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Research Article

FoxJ1-expressing cells contribute to neurogenesis in forebrain of adult rats: Evidence from in vivo electroporation combined with piggyBac transposon

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ABSTRACT

Ependymal cells in the lateral ventricular wall are considered to be post-mitotic but can give rise to neuroblasts and astrocytes after stroke in adult mice due to insult-induced suppression of Notch signaling. The transcription factor FoxJ1, which has been used to characterize mouse ependymal cells, is also expressed by a subset of astrocytes. Cells expressing FoxJ1, which drives the expression of motile cilia, contribute to early postnatal neurogenesis in mouse olfactory bulb. The distribution and progeny of FoxJ1-expressing cells in rat forebrain are unknown. Here we show using immunohistochemistry that the overall majority of FoxJ1-expressing cells in the lateral ventricular wall of adult rats are ependymal cells with a minor population being astrocytes. To allow for long-term fate mapping of FoxJ1-derived cells, we used the *piggyBac* system for *in vivo* gene transfer with electroporation. Using this method, we found that FoxJ1-expressing cells, presumably the astrocytes, give rise to neuroblasts and mature neurons in the olfactory bulb both in intact and stroke-damaged brain of adult rats. No significant contribution of FoxJ1-derived cells to stroke-induced striatal neurogenesis was detected. These data indicate that in the adult rat brain, FoxJ1-expressing cells contribute to the formation of new neurons in the olfactory bulb but are not involved in the cellular repair after stroke.

Introduction

In the adult forebrain, the ependymal layer comprises a ciliated epithelium forming the boundary between the cerebrospinal fluid-filled ventricles and the parenchyma. The ependymal cells surround the neural stem cells (NSCs) in the subventricular zone (SVZ), *i.e.*, astrocytes with single cilium [1], and provide structural, trophic and metabolic support [2]. The beating of the ependymal cells' cilia directs the migration of neuroblasts [3], whereas the disruption of ependymal cell integrity impairs neurogenesis [4]. Ependymal cells are generally considered to be post-mitotic [5–7], but have been reported to proliferate and give rise to neuroblasts and astrocytes after stroke in mice due to insult-induced suppression of Notch signaling [8]. However, none of the stroke-generated

Abbreviations: BrdU, 5′-bromo-2-deoxyuridine; GCL, granule cell layer; GFAP, glial fibrillary acidic protein; GML, glomerular layer; LVW, lateral ventricular wall; MCAO, middle cerebral artery occlusion; NSC, neural stem cell; OB, olfactory bulb; SVZ, subventricular zone; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling

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neuroblasts, derived from ependymal cells, survived to become mature neurons. Following stroke, ependymal cells delaminated from the ependymal layer and did not self-renew [8]. Another study failed to demonstrate proliferating ependymal cells and the ependymal layer remained intact after stroke in mice [9]. Whether ependymal cells can give rise to neuronal progeny after stroke in rats is unknown.

Recent studies have indicated that FoxJ1 is a characteristic marker of ependymal cells in mice [8,10,11]. FoxJ1 is a key transcription factor driving the expression of motile cilia [12]. Ependymal cells and choroid plexus epithelial cells are characterized by the presence of motile cilia [13]. Transgenic mouse models based on FoxJ1 promoter-driven GFP expression or Cre-loxP recombination systems have shown that ependymal cells express FoxJ1 [13]. FoxJ1 is required for transformation of radial glia to ependymal cells [11] and perinatal olfactory neurogenesis [10]. So far, no data are available on the expression of FoxJ1 in ependymal or other cells in the lateral ventricular wall (LVW) of the rat brain.

The objectives of the present study were two-fold: first, to evaluate whether FoxJ1 is a specific marker for ependymal cells or expressed also by other cell types in the LVW of adult rats and, second, to determine if FoxJ1-expressing cells give rise to neuronal progeny in intact or stroke-injured adult rats. To allow for fate mapping, we developed the electroporation technique combined with *piggyBac* transposon for selective targeting of FoxJ1-expressing cells in the LVW of adult rats. So far, electroporation of plasmid DNA for selective targeting has only been established for adult mice [14].

Materials and methods

Animals and experimental design

Adult male Wistar rats (Charles-River, Germany; weighing 250–300 g) were housed under 12 h light/12 h dark cycle with unlimited access to food and water. All experimental procedures were approved by the Malmö-Lund Ethical Committee for the use of laboratory animals and were conducted in accordance with European Union directive on the subject of animal rights. Four experiments were performed:

In experiment 1, plasmids expressing GFP under the CAG promoter were injected stereotaxically into the lateral ventricle of adult rats ($n=4$). One week after electroporation, rats were perfused and assessed for GFP expression in the LVW. Electroporation conditions were optimized ($n=38$ rats) for the maximal expression of GFP without deleterious effects. Six rats were administered 5′-bromo-2-deoxyuridine (BrdU; 50 mg/kg Sigma-Aldrich, Germany) at 24 h, 72 h or 1 week after electroporation, four times every 2 h and sacrificed 2 h after the last BrdU injection. TUNEL labeling was performed in this group to analyze cell death.

In experiment 2, expression of FoxJ1 in LVW was first analyzed by immunohistochemistry in intact rats ($n=2$). After establishing the FoxJ1 immunoreactivity in LVW, Cre-recombinase under FoxJ1 promoter was co-electroporated with reporter plasmid expressing GFP, without Cre activity and RFP, with Cre activity ($n=20$). Animals were sacrificed 2 weeks after electroporation and LVW was analyzed for GFP and RFP expression and whether GFP+/RFP+ cells were FoxJ1 immunoreactive.

In experiment 3, *piggyBac* transposon system expressing GFP or RFP was used to genetically label FoxJ1+ cells. Animals ($n=4$) were electroporated with pPB-UbC-eGFP (donor) and mPB (helper) plasmids and sacrificed 1 week later. Once the system had been observed to label LVW cells with GFP, animals ($n=4$) were electroporated with pPB-FoxJ1-eGFP (donor) plasmid and mPB (helper) plasmids and sacrificed two and 6 weeks thereafter. We then electroporated one group of rats ($n=2$) with pPB-UbC-DsRed2 (donor) and pFoxJ1-mPB (helper) plasmids and another group ($n=3$) with pPB-FoxJ1-eGFP (donor) and pFoxJ1-mPB (helper) plasmids. Animals were sacrificed 12 weeks after electroporation and the distribution of GFP- or RFP-expressing cells was analyzed in LVW and olfactory bulb (OB).

In experiment 4, rats were electroporated with pPB-FoxJ1-eGFP (donor), pPB-UbC-DsRed2 (donor) and pFoxJ1-mPB (helper) plasmids. One week later, stroke was induced by 30 min ($n=4$) and 2 h ($n=4$) middle cerebral artery occlusion (MCAO). One week after MCAO, BrdU (50 mg/kg) was injected i.p. twice daily for a week. Animals were sacrificed 4 weeks after the last BrdU injection and the distribution of GFP/RFP expressing cells was analyzed in LVW and OB.

In vivo electroporation

Electroporation was carried out under isoflurane anesthesia, using the CUY21 EDIT electroporator (NEPA Gene, Japan) with 7 mm platinum disc or 10 mm caliper electrodes, as described previously [14]. Briefly, electro-conductive gel (Parker Laboratories, USA) was applied to the electrodes and the head of the rat fixed to the stereotaxic frame. A saline-soaked, thin pad of 10 mm × 10 mm size sterile surgical gauze was then applied over the gel on the head. Plasmid DNA (4 μl of 3–10 μg/μl plasmid solution) was injected into the lateral ventricle (0.2 mm anterior and 1.0 mm lateral to bregma and 3.4 mm deep from surface of brain) using glass capillary needles. The electrodes were placed on the saline pad, the cathode (positive electrode) on the same side as the intraventricular injection (ipsilateral side) and anode (negative electrode) on the opposite side (contralateral side) to complete the circuit. Three to five electrical pulses (100–200 V/cm) of 50 ms duration with 1 Hz frequency were applied. Electrodes were then taken off followed by removal of the injection needle. The wound was closed with Histoacryl (Braun, Germany) and animals were allowed to recover. Grooming behavior, food and water intake and signs of burns or scars at the site of electrode application were monitored.

Plasmid constructs

Plasmids were constructed by standard molecular methods, extracted with phenol–chloroform and resuspended in 10 mM Tris chloride for electroporation. pCAX-AFP [15], expressing a variant of GFP, was used for establishing and optimizing electroporation in LVW of adult rat brain. For Cre-mediated recombination in ependymal cells, pTg-FoxJ1-CreGFP expressing Cre-recombinase and pCall2-loxP-H2B:GFP-loxP-RFP [8] reporter plasmid were used. Fate mapping experiments were carried out with *piggyBac* transposon. The helper plasmid mPB with donor plasmid pPB-UbC-eGFP (UbC: Ubiquitin-C promoter; [16]; kind gift of A. Bradley, The Wellcome Trust Sanger Institute, UK) were used and transposon activity was assessed by GFP expression. The

FoxJ1 promoter from pTg-FoxJ1-CreGFP was amplified by PCR and cloned to generate the pPB-FoxJ1-eGFP and pFoxJ1-mPB donor and helper plasmids, respectively. pPB-UbC-DsRed2 was generated from pPB-UbC-eGFP by replacing the eGFP with DsRed2 from pIRES2-DsRed2 (Clontech). The plasmids pFoxJ1-mPB with pPB-UbC-DsRed2 or pPB-FoxJ1-eGFP was used in 1:1 ratio for fate mapping experiments in intact animals. The plasmids pFoxJ1-mPB with pPB-UbC-DsRed2 and pPB-FoxJ1-eGFP were electroporated in 1:1:1 ratio, (total DNA concentration 36 μg) for fate mapping experiments following MCAO.

Middle cerebral artery occlusion

Rats were subjected to experimental stroke 1 week after electroporation. Stroke was induced, on the electroporated side (ipsilateral side), by intraluminal filament MCAO model as described previously [17]. Briefly, under isoflurane anesthesia, the right common carotid artery (CCA) and its proximal branches were isolated. The CCA and external carotid artery (ECA) were ligated, while the internal carotid artery (ICA) was temporarily occluded using a metal microvessel clip. A nylon monofilament was inserted and advanced through the CCA and ICA until resistance was felt, past the origin of middle cerebral artery (MCA). The nylon filament was carefully removed 30 min or 2 h after the start of occlusion, and ECA was ligated permanently.

Immunohistochemistry

Animals were perfused transcardially and brains were removed, post-fixed overnight in PFA, and cryopreserved in 20% sucrose solution. Free-floating frozen sections (30 μm thick) were cut on a sliding microtome (Leica, Germany) in the coronal plane. Sections were pre-incubated in 0.25% Triton X-100 in phosphate-buffered saline (PBS) containing 5% donkey or goat serum for 1 h. Sections were then incubated with rabbit anti-RFP (1:1000; ab62341, Abcam, UK) and/or chicken anti-GFP (1:5000; ab13970, Abcam) and goat anti-DCX (1:400; SC8066, SantaCruz Biotechnology, CA, USA), mouse anti-GFAP (1:400; G3893, Sigma, Germany), mouse anti-FoxJ1 (1:1000; 14-9965-82, eBioscience, UK), rabbit anti-S100β (1:200; 04-1054, Millipore, Sweden), mouse anti-Vimentin (1:200; M0725, DAKO, Denmark), rabbit anti-Iba1 (1:400; 019-19741, Wako, Japan), mouse anti-NeuN (1:400; MAB377, Millipore), rabbit anti-Ki67 (1:200; NC-Ki67p, Leica, Germany) or rabbit anti-βCatenin (1:200; 06-734, Millipore) primary antibodies overnight at 4 °C. Antigen retrieval with sodium citrate buffer (pH 6.0, at 65 °C for 20 min) was performed for βCatenin (βCat) immunostaining. Sections were then incubated for 2 h with Cy2-, Cy3- or Cy5-conjugated donkey or goat secondary antibodies (1:200; Jackson ImmunoResearch, UK) at room temperature (RT). Biotin-conjugated secondary antibody (1:200; Vector Labs, UK) followed by Alexa 647 Streptavidin conjugate (1:1000; Invitrogen, Sweden) was used to label GFP+ and RFP+ cells in the MCAO group. Sequential staining was carried out if two of the primary antibodies were made in the same species. For BrdU staining, sections stained for other markers were fixed in 4% PFA for 10 min at RT and washed in PBS. DNA was denatured with 2 N HCl for 1 h at +37 °C, washed three times with PBS, and incubated overnight with rat anti-BrdU (1:100; OBT0300, AbD Serotec, Germany). Biotin-conjugated donkey anti-rat (1:200; Jackson ImmunoResearch) secondary antibody was incubated for 2 h at RT followed by incubation with Streptavidin-conjugated Alexa-647 for 2 h at RT.

TUNEL labeling

In order to check for apoptotic cell death caused by electroporation, four evenly spaced sections including LVW, from intact and electroporated (after 24 h, 72 h and 1 week) rat brains were subjected to TUNEL labeling (*In situ* Cell Death Detection kit, TMR Red; Roche Applied Science, Germany) according to the manufacturer's instruction. Briefly, GFP-stained sections were mounted onto slides and air-dried. The sections were rinsed three times in ice-cold PBS and then in ice-cold permeabilization solution (0.1% Triton X-100 in 0.1% Sodium Citrate) for 2 min. Sections were rinsed three times in PBS and incubated in TUNEL enzyme and label mix for 1 h at +37 °C. After rinsing once in PBS, nuclei were stained with Hoechst 33357 (10 μg/ml). The sections were then rinsed twice in PBS, air-dried and coverslipped with PVA-DABCO mounting medium.

Microscopical analysis

Cell counts were performed in four evenly spaced coronal sections comprising LVW (between +1.70 and −0.80 mm in relation to bregma) or eight evenly spaced OB sections (between +7.00 and +5.70 mm antero-posteriorly in relation to bregma), by an observer blinded to sample identity. Unless otherwise stated, cell counting was carried out in LVW and OB on the ipsilateral side (electroporated side). Immunofluorescence was analyzed in an Olympus BX51 (Olympus, Germany) epifluorescence microscope and images were obtained with CellSens software (Olympus, Germany). The distance between the LVW and geometric center of the cell body of GFP+ or RFP+ cells in striatum was measured using the CellSens software. For MCAO group with Cy5 or ALexa-647 fluorochrome stained sections, confocal images of whole ipsilateral LVW were obtained with Leica TCS SP2 (Leica, Germany) laser scanning confocal microscope. Counting of GFP- and RFP-labeled cells expressing the phenotypic markers in LVW or OB was made on confocal images using Leica-lite software. Brightness and contrast were adjusted minimally with similar settings for images using Adobe Photoshop CS 6.0.

Statistical analysis

All statistical analysis and graphs were made in Prism 5.0c software (GraphPad). Paired Student's *t*-test was used for comparing the contralateral and ipsilateral sides for number of Ki67+, BrdU+ and TUNEL+ cells. Unpaired Student's *t*-test was used for comparing GFP+ and RFP+ cell phenotype in the 30 min and 2 h MCAO groups. Data are presented as number of cells per section, while subpopulation cell analysis is presented as percentage of a given population of cells. Differences are considered significant with $P<0.05$.

Results

Electroporation can target ependymal cells in adult rat brain

We first explored whether electroporation is a useful approach for delivering DNA to the ependymal cell layer and SVZ in the LVW of

adult rats. Electroporation (Fig. 1A) was performed after intraventricular injection of the GFP variant pCAX-AFP [15] plasmid (5 μg/μl; Fig. 1B1) using five 100 V/cm pulses, at 1 Hz frequency with 50 ms pulse duration [14]. The electroporated animals exhibited normal grooming behavior. There were no persistent seizures and no tissue damage in the areas where the electrodes had been applied. However, we detected only a few GFP+ cells in the ipsilateral LVW at 1 week after electroporation (data not shown). There was no labeling on the contralateral side.

In order to increase labeling, we optimized several electroporation variables, including the amount of applied electrical potential, DNA concentration, and number of pulses (Table 1). Few or no labeled cells were detected in the LVW with 50 V/cm and 100 V/cm, and animals did not recover after electroporation with 200 V/cm. In contrast, there were many labeled cells in the 150 and 175 V/cm groups (Fig. 1C–D; Table 1). None of the tested amounts of electrical potential (50, 100, 150 and 175 V/cm) caused behavioral disturbances or scalp problems. The total amount of electrical potential applied with 175 V/cm, *i.e.*, 430 V (430.1 ± 12.6 V), was used for subsequent electroporations with 7 mm platinum disc electrodes covering rostro-caudally and dorso-ventrally the LVW between +2.20 and −1.80 mm in relation to bregma.

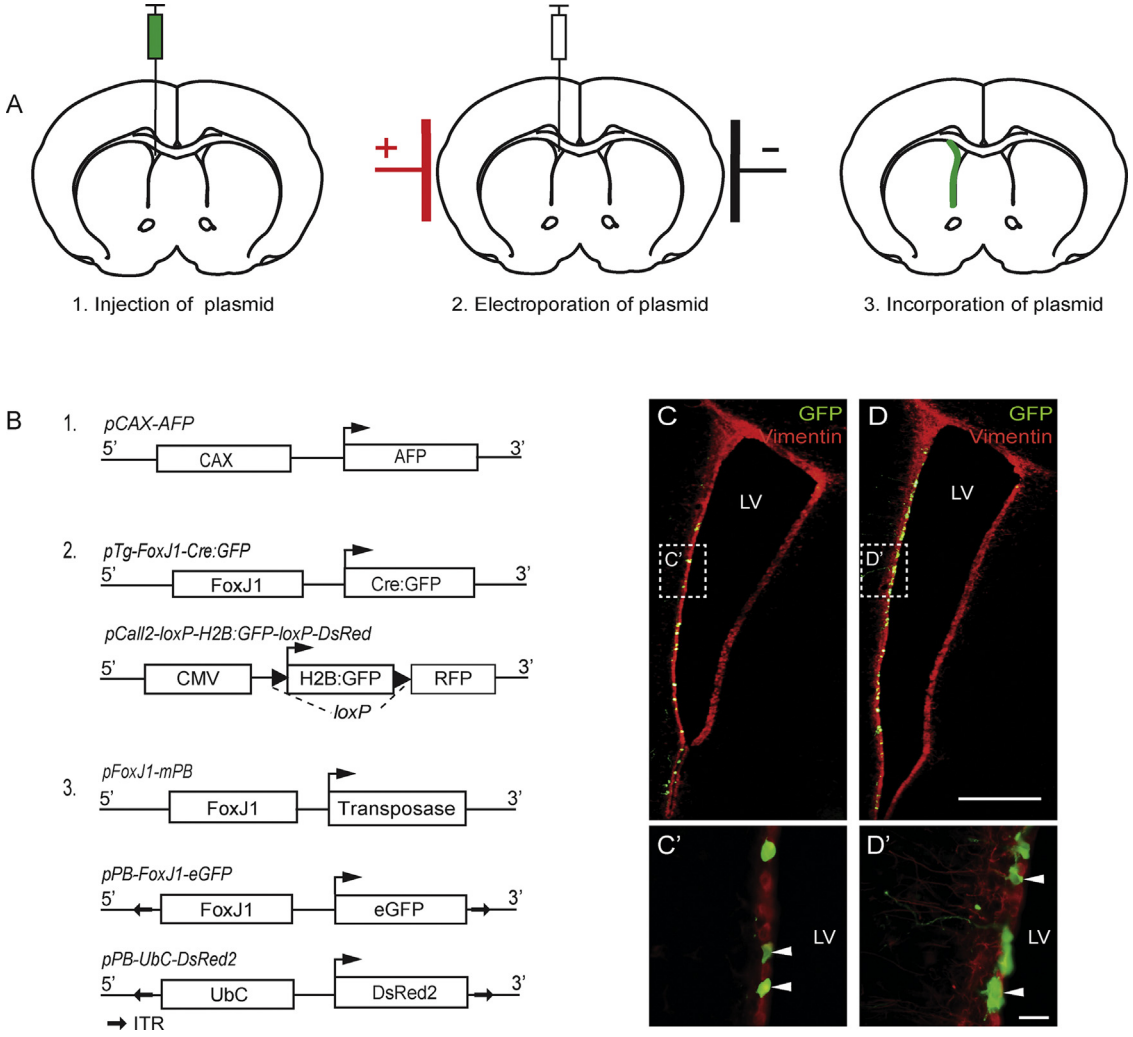

Fig. 1 – Electroporation of plasmid DNA into lateral ventricular wall (LVW) of adult rat brain. (A) Outline of the electroporation procedure. (A1) Plasmid DNA is injected into the lateral ventricle. (A2) Electrodes connected to the electroporator are applied with cathode on the plasmid-injected side. Three electrical pulses of 175 V/cm and 1 Hz are applied. (A3) Plasmid is incorporated into LVW and reporter expression can be analyzed by immunohistochemistry. (B) Plasmids used in the different electroporation experiments. (B1) pCAX-AFP plasmid used in initial experiments for validation and optimization of electroporation. (B2) Cre-recombinase and reporter plasmids used in determining the localization of FoxJ1-expressing cells. (B3) *piggyBac* transposon helper and donor plasmids used for tracing the progeny of FoxJ1+ cells. pFoxJ1-mPB is the helper plasmid expressing *piggyBac* transposase that helps in integrating the sequences within the inverted terminal repeats (ITR) of donor plasmid to the genome. pPB-FoxJ1-eGFP and pPB-UbC-DsRed2 are the donor plasmids that have the genome-integrating sequences within the ITR. (C and D) The LVW labeled by electroporation with 150 V/cm and 175 V/cm, respectively, potential applied across the electrodes and immunostained for GFP and Vimentin. The representative images show more GFP+ cells with 175 V/cm as compared to 150 V/cm. (C′ and D′) show higher magnification images of boxed areas in (C and D). Arrowheads (C′ and D′) depict cells that are GFP+/Vim+. Scale bars: C and D – 200 μm; C′ and D′ – 20 μm. Abbreviation: LV – Lateral ventricle.

Table 1 – Variables for optimization of electroporation of plasmid DNA to lateral ventricular wall in adult rat brain.

Variable		GFP+cells per section
Potential applied (V/cm)	50	0.9±0.9
	100	1.8±1.4
	150	17.3±3.4
	175	23.6±4.3
DNA concentration (μg/μl)	3	23.6±4.3
	5	22.0±5.6
	10	31.5±6.1
Number of pulses	3	17.3±3.4
	5	13.3±0.4

The pCAX-AFP plasmid was used for electroporation and GFP+ cells in lateral ventricular wall were counted in four sections. Data are mean±SEM.

Different concentrations of plasmid DNA (4 μl of 3, 5, and 10 μg/μl plasmid solution) were then injected into the lateral ventricles. We observed precipitation of DNA during injection with the highest concentration (10 μg/μl). Both 3 and 5 μg/μl of DNA gave rise to similar labeling (Table 1) and we, therefore, used 3 μg/μl for subsequent electroporations. Finally, we varied the number of pulses delivered with 50 ms duration and 1 Hz frequency, but found no differences in number of labeled cells between 3 and 5 pulses.

Using optimum parameters for electroporation (3 μg/μl plasmid DNA, 430 V (175 V/cm), 3 pulses, 50 ms duration, 1 Hz), we observed GFP+ cells in the LVW (37.9±0.9 cells per section) from +2.20 mm to −1.80 mm in relation to bregma. Most of the GFP+ cells co-expressed a marker for ependymal cells, vimentin (30.4±2.4 cells per section), with very few cells being astrocytes (GFAP+, 0.3±0.4 cells per section) or microglia (Iba1+, 0.8±0.1 cells per section). We found no significant differences between the ipsilateral and contralateral side, in numbers of cells expressing markers for cell death (TUNEL) or cell proliferation (Ki-67 and BrdU) during the first week after electroporation (TUNEL+ cells per section: ipsilateral 3.2±0.5 and contralateral 3.7±0.5; BrdU+ cells per section: ipsilateral 39.3±6.7 and contralateral 44.7±5.3; Ki67+ cells per section: ipsilateral 30.7±2.8 and contralateral 26.8±4.2). Taken together, these results show that electroporation can be used to target the ependymal layer in the rat brain without causing cell death or change in cell proliferation in the LVW.

FoxJ1 is expressed in ependymal cells in the lateral ventricular wall of adult rat

Using immunohistochemistry, we found FoxJ1 expression in nuclei of cells distributed along the LVW (Fig. 2A–D) and choroid plexus. In order to determine the phenotype of these FoxJ1+ cells, we co-stained for markers of ventricular wall lining cells such as ependymal cells (S100β and βCat), neuroblasts (DCX) and astrocytes (GFAP). We found that 83%, 97.5% and 3.6% of FoxJ1+ cells were S100β+, βCat+ and GFAP+ respectively, whereas no FoxJ1+ cells were DCX+ in the LVW. Our findings confirmed that FoxJ1 expression was largely confined to ependymal cells (Fig. 2E and F).

We then used Cre-loxP recombination system to label the FoxJ1+ cells in the LVW. Specifically, we used the pTg-FoxJ1-CreGFP plasmid (Fig. 1B2) with the human FoxJ1 promoter driving the expression of Cre-GFP fusion protein and where GFP will label only the nucleus. A GFP+ nucleus indicates that the cell expresses FoxJ1. The pCall2-loxP-H2B:GFP-loxP-RFP reporter plasmid will label the nuclei with GFP in the absence of Cre. Cells without active FoxJ1 expression express GFP from the reporter plasmid. When cells express Cre, the H2B:GFP in the reporter plasmid is floxed and cells will have RFP expression. Thus, RFP expression shows that recombination has occurred and FoxJ1 is active in the cells. This is ectopic recombination, occurring in the plasmid outside the genome. The ectopically recombined (RFP+) cells can be traced even if they lose FoxJ1 expression. Thus, FoxJ1 is active in GFP+/RFP+ cells whereas RFP+ cells are derived from FoxJ1+ cells but have changed their phenotype. The two plasmids, pTg-FoxJ1-CreGFP and pCall2-loxP-H2B:GFP-loxP-RFP, were co-electroporated and animals were sacrificed 2 weeks thereafter. We found GFP+ (14.7±1.2 cells per section) and GFP+/RFP+ (6.4±1.0 cells per section) cells in the LVW. Recombination efficiency, as estimated by the ratio between GFP+/RFP+ and GFP+ cells, was 42.8±3.1%. Among the GFP+/RFP+ cell population. None of the GFP+/FoxJ1- cells were RFP+, 51.0±7.8% and 10.1±2.4% of the cells were GFP+/RFP+/FoxJ1+ (Fig. 2G–J) and GFP+/RFP+/GFAP+ (Fig. 2K–N), respectively. Since, RFP cannot be expressed without Cre-recombinase (under the FoxJ1 promoter) floxing the H2B:GFP in the reporter plasmid, these observations raise the possibility that FoxJ1+ cells give rise to progeny or alter their phenotype.

Our data show that Cre-recombinase-based labeling of ependymal cells is possible in the rat LVW and two plasmids can be electroporated together to target FoxJ1+ cells. However we did not pursue this method for tracing the progeny of FoxJ1+ cells, first, due to possible toxic effects of Cre [18] and, second, because in ectopic recombination the cell may lose or stop transcription of the recombined plasmid and RFP expression, rendering the cell untraceable.

FoxJ1-expressing cells give rise to olfactory bulb neurons in intact rat brain

To overcome the problem with ectopic recombination and induce stable labeling of the genome of the FoxJ1+ cells, allowing for the visualization of the progeny of these cells for a long time, we decided to test the *piggyBac* transposon system. This system [16,19,20] stably integrates into the genome without adverse effect. The *piggyBac* system consists of a helper plasmid expressing 'transposase' and a donor plasmid that has the targeting construct within inverted terminal repeats (ITR) (Fig. 1B3). The transposase inserts the sequence, in our case UbC-eGFP, UbC-DsRed2 or FoxJ1-eGFP, within the ITR of the donor plasmid into the genome of targeted cells. To test the feasibility of using this *piggyBac* system, mPB (helper) and pPB-UbC-eGFP (donor) plasmids [16] were co-electroporated in a 1:1 ratio of helper: donor plasmid DNA concentration (μg/μl). Animals were perfused 1 week after electroporation and GFP expression (34.8±7.6 GFP+ cells per section) was observed in the LVW. The labeling efficiency by *piggyBac* system was consistent with the results from electroporation with pCAX-AFP plasmid used for optimizing electroporation parameters.

Having established that *piggyBac* could be used to label LVW cells, we constructed a pPB-FoxJ1-eGFP donor plasmid (Fig. 1B3) by replacing the UbC promoter with the human FoxJ1 promoter,

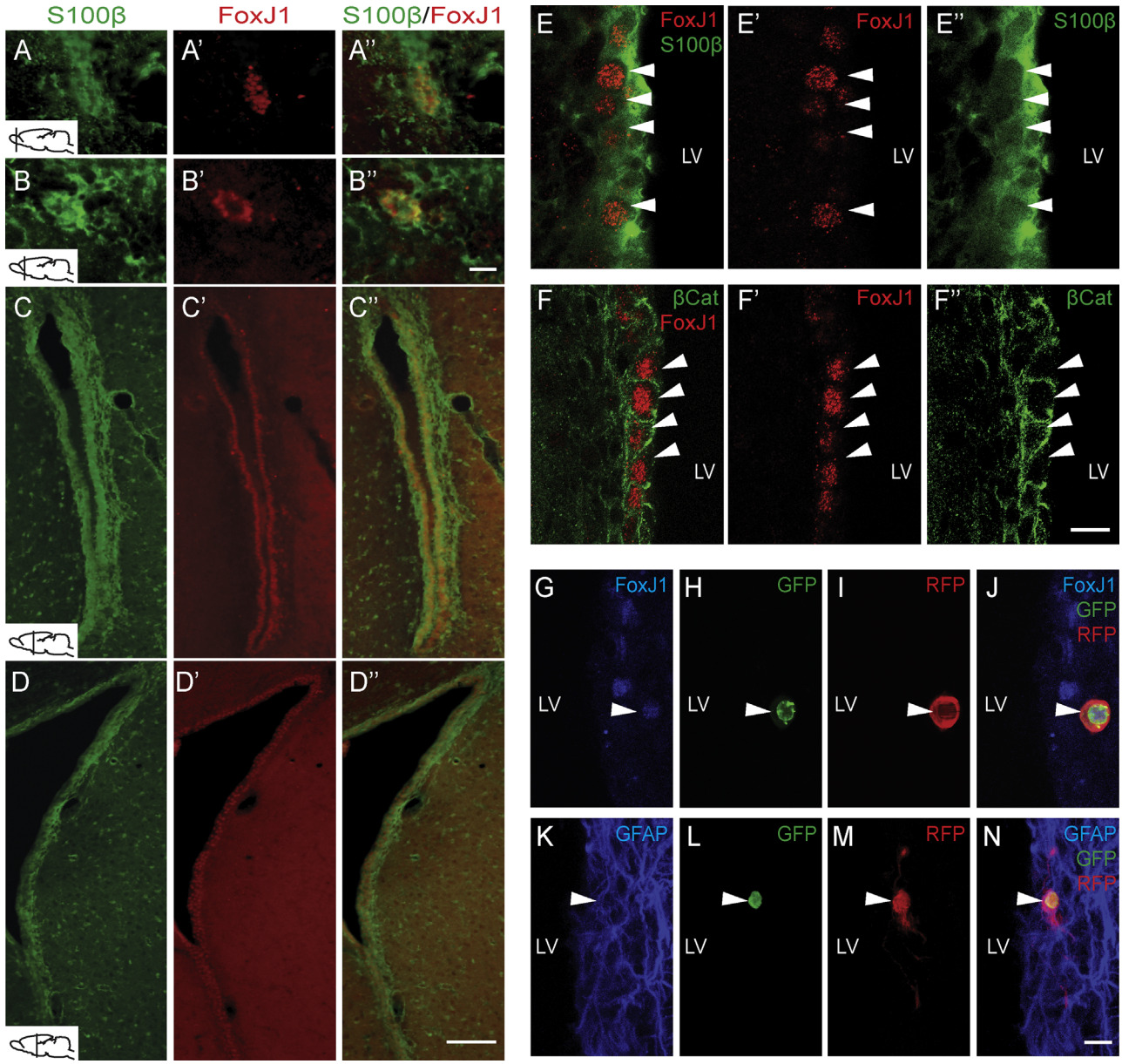

Fig. 2 – FoxJ1 is expressed in ependymal cells in the lateral ventricular wall (LVW) of adult rat brain. Coronal brain sections immunostained for S100β (A–D), FoxJ1 (A′–D′) and merged FoxJ1/S100β immunostaining (A″–D″). The inset in (A–D) depicts the levels at which immunostainings are shown. FoxJ1 extends throughout the LVW and colocalizes with other ependymal markers S100β (E) and βCatenin (βCat) (F). This is clearly seen in the higher magnification images where FoxJ1 is expressed in the nucleus (E′ and F′), S100β is expressed in the cytoplasm (E″) while βCat is confined to the cell membrane (F″). Arrowheads point to some cells that are FoxJ1+/S100β+ or FoxJ1+/βCat+. (G–N) Confocal images of results obtained from electroporation with Cre and reporter plasmids (see Fig. 1B2). Cells with FoxJ1 expression would have nuclear GFP and cytoplasmic RFP expression due to recombination, as observed in the FoxJ1+/GFP+/RFP+ cell indicated in (G–J) by arrowhead. (K–N) Arrowhead indicates a GFAP+/GFP+/RFP+ cell. Scale bars: 50 μm in A–B, A′–B′ and A″–B″; 100 μm in C–D, C′–D′, C″ D″; 10 μm in E–F, E′–F′, E″–F″ and G–J. Abbreviation: LV – Lateral ventricle.

to selectively label FoxJ1+ cells. This donor plasmid was electroporated along with the mPB helper plasmid (Fig. 3A). At 2 weeks after electroporation among the GFP+ cells in the LVW we found that 79.9% were FoxJ1+, 19.4% were GFAP+ and 12.5% were DCX+ by using immunohistochemistry. At 6 weeks after electroporation among the GFP+ cells in the LVW, 81.3% were FoxJ1+, 14.6% were GFAP+ and 5.5% were DCX+ as shown by immunohistochemistry. We varied the ratio of donor to helper plasmid concentrations from 1:1, 1:2, 2:1 to 3:1. There were no differences in mean number of GFP+ cells between the different ratios (data not shown), and the 1:1 donor to helper plasmid ratio was used in subsequent experiments.

In progeny of FoxJ1+ cells, FoxJ1 expression might be down-regulated and GFP expression would then be lost. A constitutively expressed reporter in the FoxJ1+ cells would be required to trace their progeny. Transposase is necessary to insert the reporter

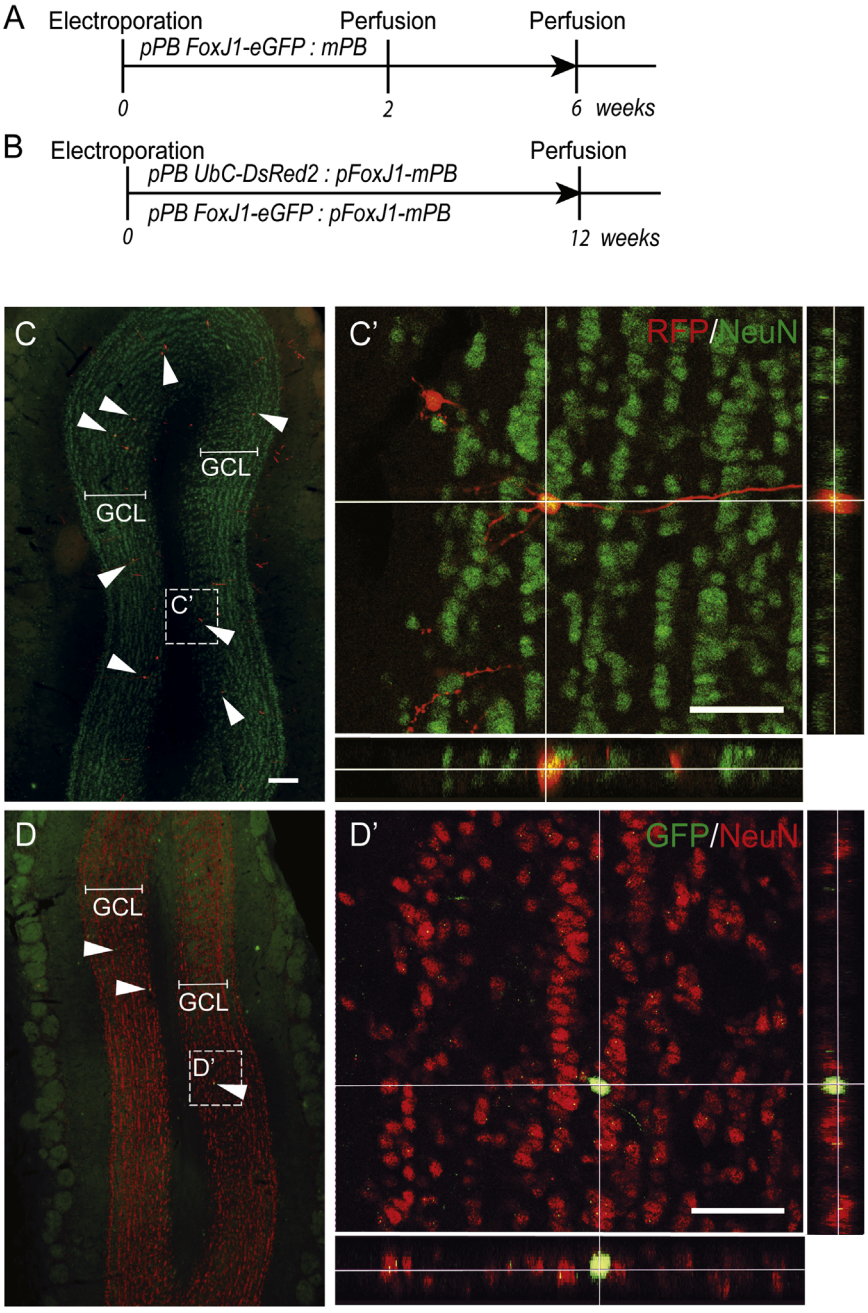

Fig. 3 – FoxJ1-expressing lateral ventricular wall cells give rise to olfactory bulb (OB) neurons. (A) and (B) show experimental paradigm used for electroporating *piggyBac* transposons. (A) Donor plasmid pPB-FoxJ1-eGFP was co-electroporated with helper plasmid mPB expressing transposase. Animals were sacrificed 2 and 6 weeks after electroporation. (B) Donor plasmid pPB-UbC-DsRed2 or pPB-FoxJ1-eGFP was co-electroporated with pFoxJ1-mPB helper plasmid (see Fig. 1B3) and animals were perfused 12 weeks after electroporation. (C and D) show the progeny of FoxJ1+ cells that have become mature neurons in the granule cell layer (GCL) of OB. (C) Image of OB immunostained for RFP and NeuN in a rat electroporated with pPB-UbC-DsRed2:pFoxJ1-mPB. There are numerous RFP+ neurons pointed by arrowheads, as compared to GFP+ neurons in (D). (C′) Confocal image from the boxed region in (C) showing an RFP+/NeuN+ neuron in the GCL of OB. (D) Image of OB immunostained for GFP and the mature neuronal marker NeuN in a rat electroporated with pPB-FoxJ1-eGFP:pFoxJ1-mPB. The intensity of GFP is weaker in the GFP+/NeuN+ neurons indicated by arrowheads, due to down-regulation of FoxJ1 activity. (D′) Confocal image from the boxed region in (D) showing a GFP+/NeuN+ neuron in the GCL of OB. Scale bars: 200 μm in C and D; 50 μm in C′ and D′. Abbreviation: GCL – Granule cell layer.

cassette in the donor plasmid into the genome. Hence a constitutively active promoter-based reporter cassette in the donor plasmid (pPB-UbC-DsRed2) with FoxJ1+ cell-specific transposase expression by helper plasmid (pFoxJ1-mPB) (Fig. 1B3) would allow for tracing FoxJ1+ cell progeny even if FoxJ1 expression is down-regulated or lost. We constructed the pFoxJ1-mPB helper plasmid, expressing *piggyBac* transposase under FoxJ1+ promoter. Electroporations were done with pPB-UbC-DsRed2:pFoxJ1-mPB in

one group (Group A) and pPB-FoxJ1-eGFP:pFoxJ1-mPB as control in another group (Group B) (Fig. 3B). pPB-FoxJ1-eGFP:pFoxJ1-mPB would only label cells expressing FoxJ1 and would lose GFP immunoreactivity if the cells down-regulate or lose FoxJ1 expression. Animals were perfused 12 weeks after electroporation.

Using immunohistochemistry we found that among the RFP+ cells in the LVW, 80.3±2.4% were FoxJ1+, 76.6±1.6% were S100β+, 19.6±3.8% were GFAP+ and 22.7±1.5% were DCX+. Among the GFP+ cells, 76.1±5.3% were FoxJ1+, 83.0±4.8% were S100β+, 11.1±0.7% were GFAP+ and 4.4±0.4% were DCX+. Importantly, we detected a higher percentage of RFP+/DCX+ as compared to GFP+/DCX+ cells, supporting that the constitutively active promoter-based construct is necessary to trace FoxJ1+ progeny.

We then characterized the GFP+ and RFP+ cells in OB using the mature neuronal marker NeuN (Fig. 3C, C′ and D, D′). We found that 85.2% of the RFP+ cells (40.8 cells) were NeuN+ in the olfactory granule cell layer (GCL). Only a few RFP+/NeuN+ cells were detected in the olfactory glomerular layer (GML). 80.7±4.1% of GFP+ cells (18.4±4.8 cells) were NeuN+ in the GCL, but the GFP intensity was faint. Taken together, these findings provide evidence that FoxJ1+ cells in LVW contribute to neurogenesis in the OB of intact adult rat brain.

FoxJ1+ cells give rise to few striatal cells in stroke-damaged rat brain

Since we observed that FoxJ1+ cells could give rise to OB neurons, we then explored if FoxJ1+ cells are also involved in striatal neurogenesis after stroke (Fig. 4A). We electroporated pPB-FoxJ1-eGFP and pPB-UbC-DsRed2 donor plasmids with pFoxJ1-mPB helper plasmid (Fig. 1B3) in a 1:1:1 DNA concentration ratio. FoxJ1+ cells would be both GFP+ and RFP+, whereas their progeny would be RFP+ but lose GFP+ labeling if FoxJ1 activity is down-regulated. One week after electroporation, animals were subjected to 30 min or 2 h of MCAO. Two weeks after electroporation, BrdU (50 mg/kg, i.p.) was administered twice daily for 1 week. Animals were perfused 6 weeks after MCAO. GFP+ and RFP+ cells were observed in the LVW and most of them were GFP+/RFP+ in both groups. To determine the phenotype of GFP+ and/or RFP+ cells in the LVW, sections were analyzed for FoxJ1, DCX, GFAP and BrdU immunoreactivity (Fig. 4B–I) as summarized in Table 2.

We found that the ischemic lesion was confined to striatum and that SVZ and LVW were spared following 30 min MCAO. In the 2 h MCAO animals, the lesion involved both striatum and neocortex. The LVW was damaged mainly in its dorsolateral part. It is well established that MCAO gives rise to striatal neurogenesis with DCX+ cells migrating from the SVZ [21,22] towards the injured striatum. We observed a similar neurogenic response using DCX immunostaining in both animal groups, which seemed unaffected by the electroporation procedure. Very few GFP+/RFP+ cells were detected in the striatum (Fig. 4J, J′ and K, K′) of BrdU-immunostained sections: 1.2±1.0 cells per section, 68.7 μm to 147.2 μm from the LVW in the 30 min group, 3.25 cells per section in one animal and less than 0.3 cells per section in the rest. In the 2 h group we observed 0.6±0.4 GFP+/RFP+ cells per section in the striatum 48 μm to 225.2 μm from the LVW. A few of these GFP+/RFP+ cells in the striatum resembled neuroblasts morphologically but were not detectable in DCX-immunostained sections. None of the GFP+/RFP+ striatal cells stained with NeuN antibody.

In agreement with our findings in intact rats, we observed RFP+ cells in 30 min (23.3±10.0 cells) and 2 h (22.3±8.7 cells) groups in the GCL and GML of the OB also after stroke. We analyzed the RFP+ cells in OB for NeuN and BrdU immunoreactivity. Among these RFP+ cells, 81.9±9.9% were NeuN+ and 27.6±7.6% were BrdU+ in the 30 min group, while 72.6±10.1% were NeuN+ and 40.1±5.2% were BrdU+ in the 2 h group. The presence of BrdU+/RFP+ cells suggests that FoxJ1+ cells divided after MCAO and gave rise to progeny that migrated to the OB.

Discussion

Here we have used genetic lineage tracing with electroporation to explore whether FoxJ1-expressing cells in the LVW contribute to neurogenesis in the intact and stroke-injured adult rat forebrain. Immunohistochemical stainings indicated that the overall majority of the FoxJ1-expressing cells in LVW were ependymal cells with a minor population being astrocytes. We optimized electroporation of reporter plasmids to the LVW and validated the FoxJ1 promoter-based plasmid constructs with Cre-recombinase system. We genetically labeled FoxJ1+ cells with the *piggyBac* transposon system and found that FoxJ1-expressing cells gave rise to neuroblasts and mature neurons in the olfactory bulb, both in intact and stroke-damaged brains. Only few cells derived from FoxJ1-expressing cells were detected in the striatum after stroke.

Identifying ependymal cells and their progeny has been a challenge because available markers are not unique to these cells. Astrocytes also express S100β and Vimentin [23], whereas CD24 and CD133 are expressed by both ependymal cells and NSCs [24–27]. Markers reported to distinguish ependymal cells from other cell types in LVW are acetylated tubulin (labels ciliary processes), γ-tubulin clusters (labels basal bodies of cilia) and β-Catenin (β-Cat; sequestered in ependymal cell membrane) [1]. Here, we explored whether FoxJ1 is a characteristic marker of ependymal cells in rats similar to what has been reported previously for mice [8,10,11]. In our immunohistochemical stainings, ependymal cells in the LVW were defined as S100β+/FoxJ1+ or β-Cat+/FoxJ1+. We observed that the absolute majority of FoxJ1+ cells in the LVW were ependymal cells (83% and 97.5% being S100β+ and β-Cat+, respectively), but that there was also a minor population (3.6%) of GFAP+/FoxJ1+ astrocytes. In contrast, we did not observe any FoxJ1+ cells co-labeled with the neuroblast marker DCX, which is in agreement with the involvement of FoxJ1 in ciliogenesis [11]. The distribution of FoxJ1+ cells based on immunostaining and the occurrence of a small subset of FoxJ1+ astrocytes, as detected here in rat LVW, are consistent with observations using a FoxJ1^{Egfp} reporter mice [11]. Taken together, these findings indicate that FoxJ1 is a specific marker of ependymal cells in the rat LVW, but with a cautionary note that similar to mouse brain, FoxJ1 is also expressed by a small population of astrocytes.

Our study shows for the first time that LVW cells can be selectively targeted in adult rats using electroporation of plasmid DNA. No evidence was obtained that the electroporation affected SVZ neurogenesis or the niche. In order to allow for long-term fate mapping, we addressed the problem that most plasmid expression vectors are ectopic and may be lost due to cell division, suppressed by the host cell or that the plasmid expression could be transient. Transposons such as *piggyBac* and *Sleeping Beauty* are genome-integrating plasmid DNAs [16,19,20], which are used

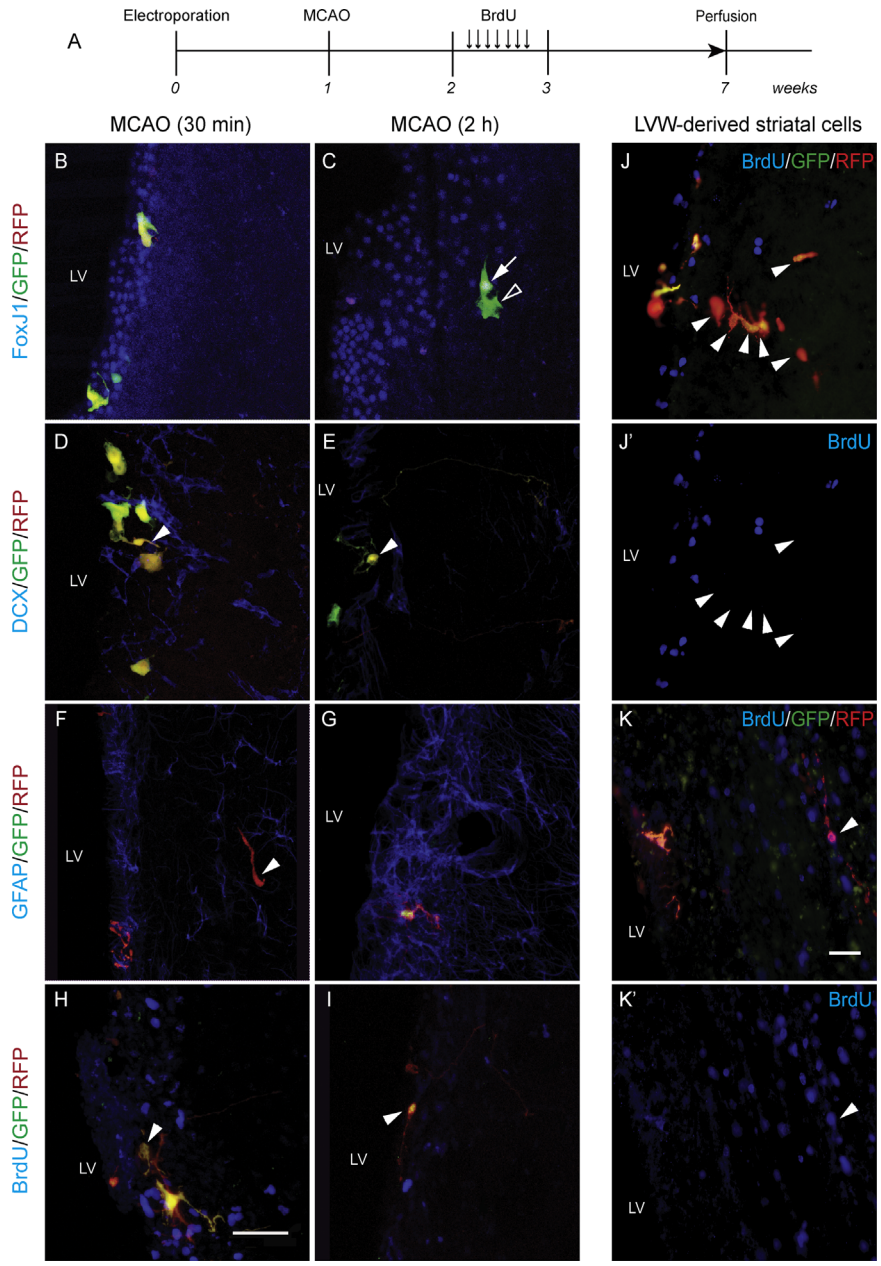

Fig. 4 – FoxJ1+ cells give rise to few striatal cells after stroke. (A) Design of the experiment to trace the progeny of FoxJ1-expressing cells in stroke-injured brain after electroporation with *piggyBac* transposon. Animals were co-electroporated with pPB-FoxJ1-eGFP, pPB-UbC-DsRed2 and pFoxJ1-mPB *piggyBac* plasmids (see Fig. 1B3). One week later, 30 min or 2 h MCAO was performed and animals were perfused 6 weeks thereafter. Confocal images of LVW in sections immunostained for GFP and RFP along with FoxJ1 (B and C), DCX (D and E), GFAP (F and G) and BrdU (H and I). Most of the labeled cells in LVW are GFP+/RFP+ indicating that FoxJ1 is still active in these cells. FoxJ1 immunostaining shows that the LVW is disturbed 2 h after MCAO (C) when compared to the intact LVW 30 min after MCAO (B). Arrow in (C) indicates a FoxJ1+/GFP+/RFP+ cell while the open-arrowhead indicates FoxJ1-/GFP+/RFP+ cells that are in the stroke-damaged striatum. (D–F, H and I) Arrowheads depict GFP+/RFP+ cells resembling neuroblasts migrating towards the striatum from LVW. (J, J′, K and K′) Progeny of FoxJ1+ cells in the striatum. Arrowheads indicate BrdU-/GFP+/RFP+ cells (J and J′) and a BrdU+/GFP-/RFP+ cell resembling a neuroblast (K and K′) migrating from the LVW into the stroke-affected striatum in BrdU immunostained sections. Scale bar: 50 μm in B–I; 20 μm in J, J′, K and K′. Abbreviation: LV – Lateral ventricle.

to stably express transgenes in preclinical studies [28]. *piggyBac* effectively transposes in human and rodent genomes both *in vitro* and *in vivo* [16,29]. With the *piggyBac* system we labeled ependymal cells using the human FoxJ1 promoter [12] in helper and donor plasmids. Importantly we observed expression of the reporter proteins for up to 12 weeks (the longest time tested) in the LVW and OB. In line with our immunohistochemical data from intact animals using the FoxJ1 antibody, GFP expression under FoxJ1 promoter was observed in S100β+ ependymal cells and GFAP+ astrocytes in LVW. The intensity of GFP expression was weak in DCX+ neuroblasts and NeuN+ OB neurons. The weak GFP staining found in the OB neurons could be attributed to the

Table 2 – Percentage of GFP+ and RFP+ cells in lateral ventricular wall expressing different phenotypic markers 6 weeks after stroke.

MCAO group	Marker	GFP+	RFP+
30 min	FoxJ1	21.8±2.1	21.7±3.5
	DCX	6.0±4.4	7.3±4.7
	GFAP	14.3±2.4	15.5±4.4
	BrdU	3.0±1.5	3.5±2.0
2 h	FoxJ1	11.1±3.9	9.2±3.1
	DCX	9.4±7.6	12.2±8.2
	GFAP	12.7±4.1	14.7±5.3
	BrdU	5.6±3.3	6.1±3.6

FoxJ1 and UbC promoter-based *piggyBac* transposons were electroporated followed by MCAO lesion after 1 week. Values are mean±SEM.

protein present in the cytoplasm when FoxJ1 was active and subsequently down-regulated when FoxJ1 is suppressed or inactivated. RFP expression under the UbC promoter was seen in S100β+ ependymal cells, GFAP+ astrocytes, DCX+ migrating neuroblasts and NeuN+ OB neurons. Thus, the *piggyBac* system is useful for *in vivo* gene transfer with electroporation and allows for long-term fate mapping experiments in the LVW of adult rat brain.

The contribution of ependymal cells to adult neurogenesis in the intact forebrain has been controversial. Some reports have suggested that forebrain ependymal cells are NSCs and give rise to OB neurons [30,31]. However, most studies have shown that the ependymal cells are terminally differentiated and that the NSCs producing OB neurons are a subset of SVZ astrocytes [5–7]. Our data with *piggyBac* transposon-mediated genetic labeling indicate that FoxJ1+ cells contribute to OB neurogenesis in intact adult rats. We observed a small number of DCX+ neuroblasts (22% of RFP+ cells) in the SVZ and RMS and NeuN+ neurons (85% of RFP+ cells) in the GCL of OB. Seemingly in contrast to our data in rats, Carlén et al., 2009 [8], could not find any contribution from the FoxJ1+ cells, identified based on the expression of lacZ and GFP reporter gene, to OB neurogenesis in intact adult mice. However, our findings are consistent with the report that FoxJ1-expressing cells produce OB neurons predominantly located in GCL in juvenile mice [10]. After day 21, the number was very low and in the postnatal mouse brain, the FoxJ1 derived cells only contributed a small fraction of the total number of OB neurons [10]. Interestingly Jacquet et al., 2009 [11], demonstrated the occurrence of a subset of mouse FoxJ1-expressing cells that gave rise to neurospheres. These neurospheres could self-renew and form astrocytes, oligodendrocytes and neurons *in vitro*. Both in mice and as shown here in rats, the majority of FoxJ1+ cells are ependymal cells but there is also a small population of astrocytes. Because ependymal cells are post-mitotic, it is tempting to speculate that both in mice and rats, the FoxJ1+/GFAP+ astrocytes can function as NSCs [11]. These cells would then give rise to OB neurogenesis as observed here.

Ependymal cells have been reported to give rise to neuroblasts in adult mouse brain [8] and transform to radial glia in rat brain [32] after stroke, and to generate progeny by asymmetric division following 6-hydroxydopamine lesion in rat brain [33]. We combined GFP (under FoxJ1 promoter) and RFP (under UbC promoter) donor plasmids with the FoxJ1-mPB helper plasmid for fate emapping in rats subjected to stroke. Both after 30 min and 2 h MCAO, we observed OB neurogenesis and a very minor contribution to cell genesis in striatum from the FoxJ1-expressing cells. In the OB, most FoxJ1-derived neurons were RFP+/GFP- or RFP+/weak GFP, indicating that FoxJ1 is down-regulated in these cells. In contrast, the FoxJ1-derived cells in striatum were GFP+/RFP+, providing evidence that FoxJ1 is still active. Most of the GFP+/RFP+ striatal cells did not stain for BrdU, suggesting that these FoxJ1+ cells had not undergone cell division but delaminated from the LVW. Our findings raise the possibility that the FoxJ1-derived striatal cells observed here are identical to the displaced ependymal cells previously detected using electron microscopy in the striatum after stroke in rats [34]. Some of the FoxJ1+ striatal cells, which were very few, exhibited morphological features of neuroblasts but we were unable to detect any expression of markers of immature or mature neurons. Whether these cells can become mature neurons in the striatum is, therefore, unclear.

In conclusion, we demonstrate here for the first time in rats FoxJ1 expression in both ependymal cells and a subset of astrocytes in the LVW. This finding raises the possibility that, similar to what has been reported in mice [11], FoxJ1 gene expression is required for the differentiation of radial glia into ependymal cells and FoxJ1-expressing astrocytes also in rats. The FoxJ1-expressing cells, presumably the astrocyte population, give rise to a very minor population of OB neurons in the adult rat forebrain. The functional relevance of these cells in rat OB is presently unclear. In the mouse, though, in which the FoxJ1-derived cells similar to the rat constitute only a fraction of the total number of OB neurons, genetic ablation of these cells suggested that they could have profound effects on the majority of neurons by acting in a paracrine manner [10]. Although OB neurogenesis from FoxJ1-derived cells was observed also after stroke, we failed to detect any significant contribution of these cells to the formation of striatal neurons. Thus, under the experimental conditions used here, neither FoxJ1-expressing ependymal cells nor astrocytes play an important role for the repair of stroke-induced brain injury.

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REFERENCES

[1] Z. Mirzadeh, F.T. Merkle, M. Soriano-Navarro, J.M. Garcia-Verdugo, A. Alvarez-Buylla, Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain, Cell Stem Cell 3 (2008) 265–278.
[2] M. Del Bigio, Ependymal cells: biology and pathology, Acta Neuropathol. 119 (2010) 55–73.
[3] K. Sawamoto, H. Wichterle, O. Gonzalez-Perez, J.A. Cholfin, M. Yamada, N. Spassky, N.S. Murcia, J.M. Garcia-Verdugo, O. Marin, J.L.R. Rubenstein, M. Tessier-Lavigne, H. Okano, A. Alvarez-Buylla, New neurons follow the flow of cerebrospinal fluid in the adult brain, Science 311 (2006) 629–632.
[4] A.J. Jimenez, J.M. Garcia-Verdugo, C.A. Gonzalez, L.F. Batiz, L.M. Rodriguez-Perez, P. Paez, M. Soriano-Navarro, R. Roales-Bujan,

P. Rivera, S. Rodriguez, E.M. Rodriguez, J.M. Perez-Figares, Disruption of the neurogenic niche in the subventricular zone of postnatal hydrocephalic hyh mice, J. Neuropathol. Exp. Neurol. 68 (2009) 1006–1020.
[5] F. Doetsch, I. Caille, D. Lim, J. Garcia-Verdugo, A. Alvarez-Buylla, Subventricular zone astrocytes are neural stem cells in the adult mammalian brain, Cell 97 (1999) 703–716.
[6] N. Spassky, F.T. Merkle, N. Flames, A.D. Tramontin, J.M. Garcia-Verdugo, A. Alvarez-Buylla, Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis, J. Neurosci. 25 (2005) 10–18.
[7] A. Capela, S. Temple, LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal, Neuron 35 (2002) 865–875.
[8] M. Carlen, K. Meletis, C. Goritz, V. Darsalia, E. Evergren, K. Tanigaki, M. Amendola, F. Barnabe-Heider, M.S. Yeung, L. Naldini, T. Honjo, Z. Kokaia, O. Shupliakov, R.M. Cassidy, O. Lindvall, J. Frisen, Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke, Nat. Neurosci. 12 (2009) 259–267.
[9] C.C. Young, J.M. van der Harg, N.J. Lewis, K.J. Brooks, A.M. Buchan, F.G. Szele, Ependymal ciliary dysfunction and reactive astrocytosis in a reorganized subventricular zone after stroke, Cereb. Cortex (2012).
[10] B.V. Jacquet, N. Muthusamy, L.J. Sommerville, G. Xiao, H. Liang, Y. Zhang, M.J. Holtzman, H.T. Ghashghaei, Specification of a Foxj1-Dependent Lineage in the Forebrain Is Required for Embryonic-to-Postnatal Transition of Neurogenesis in the Olfactory Bulb, J. Neurosci. 31 (2011) 9368–9382.
[11] B.V. Jacquet, R. Salinas-Mondragon, H. Liang, B. Therit, J.D. Buie, M. Dykstra, K. Campbell, L.E. Ostrowski, S.L. Brody, H.T. Ghashghaei, FoxJ1-dependent gene expression is required for differentiation of radial glia into ependymal cells and a subset of astrocytes in the postnatal brain, Development 136 (2009) 4021–4031.
[12] L.E. Ostrowski, J.R. Hutchins, K. Zakel, W.K. O'Neal, Targeting expression of a transgene to the airway surface epithelium using a ciliated cell-specific promoter, Mol. Ther. 8 (2003) 637–645.
[13] Y. Zhang, G. Huang, L.P. Shornick, W.T. Roswit, J.M. Shipley, S.L. Brody, M.J. Holtzman, A. Transgenic FOXJ1-Cre, System for gene inactivation in ciliated epithelial cells, Am. J. Respir. Cell Mol. Biol. 36 (2007) 515–519.
[14] F. Barnabe-Heider, K. Meletis, M. Eriksson, O. Bergmann, H. Sabelstrom, M.A. Harvey, H. Mikkers, J. Frisen, Genetic manipulation of adult mouse neurogenic niches by *in vivo* electroporation, Nat. Methods 5 (2008) 189–196.
[15] S. Inouye, H. Ogawa, K. Yasuda, K. Umesono, F.I. Tsuji, A bacterial cloning vector using a mutated Aequorea green fluorescent protein as an indicator, Gene 189 (1997) 159–162.
[16] J. Cadinanos, A. Bradley, Generation of an inducible and optimized *piggyBac* transposon system, Nucleic Acids Res. 35 (2007) e87.
[17] Z. Kokaia, G. Andsberg, A. Martinez-Serrano, O. Lindvall, Focal cerebral ischemia in rats induces expression of p75 neurotrophin receptor in resistant striatal cholinergic neurons, Neuroscience 84 (1998) 1113–1125.
[18] M. Schmidt-Supprian, K. Rajewsky, Vagaries of conditional gene targeting, Nat. Immunol. 8 (2007) 665–668.
[19] W. Wang, A. Bradley, Y. Huang, A *piggyBac* transposon-based genome-wide library of insertionally mutated Blm-deficient murine ES cells, Genome Res. 19 (2009) 667–673.
[20] K.M. Kahlig, S.K. Saridey, A. Kaja, M.A. Daniels, A.L. George, M.H. Wilson, Multiplexed transposon-mediated stable gene transfer in human cells, Proc. Natl. Acad. Sci. USA 107 (2010) 1343–1348.
[21] A. Arvidsson, T. Collin, D. Kirik, Z. Kokaia, O. Lindvall, Neuronal replacement from endogenous precursors in the adult brain after stroke, Nat. Med. 8 (2002) 963–970.
[22] J.M. Parent, Z.S. Vexler, C. Gong, N. Derugin, D.M. Ferriero, Rat forebrain neurogenesis and striatal neuron replacement after focal stroke, Ann. Neurol. 52 (2002) 802–813.
[23] K. Järlestedt, C.I. Rousset, M. Faiz, U. Wilhelmsson, A. Ståhlberg, H. Sourkova, M. Pekna, C. Mallard, H. Hagberg, M. Pekny, Attenuation of reactive gliosis does not affect infarct volume in neonatal hypoxic-ischemic brain injury in mice, PLoS ONE 5 (2010) e10397.
[24] C.V. Pfenninger, T. Roschupkina, F. Hertwig, D. Kottwitz, E. Englund, J. Bengzon, S.E. Jacobsen, U.A. Nuber, CD133 is not present on neurogenic astrocytes in the adult subventricular zone, but on embryonic neural stem cells, ependymal cells, and glioblastoma cells, Cancer Res. 67 (2007) 5727–5736.
[25] C.V. Pfenninger, C. Steinhoff, F. Hertwig, U.A. Nuber, Prospectively isolated CD133/CD24-positive ependymal cells from the adult spinal cord and lateral ventricle wall differ in their long-term *in vitro* self-renewal and *in vivo* gene expression, Glia 59 (2011) 68–81.
[26] J. Pruszak, W. Ludwig, A. Blak, K. Alavian, O. Isacson, CD15, CD24, and CD29 define a surface biomarker code for neural lineage differentiation of stem cells, Stem Cells 27 (2009) 2928–2940.
[27] J. Pruszak, K.-C. Sonntag, M.H. Aung, R. Sanchez-Pernaute, O. Isacson, Markers and methods for cell sorting of human embryonic stem cell-derived neural cell populations, Stem Cells 25 (2007) 2257–2268.
[28] T. VandenDriessche, Z. Ivics, Z. Izsvak, M.K.L. Chuah, Emerging potential of transposons for gene therapy and generation of induced pluripotent stem cells, Blood 114 (2009) 1461–1468.
[29] S.K. Saridey, L. Liu, J.E. Doherty, A. Kaja, D.L. Galvan, B.S. Fletcher, M.H. Wilson, *piggyBac* transposon-based inducible gene expression *in vivo* after somatic cell gene transfer, Mol. Ther. 17 (2009) 2115–2120.
[30] C.B. Johansson, S. Momma, D.L. Clarke, M. Risling, U. Lendahl, J. Frisén, Identification of a neural stem cell in the adult mammalian central nervous system, Cell 96 (1999) 25–34.
[31] V. Coskun, H. Wu, B. Blanchi, S. Tsao, K. Kim, J. Zhao, J.C. Biancotti, L. Hutnick, R.C. Krueger, G. Fan, J. de Vellis, Y.E. Sun, CD133+ neural stem cells in the ependyma of mammalian postnatal forebrain, Proc. Natl. Acad. Sci. USA 105 (2008) 1026–1031.
[32] R.L. Zhang, Z.G. Zhang, Y. Wang, Y. LeTourneau, X.S. Liu, X. Zhang, S.R. Gregg, L. Wang, M. Chopp, Stroke induces ependymal cell transformation into radial glia in the subventricular zone of the adult rodent brain, J. Cereb. Blood Flow Metab. 27 (2007) 1201–1212.
[33] D. Gleason, J.H. Fallon, M. Guerra, J.C. Liu, P.J. Bryant, Ependymal stem cells divide asymmetrically and transfer progeny into the subventricular zone when activated by injury, Neuroscience 156 (2008) 81–88.
[34] A.I. Danilov, Z. Kokaia, O. Lindvall, Ectopic ependymal cells in striatum accompany neurogenesis in a rat model of stroke, Neuroscience 214 (2012) 159–170.