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Retrovirally delivered Islet-1 increases recruitment of Ng2 expressing cells from the postnatal SVZ into the striatum

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Abstract

Neural stem and progenitor cells hold the promise to be used in cell-based therapies to treat both acute and degenerative neurological diseases. To date, most research has been focused on the use of in vitro propagated stem cells used as a source of cells in cell replacement therapies. However, mobilization of endogenous neural stem cells to generate a specific differentiated cell type offers an attractive alternative. In this study, we investigate the possibility to direct the formation of specific cells from the endogenous stem and progenitor cells residing in the subventricular region of the postnatal brain. With the aim to induce postnatal generation of striatal neurons, we ectopically expressed Islet-1, a LIM homeodomain transcription factor expressed by striatal progenitors during development, in cells of the subventricular zone (SVZ) of neonatal and adult rats. Ectopic expression of Islet-1 in the neonatal, but not adult, SVZ resulted in the appearance of a population of cells in the striatum. These cells were primarily located in the ventrolateral area of the striatum where they differentiate into Ng2 expressing cells. However, no neurogenesis was observed in the striatum, nor was ectopic striatal differentiation observed in any other area of the brain after retroviral expression of Islet-1 in the SVZ. Thus, although ectopic expression of Islet-1 is sufficient to direct the migration of cells into the striatum in neonatal animals, it does not specify a striatal projection neuron phenotype in cells generated from the SVZ after birth.

Keywords: GFP; Subventricular zone; Endogenous stem cells; Striatum; Rat

Introduction

Neural stem cells exist primarily in two regions of the central nervous system, the hippocampus and in the area lining the lateral wall of the lateral ventricles called the periventricular region or subventricular zone (SVZ) (Gage, 2000). Cells from the SVZ migrate through the rostral migratory stream (RMS) to the olfactory bulb (OB). Once they reach the bulb, they migrate radially into the granule and periglomerular cell layers where they mature into interneurons (Luskin, 1993; Lois and Alvarez-Buylla, 1994; Alvarez-Buylla and Garcia-Verdugo, 2002). Hence, the normal fate of the SVZ cells is OB interneurons, but their full differentiation potential is not known and remains to be elucidated. Recent studies have shown that postnatal production of other cells types, such as striatal cells can occur under experimental conditions (Pencea et al., 2001; Arvidsson et al., 2002; Parent et al., 2002; Chmielnicki et al., 2004), suggesting that a precursor with a latent potential for striatal neuron diffeentiation exists in the SVZ also after birth.

Members of the LIM homeodomain family have been shown to be involved in cell fate specification and migration (Bulchand et al., 2001; Zhao et al., 2003; Alifragis et al., 2004). During striatal development, Islet-1 (Isl1) is expressed by progenitors for projection neurons and cholinergic interneuron (Pakzaban et al., 1993; Deacon et al., 1994; Olsson et al., 1995, 1998; Wang and Liu, 2001); however, its exact role in the formation of these cell types remains to be elucidated.

To test if cell-intrinsic expression of Isl1 is sufficient to promote the generation of striatal neurons from postnatal SVZ precursors, we constructed a retroviral vector carrying the

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cDNA coding for Isl1 and infected the cells in the adult and neonatal SVZ with this retrovirus. Consequently, Isl1 protein is expressed by the proliferating cells of the postnatal SVZ, a region where it is normally not expressed at this time point. Although no new striatal neurons were observed after ectopic Isl1 expression, we show that ectopic Isl1 expression specifically promotes the migration of cells from the SVZ into the striatum. This population of cells is most prominent in the ventrolateral area of the striatum, where the cells survive for an extended time, maintain Isl1 expression but lack any markers of mature neurons and glia, and based on morphology and antigen expression most likely represent glial precursors.

Materials and methods

Retroviral construction and production

To construct CMMP.ISL1.IRES.EGFP (RV.ISL), full length *Isl1* cDNA was obtained from Stratagene cDNA library of embryonic day12.5 (E12.5) mouse using standard PCR technologies. The PCR product was sequenced to confirm full length, then cut with *Sac*I–*Xho*I, and cloned into the *sac*I–*Sal*I site in pIRESII-EGFP (Clontech). A *Bg1*II–*Not*I fragment containing ISL1-IRESII-GFP was excised and inserted into the *Bam*HI–*Not*I site of CMMP.GFP.WPRE (Rogelius et al., 2005). All retroviruses

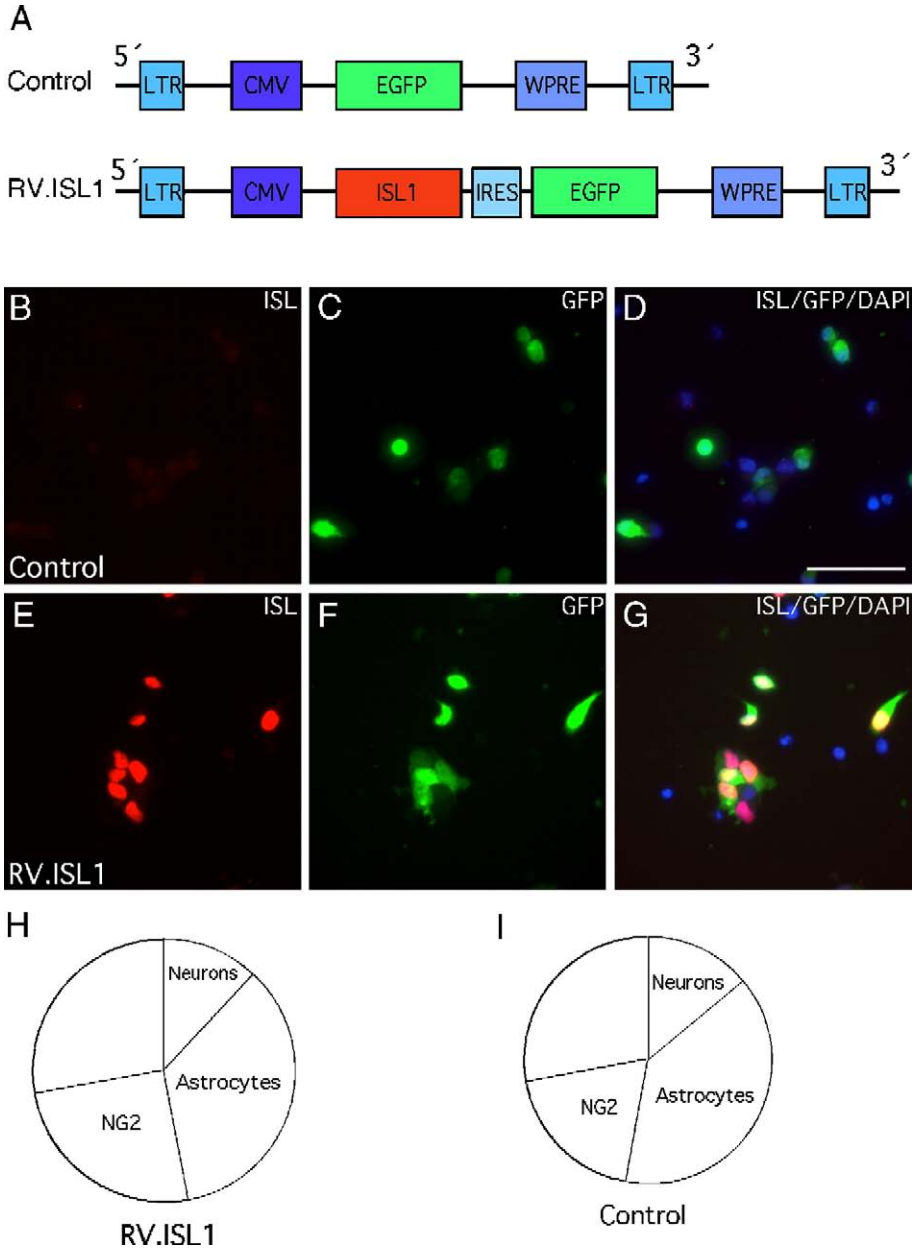


Fig. 1. The RV.ISL construct and the control vector are illustrated (not to scale) in panel A. The gene coding for GFP is used as a reporter in both construct and after infection in vitro, GFP is expressed in 40–60% of the cells (C, F) and can be reliably used as a marker for targeted cells as it is coexpressed with ISL1 in cells infected with RV.ISL (E–G). Following differentiation in vitro, GFP was detected in both neurons and glia and infection with RV.ISL did not significantly change the proportion of β-III-tubulin expressing neurons, GFAP expressing astrocytes, or NG2 expressing progenitors from neural stem and progenitor culture from the neonatal SVZ after 5 days differentiation (H–I).

used in this study (RV.ISL, RV.GFP, and RV.Ngn2) were produced as previously described (Ory et al., 1996). Briefly, the constructs were transfected using lipofectamin (Life Technologies) to the packaging cell line 239GPG (gift from Dr RC Mulligan). Virus containing media were collected every day for 7 days after transfection and the harvested supernatants were concentrated by ultracentrifugation (Beckman L-70), spun at 16 500 rpm for 1.5 h at 4°C. Viral titers were determined based on green fluorescence protein (GFP) expression, and less than 30% GFP positive cells was used to calculate transduction units (tu)/μl. The viral titer for RV.ISL was between 1.4–4.5 × 10^8 tu/μl, 4.5 × 10^8 tu/μl for the control (RV.GFP), and 5 × 10^8 tu/μl for the RV.Ngn2.

Retroviral infection of neural stem and progenitor cell in vitro

For in vitro differentiation analysis, neonatal or adult SVZ neurospheres generated using standard protocols (Parmar et al., 2002, 2003) were dissociated and plated on PLL coated 4-well chamber slides at the density of approximately 200,000 cells/well. Twenty-four hours after plating, the cells were infected (MOI 0.5) and 48 h after infection, the expansion medium was replaced by differentiation medium containing 1% serum and no growth factors and allowed to differentiate for 4–5 days.

The cells were fixed in ice-cold 4% paraformaldehyd in 0.1 M phosphate buffer pH 7.4 (PFA) for 15 min and rinsed 3 times in phosphate-buffered saline (PBS) before being processed for immunocytochemistry.

Retroviral injections and tissue processing

Sprague–Dawley rats received unilateral 1 μl injections of RV.ISL or the control vector into the anterior SVZ of the adult animals and into the lateral ventricle (LV) for the rat pups (P1). Using a stereotactic frame, the rats were injected at the following

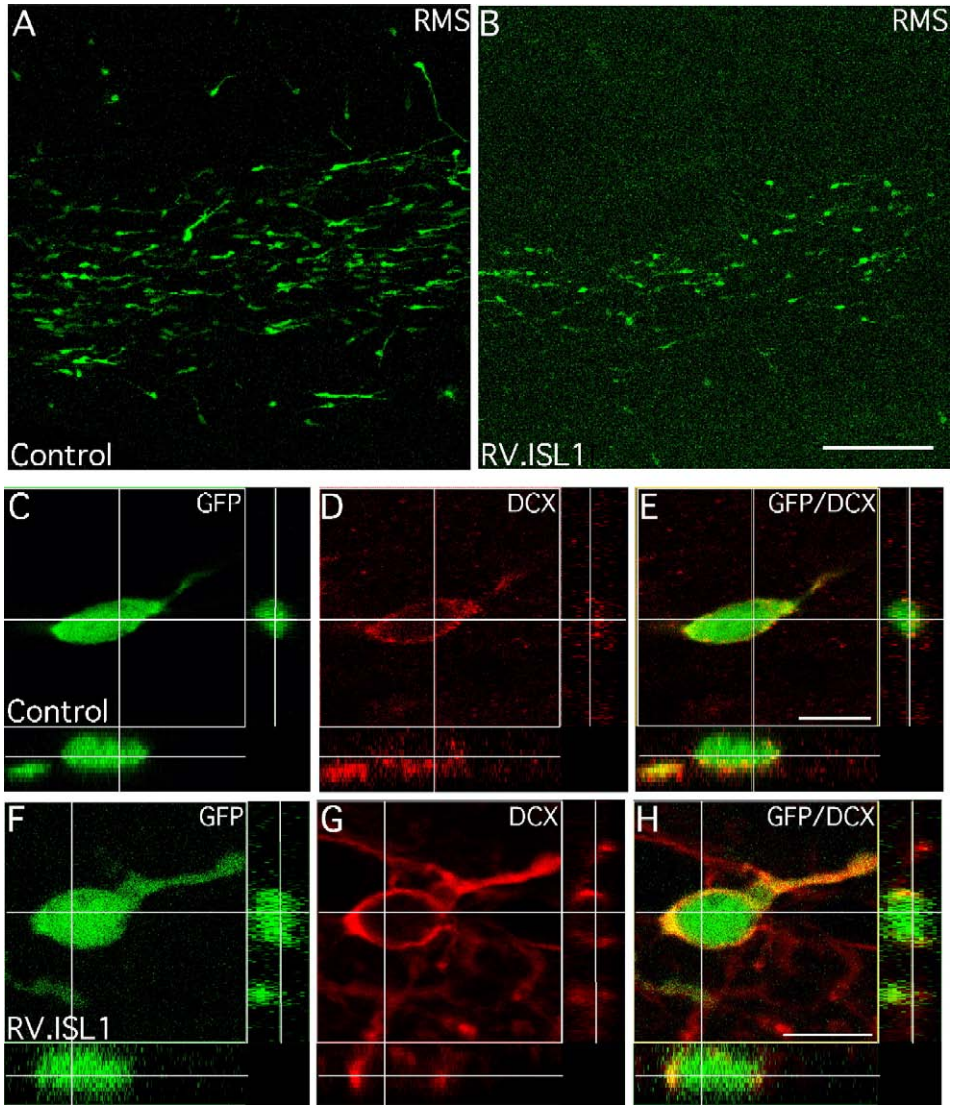


Fig. 2. Ectopic Isl1 expression in the neonatal SVZ cells does not effect the migration of cells in the RMS. In both the control (A) and RV.ISL (B)-injected groups, migrating cells in the RMS detected by GFP immunohistochemistry appeared phenotypically normal with morphology of migrating neuroblasts (A–B). The transduced cells from both groups expressed DCX, a marker for migrating neuroblasts and newly formed neurons (C–H). All images are from the RMS. Scale bar: 100 μm (A, B) and 10 μm (C–H).

coordinates measured from the dura; adult SVZ TB = −3.3 mm AP = +0.7 mm ML = −1.5 mm DV = −3.0 mm (Paxinos and Watsson, 1986) neonatal LV ML = −1.0 mm VD = −1.5 mm (Altman and Bayer, 1995). Thirty-four adult animals (12 control animals and 22 RV.ISL animals) were injected in two separate surgical sessions. For the neonatal injections, six litters were used on four separate surgical ses2sions. The animals were divided into groups with different survival times: 2 days, 1, 2, 3, 6, and 12 weeks. In all experiments, control animals were injected with vector containing only GFP, and control-injected animals were always processed in parallel with the RV.ISL-injected animals.

The brains were fixed in 4% PFA. For the neonatal groups with 2 days and 1 week survival times, the brain were immersion fixed, in all other groups the animals were perfused through the ascending aorta with isotonic saline, followed by ice-cold 4% PFA. The brains were then postfixed for 6 h and placed in 25% sucrose in 0.1 M phosphatase buffer. The brains were cut in 40 μm-thick sections on a freezing microtome (1400 Leitz, Germany). The cerebral hemispheres were cut as coronal sections and the OB (including the rostral part of the RMS) into sagittal sections.

Immunohistochemistry

After rinsing in PBS, cultures or sections were preincubated in 5% normal serum (Chemicon International, UK and Göteborgs Thermometerfabrik, Sweden) and 0.25% triton-X100 (Sigma-Aldrich, USA) for 1 h at room temperature. Primary antibodies used were: chicken anti-GFP (1:5000 Chemicon International, UK), rabbit anti-ISL1 (1:500 gift from T. and H. Edlund), mouse anti-S100β (1:500, Sigma) rabbit anti-GFAP (1:500 DAKO, Denmark), rabbit anti-β-III-tubulin (1:500, Covance), rabbit anti-Ng2 (1:500, Chemicon International, UK), rabbit anti-Nestin (1:500 gift from Dr. R. McKay), rabbit anti-DARPP-32 (1:1000 Chemicon International, UK), mouse anti-DARPP32 (1:20,000 gift from Dr. P. Greengard), goat anti-DCX (1:3000 Santa Cruz, USA), and rabbit anti-Er81 (1:2000 Gift from Drs. T. Jessell/S. Morton). Incubations in primary antisera were carried out overnight at room temperature. The FITC and Cy3 conjugated secondary antibodies (1:400 Jackson ImmunoResearch, USA) were diluted in the preincubation solution and incubated for 2 h at room temperature. The sections were mounted on chrome-alum coated slides and coverslipped using PVA-DABCO.

Quantification and statistical analysis

A total of 15 animals (5 control-injected and 10 RV.ISL-injected) were used to quantify the transduced cells in vivo. Successful injection was determined based on the presence of cells in the OB and one animal from the RV.ISL group was excluded using this criteria. In agreement with a previous study using the same virus (Rogelius et al., 2005), the rostro-caudal migration of transfected cells was limited. Thus, to estimate the number of cells in the striatum of each animal, three adjacent sections covering virtually all of the transduced cells (one central section representing the injection site, one more rostral and one more caudal section) through the striatum of individual RV.ISL and control-infected animals were selected. The sections were randomized and further processed without the observer having any knowledge of which group they belonged to. The width of the striatum (defined as the area between the ventricular wall and external capsule) was measured for each section and subsequently divided into 3 equally wide zones (Zone I, II, and III, see illustration Table 2). The total number of GFP-expressing cells in each zone per section was counted and from this the number of cells in each zone of the striatum of each animal was estimated.

Microscopy and schematic diagram

Cell cultures and sections were analyzed using a fluorescence microscope (Leica, Germany) and Open Lab software

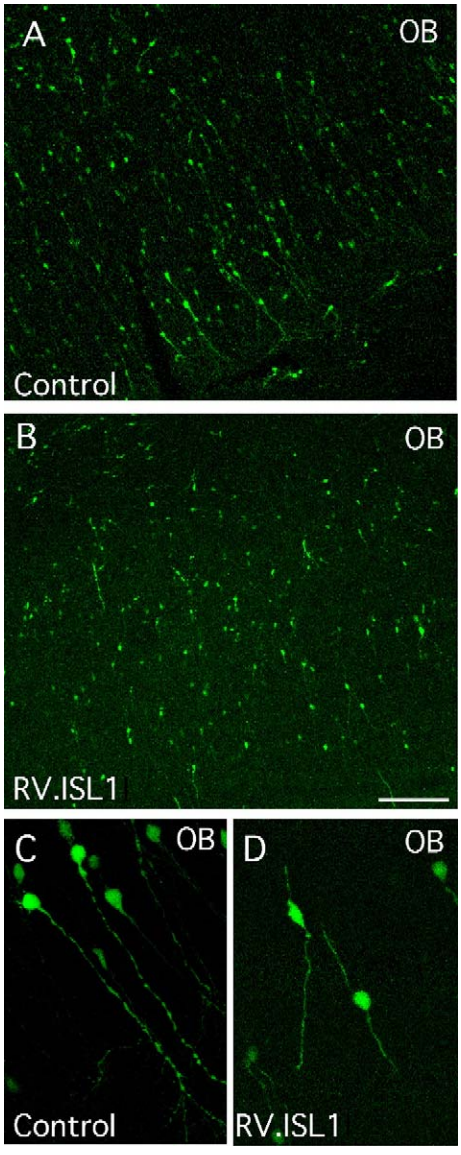


Fig. 3. Ectopic ISL1 expression in the neonatal SVZ does not interfere with OB differentiation. In both control (A) and RV.ISL (B)-injected groups, GFP-expressing progeny of transduced SVZ cells could be detected differentiating in the OB 2 week postinjection. All of the cells in the OB had a morphology typical of newly formed OB interneurons (C, D). All images are from the OB. Scale bar: 10 μm.

(Improvision, USA). All analyses of double-labeled cells were performed using a Leica confocal microscope, and Z-stacks were always generated to confirm the double labeling.

Schematic diagrams were constructed to show the typical distribution and density of cells 2 weeks after viral injection. The schematics are based on 4 animals in the control group, 6 animals in the RV.ISL group, and 11 animals in the RV.Ngn2 group.

Results

A retroviral vector (RV.ISL) was constructed by subcloning the complete open reading frame of the *Isl1* cDNA and *egfp* joined by an IRES sequence into the backbone of the control vector (Fig. 1A). As previously reported, the vector efficiently transduces the actively proliferating cells in the postnatal SVZ, which includes primarily the rapidly proliferating type A and type C cells, but also low numbers of the slowly dividing GFAP expressing B cells (Rogelius et al., 2005). As both the control and the RV.ISL constructs contain *egfp*, GFP expression can be used to detect the transduced cells.

After transfection in vitro, 40–60% of the cells in the RV.ISL or control-infected cultures expressed GFP (Figs. 1C, F), whereas ISL1 protein could only be detected in RV.ISL-infected cultures (Fig. 1E). There was a good overlap between GFP and ISL1 in the RV.ISL-infected cultures; however, some cells expressed ISL1 but not GFP (Fig. 1G). No obvious effect of ISL1 on the phenotype of the cells was observed after differentiation in vitro. In cultures generated from the neonatal SVZ, approximately the same proportion (34% in control and 40% in ISL1 cultures) of GFP-expressing cells could be detected after 5 days of differentiation. Out of the GFP-expressing cells in the RV.ISL-infected cultures, 12% expressed β-III-tubulin, 35% expressed GFAP, 23% expressed NG2, and the rest remained undifferentiated (Fig. 1H). This proportion was identical to that in control-infected neonatal cultures where 14% of the GFP-expressing cells had differentiated into β-III-tubulin expressing neurons, 39% of the cells into GFAP expressing astrocytes, and 21% expressed NG2 (Fig. 1I). Thus, GFP is expressed in both neurons and glia and expression of ISL1 does not seem to have any fundamental role in neuronal or glial fate specification in vitro.

Table 1
Migration of cells from the SVZ

	SVZ	OB	STR	CE	SPT
RV.GFP	+	+	–[a]	–	–
RV.ISL	+	+	+	–	–
RV.Ngn2	+	–	+	+	+

After RV.GFP injections into the neonatal SVZ, cells could be detected in the normal migration route, the RMS and OB. After RV.ISL injections, an additional population of cells could also be detected in the ventrolateral striatum but no cells were present in any other region of the brain. This is in contrast to animals receiving RV.Ngn2 injections where cells could be detected in various brain regions including the striatum, external capsule, and septum. SVZ = subventricular zone, OB = olfactory bulb, STR = striatum, CE = external capsule, SPT = septum.

[a] cells could be detected in striatum close to ventricle after 2 weeks, but no cells at all could be detected in the striatum after 6 or 12 weeks.

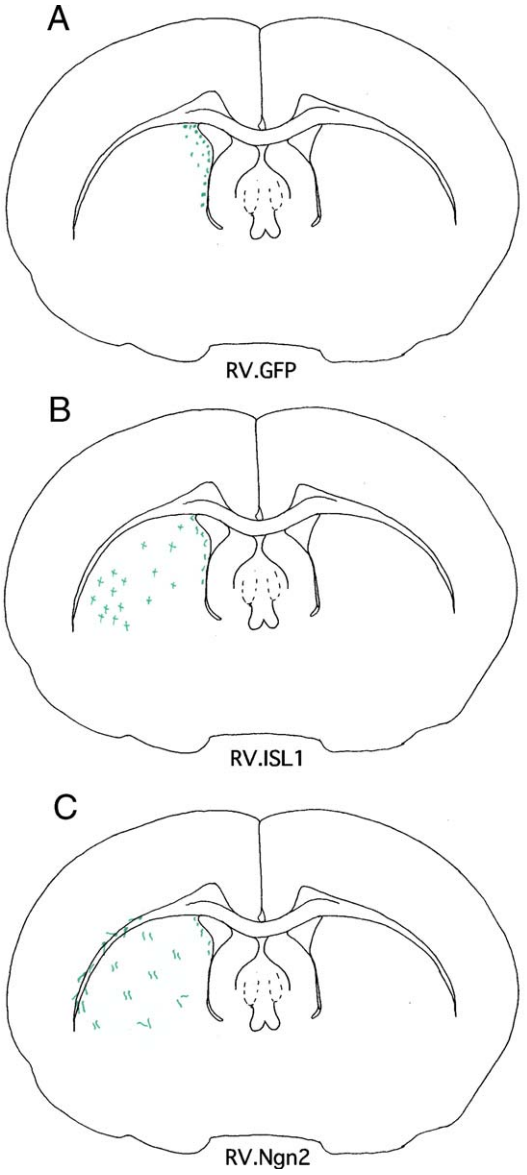
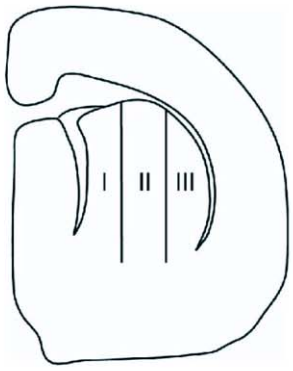


Fig. 4. The distribution of cells in the striatum is different when animals are infected with RV.ISL and RV.Ngn2. Schematic diagrams showing the distribution of cells 2 weeks after viral injection. With the control virus, cells could be detected in the striatal parenchyma but in close proximity to the SVZ (A). In animals receiving RV.ISL injections, the majority of cells in the striatum were found clustered in the ventrolateral area of the striatum (B) in contrast to animals receiving RV.Ngn2 where cells were evenly and randomly distributed within the striatal parenchyma (C). Also note the absence of cells in the external capsule in RV.ISL-injected animals.

Ectopic expression of Isl1 in the neonatal SVZ does not disrupt the migration of cells in the RMS or the generation of OB interneurons

The RV.ISL or control virus were injected unilaterally into the LV of neonatal rats, thus infecting only the actively dividing cells in the ipsilateral SVZ (Rogelius et al., 2005). The injections were performed on four separate occasions, and a total of 41 and 28 animals were injected with RV.ISL and control virus, respectively. One and two weeks after injection, large numbers of both control and Isl1 transduced

Table 2
Quantification of GFP positive cells recruited to the striatum after ectopic Isl1 expression



	Striatum Zone I	Striatum Zone II	Striatum Zone III	OB
RV.GFP 2w				
#7	840	315	0	+
#8	495	270	15	+
RV.ISL1 2w				
#9	255	585	330	+
#10	165	480	285	+
#11	585	1965	885	+
#12	285	1095	300	+
RV.GFP 6w				
#1	0	0	0	+
#2	0	0	0	+
#3	0	0	0	+
RV.ISL1 6w				
#4	225	585	195	+
#5	210	330	345	+
#6	180	315	240	+
#7	180	1110	1710	+
#8[a]	0	0	0	−
#9	0	45	0	+

The Tstriatum was divided in three equally wide zones as illustrated in the schematic picture, and the number of GFP positive cells per zone in each animal was quantified.
+ = presence of GFP-expressing cells, − = no GFP-expressing cells present. OB = olfactory bulb.
[a] Excluded from statistical analysis.

cells migrating in the RMS on their way from the SVZ to the OB could be detected (Figs. 2A and B). In both groups, infected cells migrating in the RMS appeared phenotypically normal: the cells displayed morphologies characteristic of migrating neuroblasts and they expressed DCX (Figs. 2C–H), a protein normally expressed by migrating neuroblasts in the SVZ and RMS (Gleeson et al., 1999; Nacher et al., 2001). Two weeks after injection of the viruses, many GFP-

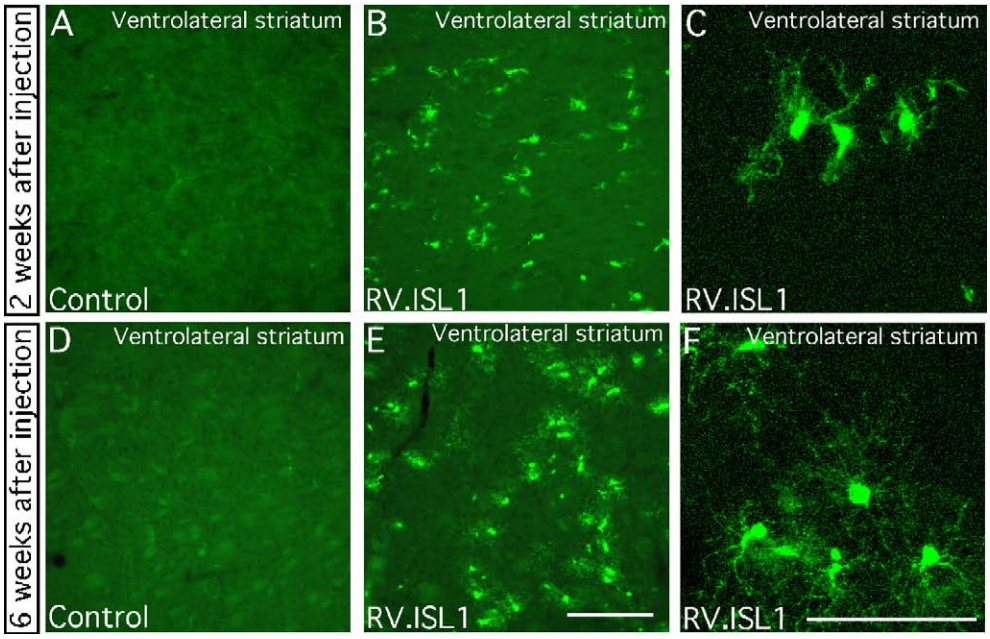


Fig. 5. Ectopic ISL1 expression in neonatal SVZ cells promotes striatal recruitment. Two weeks after injection of the control vector, virtually no GFP positive cells could be detected in the ventrolateral striatum (A). In contrast, in the RV.ISL-injected group, numerous GFP positive cells could be detected in this area at the same survival time point (B). The cells within this population displayed immature morphology (C). Six weeks after RV.ISL injection, the striatal cell population was still present (E, F) whereas no cells at all could be detected in the control-injected animals (D). All images are from the ventrolateral striatum. Scale bar: 100 μm.

labeled cells could be found in the OB of both control and RV.ISL-infected animals (Figs. 3A and B). No obvious difference in the distribution of the cells in the different layers of the bulb between the two groups (Figs. 3A and B) was apparent. All GFP cells in both groups displayed a morphology characteristic of newly formed OB interneurons (Figs. 3C and D), and some also coexpressed Er81 (not shown) a protein normally expressed by OB interneurons and their progenitors (Stenman et al., 2003). None of the GFP-expressing cells had immature progenitor or glia like morphology and none of the GFP-expressing cells expressed Nestin (not shown). Thus, in the context of the neonatal environment, ISL1 does not seem to disrupt the migration of SVZ-derived neuroblasts in the RMS, or their subsequent differentiation into OB interneurons.

As Isl1 is expressed by striatal progenitors during development, we analyzed the expression of DARPP-32, a protein normally present in striatal projection neurons, to see if viral-driven Isl1 expression could confer an ectopic striatal phenotype in the OB. However, DARPP-32 could not be detected in the OB of either control or RV.ISL-infected animals (not shown). That ISL1 alone is not sufficient to drive DARPP-32 expression was confirmed by in vitro experiments. Many β-III-tubulin positive neurons were formed after differentiation of embryonic and postnatal neural stem and progenitor cultures infected with RV.ISL; however, none of the neurons expressed DARPP-32 (not shown). Thus, viral-driven Isl1 expression does not seem to be sufficient to confer an ectopic striatal phenotype, either in vitro or in OB neurons.

Striatal recruitment of cells from the neonatal SVZ after ectopic expression of Isll1

Interestingly, in all neonatal animals receiving RV.ISL injections, an additional population of GFP-expressing cells could be detected outside the normal migration route in the RMS and SVZ. These cells were exclusively found within the ipsilateral striatal parenchyma and they were not present in the control animals (Table 1). In contrast, this specific recruitment to the striatum was not observed in control-infected animals or when neonatal animals (n = 11) were injected with a similar retrovirus construct encoding the proneural gene Neurogenin-2 (RV.Ngn2) (Falk et al., 2002). In the case of RV.Ngn2, GFP-expressing cells were found in many different areas of the brain including the SVZ and ipsilateral striatum, as well as septum, external capsule, and contralateral striatum, but not in the RMS or OB (Table 1). The distribution of cells within the striatum

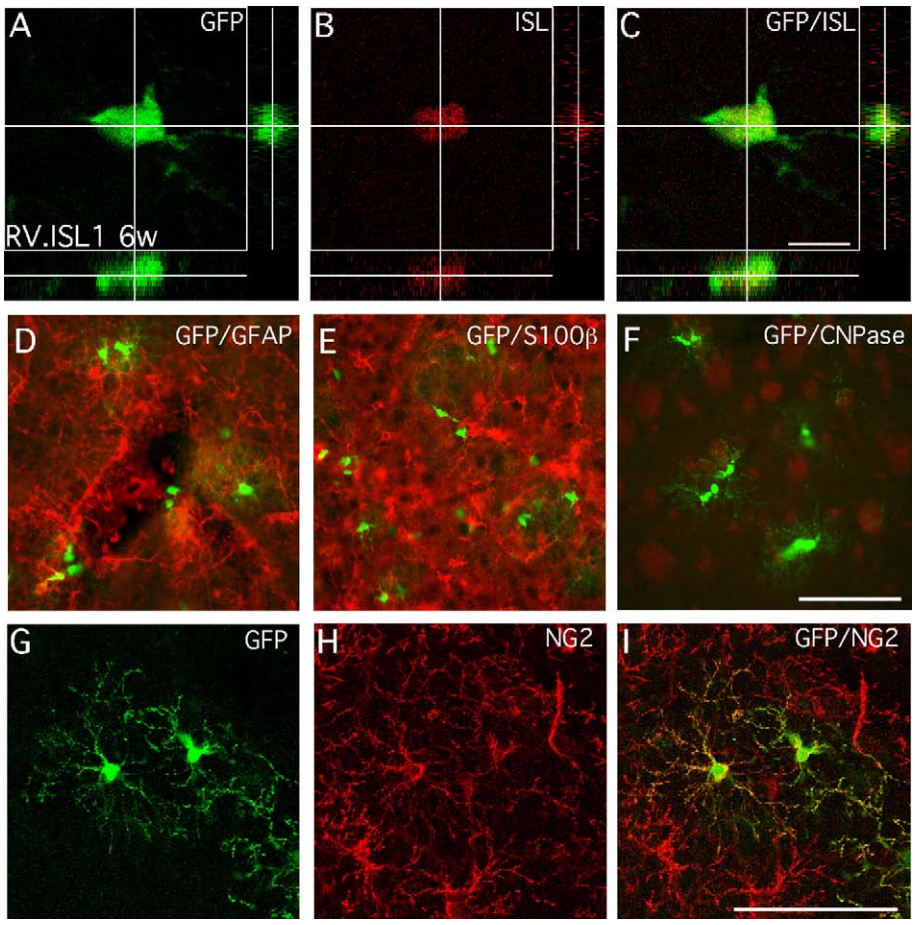


Fig. 6. Phenotypic characterization of the cells recruited to the striatum 6 weeks after RV.ISL injections into the neonatal SVZ. All of the transduced cells still coexpressed ISL1 and GFP (A–C). The RV.ISL transduced cells in the ventrolateral striatum all had a glia-like morphology, but did not express GFAP (D), S100β (E), or CNPase (F). The great majority of cells, however, expressed NG2 (G–I). All images are from the ventrolateral striatum. Scale bar: 10 μm (A–C) and 100 μm (D–I).

also differed between the groups. In control-injected animals, GFP-positive cells could only be detected close to the ventricle (Fig. 4A). After RV.ISL injections, the majority of the cells appeared clustered and were located in the ventrolateral area of the striatum (Fig. 4B), whereas after RV.Ngn2 injections, cells were evenly and homogenously distributed within the striatal parenchyma (Fig. 4C).

Although the number of GFP-expressing cells in the striatum varied between animals and surgical sessions, the distribution pattern of the cells was always similar. The cells in the striatum after RV.ISL injections were quantified in all animals from one representative surgical session. To make an unbiased evaluation of the distribution of the cells, we divided the striatum into three equal zones: Zone I, II, and III (See schematic in Table 2) where Zone I includes the SVZ and the ventral aspect of Zone III corresponds to the ventrolateral striatum. Two weeks after infection, more GFP-expressing cells (1804 ± 1132 compared to 967 ± 265) were present in the striatum of RV.ISL-infected animals. In control-injected animals, the majority of the GFP-expressing cells were found in Zone I close to the ventricular wall and very few, if any, cells were present in Zone III (Table 2). In contrast, a significant number of GFP-expressing cells were found in all three zones of the RV.ISL-injected animals with the majority of cells residing in Zone II (Table 2). Six weeks after Isl1 infection, many of the cells had migrated further in the striatal parenchyma and now proportionally more cells resided in the ventrolateral-most area (ventral half of Zone III) (Table 2). At this time point, no GFP-expressing cells at all could be detected in any of the striatal zones in control animals (Table 2).

The cells recruited to the striatum after Isl1 expression are Ng2-positive and display morphologies of glial precursors but do not further differentiate into mature glial cells

Closer examination of the cells recruited to the ventrolateral area of the striatum in response to ectopic Isl1 expression in the SVZ showed that their morphology was different from the GFP-expressing neural precursor/immature neurons migrating in the RMS or residing in the OB (Figs. 5C and F compared to Figs. 2C–H and 3C and D), suggesting that they were glia or glial precursors rather than immature neurons or neuronal precursors.

Microscopic analysis of the cells at 2 weeks after injection showed that the majority of the GFP-expressing cells in the striatum displayed immature morphologies without characteristics of either mature neurons or glia (Fig. 5C). Unlike the cells migrating in the OB, none of the cells recruited to the striatum expressed DCX (not shown), thus are unlikely to represent neuronal precursors (Couillard-Despres et al., 2005). Further, with the exception of a few cells (less than 5%) that express Ng2, immunohistochemical analysis of the cells showed that no markers characteristic of mature glial cells or their progenitors were coexpressed with GFP 2 weeks after infection (not shown). Six weeks after viral injection, ISL1 protein was still produced in virtually all of the targeted cells (Figs. 6 A–C) but still no cells with neuronal morphologies could be detected. Although the cells had a glia-like morphology (Fig. 5 F), they did not express GFAP, S100β, or CNPase (Figs. 6D–F), that are normally expressed in mature astrocytes and oligodendrocytes, but virtually all of the cells now expressed Ng2 (Figs. 6G–I). Twelve weeks after injection, many GFP-expressing cells could still be detected in the striatum of Isl1-injected animals but none of the cells had the morphology of mature oligodendrocytes, suggesting that the Ng2 positive cells do not further differentiate even after extended time in the striatum (not shown).

Ectopic Isl1 expression in the adult SVZ does not promote striatal recruitment

In two separate experiments, a total of 34 adult rats were injected with RV.ISL (n = 22) and control (n = 12) virus into the SVZ. The rats were sacrificed 2 days, 1 week, 2 weeks, and 3 weeks after injection. One to three weeks after injection, GFP-expressing cells could be detected at the injection site, as well as in the RMS, and in the OB of the control animals as previously reported (Rogelius et al., 2005). In the animals that received the RV.ISL injections, however, infected cells could only be detected at the site of injection and not in the OB, striatum, or any other area of the brain at any of the time-points examined. Thus, ectopic expression of Isl1 in the progenitor cells of the adult SVZ is not sufficient to direct the migration of cells into the striatum; however, it interferes with the normal migration into the OB.

Discussion

Today, most strategies for brain repair focus on replacing lost or injured neurons and commonly involve in vitro expansion of neural stem and progenitor cells followed by transplantation into the damaged brain(Bjorklund et al., 2003). An alternative strategy would be to mobilize endogenous neural stem and progenitor cells to generate new neurons in situ (Leker and McKay, 2004). However, much remains be learned about the differentiation potential and basic biology of these cells before one can attempt to learn how to manipulate them towards a specific cell type.

Postnatal generation of striatal neurons, both after in vivo models of stroke (Arvidsson et al., 2002; Parent et al., 2002) and after exposure to BDNF (Benraiss et al., 2001; Pencea et al., 2001; Chmielnicki et al., 2004), has recently been reported. These studies and the maintained expression in the adult SVZ of transcription factors such as Gsh2 and Dlx (Doetsch et al., 2002; Parmar et al., 2003; Saino-Saito et al., 2003) suggest that striatal progenitors that are present during development may be maintained in a quiescent state also in the adult brain. During embryonic development, striatal projection neuron progenitors as well as progenitors for cholinergic interneurons express Isl1. With the exception of the cholinergic interneurons, Isl1 is down-regulated around birth and at the same time the production of striatal projection neurons ceases (Toresson et al., 2000; Wang and Liu, 2001; Stenman et al., 2003). In this study, we ectopically

expressed Isl1 in cells of the neonatal and adult SVZ, with the aim to induce postnatal generation of striatal neurons. However, no neurogenesis was observed in the striatum of Isl1-transfected animals. Further, after Isl1-transfections in neonatal animals, many targeted cells could still be found in the OB. These cells showed no evidence of respecification into cells with striatal characteristics, but were normally distributed within the OB, and displayed morphologies and protein expressions similar to that of unmanipulated OB interneurons. Likewise, neural stem and progenitor cell cultures from the developing or postnatal striatum failed to differentiate into DARPP-32 expressing neurons after Isl1 infection in vitro. Thus, Isl1 expression alone does not seem to be sufficient to specify a striatal neuron phenotype in neurons generated from the SVZ after birth.

Interestingly, however, ectopic expression of Isl1 in the neonatal SVZ resulted in the appearance of a population of cells in the striatal parenchyma that is not present in control animals. The spatial and temporal distribution of the cells suggests that they had migrated through the striatum in a direction away from SVZ. The cells had a non-neuronal morphology and are most likely glia progenitors since they express Ng2 but not markers of more mature neurons, astrocytes, or oligodendrocytes. It is conceivable that Isl1 either confers a glial fate in a subpopulation of postnatal SVZ cells which in turn results in altered migration, or alternatively that Isl1 directly affects the migration of the cells. Although not conclusive, our in vitro differentiation analysis and the fact that no glial differentiation could be detected in the OB after Isl1 infections, suggest that Isl1 does not bias neonatal SVZ progenitors towards a glial fate. Thus, Isl1 is likely to regulate migration of the postnatal SVZ cells. As the striatum is the only structure of the brain that GFP-positive cells could be found outside the SVZ/RMS/OB system, in contrast to the widespread migration observed when the proneural gene *Ngn2* is expressed in the same cells, it is not likely that Isl1 increases migration in general. Instead, Isl1 expression seems to be directing the migration of the cells specifically into the striatum. During development, this also seems to be the case as the Isl1-expressing cells in the medial ganglionic eminence migrate to the striatum where they differentiate into cholinergic interneurons, in contrast to cortical interneurons not expressing Isl1 but derived from the same structure which is repelled by the striatum (Marin et al., 2000).

Factors involved in and mechanisms controlling the migration of cells to the striatum are largely unknown but it is interesting to note that ectopic Isl1 expression in the postnatal SVZ only directs migration of cells to the striatum perinatally when the expression of factors such as Eph4A and netrin, factors important for migration during the embryonic period, are maintained at a high level (Livesey and Hunt, 1997; Greferath et al., 2002). These migratory cues are then down-regulated after the first postnatal weeks. This down-regulation coincides with, and could explain, the lack of striatal recruitment after ectopic Isl1 expression in the adult SVZ observed in this study. It cannot be ruled out, however, that the cells in the neonatal SVZ are more plastic and thus more malleable than their adult counterparts, such that only neonatal but not adult SVZ cells are able to respond to Isl1 expression.

The spatial and temporal expression of Isl1 in the embryonic brain suggests that it plays a role in the production of striatal projection neurons and cholinergic interneurons during development. However, as mice with a targeted disruption of *Isl1* die earlier than the phenotype in the forebrain can be studied (Pfaff et al., 1996), the exact role of Isl1 in striatal neurogenesis remains to be determined. Our results suggest that at least in the postnatal brain, Isl1 does not seem to play a fundamental role in specifying a striatal neuron phenotype. Instead, we suggest that ectopic expression of Isl1 in the postnatal brain reprograms a subpopulation of the SVZ progenitors such that they can respond to migratory cues present in the neonatal striatum. Once the cells reach the neonatal striatum, however, they do not receive the cues that are present in the developing striatum during embryogenesis that are necessary to direct neuronal differentiation, but are exposed to a purely gliogenic environment and at least partly differentiate according to these cues.

The full differentiation potential of the Ng2 expressing cells recruited to the striatum after Isl1 expression remains to be determined. Ng2 expressing cells often differentiate into oligodendrocytes in vitro and in vivo (Nishiyama et al., 2002; Dawson et al., 2003; Bu et al., 2004) but this does not seem to be the case in this study as no cells with characteristic oligodendrocyte morphologies or protein expression can be detected even after extended timepoints. Another possibility is that the Ng2/GFP positive cells in the striatum arise from Ng2 positive cells residing in the early postnatal SVZ. These cells have recently been shown to form self-renewing multipotent neurospheres in vitro and to generate neurons in vivo and in vitro (Belachew et al., 2003; Aguirre and Gallo, 2004; Aguirre et al., 2004). As these Ng2 cells are actively dividing in the SVZ (Aguirre et al., 2004), they are potential targets of the retrovirus and it is conceivable that Isl1 expression in these cells may direct their migration into the striatum instead of to the OB. From our results, it is not clear weather the Ng2 expressing cells recruited to the striatum represent such progenitors that are arrested in their further development but that harbors a potential to, given the proper differentiation cues, fully differentiate into neurons or other mature cell types reported to be the progeny of Ng2 cells (reviewed in Sellers and Horner, 2005) or weather they represent terminally differentiated Ng2 expressing glia as described by Peters (2004).

Besides giving insight into possible functions of Isl1, this study shows that by genetically manipulating the cells in the SVZ to express a developmentally important transcription factor, the differentiation of the cells can be changed. In this case, Isl1 switches the fate of the progenitors from generating OB interneurons to generation of striatal glia, most likely by affecting the migration of the cells. These results take us one step closer to elucidating the full differentiation capacity of the endogenous neural stem and progenitor cells in the postnatal brain. An increased understanding of the plasticity and developmental potential of the SVZ cells, and how to manipulate them, may help in designing new strategies for brain repair utilizing these resident precursor cells.

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