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Identification of Factors Involved in Neurogenesis Recovery After Irradiation of the Adult Mouse Subventricular Zone: A Preliminary Study

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1. Introduction

Neurogenesis insures the production of functional neurons throughout life and occurs in two narrow regions of the adult mammalian brain, the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone in the hippocampus (Alvarez-Buylla&Lim,2004). The adult SVZ, which is separated from the lateral ventricle by a layer of epithelial cells known as ependymal cell, contains three main populations of neural progenitors: neural stem cells (NSCs), transit-amplifying cells (TAPs) and neuroblasts. NSCs are undifferentiated cells generally characterized by their functional capacities to both self-renew and to generate a large number of differentiated progeny cells (Song, et al.,2011). Adult NSCs have an astrocyte-like phenotype (type B cells) and represent only 0.2-0.4 % of the SVZ cells, they are relatively quiescent and divide very slowly *in vivo* with a cell cycle length of 14 days (Morshead, et al.,1994). These cells are the precursors of rapidly dividing TAPs, or type C cells, which have the capacity to differentiate into neuroblasts (type A). Neuroblasts are organized as migratory chains along the rostral migratory stream (RMS) and integrate the Olfactory bulb (OB) to become interneurons (Fig. 1).

The highly organised cytoarchitecture of the SVZ constitutes a niche for NSCs (Ihrie&Alvarez-Buylla, 2011). Cells localized in this specific microenvironment secrete a variety of factors involved in NSC proliferation, migration and/or differentiation

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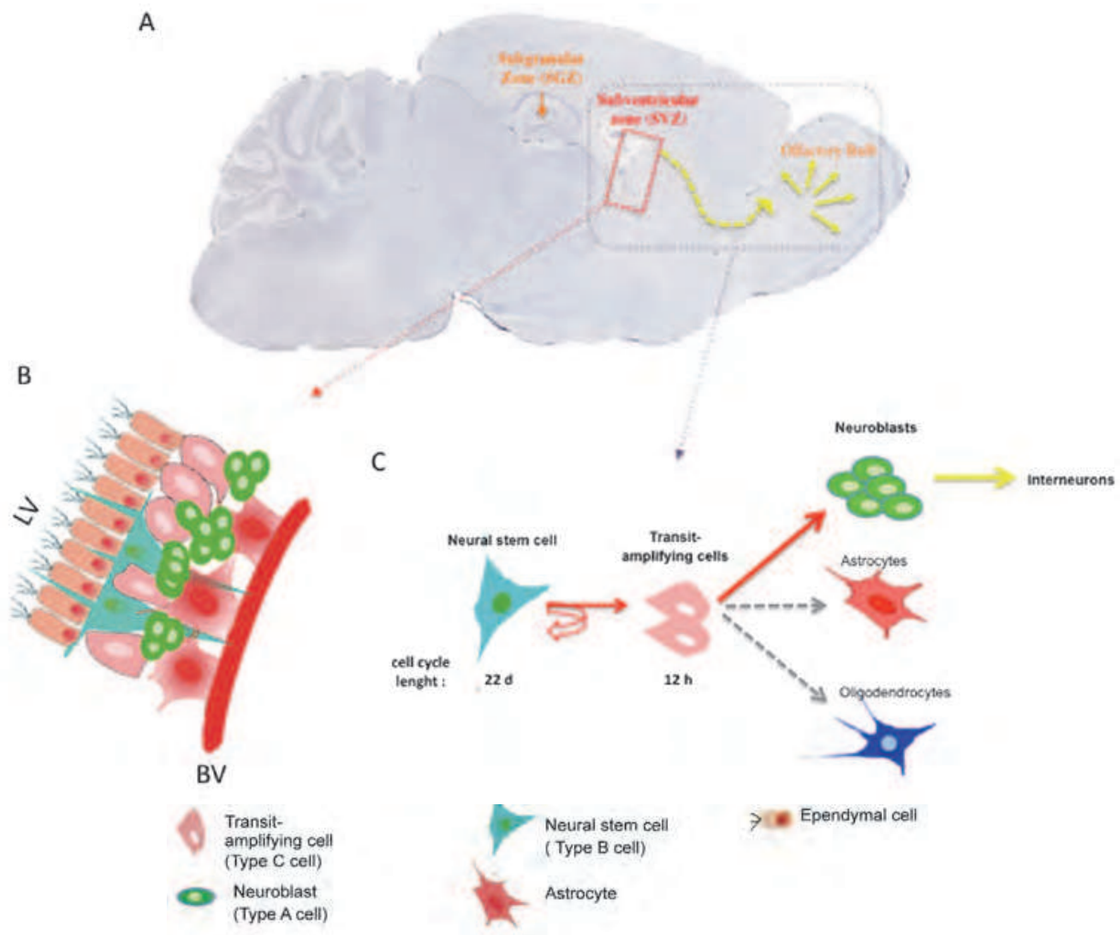


(A) In adult mouse brain, neurons are continuously produced in two restricted regions: the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) lining the lateral ventricles (LV). The former produces neurons that functionally integrate into the granular cell layer of the hippocampus, whereas the SVZ produces neuroblasts, which migrate along the rostral migratory stream (RMS) to integrate the olfactory bulbs (OB) and differentiate into interneurons.
(B) The SVZ is an highly organized neurogenic area which contains three cell types of neural cells: neural stem cells (NSCs), transit-amplifying progenitors (TAPs) and neuroblasts. Ependymal cells that contact the NSCs are organized as a single layer of epithelial cells separating the SVZ from the ventricles.
(C) NSCs are defined as slow dividing cells having capacities to self-renew and to generate multiple cell types (neurons, astrocytes and oligodendrocytes). NSCs give rise to highly proliferating TAPs, which differentiate mostly in neuroblasts. NSCs are also able to produce oligodendrocytes and astrocytes according to development stages, in response to brain injury or *in vitro* conditions. The length of the cell cycle for NSCs and TAPs lasts 22 days and 12 h, respectively (Doetsch, et al.,1999, Morshead, et al.,1994). BV (blood vessel).

Fig. 1. Neurogenesis in the adult mouse brain

(Doetsch,2003). For example, GABA released from neuroblasts provides a feedback mechanism to control proliferation of NSCs in the SVZ (Liu, et al.,2005). Bone morphogenetic proteins signalling can direct neural progenitors to glial fate in the adult brain (Lim, et al.,2000), whereas the secretion by ependymal cells of Noggin, a polypeptide

antagonist of bone morphogenetic proteins, antagonizes Bone morphogenetic protein signalling and stimulates neurogenesis (Lim, et al.,2000). Brain endothelial cells are thought to be crucial for the NSC niche because they lie in close proximity to NSCs (Tavazoie, et al.,2008), where they probably regulate self-renewal of NSCs and their differentiation into neurons (Ramirez-Castillejo, et al.,2006). Brain endothelial cells may also balance proliferation/quiescence of NSCs by secreting Bone morphogenetic proteins (Mathieu, et al.,2008).

A number of studies indicate that brain injury can induced SVZ cells to migrate towards non-OB areas especially towards lesions and participate in neuronal and glial repair (Alonso,1999, Cayre, et al.,2006, Goings, et al.,2004, Jankovski, et al.,1998, Macas, et al.,2006, Picard-Riera, et al.,2002, Yamashita, et al.,2006). Adult NSCs may therefore promise hopes in the repair of damaged central nervous system (Dubois-Dalcq,2005).

Adult NSCs survive anti-mitotic cytosine arabinoside treatment *in vivo* unlike actively dividing TAPs and neuroblasts, which rapidly disappear. This assay has been used by Doetsch et al. to demonstrate that quiescent NSCs are able regenerate to the neurogenic SVZ niches (Doetsch, et al.,1999). Exposition of the brain to ionizing radiation induces apoptosis of proliferating cells in the SVZ (Shinohara, et al.,1997). For high doses of irradiation an incomplete repopulation of the SVZ occurred and neurogenesis is collapsed for long term (Tada, et al., 1999).

However, exposition to low doses allows SVZ to be replenished. A recovery of proliferating cells is observed starting 3 days after radiation and peaking at day 7 (Hopewell and Cavanagh 1972; Tada, et al.,1999).

In light of the recovery capacity of SVZ, we developed a model of low dose irradiation (2 Gy) of adult mouse brain, which provokes a transient collapse of neurogenesis followed by a rapid recovery of the SVZ. The repopulation of the SVZ was most probably due to the stimulation of relatively quiescent NSCs and their proliferation (Morshead, et al.,1994, Pastrana, et al.,2009).

Seek for factors involved in the stimulation of neurogenesis and production of new neurons remains a challenging task. We assume that our *in vivo* irradiation model will be helpful to identify proteins coinciding with SVZ reconstitution, i.e. those involved in NSC proliferation.

Only few global proteomic analyses on rodent brains have been reported, attempting to identify proteins that are involved in brain injury such as middle cerebral artery occlusion ischemia (Sung, et al. 2010), or to gain further insight into the molecular mechanisms of neurodegenerative diseases (Broadwater, et al., Castegna, et al.,2002). Others proteomic studies aimed to identify crucial proteins by comparison between different brain developmental stages and during brain aging (Shoemaker, et al. 2010, Yang, et al.,2008). Therefore, we performed a global proteomic analysis based on 2 dimensional-gels and identification by mass spectroscopy in our SVZ reconstitution model. We decided to work with proteins extracted from SVZ, instead of cells isolated using a specific marker. Indeed, with all SVZ extract, including NSC, progenitor cells and the SVZ extracellular matrix, it was possible to search for protein and secreted factors potentially involved in NSC proliferation, migration and/or differentiation.

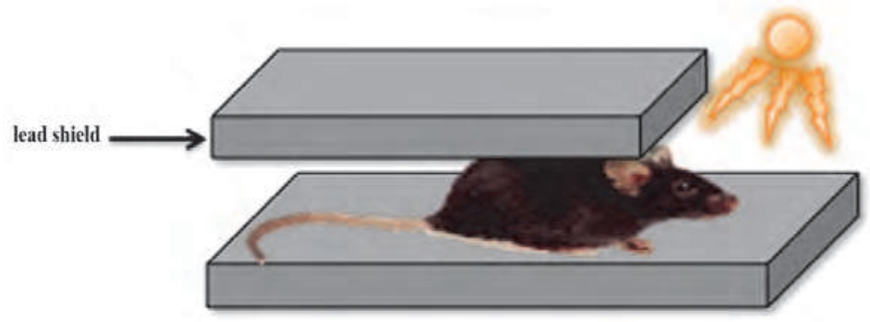
In this preliminary study, an accurate analysis of 2D-gel revealed that several proteins from SVZ appeared as modulated following brain radiation. Important issues of this study are the identification of candidates possibly involved in the stimulation of quiescent NSCs.

2. Material and methods

2.1 Mouse irradiation

We used eight-week old male C57BL/6J mice (Janvier, Le Genest-Saint-Isle, France). All experimental procedures complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and European Union guidelines.

Mice were irradiated with a medical Alcyon irradiator (γ-rays ^{60}Co) (Fig. 2). Prior to radiation, mice were anesthetized with ketamine (75 mg/kg, Merial, Lyon, France) and medetomidine (1 mg/kg, Pfizer, Paris, France) by intraperitoneal (i.p.) route. Immobilized mice were placed under a lead shield in order to expose the head and to protect the rest of the body. A total dose of 2 Gy was delivered with a dose rate of 1 Gy/mn. After exposure, mice were woken up by i.p. injection of antipamezole (1 mg/kg, Pfizer, Paris, France).



Irradiation was performed with a medical irradiator (Alcyon, ^{60}Co source). Irradiation window was focused at the level of the head of the anesthetized mouse. The rest of the body was protected by a lead shield.

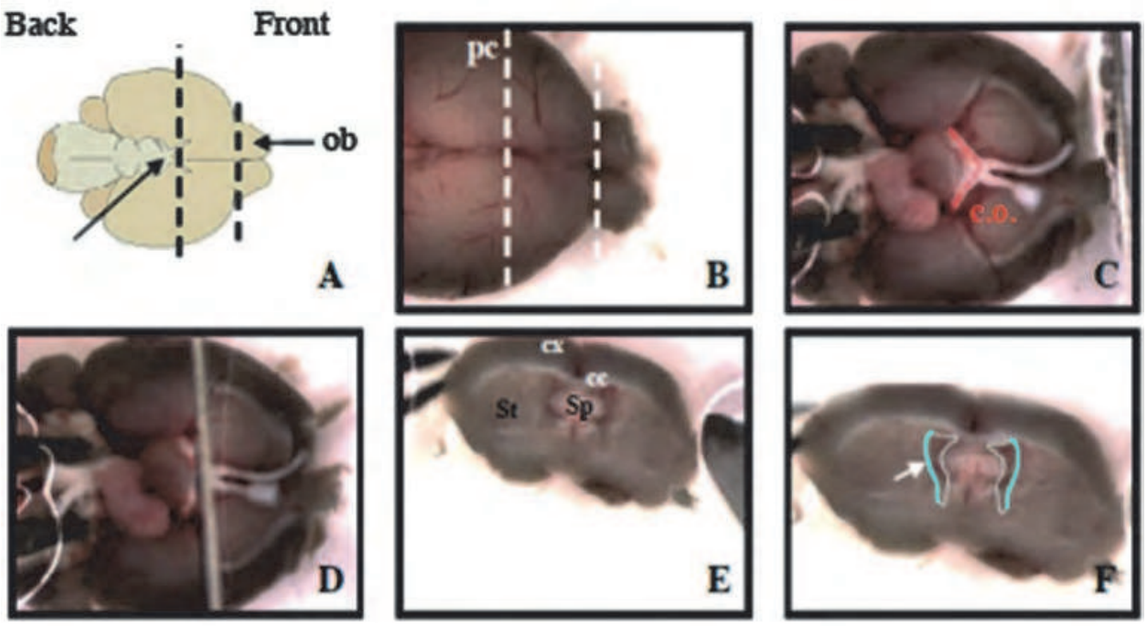
Fig. 2. Schematic representation of brain irradiation

2.2 Micro-dissection of the brain

At different time points after radiation, mice were euthanatized and SVZ and striatum (STR) were micro-dissected (Fig. 3). The micro-dissection method used to isolate SVZ is very tricky since SVZ is a very tiny part of brain tissue. Briefly, skull was cut with scissors at the midline and carefully removed with forceps. The brain was transferred into a Petri dish containing phosphate buffered saline and 6g/L of glucose. OBs were removed and a coronal cut was made at the optical chiasma. Then, under a stereomicroscope, septum was removed from the fore part of the brain with small forceps. Lateral walls of ventricle, containing the SVZ, were microdissected using small forceps and cleared out of contaminating corpus callosum and STR. A piece of STR adjacent to SVZ was taken. Tissue pieces were immediately frozen in liquid nitrogen.

2.3 Protein extraction

Proteins were extracted from SVZ and STR of control (Ctr) and irradiated mice. Tissues were homogenized in buffer containing 9M urea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT and a protease inhibitor cocktail (Roche) with a small Teflon pestle and cell debris were removed by ultra-centrifugation at 100 000g for 1 hour (TL100, Beckman). The protein



(A) A schematic representation of ventral face of the brain with the two cuts: one at the front just behind the OB and one at the level of optic chiasma. (B) Representative photographs of dorsal face and (C, D) ventral face of the mouse brain. (E, F) Coronal views of brain slices after cuts. (E) Lateral ventricles are visible from each part the septum. The ventricular walls to be dissected out containing the SVZ are indicated by arrow and light blue lines.
Cx: cortex; cc: corpus callosum; St: striatum; Sp: septum.

Fig. 3. Process for micro-dissection of the SVZ

content was estimated in the supernatant using the Bradford assay. To limit variability, tissue pieces from mice with the same treatment were mixed together in proteomic sample buffer.

2.4 Two-dimensional electrophoresis

Two-dimensional electrophoresis was performed with at least 5 technical replicates. Briefly, precast 18 cm strips, pH range 3-10 NL (GE), were rehydrated in the presence of 100 µg of protein extract. Isoelectric focusing was carried out using a Protean IEF Cell (Bio-Rad, Hercules, CA, USA) isoelectric focusing system until 80 KV h-1. The strips were then incubated in the first equilibration solution (50 mM Tris–HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS) with 130 mM DTT and then in the second equilibration solution (50 mM Tris–HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS) with 130 mM iodoacetamide. Strips were then embedded using 1% (w/v) low-melting agarose on the top of the acrylamide gel. SDS-PAGE was carried out on a 12% acrylamide SDS-polyacrylamide gel, using the Dodeca Cell electrophoresis unit (Bio-Rad).
Gels were stained with Sypro-Ruby and scanned to images, which were digitized with a Typhoon 9400 fluorescent scanner (Typhoon 9400 GE) using the 532 nm excitation laser and the 610BP emission filter. Image were acquired at a 100 µm resolution with a 550 voltage applied to the photomultiplier tube.

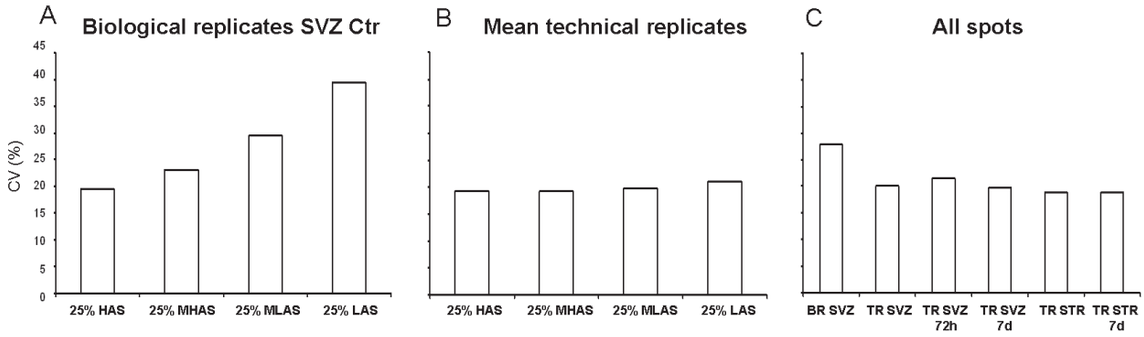
2.5 Image analysis

Images from stained gels were analyzed using the Samespots software v4.1 (Non-linear Dynamics, UK). All pictures were first aligned and gel replicates were then grouped to create a global analysis with all conditions. Spots of each sample were compared between control and irradiated conditions. A multivariate statistic analysis was performed using the statistic mode of the Samespots software v4.1 (Non-linear Dynamics, UK). Spots with significant differences between control and irradiated cells (Anova t-test $p<0.05$) were first extracted. Then, only spots with a P value < 0.05 and a power > 0.8 were finally selected. Spots of interest were selected for subsequent protein identification by mass spectrometry analysis.

2.6 Two-D gel reproductibility

Using gel-based proteomics analysis, it is important to estimate the contribution of biological and technical variations. We assessed the degree of biological variation inherent to the 2-DE process. SVZ were extracted separately from 9 mice. For each mouse, about 150 µg proteins were extracted in buffer containing 9M urea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT and a protease inhibitor cocktail (Roche) then were used to produce a corresponding 2D-gel. Protein spot volume was determined for all spots matched in an experimental set using the Samespots software packages with default settings, as used for all the study. Coefficients of variation (CV) were calculated for each protein sample, or as a function of spot intensity. CV was calculated as a percentage of standard variation as related to mean: (SD/mean) x100 (Anderson, et al., 1985).

The SVZ of each animal were used to perform a single 2-D gel. All spots of the corresponding 9 gels were compared as biological replicates (Fig. 4 A).



(A) coefficient of variation of SVZ Ctr samples between 9 biological replicates, as a function of spot intensity (HAS: high abundant spots; MHAS: medium high abundant spots; MLAS: medium low abundant spots; LAS: low abundant spots).
(B) coefficient of variation of all technical replicates, as a function of spot intensity.
(C) comparison between coefficients of variation of biological replicates (BR) and technical replicates (TR) of SVZ and STR samples.

Fig. 4. Biological and technical variability of proteomes as measured by 2-D gel electrophoresis.

Coefficient of variation grew up from 20% for high abundant spots to about 40% for low abundant spots. As a comparison, the degree of technical variation inherent to the 2-DE process was estimated for each SVZ and STR samples. Biological samples corresponding to the same treatment were mixed and the resulting protein samples were used to perform

technical 2-D gel replicates (Fig. 4 B). In this case, a constant coefficient of variation was observed (about 20%) whatever spot abundance (Fig. 4 B) and sample treatment (Fig. 4 C). This analysis of 2-D gel reproducibility clearly showed the advantage to perform technical replicates with mixed biological samples.

2.7 MALDI-TOF MS analysis

Spots were excised from preparative two-dimensional electrophoresis gels by hand, and processed using a Packard Multiprobe II liquid-handling robot (Perkin Elmer, Courtaboeuf, France). After washing successively with water, 25 mM ammonium bicarbonate, acetonitrile / 25 mM ammonium bicarbonate (1:1, v/v) and acetonitrile, gel fragments were dried at 37°C. Protein digestion was carried out at 37°C for 5 hours following addition of 0.125 µg trypsin (sequencing grade, modified, Promega, Charbonières, France), and resulting fragments were extracted twice with 50 µL acetonitrile / water (1:1, v/v) containing 0.1 % trifluoroacetic acid for 15 min. Pooled supernatants were concentrated with a speedvac to a final volume of 20 µL. Peptides were simultaneously desalted and concentrated with C18 Zip-Tip micro-columns to a final volume of 3 µL, an aliquot of each sample was mixed (1/1) with the alpha-cyano-4- hydroxycinnamic acid matrix at half saturation in acetonitrile/water (1:1, v/v) and the mixture was immediately spotted on the MALDI target by the Multiprobe II robot. Mass spectra were recorded in the reflector mode on a UltraFlex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Automatic annotation of monoisotopic masses was performed using Bruker's SNAPTM procedure. The MASCOT search engine software (Matrix Science, London, UK) was used to search the NCBInr database.

The following parameters were used: mass tolerance of 30 to 100 ppm, a minimum of five peptides matching to the protein, carbamidomethylation of cysteine as fixed modification, oxidation of methionine as variable modification, and one missed cleavage allowed.

2.8 Nano LC MS analysis

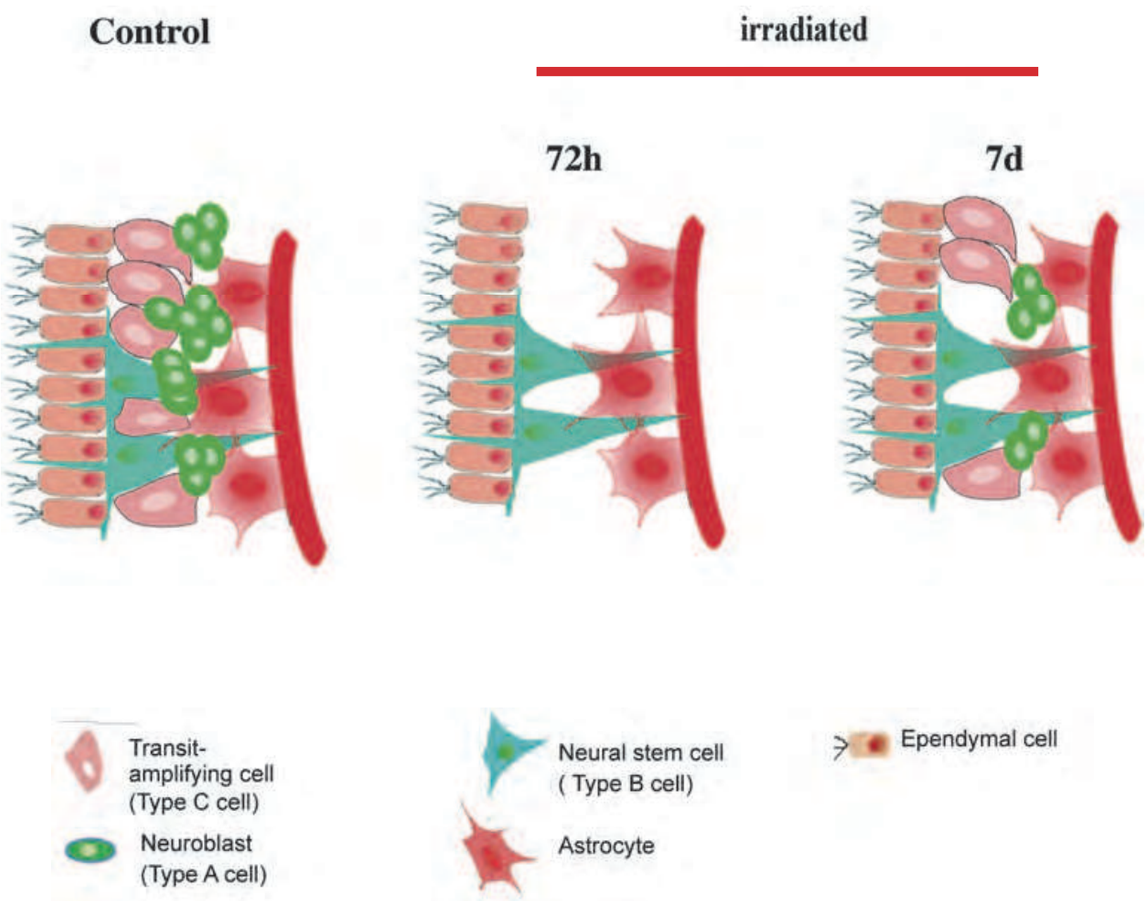
When low abundant spots could not be identified by PMF, LC-MS/MS analysis was conducted. Stained protein spots were excised manually, washed, digested with trypsin and extracted using formic acid. Protein digests were analysed using an ion trap mass spectrometer (Esquire HCT plus; Bruker, Billerica, MA, USA) coupled to a nano-chromatography system (HPLC 1200, Agilent, Santa Clara, CA, USA) interfaced with an HPLC-Chip system (Chip Cube, Agilent). MS/MS data were searched against NCBI (National center for Biotechnology information) and MSDB databases using Mascot software.

3. Results and discussion

3.1 Regeneration of the SVZ after radiation

We have developed a model of SVZ reconstitution after low dose irradiation (2 Gy) of adult mouse brain. Radiation exposure provoked a transient collapse of neurogenesis followed by a rapid recovery of the SVZ (Fig. 5).

Cell proliferation was assessed in the SVZ by injection with the thymidine analog 5-bromo-2'-deoxyuridine (BrdU). We observed a sharp decrease in BrdU incorporation 72h after radiation, however, BrdU labelling subsequently recovered within 7 days after exposure (Fig. 6A). BrdU positive cells were scarce 72h after irradiation but most of them expressed

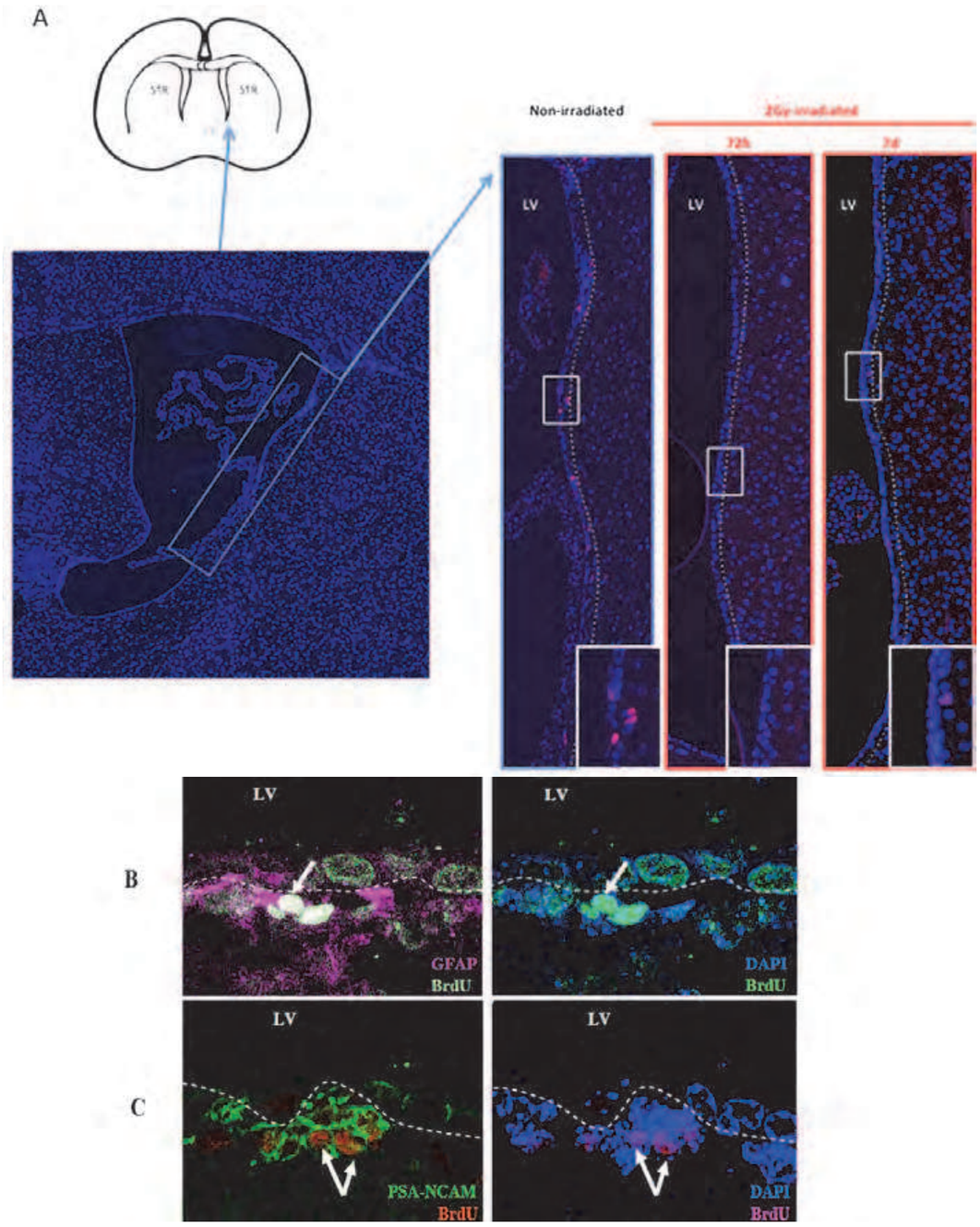


Radiation exposure of mice at 2Gy provoked a transient disappearance of progenitors of the subventricular zone (SVZ) such as transit-amplifying cells (Type C cell) and neuroblasts (Type A cell) at 72 hours. Four days later, those cells had almost completely replenished the SVZ.

Fig. 5. Schematic representation of SVZ reconstitution model after adult mouse brain irradiation.

Glial Fibrillary acidic protein (GFAP), a marker for NSCs, suggesting that quiescent NSCs are activated to re-enter cell cycle. Seven days after radiation, more numerous BrdU positive cells were observed and expressed the neuroblast marker PSA-NCAM arguing that neurogenesis recovered (Fig. 6B).

On the basis of these data and with the aim of finding proteins, such as growth factors secreted in the microenvironment involved in regeneration of the cells of the SVZ after radiation, we extracted proteins of non-irradiated SVZ and 2 Gy-irradiated SVZ at 3 and 7 days after exposure.



Mice were injected with BrdU 3 hours before sacrifice to label proliferating cells in control mice and after 2Gy-irradiation. Brain slices were counterstained with 4',6-diamidino-2-phenylindole (DAPI). (A) BrdU positive cells almost completely disappeared 72h post irradiation then they subsequently recovered in the SVZ niches.(B) 72 hours post-irradiation, some GFAP positive cells (pink), corresponding to candidate NSCs, have incorporated BrdU positive (green). At that time after irradiation, essentially NSCs proliferated in the SVZ niche. This result might underlie an activation of relatively quiescent NSCs 72h after irradiation. (C) 7 days after irradiation, the SVZ regeneration is evidenced by numerous neuroblasts PSA-NCAM postivie (green) and BrdU positive (red) in the SVZ.

Fig. 6. Regeneration of the SVZ after irradiation

3.2 Proteomic strategy

We combined 2D-gel electrophoresis and MS analyses of SVZ samples to determine proteins that are altered following radiation in adult neurogenic niches in comparison to a non-neurogenic brain region, i.e. STR. Protein samples were separated using two-dimensional electrophoresis in 3-10 non-linear pH gradient strips and 12% acrylamide gels.

SVZ samples obtained from irradiated mice at 3 and 7 days after radiation were compared with SVZ samples from non-irradiated control mice (Fig. 7). Along with micro-dissection procedures, SVZ can be contaminated with STR. For that reason and because the striatum is not a neurogenic zone, non-irradiated and 7 days-irradiated striatum samples were also taken.

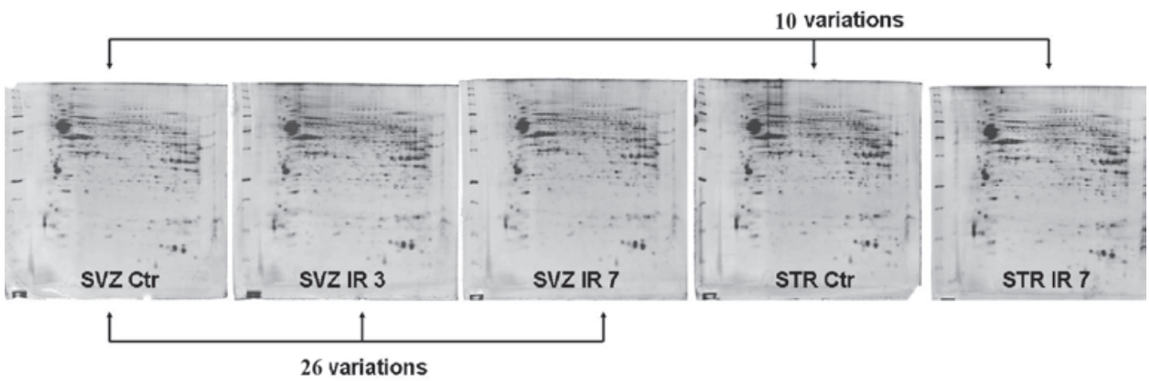


Fig. 7. Proteomic strategy of SVZ and STR samples comparison with no radiation (Ctr) and 3 days (IR 3) or 7 days (IR 7) after radiation.

3.3 Proteomic map analysis

As it can be observed in Fig. 7, pictures corresponding to the different samples analysed were very close and only minor differences can be observed, using a dedicated image analysis software.

From the 871 spots observed, a total of 36 spots were significantly modified for all the comparisons (Fig. 8). Thirty-two spots were modulated after irradiation and 4 spots were different between SVZ and STR. From these last spots, only one spot was more abundant in the SVZ than in STR suggesting a protein specifically expressed in the SVZ.

Using non-irradiated SVZ as control, 5 spots were modulated 3 days after radiation, 16 spots were modulated at 7 days (Table 1). Sixteen spots were altered at day 7 after radiation when compared with day 3. In addition, SVZ samples were also compared with STR and 4 spots appeared as modulated 7 days following radiation. The variation in spot intensity was ranged between 1.2 and 1.9 that was in agreement with previously published data using similar 2D-gel approach (Broadwater, et al. 2011, Gasperini, et al. 2011).

These spots, according to their localisation on the 2D reference map (Fig. 8), displayed an heterogeneous repartition on the gel, with a large range of iso-electric points (from 4 to 8) and of molecular weights (from 13 to 46 kDa).

Different profiles were established according to spot modifications, as illustrated in the Fig. 9.

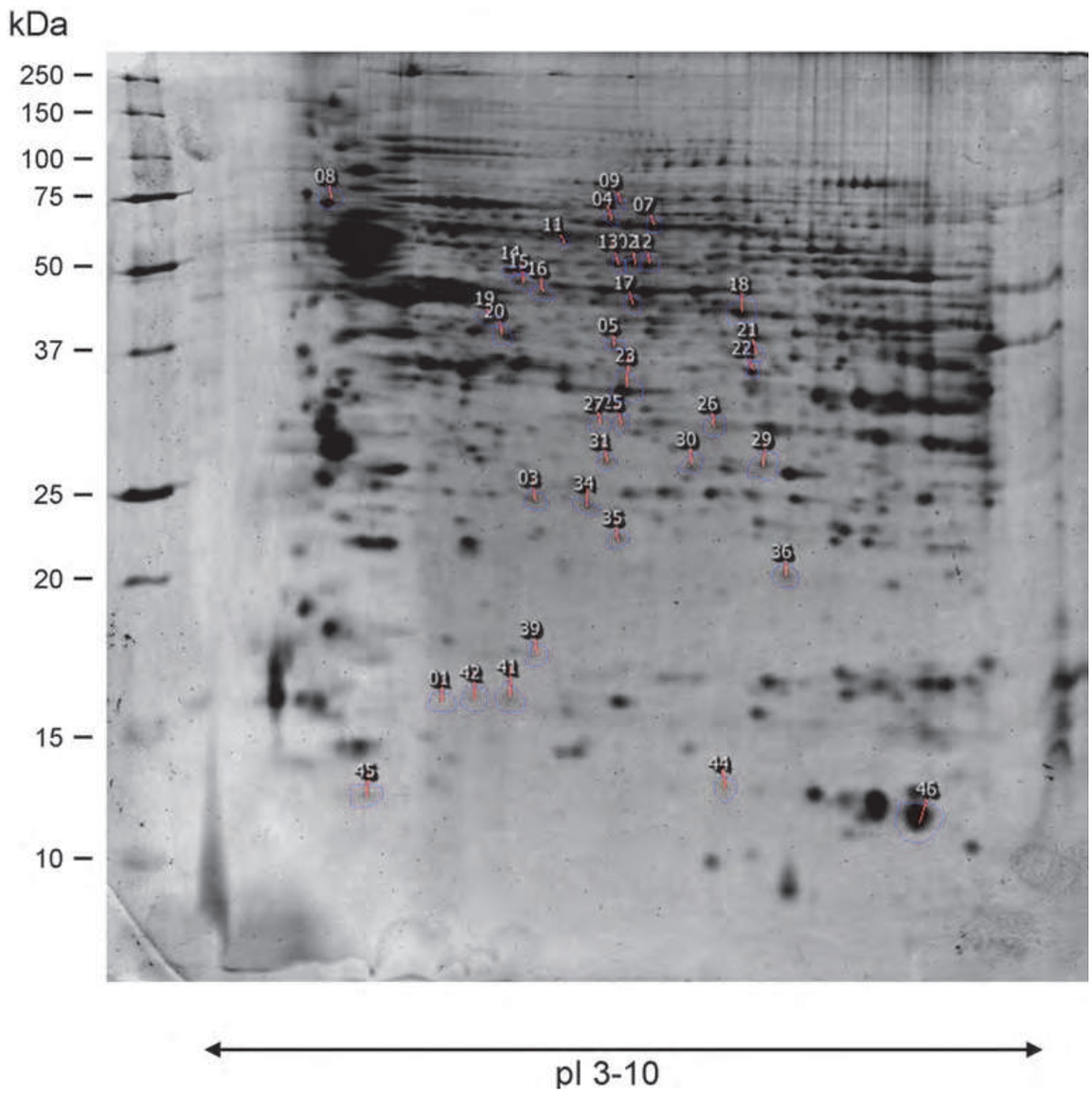
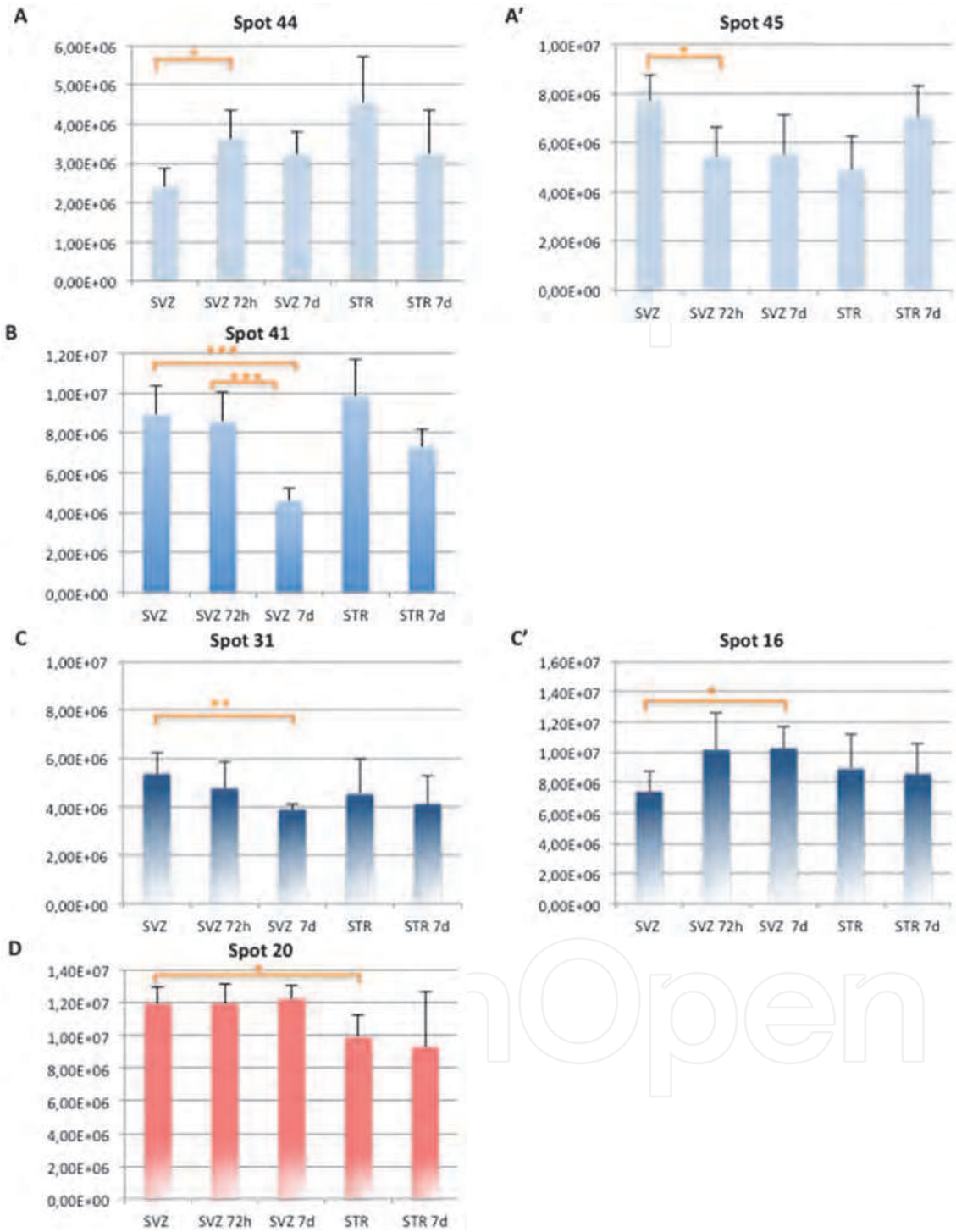


Fig. 8. Two-dimensional electrophoresis of SVZ proteins, separated under reducing conditions using 18-cm pH 3-10 strips for the first dimension, and a 12% acrylamide gel for the second dimension.



In comparison to non-irradiated SVZ: (A,A') early modified from 3 days after radiation, (B) downregulated by irradiation from 3 days to 7days or (C,C') late response to radiation at 7 days. In comparison to STR (D): no response to radiation. Numbers of spots corresponded to those listed in Fig. 3 and Table 1. Results are representative of volume intensity of the spot ± standard deviation from 5 technical replicates.

Fig. 9. Intensity profiles of spots specifically modified in the SVZ

Interestingly, 79% of the spot variations were obtained from comparison between non-irradiated SVZ and irradiated SVZ and appeared to be specific of the SVZ. Forty three percent of these spots were found modified between 3 and 7 days post-irradiation that might underlie functions of these proteins during the intense proliferation phase. Thirteen percent of them were modified 3 days after radiation as compared with control SVZ suggesting a role for the proteins in the activation of quiescent NSCs.
The rest of them (21%) corresponded to spots altered in both regions after radiation in comparison to respective region controls, suggesting a role of these proteins in the response to radiation.
Among the 32 spots identified with a significant response to radiation, we eliminated 9 abundant spots (more than 0.1% of the total volume of spots) because they might not correspond to proteins involved in proliferation processes but rather have a cytoarchitecture function in the tissue. In a first round of analysis, among the 21 remaining spots, only 15 were abundant enough and well separated in the gel to be excised allowing identification by MS (Table 1).

3.4 Biological functions of the proteins

We verified the expression of the genes corresponding to the identified proteins (Table1) using the Allen Brain Atlas database (Fig. 10). This database contained a thorough list of genes expression in the mouse brain by RNA hybridization (http://mouse.brain-map.org) (Lein, et al.,2007). We found that indeed 47% of our identified proteins have its gene expressed in the SVZ; the others being at least expressed in the adjacent regions, i.e. striatum or in the corpus callosum (**Fig. 10**).
Identified proteins were classified according to their known biological activity (Fig. 11). Surprisingly, we did not identify proteins matching with growth factors or cell cycle regulation, as they may be present in too small quantity to be identified. Otherwise, we cannot exclude that gel resolution might not be optimized for the identification of this type of proteins.
Myelin basic protein (MBP) that wraps axons has been identified in two adjacent spots (41, 42) that varied according to Pi but not in their MW indicating posttranslational modifications. Strikingly, the amount of these spots decreased after radiation, which suggested a degradation of myelin sheets by radiation. This is of importance because MBP modifications have never been reported with such a low radiation dose (Tian, et al.,2008).
Otherwise, this current proteomic analysis of SVZ demonstrates that a 2 Gy-radiation exposure affected major cellular functions such as proteasome, energy production, vesicle trafficking and cytoskeletal maintenance.
Thirteen percent of the spots belong to the proteasome system known to be involved in the degradation of unneeded or damaged proteins by proteolysis (Mcbride, et al.,2003). The intensity of these spots were decreased between 3 and 7 days (spot) 34 or only 7 days post irradiation (spot 3) that might underscore a decrease of proteasome activity that has already been reported after irradiation in a variety of cell types (Mcbride, et al.,2002, Pajonk&Mcbride,2001). This reduction of proteasome activity has been proposed to be related, at least in part, to an increased expression of proteasome inhibitors (Conconi&Friguet, 1997, Zaiss, et al., 2002).
Twenty percent of the spots corresponded to proteins having functions in metabolism pathways. Two of them (5, 44) were modified within 3 days after radiation. Another was increased 7 days after radiation and the identified protein have been implicated in

Spot	Observed pI	MW	Abundance	Variations	reference sample	ANOVA (p)
1	5.28	16	0.03899	-1.9 in SVZ IR 7 -1.8 in SVZ IR 7	SVZ CT SVZ IR 3	0.008 0.013
2	**6.53**	**49**	**0.09853**	**-1.3 in SVZ IR 7**	**SVZ IR 3**	**0.022**
3	5.95	25	0.05079	+1.3 in SVZ IR 7	SVZ CT	0.033
4	**6.4**	**62**	**0.06269**	**+1.3 in SVZ IR 7**	**SVZ IR 3**	**0.004**
5	6.41	36	0.04725	-1.3 in SVZ IR 3	SVZ CT	0.012
7	**6.68**	**60**	**0.27306**	**+1.3 in SVZ IR 7**	**SVZ IR 3**	**0.043**
8	**4.3**	**68**	**0.19432**	**+1.4 in STR CT**	**SVZ CT**	**0.05**
9	**6.44**	**68**	**0.01876**	**+1.3 in SVZ IR 7**	**SVZ IR 3**	**0.019**
11	**6.12**	**57**	**0.04227**	**-1.3 in STR IR 7**	**STR CT**	**0.031**
12	**6.61**	**49**	**0.10724**	**+1.2 in SVZ IR 7** **+1.2 in SVZ IR 7**	**SVZ CT** **SVZ IR 3**	**0.05** **0.010**
13	**6.43**	**49**	**0.06881**	**+1.2 in SVZ IR 7** **+1.3 in STR CT**	**SVZ IR 3** **SVZ CT**	**0.028** **0.007**
14	**5.74**	**48**	**0.08356**	**+1.2 in SVZ IR 7**	**SVZ IR 3**	**0.004**
15	5.82	46	0.08980	+1.3 in SVZ IR 7	SVZ CT	0.02
16	5.98	44	0.06190	+1.4 in SVZ IR 7	SVZ CT	0.021
17	**6.53**	**43**	**0.10764**	**+1.3 in SVZ IR 7**	**SVZ IR 3**	**0.003**
18	**7.18**	**41**	**0.25750**	**+1.2 in STR CT**	**SVZ CT**	**0.018**
19	5.58	40	0.07969	-1.2 in SVZ IR 3	SVZ CT	0.012
20	5.72	37	0.09955	-1.2 in STR CT	SVZ CT	0.012
21	**7.28**	**36**	**0.03971**	**-1.4 in SVZ IR 7**	**SVZ CT**	**0.038**
22	**7.29**	**34**	**0.12789**	**+1.2 in SVZ IR 3** **+1.4 in STR IR 7**	**SVZ CT** **STR CT**	**0.021** **0.001**
23	**6.47**	**33**	**0.38047**	**+1.2 in STR CT**	**SVZ CT**	**0.011**
25	6.47	30	0.03257	-1.7 in SVZ IR 7 -1.6 in SVZ IR 7	SVZ CT SVZ IR 3	0.027 0.047
26	**7.04**	**30**	**0.05344**	**-1.5 in SVZ IR 7**	**SVZ CT**	**0.04**
27	**6.35**	**30**	**0.02708**	**-1.6 in SVZ IR 7** **-1.6 in SVZ IR 7**	**SVZ CT** **SVZ IR 3**	**0.01** **0.015**
29	**7.33**	**27**	**0.10550**	**-1.3 in SVZ IR 7** **+1.4 in STR IR 7**	**SVZ IR 3** **STR CT**	**0.041** **0.004**
30	6.88	27	0.05109	-1.3 in SVZ IR 7	SVZ CT	0.038
31	6.37	27	0.04457	-1.4 in SVZ IR 7	SVZ CT	0.01
34	6.24	24	0.05691	-1.3 in SVZ IR 7 -1.3 in SVZ IR 7	SVZ CT SVZ IR 3	0.017 0.036
35	**6.45**	**22**	**0.03776**	**-1.4 in SVZ IR 7**	**SVZ CT**	**0.016**
36	7.49	20	0.06499	-1.6 in SVZ IR 7	SVZ IR 3	0.046
39	**5.92**	**17**	**0.02929**	**-1.4 in SVZ IR 7** **-1.4 in SVZ IR 7**	**SVZ CT** **SVZ IR 3**	**0.015** **0.018**
41	5.74	16	0.07440	-1.9 in SVZ IR 7 -1.9 in SVZ IR 7	SVZ CT SVZ IR 3	0.001 0.001
42	**5.5**	**16**	**0.04760**	**-1.6 in SVZ IR 7** **-1.5 in STR IR 7**	**SVZ CT** **STR CT**	**0.002** **0.013**
44	7.09	13	0.02003	+1.5 in SVZ IR 3	SVZ CT	0.018
45	4.63	13	0.06425	-1.4 in SVZ IR 3	SVZ CT	0.028
46	**8.48**	**12**	**0.94660**	**+1.4 in STR IR 7**	**STR CT**	**0.002**

Table 1. List of spots significantly modified in: control SVZ; 3 days following radiation SVZ, 7 days following radiation SVZ, control STR; 7 days following radiation STR. Spot number; experimental protein molecular weight and pI; variation level, under-expressed (-) or over-expressed (+) in the corresponding sample, *vs* reference sample; and ANOVA, (significant when $p<0.05$). Spots indicated in bold were excised from the gels and analysed by MS to allow the identification the proteins. The abundance corresponds to the ratio (%) of the volume one spot on the total volume of spots.

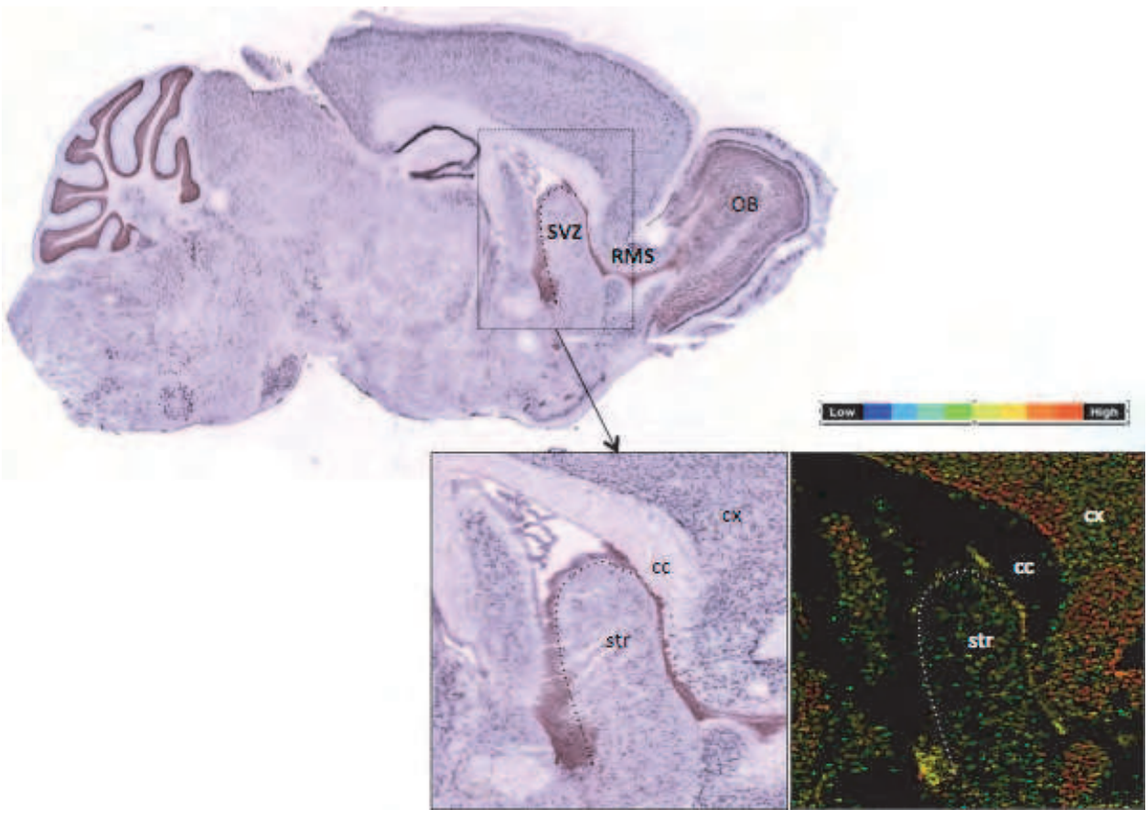
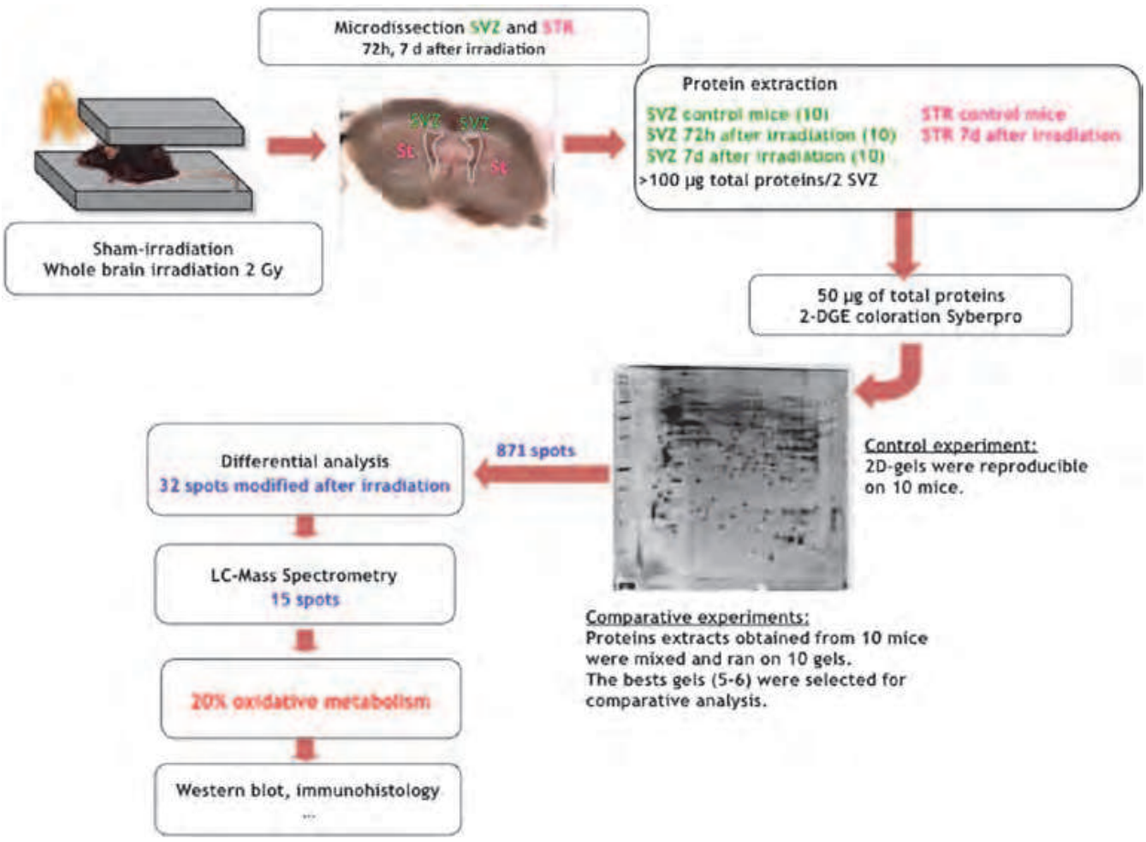


Fig. 10. An example of the expression of one gene corresponding to an identified protein using the Allen Brain Atlas database. This gene is expressed along the SVZ, a part of the RMS and also in the cortex (cx) but not in the corpus callosum (cc).

Biological functions	Spot number	Response to radiation
Mitochondrial respiratory chain	3 15 45	Late Late Early
Intracellular transport	20 30	No response Late
Proteasome	31 34	Late Mid
Metabolism	5 16 44	Early Late Early
Cytoskeleton	1 19	Mid Early
Axogenesis/myelination	25 41 42	Early Mid Late

Fig. 11. Distribution of the proteins identified by MS according to their biological function.

phospholipid biosynthesis that might correspond to proteins involved in the organisation of membrane structure during cell proliferation. In our context, these identified proteins might interfere with the proliferation wave during SVZ regeneration.
Thirteen percent of the modulated proteins (spots 34, 30, 20) are central effectors in intracellular signal transduction pathways like GTPases mediating the formation of vesicles in neural cells, a fundamental process of neurotransmitter release (De Camilli, et al.,1995).
Thirteen percent of the spots referred to cytoskeleton proteins (spots 1, 19) showing alteration from day 3 to 7. Thus, this proteomic analysis revealed that radiation exposure influence the expression of proteins involved the reorganisation of actin cytoskeleton probably associated with proliferation and/or migration.
It appeared that 20% of the modulated proteins were known for being implicated in oxidative metabolism. One of them (spot 45) decreased early after radiation that might reflect a radio-induced defect of mitochondrial metabolism. The two other spots (3, 15) were



From 871 spots, 32 spots showed variations of intensity after irradiation. Among the last spots, we analysed and identified 17 spots. Twenty-six percents of the identified proteins matched with proteins already described in oxidative metabolism. The next step of this work will consist firstly, in confirming these variations of intensity by other technical approaches such as western blot and immunohistochemistry and secondly, in demonstrating their involvement in the neurogenesis stimulation after SVZ irradiation.

Fig. 12. Schematic representation of the current comparative proteomic analysis from control SVZ and 2Gy-irradiated SVZ at 3 and 7 days after exposure.

increased 7 days after radiation probably as a consequence of an increased metabolic activity of proliferating SVZ cells.
The approach of the current proteomic analysis and the main results are represented in the Fig. 12. The next step of this work will be the validation of the variation of identified proteins and a deep analysis to demonstrate their involvement in neurogenesis stimulation following SVZ radiation and their potential use to stimulate neurogenesis in aged brains and/or neurodegenerative diseases.

4. Conclusion

This preliminary study demonstrates that 2D-gel electrophoresis is an accurate global proteomic analysis to analyse modifications in very small brain regions. In our model of neurogenic niche regeneration after 2Gy irradiation, which is associated with stem cell proliferation, we have identified proteins, which have major cellular functions such as energy production, cytoskeletal maintenance and vesicle trafficking. Interestingly, identified proteins have functions known to be involved in pathways previously reported to be altered by radiation which underscores the reliability of our proteomic approach. Especially, proteins involved in mitochondrial respiratory chain have been identified in our study; they produce energy and generate reactive oxygen species. Acharya et al. have shown that human NSCs significantly increased their oxidative and nitrosative stresses after radiation (Acharya, et al. 2010). The importance of endogenous oxygen reactive species to control NSC proliferation has been reported as well (Le Belle, et al. 2010). Moreover, oxidative stress is implicated in the progression of aging and neurodegenerative disorders (Berlett&Stadtman, 1997, Butterfield, et al.,1997, Lauderback, et al.,2002, Richardson, 2009). In light of theses studies, our data concerning mitochondrial respiratory chain are very promising. This chapter describes the first part of our project, including a validation of the technical strategy, with examples of protein functions, associated with SVZ regeneration following 2Gy irradiation. The characterization of identified proteins is still under investigation, and needs further biological validations.

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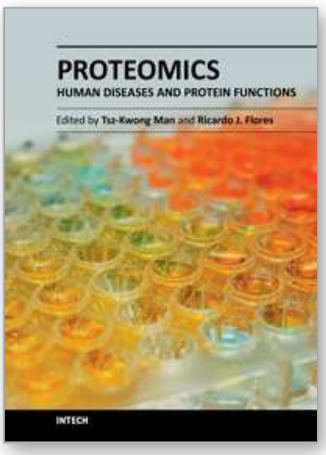

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