figure 3 Mori et al.

Cre expression in the glial cells of the retina in GLAST::Cre-IRES-hrGFP mice



Supplemental Figure 4 Mori et al.

Induction of Cre activity in Müller Glia and astrocytes of the retina in adult GLAST::CreERT2 mice



Supplemental Figure 5 Mori et al.

Progeny of radial glia induced to recombine at embryonic day 12