Development of GABAergic neurons

in mouse superior colliculus

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Contents

ABSTRACT

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS). The levels of GABA and its synthetic enzyme glutamate decarboxylase (GAD) in superior colliculus (SC) are high. We investigated the early development of SC, including early GABAergic neurons, using GAD67-GFP knock-in (GAD67+/- and GFP+/-) mice to mark GABAergic neurons and observe their migration.

It was revealed by observation of cells labeled with BrdU that neurons in mouse SC generated from E 11.5 to E 15.5 with a peak at E 13.5. The generation of GABAergic neurons had a peak at E 12.5 and they migrated and spread throughout SC by E 16.5. By BrdU and DsRed2 labeling, the inside-out cell migration pattern was not evident in SC compared with that in neocortex. We observed the shape of cell bodies, arrangements, nestin-immunoreactivity of SC cells, and time-lapse of living GABAergic neurons. These observations indicated radial migration of GABAergic neurons and generation in the ventricular zone. We made cuts at an early stage that intercept either or both radial and tangential cell migration in SC, and the result of this experiment also supported our conclusion. It was also noticeable that the surface of the

SC was covered with GABAergic fibres at an early stage, when GABAergic cells were

not yet differentiated in SC.

INTRODUCTION

γ -Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS). GABAergic inhibitory neurons take on various forms and distribution in every region of the CNS. Recently it has been demonstrated that the mode of development of GABAergic neurons is distinct from that of other types of neurons in the cerebral cortex. In general, cortical neurons are produced at the ventricular zone and then migrate radially along the radial glial fibres to each layer (Rakic, 1972). In contrast, GABAergic interneurons are generated in the remote area called the ganglionic eminence and are distributed to the cortex by tangential migration (Anderson et al., 1997; Tamamaki et al., 1997). Resultingly clarification of whether such developmental patterns for GABAergic neurons is common to other layered structure is interesting.

The superior colliculus (SC) consists of 7 layers which have characteristic synaptic organization individually. SC integrates visual and other sensory inputs, and relays its information to various regions (Huerta and Harting, 1984). The levels of GABA and its synthetic enzyme glutamate decarboxylase (GAD) are high in SC. Especially, the

GABA content is highest next to that of the substantia nigra, globus pallidus and hypothalamus in the CNS (Fahn and Cote, 1968; Okada et al., 1971). GABA is considered to be involved in the control of attention, consciousness, and saccade eye movements in SC. For example, injection of a GABA antagonist, bicuculline into SC facilitated the initiation of the saccade (Hikosaka and Wurtz, 1985).

Previous investigations have disclosed the following processes in development of the SC. Most collicular neurons are already generated between embryonic day (E) 11 and 14 in the mouse (Edwards et al., 1986a). Cooper and Rakic (1981) and Edwards et al. (1986b) have suggested the inside-out pattern of neuron development in SC as observed in the neocortex (Angevine and Sidman, 1961). Optic axons are distributed to the SC between E 15 and 19 in the mouse and then organized to the retinotopic pattern together with the layer formation (Edwards et al., 1986b). Recently, Tan et al. (2002) demonstrated the radial migration and lateral dispersion of SC cells expressing β-gal using chimeric mice. Development of GABAergic interneurons in the SC, however, has not fully been investigated.

The present investigation was, therefore, addressed this question using GAD67

knock-out/GFP knock-in mice in which glutamic acid decarboxylase (GAD67) gene

was inactivated by insertion of green fluorescent protein (GFP) gene into GAD67 exon

1 (Yanagawa et al., 2001). GABAergic neurons, which always GAD67, could therefore

be identified by expression of GFP in these mice. Although GAD67-/- mice die

immediately after birth because of cleft palate (Asada et al., 1997), the GAD67+/- and

GFP+/- mice used in the present study did not show any marked reduction of brain

GABA content nor any serious abnormal phenotype. BrdU labeling was conducted to

determine the birth of the collicular neurons, including GABAergic neurons.

Immunohistochemistry for several cell markers was performed to discriminate cell

types. Together with other *in vitro* and *in vivo* manipulations, tangential migration of

GABAergic neurons from any discrete region in SC was not observed but rather radial

migration from the underlying ventricular zone was seen. It was also noticeable that the

surface of the SC was covered with GABAergic fibres at an early stage, when

GABAergic cells were not yet differentiated in SC.

MATERIALS AND METHODS

GAD67-GFP knock-in mouse

The generation of GAD67-GFP knock-in mice was described previously (Fig.1:

Yanagawa et al., 2001; Tamamaki et al., 2003).   In these mice a cDNA encoding an

enhanced GFP (EGFP; Clonetech, Alto, CA, U.S.A) was targeted to exon 1 of GAD67

gene by homologous recombination.   Since GAD65 and GAD67 coexist in all

GABAergic neurons for GABA biosynthesis, they are useful as markers for GABA

neurons.   We focused on GAD67, because production of GABA at embryonic stages is

synthesized predominantly by GAD67 (Asada et al., 1997).   The mice were

maintained with a genetic background of C57BL/6. Heterozygous male mice were

mated with ICR female mice (Chubu-Kagaku, Nagoya, Japan) to obtain the offspring

for the present study, because ICR strain female had much better birth rates. The day

vaginal plug was observed in the morning was designated embryonic day (E) 0.5 and

the day of birth was postnatal day (P) 0.5. These experiments were approved by the

Animal Research Committee of the Okazaki National Research Institutes and followed

the guidelines of National Institute of Health (USA) and Japan Neuroscience Society.

Observation of GFP Fluorescence

Under anesthesia with pentbarbital, E 10.5-E 16.5 fetuses were removed and GFP-positive fetuses were selected in order to visualize GFP-positive cells in tissue specimen. The brains were fixed by immersion in 4% paraformaldehide in 0.1M phosphate buffer (PB, pH 7.4) overnight at 4 °C. Heads or brains from E 10.5-E 12.5 embryos were embedded in gel of low-melting-point agarose (3.5%, FMC Bioproducts, Rockland, ME, U.S.A) for adjustment of orientation at sectioning. After cryoprotection with 30% sucrose in phosphate buffered saline (PBS), frozen sections 14μm thick were obtained using a cryostat microtome (Frigocut 2800 Reichert-Jung, Heidelberg, Germany), and mounted on poly-L-lysine-coated glass slides for observation under a fluorescence microscope. In confocal microscopy (LSM510, Zeiss, Jena, Germany). Z series of confocal images of the sections were assembled as a single image with the LSM 510 software program. All digital files were imported into Adobe Photoshop 5.5. After minor adjustment of contrast and brightness, montages of images were constructed.

Immunohistochemistry

Embryos under E 16.5 were taken from the pregnant mice under diethylether anesthesia and fixed by immersion in 4% paraformaldehide in 0.1M PB (pH 7.4). Fetuses over E 17.5 were artificially born by Caesarean section. Under anesthesia in crushed ice, these embryos and postnatal pups were fixed by transcardial perfusion with the same fixative and then the brains were excised and post-fixed. The transcardial fixation was also performed on adult mice under pentobarbital anesthesia (50 mg/kg, i.p.). For immunostaining of GABA, the above fixative was supplemented with 0.25% glutaraldehyde. Frozen sections 14μm thick were prepared as above. Immunohistochemistry was performed with antibodies against GFP (a gift of Dr. N. Tamamaki, Kyoto University), GABA (A2052, Sigma, St. Louis, MO, U.S.A), BrdU (FITC-labeled OBT0030F, Oxford Biotechnology, Oxford, U.K.; BD-555627, Becton Dickinson, Franklin Lakes, NJ, U.S.A), neurofilament M (AB1987, Chemicon, Temecula, CA, U.S.A), nestin (BD-556309, Becton Dickinson, Franklin Lakes, NJ, U.S.A) and vimentin (Ab-2, NeoMarkers, Fremont, CA, U.S.A).

5-Bromo-2'-deoxyuridine (BrdU) labeling

BrdU (6mg/ml in sterile physiological saline and 0.007N NaOH) at 20 mg/kg body weight was injected intraperitoneally in the maternal mice at each different gestational stage (E 10.5, E 12.5, E 13.5, E 14.5, E 15.5 and E 16.5). After BrdU loading E 14.5-E 15.5 embryos and 4-week-old mice were fixed as above with 4% paraformaldehyde in 0.1 M PB. Frozen sections were cut at 14μm thickness with the use of a cryostat microtome and mounted on poly-L-lysine-coated glass slides.

For BrdU immunostaining, sections were pretreated with 2 N HCl for 30 minutes at 37 °C and neutralized with 0.15 M borate buffer, pH 8.4.

DsRed2 labeling

An expression plasmid vector of DsRed2 was produced by ligation of DsRed2 cDNA (Clontech, Palo Alto, CA, U.S.A) with a CAG promoter which consisted of a cytomegalovirus enhancer and chicken β-actin promoter (Niwa et al., 1991). The vector was introduced into SC cells at the ventricular wall of living embryos by in utero electroporation.

Manipulation of the embryos in the uterus was carried out as follows. At E 12.5 or

E 14.5, pregnant mice were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p., Dainippon, Osaka, Japan), and the uterine horns were exposed. Plasmid DNA purified with the plasmid maxi or mega kit (QIAGEN, Hilden, Germany) was dissolved in PBS at a concentration of 5-10!mg/ml, and Fast Green solution (0.1%) was added to the plasmid solution in a ratio of 1:10 to monitor the injection. Approximately 1-2 µl of the solution was injected into the cerebral aqueduct through a glass micropipette with a tip of 2-3 µm. The embryo in the uterus was placed between the tweezers which had disc electrode of 5!mm in diameter at each tip (CUY650-5, BEX, Tokyo, Japan). The vector was introduced into the cells facing the ventricular wall cells by delivering 5 squere pulses (50V; pulse duration, 50 msec) at an interval of 75 msec with a square-pulse electroporator (CUY21, BEX, Tokyo, Japan). Frozen sections of SC were prepared from 4-8-week-old wild mice and DsRed2 fluorescence was examined.

Cutting of SC

Pregnant mice carrying E 11.5-E 13.5 fetuses were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and after laparatomy, GAD67-GFP knock-in fetuses were

identified through the uterus by their fluorescence. A tungsten microelectrode (tip

diameter < 1 micron, FHC, Bowdoinham, ME, U.S.A) was inserted through the uterine

wall to the presumptive SC and injury was applied by hand to split the tissue. The

fetuses were maintained alive for 1 to 3 days. The embryos were fixed with 4%

paraformaldehyde in 0.1 M PB and treated as above for histological analysis.

Time lapse observation of living GABA neurons

Under anesthesia with pentbarbital, E11.5 or E12.5 fetuses were removed and GFP-

positive fetuses were selected. Their midbrain, containing SC, were embedded in gel of

low-melting-point agarose (3.5%) for adjustment of orientation at sectioning and sliced

at 200 μm thickness in Hanks' solution and mounted on Millicell membrane (Millicell-

CM, Millipore, Bedford, MA, U.S.A) coated with Cell-Tak (Collaborative BioMedical

Products, Bedford, MA, U.S.A) for enhanced tissue-slice attachment. The preparation

was kept at 37 °C in a humidified box on a stage of a fluorescence microscope. GFP-

positive cells in the SC were observed and photographed intermittently at the interval of

5-10 min for up to 2 h. Displacement of each cell in the slice was analyzed on

photographs.

DiI labeling

E 13.5 and E 16.5 embryos were put under diethiylether anesthesia and fixed

overnight with 4% paraformaldehyde in 0.1 M PB (pH7.4). The heads were embedded

in gel of low-melting-point agarose (3.5%) for adjustment of orientation at sectioning.

The midbrain was cut coronally into 200 μm-thick slices with a vibrating blade

microtome (Series 1000, TPI, Lowell, CA, U.S.A) in PBS. A small powder of

1,1dioctadecyl-3,3,3-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes,

Eugene, OR, U.S.A) was placed on the SC. The slices were kept in PBS in the dark for

2-7 days and then observed under a fluorescence microscope.

RESULTS

Distributions of BrdU labeled cells

BrdU labeling of the neurons during terminal division revealed that neurons in the mouse SC were generated mostly during the 4-day period of E 11.5-E 15.5.

Few cells were labeled with BrdU at E 10.5 or E 16.5, while the cells labeled with BrdU at E 13.5 were abundant in SC (Fig. 2). Neuronal generation had a peak at E 13.5, and the neurons labeled at E 13.5 were found throughout the SC. We compared the distribution of labeled cells for the stage of labeling but did not find any significant difference. This suggests that the inside-out generation, as observed in the neocortex, is not evident in the SC.

To examine for another method of cell generation, we labeled the cells in the ventricular zone with DsRed2 by gene transfer at E 12.5 and E 14.5, and then studied the location of the labeled cells in the adult SC.

Cells labeled at E 14.5 were distributed rather in the superficial layer, while cells labeled at E 12.5 were concentrated in the deeper layer, though not in any specific narrow band (Fig. 3). These results indicates that a group of cells born at the same stage

migrated into the same layer and the location of early to lately born neurons was from inside to outside in order.

Birth of GABA neurons

GFP fluorescence was first observed in the cerebrum, midbrain and spinal cord at E 10.5 and increased in extent and intensity thereafter. Fig. 4 shows immunostaining of GABA together with GFP fluorescence on E 12.5 SC. GFP-positive cells and fibres are also GABA positive. GABA immunostaining of the cell soma was not as extensive as in the adult CNS but was detected even at E 12.5. Therefore, GFP-positive cells and fibres may be called GABAergic. The cells in the ventricular zone lacked GFP fluorescence except for several faintly labeled cells, which had probably differentiated to GABAergic (Figs. 4 and 5). GABAergic neurons were observed only in the lateral part of mouse SC before E 11.5, and then in the medial part at E 13.5. The density of GFP-positive cells reached a maximum at E 16.5 (Fig. 5). Double labeling with BrdU and GFP should demonstrate the birth date of GABAergic neurons. BrdU administration to pregnant mice carring E 8.5-16.5 fetuses showed that GABAergic neurons were

produced between E 11.5-E 15.5 with a peak at E 12.5 (Fig. 6)

GFP- and GABA-positive fibres were most obvious in the uppermost layer even in the

absence of GFP- and GABA-positive cells in E 10.5-12.5 SC (Fig. 5). These fibres

crossed the midline (see below).

Shape and arrangement of GABAergic neurons

When E 12.5-13.5 SC was observed under a confocal laser scanning microscope or

in a cell-scarce area, individual GFP-positive cells were spindle-shaped with fairly long

processes at both poles. The cells and processes were oriented radially from the

ventricular wall to the outer surface (Fig. 7).   A small number of cells expressed GFP

weakly in the ventricular zone and they showed the same appearance. Other types of

cells were visualized by immunohistochemistry for nestin and vimentin. They are

considered as markers of proliferating progenitor cells and radial glial cells,

respectively, at the early stage in the central nervous system (Hockfield and McKay,

1985; Pixley and de Vellis, 1984). As shown in Fig. 8 anti-nestin antibody stained

apparently every cell at E 12.5.   The labeled cells were oriented similarly to GFP-

positive cells and closely packed against each other, suggesting that all neurons in SC

migrated or spread radially from the ventricular zone. Anti-vimentin antibody stained

only a few radial glia-like structures in SC at this stage and close association of radial

glia with migrating neurons was not demonstrated.

Cutting of SC

Tamamaki et al. (1997) concluded that GABAergic neurons migrated tangentially

after making a horizontal cut in the neocortex. Making a cut and intercepting the

migratory pathway of GABAergic neurons results in a disturbance of their distribution.

With this strategy we studied the migration of GABAergic neurons in the SC. We

manipulated over 300 embryos to make a cut in the SC and obtained three embryos in

which the operation was successful.

Figs. 9A and B show the SC of an E 13.5 embryo which was cut obliquely in the

lateral direction from the surface to the deep layer at E 12.5 and kept alive for another

day. The density of GFP-positive cells was much higher in the ventricular side of the

cut. When the number of GFP-positive cells was quantified, the areas below and above

the cut contained 113!and 50 GFP-positive cells per $0.02mm^2$, respectively. These

results indicate that the tangential migration from the lateral part was not active and,

instead the presence of radial migration from the ventricular zone.

Two embryos were kept alive for 3 days after the cut was made at E 12.5. One was

cut parallel to the ventricular wall (Fig. 9C) and the other had two cuts which were

parallel and vertical to the ventricular wall (Fig. 9D). They showed the distinguishable

accumulation of GFP-positive neurons on the ventricular side of the parallel cut. When

observed in cresyl-violet stain the distribution of all cells was similar on the both sides

of the cut (not illustrated). These results from 3 cases support that radial migration was

predominant for GABAergic neurons in the mouse SC.

Time lapse observation of living GABAergic neurons

Using time-lapse video microscopy, Nakaya et al. (2003) demonstrated the

tangential migration of GABAerugic neurons in the neocortex of the GAD67-GFP

knock-in mice. Several GFP-positive cells move radially in the same tissue although it

is not known whether they do so from the ventricular zone or after/during the tangential

migration. In order to similarly observe the migration of living GFP-positive cells, we

prepared the coronal slices of E 11.5 or E 12.5, when the development of GFP-positive

neurons was highly active, and examined GFP-positive cells in a time lapse manner

(Fig. 10).

Many GFP-positive neurons did not move in the slice preparation during

observation up to 2 h. However, 24 % of the cells moved radially at a velocity of 7.0

μm/ 15 min (Table 1).

Commissural GABAergic fibres

At E 11.5 the SC still consisted mostly of the ventricular zones occupied by

undifferentiated cells. As described above, GFP-positive fibres were located at the

surface, even at this stage (Figs. 5, 11 and 12).   They were also stained with anti-

GABA and anti-neurofilament antibodies, indicating that they were GABAergic axons

(Fig. 13). They formed bundles with several isolated leading axons and traversed the

midline, extending further laterally (Figs. 11 and 12). Their cell bodies could not be

identified because GFP-positive cells were abundant at the lateral part of the midbrain.

To identify the origin of the commissural fibres, slice preparations at 150-200 μm thickness were prepared from the fixed tissue of E 12.5, E 15.5 and P 0.5 mice, and DiI particles were placed on the slices close to the midline of SC and maintained for 2-7 days to distribute the dye throughout the labeled elements. As exemplified for E 12.5 SC in Fig. 14, DiI-labeled fibres ran at the superficial layer of the contralateral side and a group of labeled cells were distant from SC primordium. When DiI was placed at E 15.5 and P 0.5, a small number of the labeled fibres were observed in deep layers at the contralateral side and the cells stretching these fibres were situated in SC (Fig. 15). In addition to the commissural axons, a group of radial glial cells with typical endfeet at the outer surface were labeled with DiI at E 15.5. Identification of DiI-labeled GABAergic commissural fibres was not possible because the fluorescence of DiI was too intense compared with GFP fluorescence. However, the DiI-labeled path suggests that most GFP-positive commissural fibres at the early stage originated from the cell bodies located at the ventrolateral of the midbrain.

DISCUSSION

In the development of the forebrain, a large pool of GABAergic neurons appears in the ganglionic eminence at the ventral wall of the lateral ventricle, and a portion of them migrate tangentially to the neocortex to form cortical GABAergic interneurons (Anderson et al., 1997; Tamamaki et al., 1997). In the spinal cord, neurons are generated in the ventricular zone surrounding the central canal. Here, GABAergic neurons in the ventral cord are not generated uniformly but at the restricted area named p1 (Pierani et al., 2001). In the midbrain, SC is a layered structure as the cerebral cortex is and a prominent pool of GABAergic neurons is observed at the lateral part of the midbrain during development (Tamamaki et al., 2003). The present analyses, however, did not support the idea that GABAergic neurons in SC are generated in the restricted area of the ventricular zone, e.g. the lateral midbrain, but indicated generation in the underlying ventricular zone and the subsequent radial migration. All GFP-positive cells appeared to be oriented in the direction from the aqueduct to the surface together with other cells, as revealed by nestin immunoreactivity. Radial glial fibres, which guide neurons for radial migration in the cerebral cortex, were abundant in the embryonic SC

(not illustrated). When tissue in which the cell migration is taking place in one direction

is severed, migrating cells will accumulate on one side of the injury and disappear on

the other side. The distribution of GFP-positive cells was dominant on the ventricular

side of the injury. The migration in the sliced SC was radial for GFP-positive cells. It

has been demonstrated recently that in the human neocortex, GABAergic interneurons

are derived mainly from the underlying ventricular zone and a smaller population are

derived from the ganglionic eminence (Letinic et al., 2002). Their velocity of cell

migration in the SC is lower than that of the tangential migration observed in the mouse

neocortex (Nakaya et al., 2003). This may be due to the difference in distance, direction

or the mechanism of migration.

Commissural tectotectal fibres in the adult SC are implicated in the control of eye

saccades and distributed chiefly in the rostral part of SC: in the cat the tectotectal cells

are located in the deeper layers (from stratum griseum intermediale to profundum) and

are glutamatergic, GABAergic or undetermined in their neurotransmitters (Rhoades et

al., 1986; Olivier et al., 2000). Some extrinsic GABAergic fibres in the SC originate

from the external nucleus of the contralateral inferior colliculus and the dorsal and

ventral nuclei of the lateral lemniscus (Appell and Behan, 1990). At E 10.5-11.5 GFP-positive fibres in SC were restricted within the superficial layer. They formed fiber bundles, with several isolated leading fibres traversing the midline of the surface of the tectal primordium. At this stage, the SC consisted mostly of undifferentiated cells in the ventricular zone and GFP-positive cells were found only in the most lateral part of SC. Their origin and function are of interest. Furthermore, retrogradely DiI-labeled cells were located facing the aqueduct, most likely outside of SC at E 12.5. The superficially located GABAergic fibres were not observed at the later stage. GFP-positive or neurofilament-immunoreactive commissural fibres observed in the whole layer of E 15.5-P0.5 SC might correspond to the commissural fibres found in the adult. DiI-labeled cell bodies in P0.5 were found only in SC. It is possible that the superficial GFP-positive fibres changed their neurotransmitter phenotype or were degenerated after transient existence. In the still-developing neocortex, Cajal-Rtzius cells are temporarily situated at the superficial layer for the guidance of other cells and a portion of them are GABAergic (Imamoto et al., 1994). In the embryonic SC, no immunoreactive cells were observed against Cajal-Retzius cell-specific Reelin (not illustrated). In the

embryonic spinal cord the commissural fibres probably play some role in guidance for

other axons or cells (Jessell and Sanes., 2000)   Any developmental roles of the

commissural fibres or GABA is a matter of future investigation, considering that GABA

deficiency in GAD67 and GAD67/65 knockout mice did not result in serious defects in

histogenesis of the brain including SC (Asada et al., 1997; Ji et al., 1999)

ACKNOWLEDGEMENTS

REFERENCES

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal

forebrain to neocortex: dependence on Dlx genes. Science 278(5337): 474-476, 1997.

Angevine JR Jr, Sidman RL. Autoradiographic study of cell migration during

histogenesis of cerebral cortex in the mouse. Nature 192: 766-768, 1961.

Appell PP, Behan M. Sources of subcortical GABAergic projections to the superior

colliculus in the cat. J Comp Neurol 302(1): 143-158, 1990.

Asada H, Kawamura Y, Maruyama K, Kume H, Ding RG, Kanbara N, Kuzume H,

Sanbo M, Yagi T, Obata K. Cleft palate and decreased brain gamma-aminobutyric

acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. Proc Natl

Acad Sci U S A 94(12): 6496-6499, 1997.

Cooper ML, Rakic P. Neurogenetic gradients in the superior and inferior colliculi of the

rhesus monkey. J Comp Neurol 202(3): 309-334, 1981.

Edwards MA, Caviness VS Jr, Schneider GE. Development of cell and fiber lamination

in the mouse superior colliculus. J Comp Neurol 248(3): 395-409, 1986a.

Edwards MA, Schneider GE, Caviness VS Jr. Development of the crossed

retinocollicular projection in the mouse. J Comp Neurol 248(3): 410-421, 1986b.

Fahn S, Cote LJ. Regional distribution of gamma-aminobutyric acid (GABA) in brain

of the rhesus monkey. J Neurochem 15(3): 209-213, 1968.

Hikosaka O, Wurtz RH. Modification of saccadic eye movements by GABA-related

substances. I. Effect of muscimol and bicuculline in monkey superior colliculus.

J Neurophysiol 53(1): 266-291, 1985.

Huerta MF, Harting JK Connectional organization of the superior colliculus. Trends

Neurosci 286-289, 1984.

Hockfield S, McKay RD. Identification of major cell classes in the developing

mammalian nervous system. J Neurosci 5(12): 3310-3328, 1985.

Imamoto K, Karasawa N, Isomura G, Nagatsu I. Cajal-Retzius neurons identified by

GABA immunohistochemistry in layer I of the rat cerebral cortex. Neurosci Res

20(1): 101-105, 1994.

Jessell TM, Sanes JR. Development. The decade of the developing brain. Curr Opin

Neurobiol 10(5): 599-611, 2000.

Letinic K, Zoncu R, Rakic P.   Origin of GABAergic neurons in the human neocortex.

Nature 417(6889): 645-649, 2002.

Nakaya Y, Tanaka D, Yanagawa Y, Obata K and Murakami F. Dynamics and Zone-

Specific Migratory Behavior of Neocoritical GABAergic Neurons. Submitted, 2003.

Niwa H, Yamamura K, Miyazaki J. 1991. Efficient selection for high-expression

transfectants with a novel eukaryotic vector. Gene 108: 193-199, 1991.

Okada Y, Nitsch-Hassler C, Kim JS, Bak IJ, Hassler R. Role of gamma-aminobutyric

acid (GABA) in the extrapyramidal motor system. 1. Regional distribution of GABA

in rabbit, rat, guinea pig and baboon CNS. Exp Brain Res 13(5): 514-518, 1971.

Olivier E, Corvisier J, Pauluis Q, Hardy O. Evidence for glutamatergic tectotectal

neurons in the cat superior colliculus: a comparison with GABAergic tectotectal

neurons. Eur J Neurosci 12(7): 2354-2366, 2000.

Pierani A, Moran-Rivard L, Sunshine MJ, Littman DR, Goulding M, Jessell TM.

Control of interneuron fate in the developing spinal cord by the progenitor

homeodomain protein Dbx1. Neuron 29(2): 367-384, 2001.

Pixley SK, de Vellis J. Transition between immature radial glia and mature astrocytes

studied with a monoclonal antibody to vimentin. Brain Res 317(2): 201-209, 1984.

Rakic P. Mode of cell migration to the superficial layers of fetal monkey neocortex. J

Comp Neurol 145(1): 61-83, 1972.

Rhoades RW, Mooney RD, Szczepanik AM, Klein BG. Structural and functional

characteristics of commissural neurons in the superior colliculus of the hamster. J Comp

Neurol 253(2): 197-215, 1986.

Tamamaki N, Fujimori KE, Takauji R. Origin and route of tangentially migrating

neurons in the developing neocortical intermediate zone. J Neurosci 17(21): 8313-

8323, 1997.

Tamamaki N, Nakamura K, Furuta T, Asamoto K, Kaneko T. Neurons in Golgi-stain-

like images revealed by GFP-adenovirus infection in vivo. Neurosci Res. 38(3): 231-

236, 2000.

Tamamaki N., Yanagawa Y., Tomioka R., Miyazaki J., Obata K., Kaneko T. Green

fluorescent protein expression and colocalization with calretinin, palbalbumin, and

somatostatin in the GAD67-GFP knock-in mouse. Submitted, 2003.

Tan SS, Valcanis H, Kalloniatis M, Harvey A. Cellular dispersion patterns and

phenotypes in the developing mouse superior colliculus. Dev Biol 241(1): 117-131,

2002.

Yanagawa Y., Kaneko K, Kanbara N, Totsuka M, Yagi T, Obata K. Development of

mouse expressing GFP in GABAergic nerons. Neurosci Res Suppl 25, S77, 2001.

figures and table
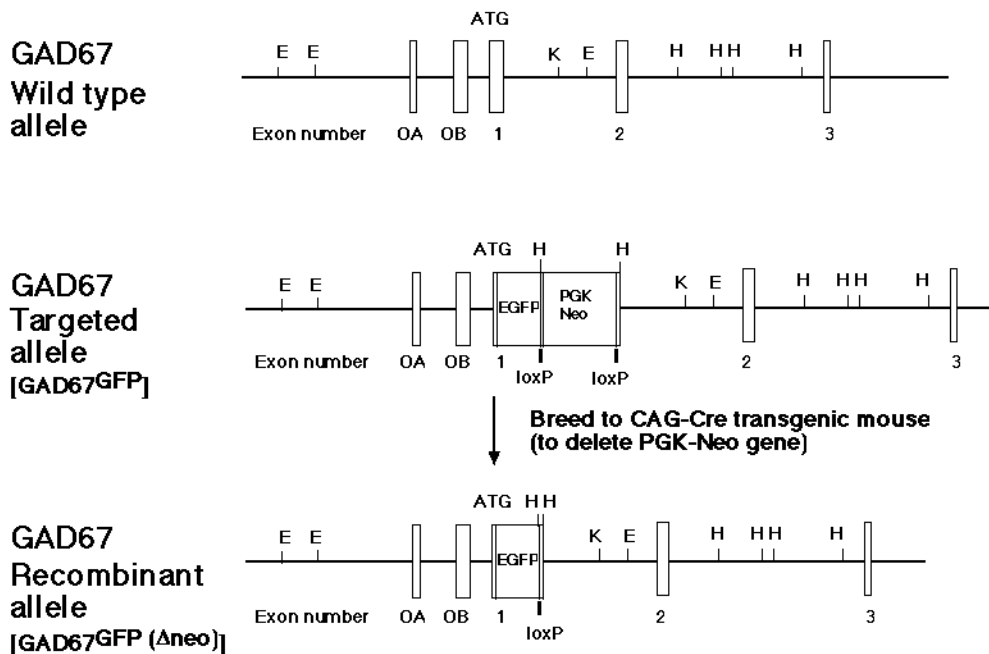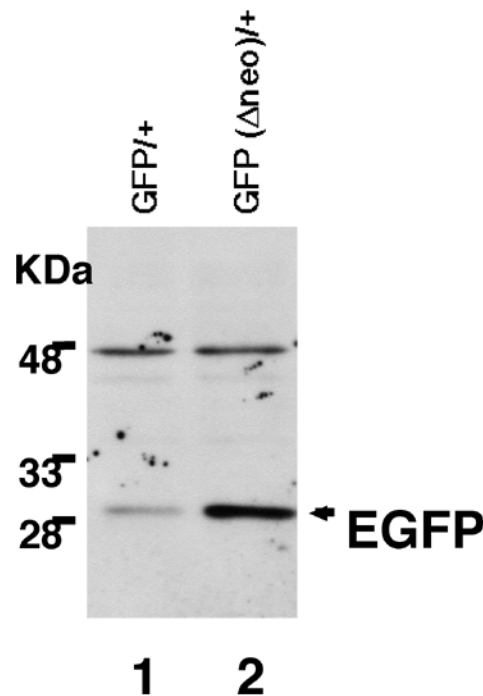
Fig. 1 Generation of GAD67-GFP (△neo) mice.

A: Schematics of the wild-type, targeted, and recombinant alleles of GAD67. The original GAD67-GFP knock-in mice retained a loxP-flanked neomycin-resistance cassette (PGK-Neo). The knock-in mice were bred with CAG-Cre transgenic mice to eliminate the neomycin-resistance cassette from the GAD67 locus. The recognition sites of EcoRI (E), HindIII (H), and KpnI (K) are indicated. B: Immunodetection of GFP protein. Lysate (10 μg of protein) from the cerebrum was subjected to electrophoresis. Lane 1, lysate from GAD67-GFP mouse (GFP/+). Lane 2, lysate from GAD67-GFP(△neo) mouse [GFP(△neo)/+]. The arrow indicates the band of EGFP at 28kDa. From Tamamaki et al. (2003).
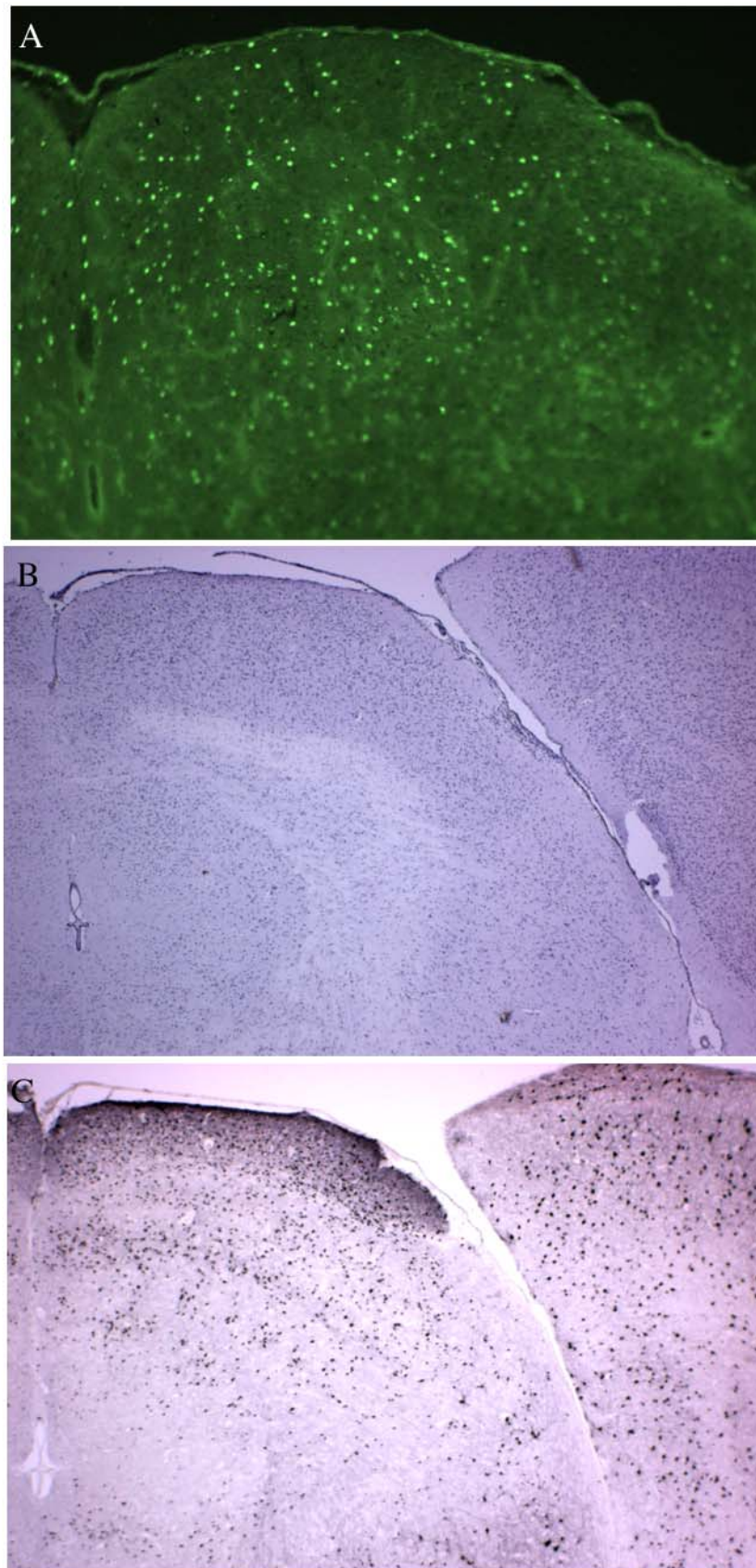
Fig. 2 Cells in adult mouse SC. Coronal section. A: BrdU labeled cells in SC. BrdU injection at E13.5. B: cresyl-violet staining. C: GFP-positive cells stained with an antibody against GFP (from Tamamaki et al., 2000).
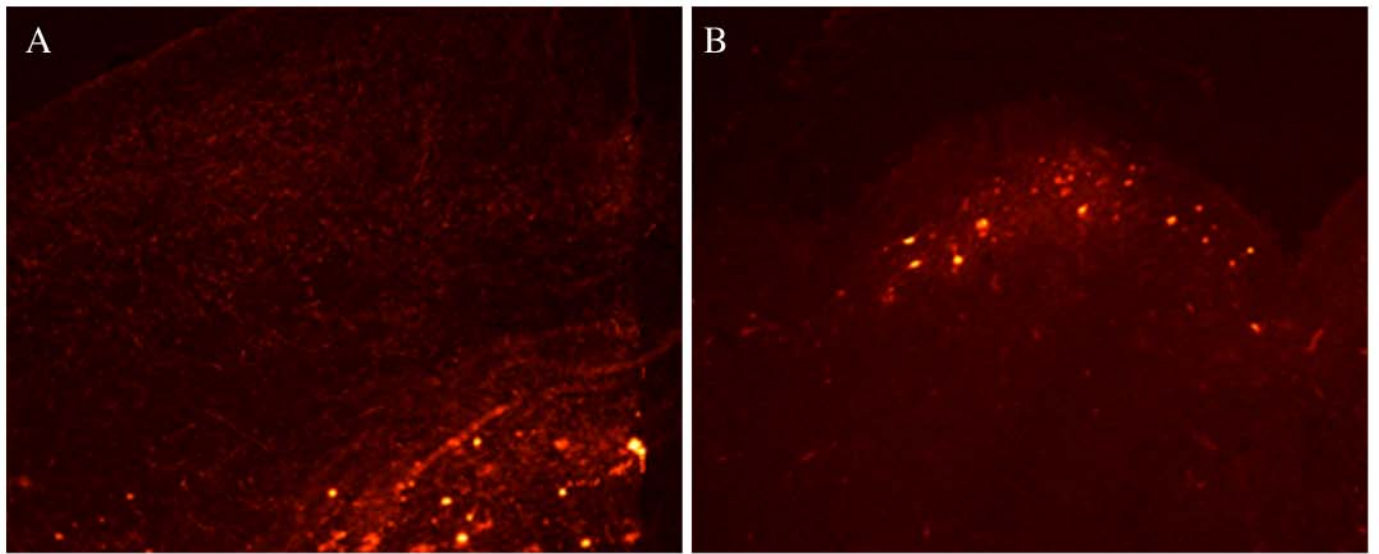
Fig. 3 DsRed2 positive cells in 4-week-old mouse SC. The DsRed2 vector was electroporated at E12.5 (A) and E14.5 (B). Many DsRed2-positive cells are located close to the aqueduct (lower right, outside of image) in A, while DsRed2-positive cells in B are distributed relatively close to the surface of SC.
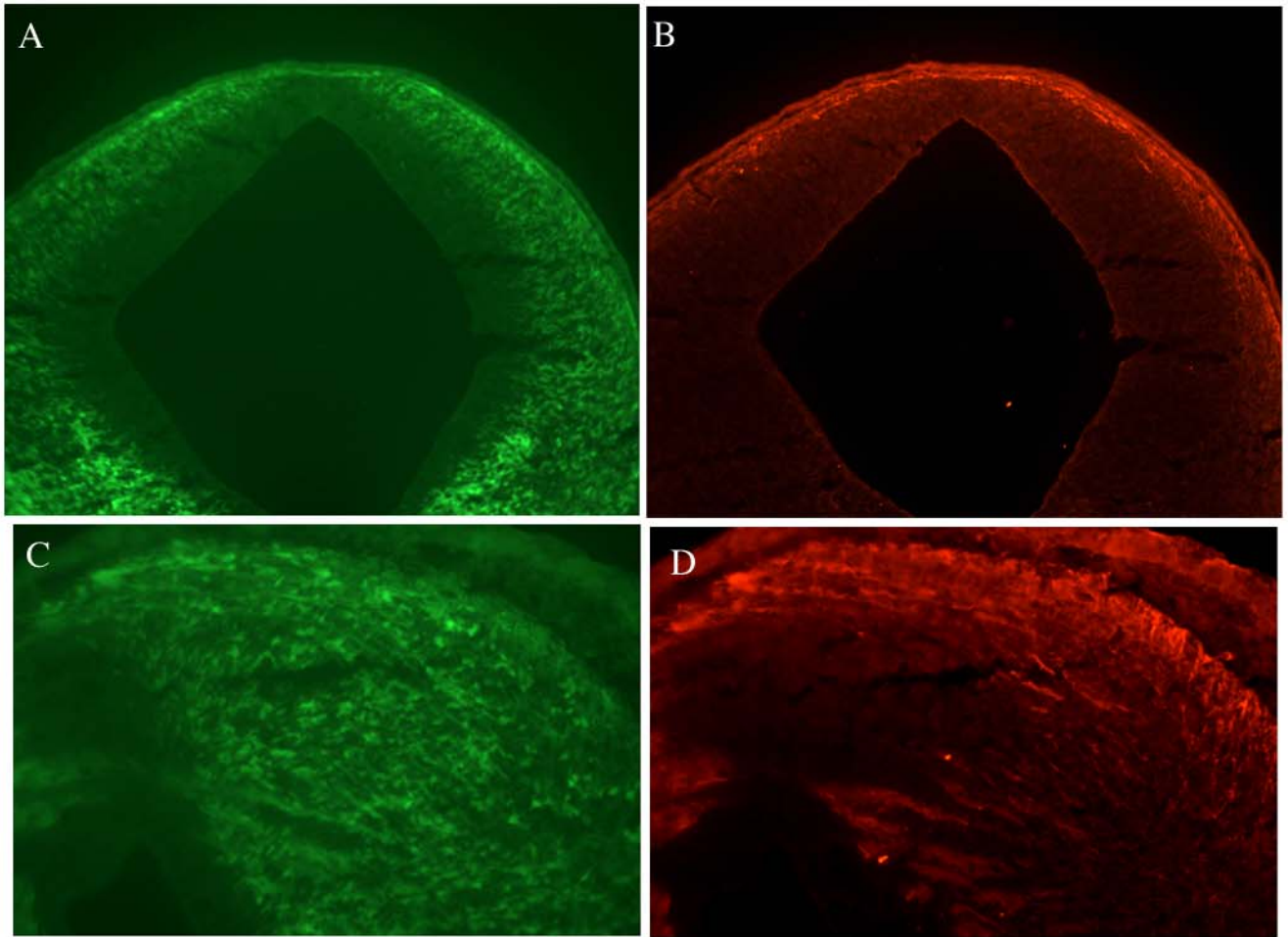
Fig. 4 GFP fluorescence (A and C) and GABA immunoreactivity to GABA (B and D) of E12.5 GAD67-GFP mouse SC. A and B, and C and D are the same section.
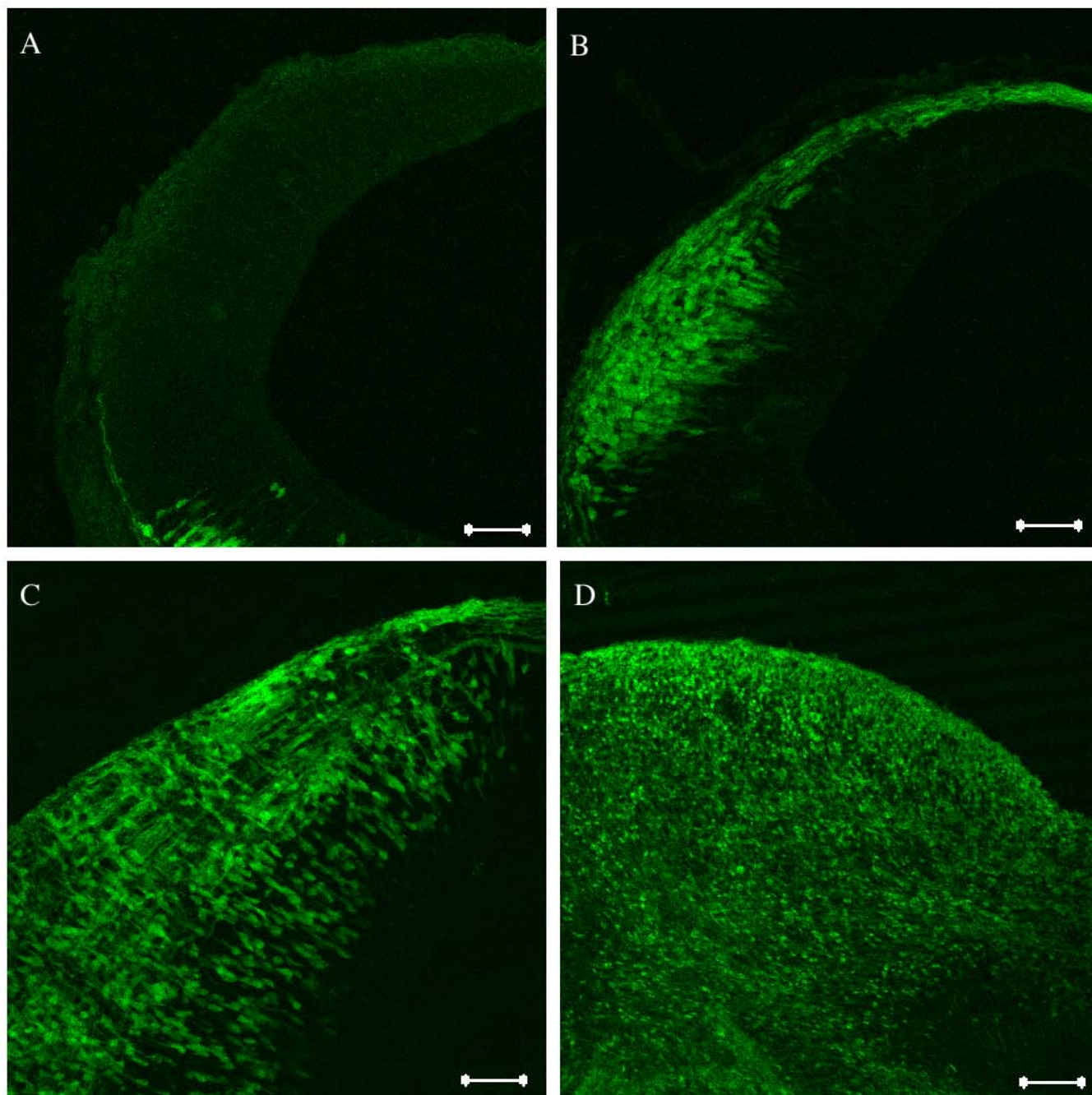
Fig. 5 GFP fluorescence in SC of GAD67-GFP knock-in mice. A: E10.5, B: E11.5, C: E13.5 and D: E16.5. Coronal section. Midline of SC is at the right-hand edge of each figure. Scale bars in A-C are 50 μm and in D is 100 μm.
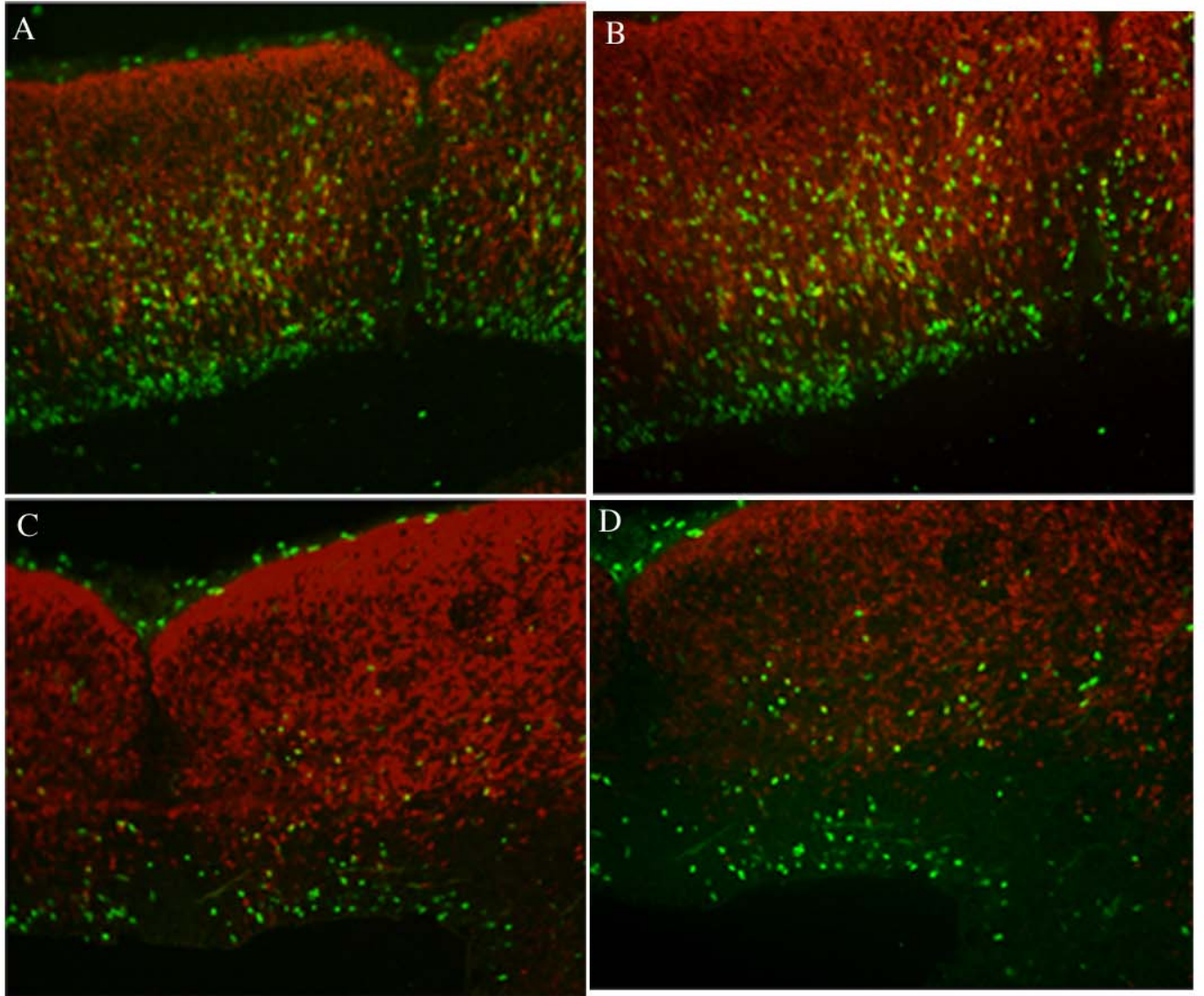
Fig. 6 Double labeling of embryonic SC with BrdU (green) and GFP (red). GFP was stained with its antibody and RITC-conjugated second antibody and then BrdU with FITC-conjugated anti-BrdU antibody. GFP fluorescence was lost during acid treatment for BrdU staining. A and B: E15.5. BrdU injection at E13.5. B and D: E16.5. BrdU injection at E14.5. Coronal sections close to the midline.
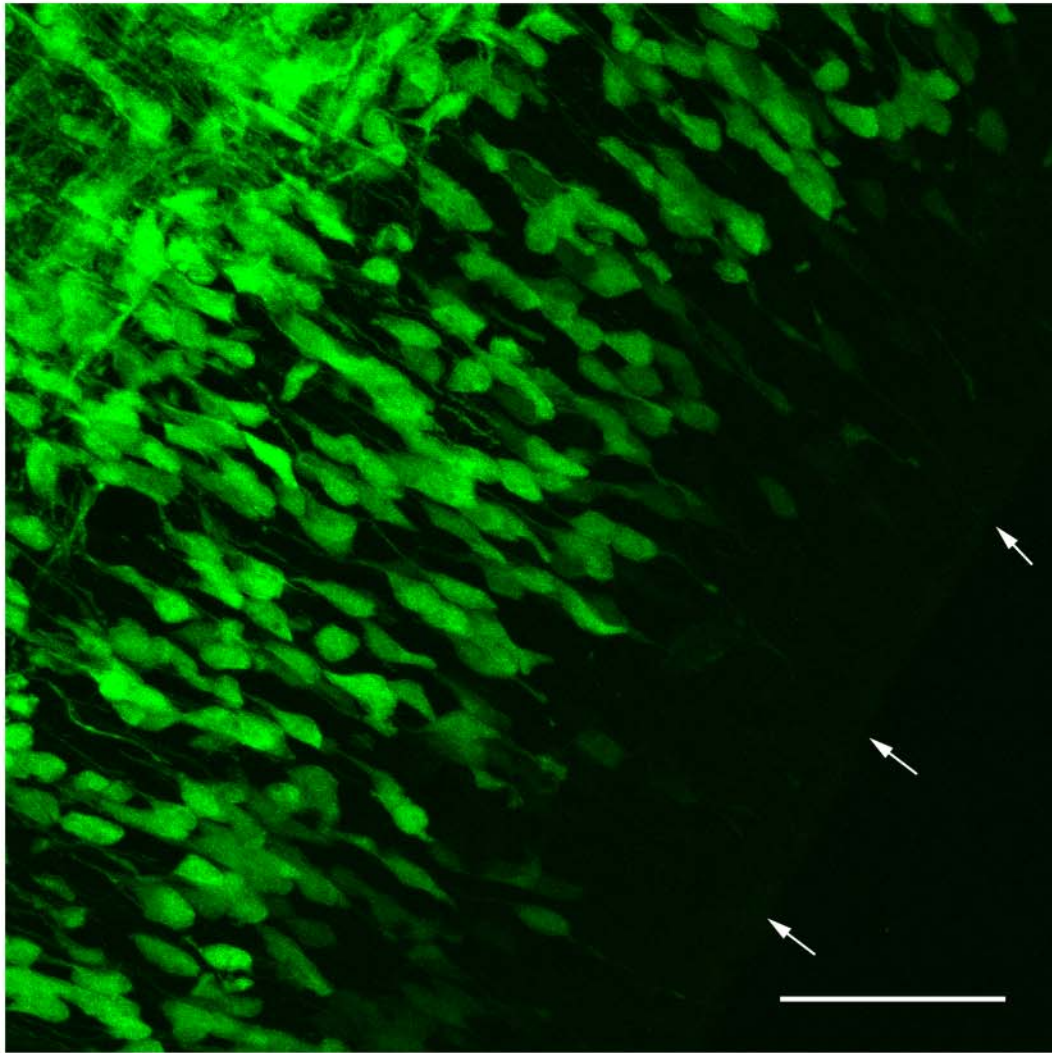
Fig. 7 A laser scanning confocal fluorescence micrograph of GFP-positive cells. Coronal section in SC of E 13.5 GAD67-GFP knock-in mouse. Arrows indicate the ventricular wall. Scale bar is 50 μm.
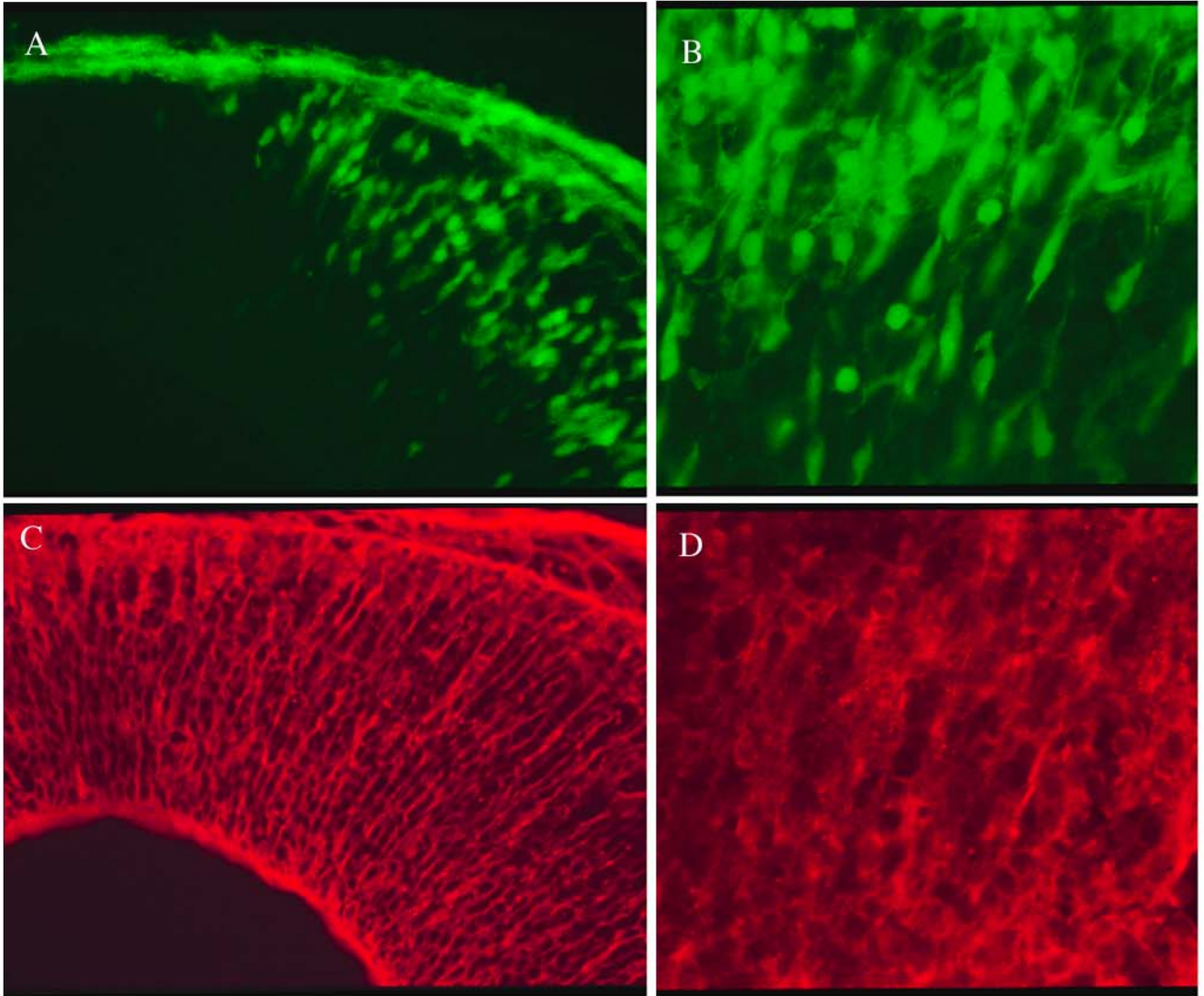
Fig. 8 GFP and nestin expression in E12.5 GAD67-GFP mouse SC. A and B: GFP fluorescence. C and D: The same section was stained with anti-nestin. B and D: higher magnification of A and C, respectively.
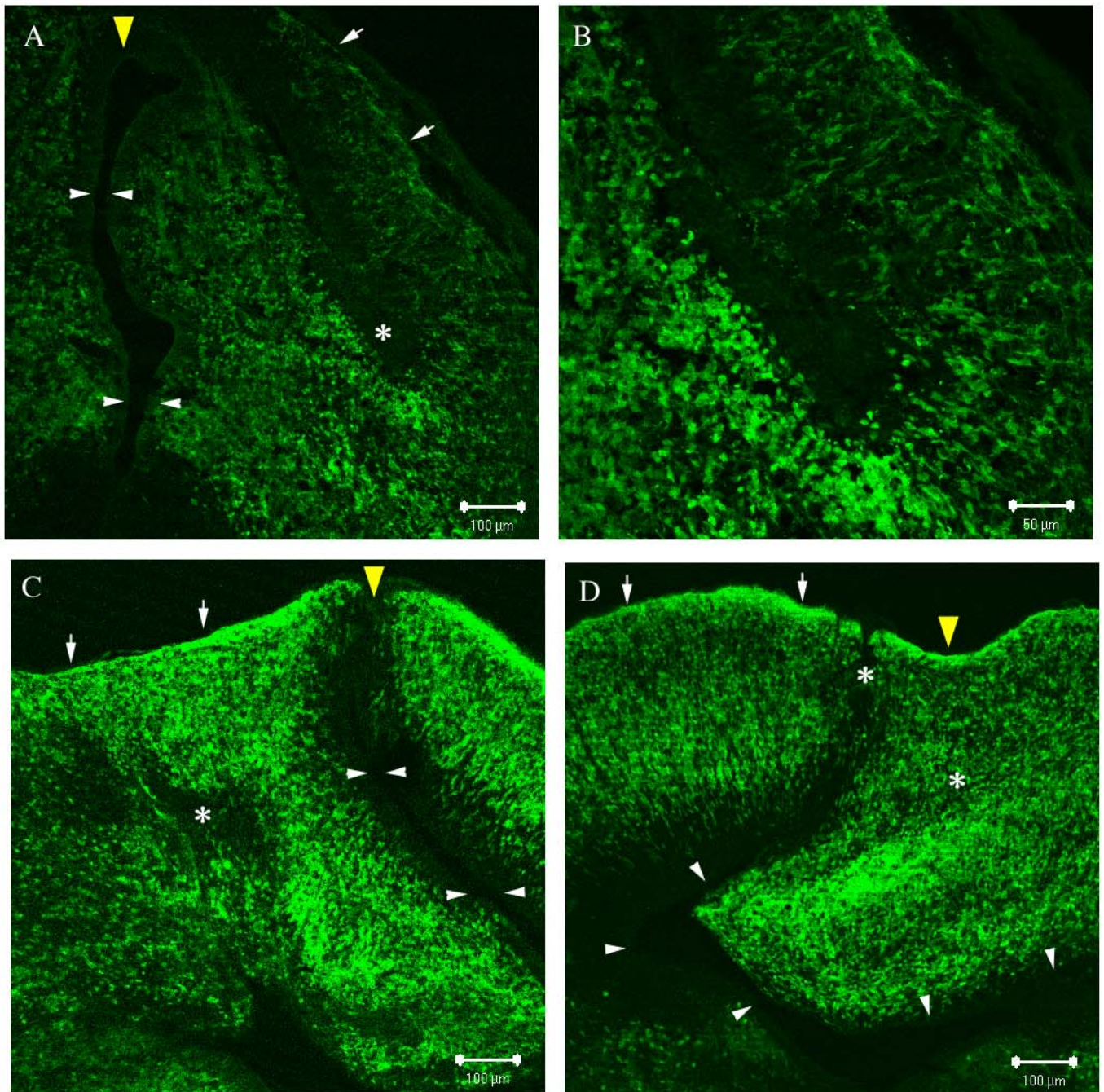
Fig. 9 GFP-positive cells in SC in which the cut was made at E12.5. A: E13.5. Coronal section.
B: high magnification image of area indicated by an asterisk in A. C: E15.5. Coronal section.
D: Another mouse at E15.5 with the two cuts. Coronal section. Arrows and arrowheads in A, C
and D indicate the surface and ventricular wall of SC, respectively. Yellow arrowheads indicate
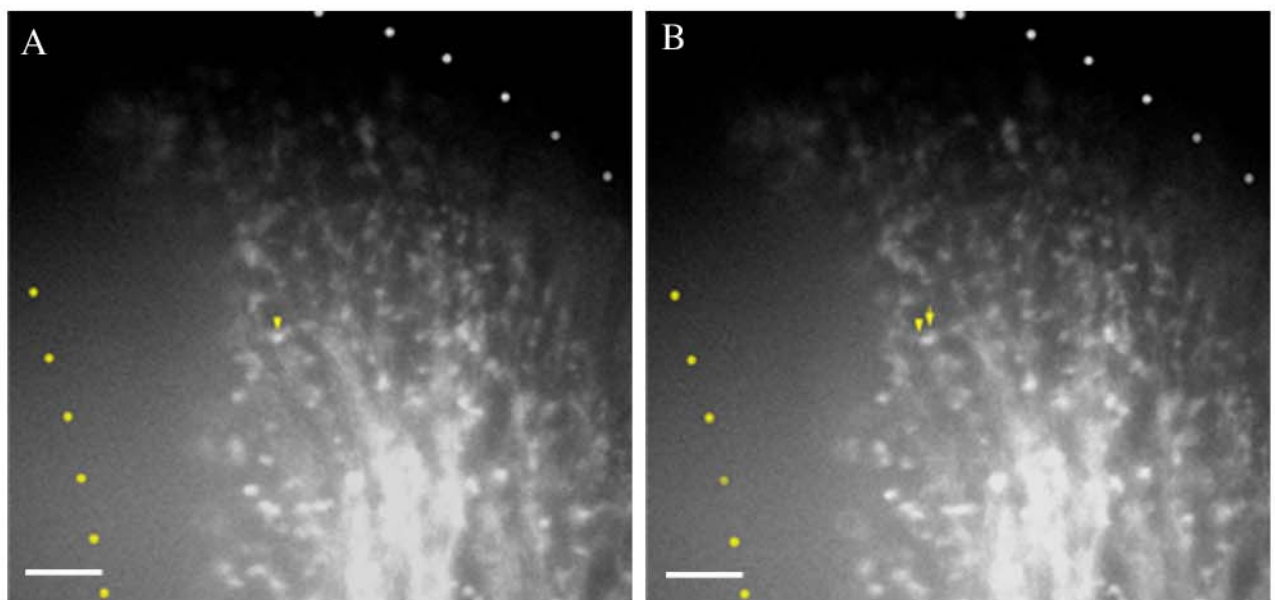the midline. Asterisks in A-D indicate the cuts. Scale bars: A, C, D, 100 μm; B, 50 μm

Fig. 10 Time lapse observation of living GFP-positive cells. Pictures of a SC slice were taken at 20 min (A) and 35 min (B) during observation. Arrowheads indicate the position of a specimen cell at 20 min and an arrow indicate its position at 35 min. Yellow and white dots indicates ventricular wall and surface of SC, respectively. The upper side is dorsal. Scale bar is 50 μm.
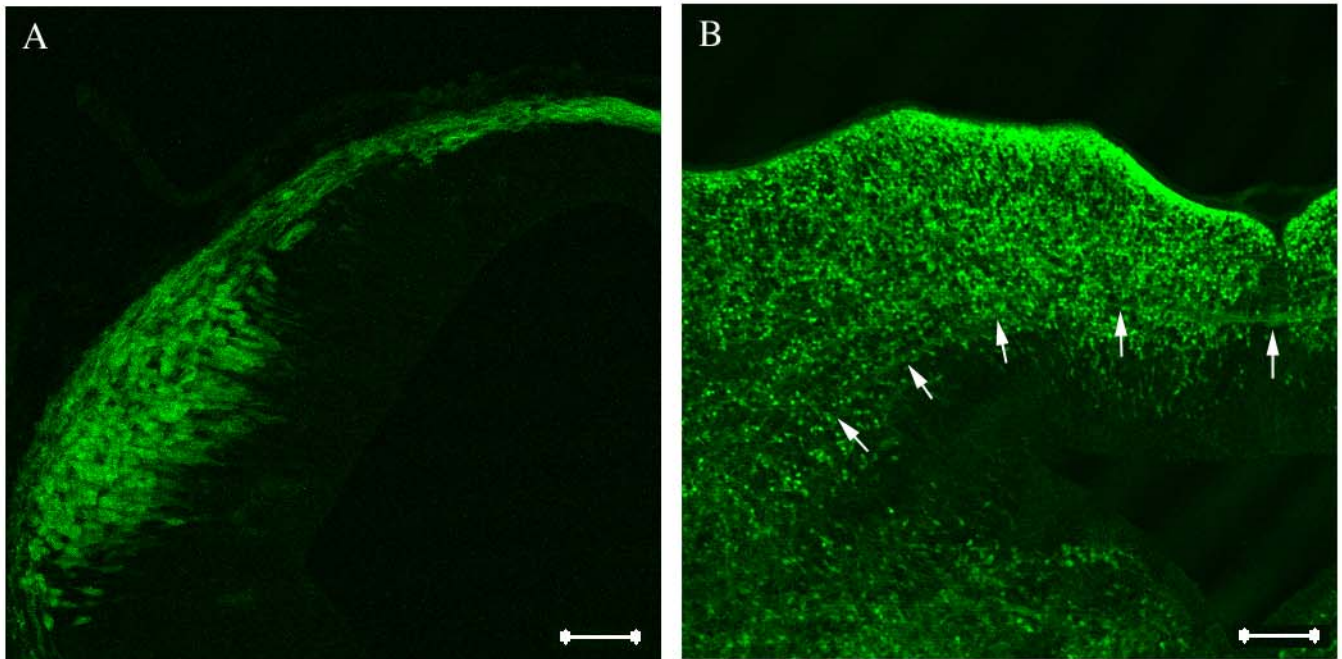
Fig. 11 GFP-positive fibers in SC from GAD67-GFP knock-in mouse. Coronal section.
A: E11.5 and B: E15.5 GFP-positive fiber bundles (arrows) in deep layers. Scale bar in A is 50 μm and in B is 100 μm.
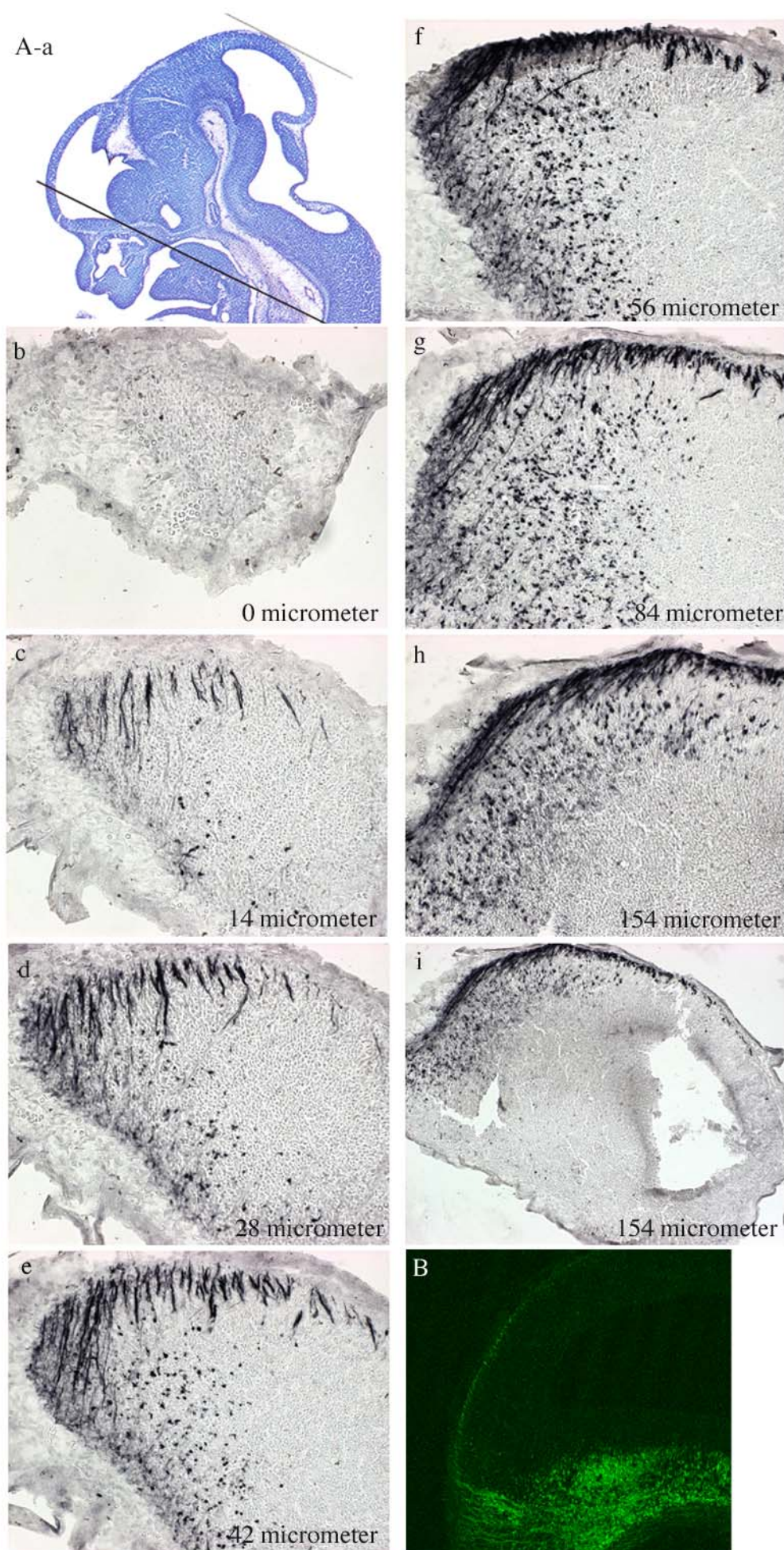
Fig. 12 GFP-positive fibers in E11.5 SC. Ab-f: serial sections from the surface were stained with an antibody against GFP (from Tamamaki et al., 2000). Figures in each image indicate the distance from b. The orientation of section was parallel to an upper line in a. i: Low magnification image of h. B: GFP fluorescence. Sagittal section. Upper and left lower side of this figure are the dorsal and anterior part of SC, respectively.
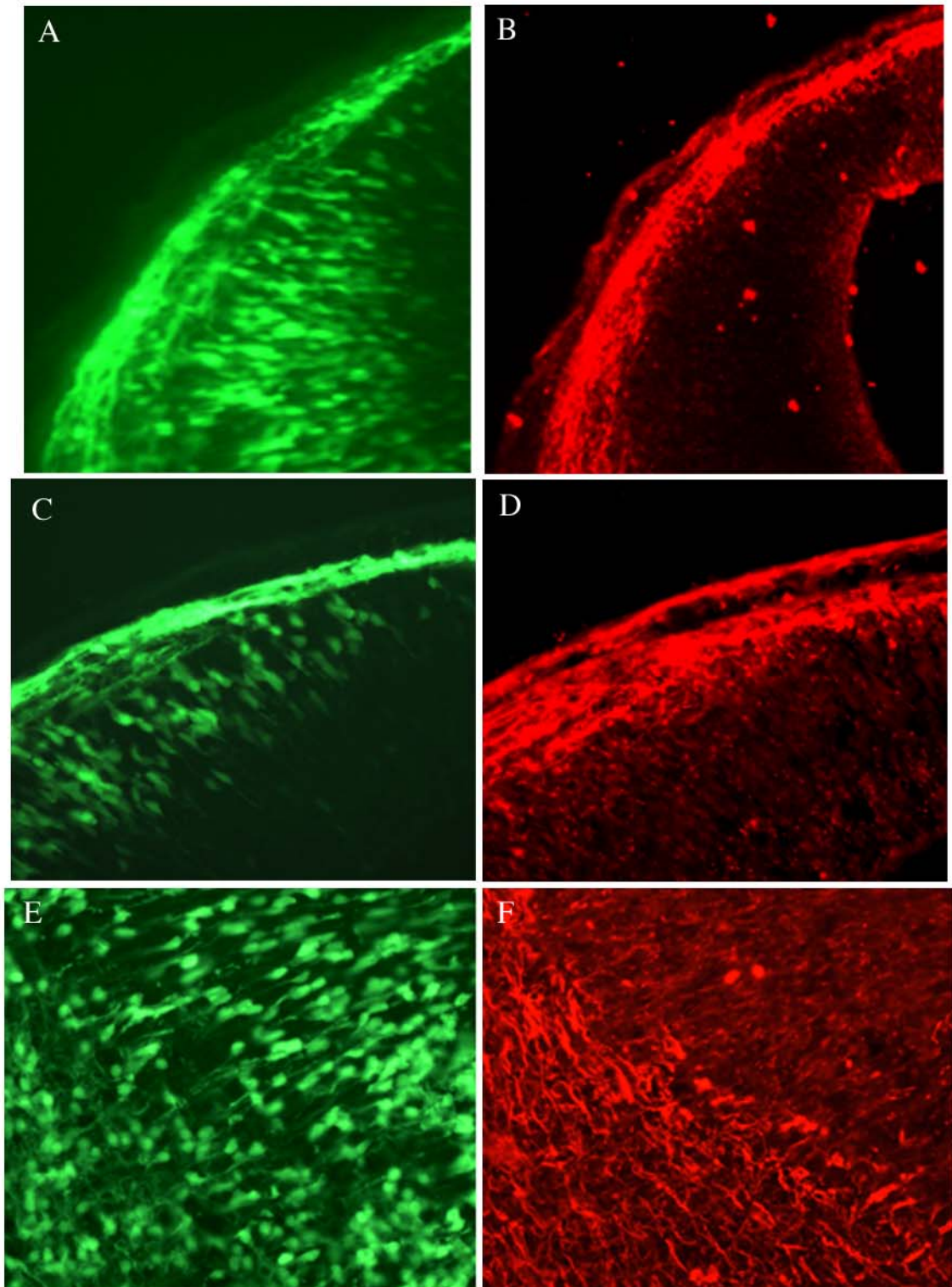
Fig. 13 Expression of GFP and neurofilament in E12.5 GAD67-GFP mouse SC. A, C and E: GFP fluorescence. B, D and F: same sections were stained with an antibody against neurofilament M. E, F: under higher magnification.
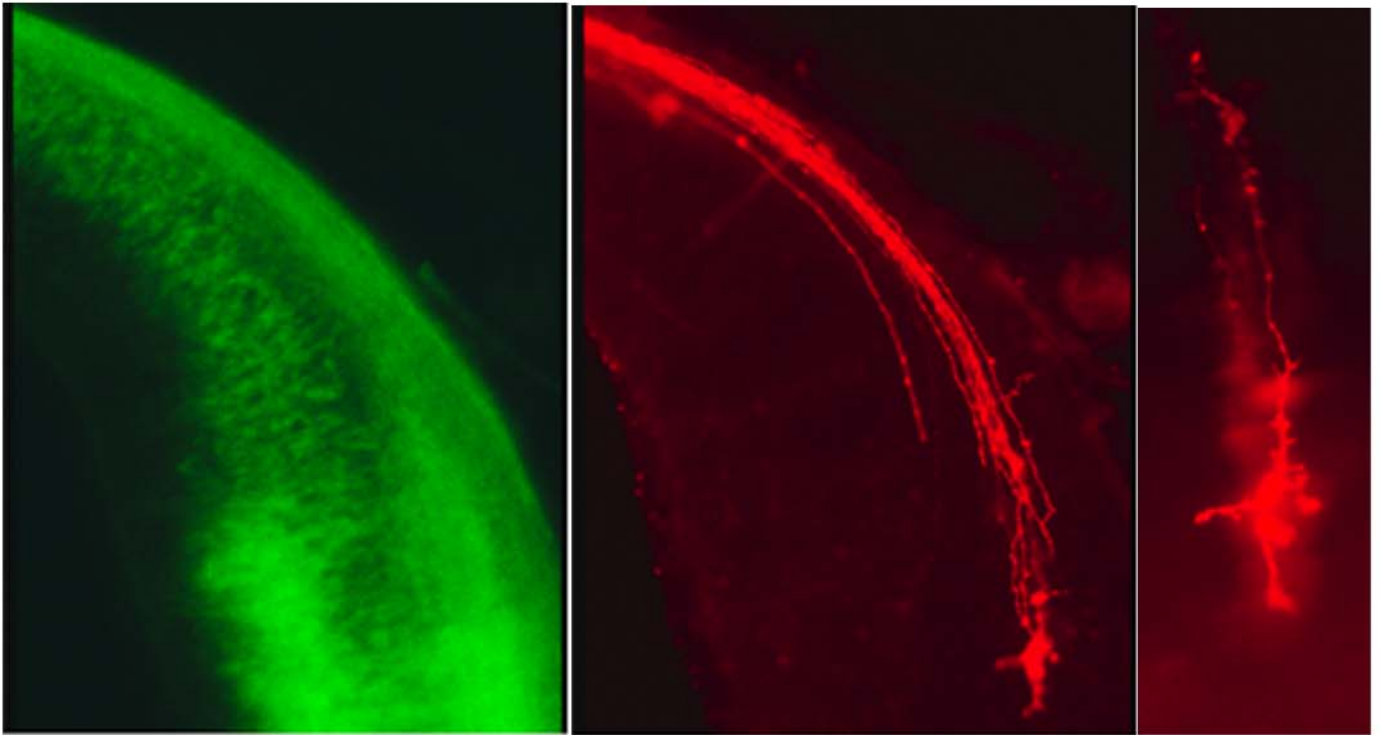
Fig. 14 DiI labeling of commissural fibers in E12.5 SC slices. A: GFP fluorescence in the same slice. B: DiI labeling. DiI particle was placed on the contralateral side (not seen on the left-hand side) of SC. A group of retrogradely labeled cell bodies are seen at the top of axons. C: Higher magnification of B.
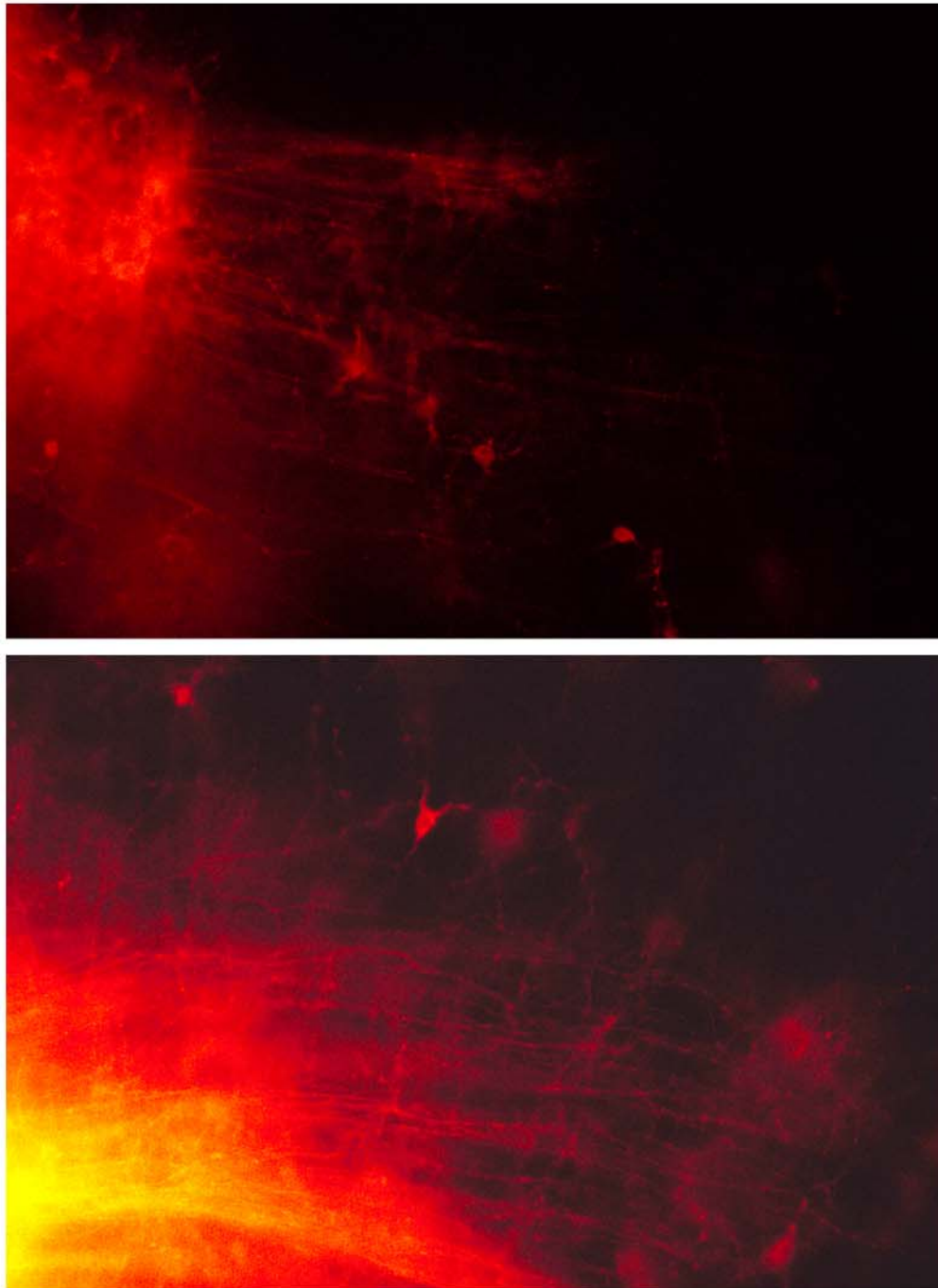
Fig. 15 DiI labeling of P0.5 SC slices. DiI particle was placed on the contralateral side of SC. Midline is at the left-hand side of each picture. Labeled commissural axons and retrogradely labeled cell bodies are seen deep in SC.

Table1 The velocity and direction of migration of GABAergic neurons in the slice preparation of SC from E11.5 or E12.5 mice.

Velocity (μm/15min)		Cells	
		Radial	Tangential
0.0	22	0	0
5.0-10.0	4	4	0
10.1-15.0	1	1	0
15.1-20.0	2	2	0
Total	29	7	0