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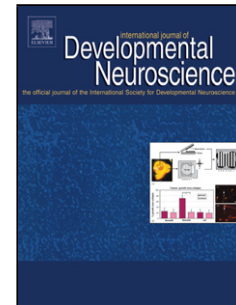
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**Title page****Title:**

Oligodendrogenesis from neural stem cells: perspectives for remyelinating strategies

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**Highlights**

- !! Spontaneous remyelination in MS fails with disease progression
- !! Remyelination decline is multifactorial
- !! Cell therapy may rely on NPCs transplantation or stimulation of local OPCs
- !! Oligodendrogenesis may be a key target in cell therapy for MS
- !! *Ex vivo* models of demyelination constitute an important tool toward MS cell therapy

**Abstract**

Mobilization of remyelinating cells spontaneously occurs in the adult brain. These cellular resources are specially active after demyelinating episodes in early phases of multiple sclerosis (MS). Indeed, oligodendrocyte precursor cells (OPCs) actively proliferate, migrate to and repopulate the lesioned areas. Ultimately, efficient remyelination is accomplished when new oligodendrocytes reinvest nude neuronal axons, restoring the normal properties of impulse conduction. As the disease progresses this fundamental process fails. Multiple causes seem to contribute to such transient decline, including the failure of OPCs to differentiate and enwrap the vulnerable neuronal axons. Regenerative medicine for MS has been mainly centered on the recruitment of endogenous self-repair mechanisms, or on transplantation approaches. The latter commonly involves grafting of neural precursor cells (NPCs) or neural stem cells (NSCs), with myelinogenic potential, in the injured areas. Both strategies require further understanding of the biology of oligodendrocyte differentiation and remyelination. Indeed, the success of transplantation largely depends on the pre-commitment of transplanted NPCs or NSCs into oligodendroglial cell type, while the endogenous differentiation of OPCs needs to be boosted in chronic stages of the disease. Thus, much effort has been focused on finding molecular targets that drive oligodendrocytes commitment and development. The present review explores several aspects of remyelination that must be considered in the design of a cell-based therapy for MS, and explores more deeply the challenge of fostering oligodendrogenesis. In this regard, we discuss herein a tool developed in our research group useful to search novel oligodendrogenic factors and to pharmacologically study oligodendrocyte differentiation in a time- and cost-saving manner.

**Keywords**

Cell therapy; neural stem cells; oligodendrocytes; multiple sclerosis; remyelination; CNS repair.

Abbreviations - MS: multiple sclerosis; NPCs: neural precursor cells; CNS: central nervous system; PDGFR $\alpha$ : platelet-derived growth factor receptor alpha; OPCs: oligodendrocyte precursor cells; PNS: peripheral nervous system; NSCs: neural stem cells; SVZ: subventricular zone; EAE: experimental autoimmune encephalomyelitis; eNSCs: embryonic neural stem cells; iPSCs: inducible pluripotent stem cells; T3: triiodothyronine; TRs: T3 receptors; MBP: myelin basic protein; PLP: proteolipid protein; MAG: myelin-associated glycoprotein; CNP: 2'3'-cyclic nucleotide-3'-phosphohydrolase; NGF: nerve growth factor; CPZ: cuprizone; DIV: days *in vitro*; eGFP: enhanced-green fluorescent protein; DG: dentate gyrus; MSCs: mesenchymal stem cells.

## 1. Introduction

Central Nervous System (CNS) plasticity and neural adaptation are concepts that have received much attention in recent years. In fact, neuronal circuitries, glial meshworks and vasculature constantly adjust to the changing needs and interact with one another. This highly dynamic activity models the brain parenchyma in response to a given insult, ageing, hormonal changes, learning or physical exercise. One remarkable example of such naturally occurring CNS responses is the extensive regenerative capacity of the demyelinated brain in early phases of multiple sclerosis (MS), the most common myelin disorder. The remyelinating activity, by native precursor cells upon acute MS lesions, results in restoration of the myelin sheaths and functional recovery. Nevertheless, at later stages of the chronic disease this spontaneous reparative process eventually fails, leading to axonal degeneration and progressive neurological deficits.

Cell therapy for MS has been an important focus in attempts to develop strategies that aim at reinstalling the normal cytoarchitecture of the CNS. This goal has been pursued by introducing cells with myelinogenic capacity, or by helping the endogenous precursors in their endeavor to reinvest nude and vulnerable axons. Accordingly, to reach these objectives, it is crucial to understand: 1) the biology of the disease and its progression; 2) why remyelination fails, and ultimately 3) how to manipulate the endogenous precursor cells, or the transplanted cells, to accomplish remyelination in the chronic non-permissive scenario. In the present review we discuss these aims and refer to available tools useful to instruct neural stem or precursor cells to differentiate into myelinating oligodendrocytes, in order to efficiently attain remyelination.

## 2. Multiple sclerosis (MS)

MS is a chronic inflammatory and neurodegenerative disease of the CNS that affects more than 2 million people worldwide, usually beginning in early adulthood (Flachenecker and Stuke, 2008). Although the etiology is unclear, it appears to be linked to both genetic and environmental factors. MS causes disability through demyelination of axons, affecting neuronal conduction of action potentials and contributing to axonal vulnerability and atrophy. The prevalent scenario involves a direct attack, by infiltrating autoreactive T cells and macrophages, against oligodendrocytes and myelin. As a result, activated macrophages and microglia release

massive amounts of inflammatory cytokines, and phagocyte myelin debris, thus amplifying the autoimmune pro-inflammatory reaction. However, in apparent contradiction, the observation of extensive oligodendrocyte apoptosis and microglia activation with only few, or in some cases, none T cells in early MS lesions has challenged this view (Barnett and Prineas, 2004). Accordingly, the latter study raises a new conceptual hypothesis for the initial formation of MS lesions. Consistently, primary oligodendrocyte cell loss triggers a progressive autoimmune cascade against myelin, resulting in widespread demyelination. It is noteworthy to mention that samples from MS patients reveal an important inter-individual heterogeneity concerning the pathogenesis of the disease, suggesting that both scenarios may be possible and are not mutually exclusive (Lucchinetti et al., 2000).

In the CNS, the myelin sheath is formed by cholesterol-rich specialized membranes of oligodendrocytes, compactly enwrapped around axons. The myelin sheath is not a continuous structure along the axons, forming internodes of myelin intercalated with nude nodes of Ranvier. This structure provides electrical insulation and clustered distribution of ion channels in the nodes and paranodes, allowing a fast and saltatory propagation of the action potential along the axon. In addition, myelin sustains and protects the axon. Mutually, healthy axons are necessary for the maintenance of myelin, suggesting a win-win symbiotic relationship (Taveggia et al., 2010). Thus, myelin integrity is essential for the rapid conduction of the neuronal impulse and also for axonal protection. Denuded, unprotected, axons become vulnerable and start degenerating as the disease progresses. Functional axonal failure and axonal loss represent the main cause of physical impairment in demyelinating diseases. Current treatments for MS are largely based in immunomodulatory agents that in essence reduce the frequency and intensity of relapse events in the most common forms of MS; the partially reversible relapsing-remitting MS, consisting on recurrent episodes of sudden symptomatic attacks, interspersed by periods of remission.

### **2.1. Remyelination in MS**

Spontaneous and robust remyelination occurs at the early stages of MS. However, under these circumstances, remyelination is incomplete – *i.e.*, faithful reconstruction is not fully attained, originating a thinner myelin sheath. As the disease progresses, eventually remyelination completely fails. Most of the remyelinating oligodendrocytes derive from resident OPCs, a special NG2-positive cell expressing

platelet-derived growth factor receptor alpha (PDGFR $\alpha$ ), widespread distributed throughout the adult brain parenchyma. Upon demyelinating injury, these proliferating cells are stimulated to divide faster and to migrate extensively towards the demyelinated area; where they mature and reinvest the denuded axons, forming new myelin sheaths (Franklin and Ffrench-Constant, 2008). Using genetic fate mapping to trace OPCs progeny during CNS demyelination, Zawadzka et al. (2010) have shown that OPCs may differentiate as well in Schwann cells, the myelin forming cells of the peripheral nervous system (PNS), which also contribute to CNS remyelination (Zawadzka et al., 2010). In addition, upon demyelination, neural stem cells (NSCs) in the rodent subventricular zone (SVZ) niche become activated and provide another source of myelinating oligodendrocytes. SVZ-derived cells expand and migrate to the nearby corpus callosum, undergo oligodendrogenesis (Nait-Oumesmar et al., 1999; Picard-Riera et al., 2002), acquiring morphology of myelinating cells and express myelin proteins (Menn et al., 2006). Consistently, analysis of *post-mortem* human tissue from MS patients detected more frequent SVZ-derived activated oligodendrocytes in and around the SVZ (Nait-Oumesmar et al., 2007). Nonetheless, given the multifocal nature of MS and the minor contribution of the endogenous SVZ cells to remyelination, as compared to the main effectors, the resident OPCs, the efficiency of SVZ cells to promote repair in MS is relatively modest. Therapeutic approaches based on stimulating the replenishment by endogenous neural precursor cells (NPCs), OPCs and SVZ cells, have been envisaged by several groups. In a different perspective, transplantation of cells with myelinogenic potential provided encouraging results in animal models of MS.

## **2.2 Remyelination decline: a multicausal scenario in chronic MS**

The success of cell-based strategies for MS requires a better understanding of the causes behind spontaneous remyelination decline. In early phases, and although partial, the multilamellar enwrapping of the demyelinated axons seems to efficiently recover normal impulse conduction and neurological deficits (Smith et al., 1979, 1981). However, it remains elusive whether the new thinner and shorter myelin sheath is more vulnerable to the next demyelinating event. Several hypotheses for impairment in remyelination across disease progression have been suggested and involve a concerted action of elements that appear to act synergistically: 1) depletion of OPCs in the lesioned site, 2) impairment of OPCs recruitment (proliferation, migration,

differentiation and/or remyelination), 3) presence of inhibitory factors or absence of permissive/conductive factors, 5) glial scar, 6) macrophages response, and 7) age.

Exhaustion of the OPCs pool in the demyelinated area have been claimed to be the main cause of remyelination failure. Although this process may cause a major impact in situations when continuous demyelination occurs in the same area (Armstrong et al., 2006; Mason et al., 2004), others have shown a great capacity of OPCs to repopulate depleted areas bearing transient episodes of demyelination (Chari and Blakemore, 2002; Penderis et al., 2003). In studies using few interspersed demyelinating lesions, recolonizing OPCs are able to remyelinate (Penderis et al., 2003). On the contrary, in experimental models of chronic lesions the recruitment of OPCs is insufficient for efficient remyelination. This can be caused by a) impaired migration, due to the lack of semaphorins in the chronic MS lesion (Williams et al., 2007b) – important molecules in OPCs migration during development and in active MS lesions; b) quiescence of available OPCs in the lesion site, being unable to differentiate (Wolswijk, 1998); c) or failure of the available and mature oligodendrocytes to accomplish remyelination of axons (Chang et al., 2002). Importantly, axons in the lesion area may no longer consent remyelination, likely due to a decrease in molecular signals supporting remyelination, or due to an increase in other negative signaling pathways affecting the reparative process (Coman et al., 2005). In general, the cellular and molecular environment in chronic lesions drastically differs from the one found in active lesions, especially concerning inflammation. Indeed, early lesions display active inflammation, which is a positive modulator of differentiation of OPCs and remyelination, while chronic lesions are comparatively devoid of active inflammatory events. Reactive astrocytes are found in acute lesions and appear to be important mediators of remyelination, by releasing growth factors that foster OPCs recruitment and differentiation. In contrast, hypertrophic astrocytes of chronic lesions form a glial scar that, instead, hampers remyelination (Albrecht et al., 2003; Williams et al., 2007a). A key determinant of remyelination decline is ageing. With ageing, a) OPCs become intrinsically less capable of undergoing recruitment towards lesions, and become specially limited in their differentiation potential (Sim et al., 2002) *via*, at least partially, changes in regulatory epigenetic mechanisms (Shen et al., 2008); b) axons become more vulnerable due to repetitive exposures; and 3) the environment itself turns less prompt to harbor myelination, namely because macrophage activity is impaired, thus entails

poor secretion of inflammatory molecules and allows the accumulation of myelin debris.

### **3. MS: a suitable candidate for cell therapy**

Among neurodegenerative diseases, MS seems to be particularly eligible for cell therapy. Albeit axonal loss occurs in MS, the disease is a primary demyelinating disease, *i.e.*, it targets oligodendrocytes and myelin, and axons are spared until later stages of the disease. Therefore, new myelinating cells (engrafted or endogenous NPCs) have “only” to re-enwrap the nude axons with their membranes. This is a far simpler scenario than that offered by using cell therapy strategies to replace neurons. In this case, grafted cells must integrate in pre-existing intricate networks and establish functional synapses with the correct counterparts. Moreover, remyelination and functional recovery spontaneously take place in the acute injured environment supporting the view that, at early stages, new NPCs encounter the proper environmental cues to differentiate and remyelinate. Thus, a cell therapy for MS requires procedures to sustain endogenous remyelination into chronic stages of the disease – boosting their survival, migration, differentiation or maturation – or the implantation of healthy NPCs previously amplified and instructed *in vitro*, to become myelinating oligodendrocytes. These strategies should be implemented before axonal loss and consequent compromised functional recovery takes places. Moreover, adjuvant strategies to manipulate the chronic lesion environment turning it more permissive for remyelination are pivotal. These may encompass treatments to confer axonal protection along disease progression, and cues to reverse at least some of the age-dependent and/or injury-mediated effects. It is important to refer that the introduction of healthy myelinating cells may be the only feasible strategy to restore neurological function in the case of genetic demyelinating diseases, where the endogenous oligodendrocytes are endowed with defects in the production of myelin, like leukodystrophies.

#### **3.1. Transplantation of NPCs in MS**

Strategies relying on cell transplantation of NPCs in injured areas of the brain have been largely explored in the last decade. In the damaged areas, grafted NPCs need to 1) survive in a typically hostile environment; 2) differentiate and mature in the phenotype of appropriate cell types; and 3) incorporate in the complex network of the host tissue, successfully reconstructing the affected circuits. However, as previously



mentioned, transplantation strategy in MS focus on adjuvant approaches to enhance and sustain the existing repair resources. One cannot exclude, however, partial contribution of cell-intrinsic mechanisms regulating the remyelinating capacity.

Pioneering transplantation assays performed in the 80s, using myelinogenic cells namely OPCs, Schwann cells and NSCs (Blakemore and Crang, 1985, 1988; Crang and Blakemore, 1989) raised important expectations by showing evidences of grafted cells-induced remyelination of demyelinated areas. The loss of glial cells occurring in MS may thus be counteracted by the introduction of new precursor-derived oligodendroglial cells that are previously expanded *in vitro*. NPCs from SVZ or OPCs pools can be propagated in culture, providing cellular resources for transplantation, in order to generate myelinating oligodendrocytes. Although in spite of evidences that, in demyelinated conditions, implanted OPCs elicit remyelination and promote functional recovery (Groves et al., 1993; Lachapelle et al., 1983; Utzschneider et al., 1994), in the healthy brain supranumerary OPCs are not able to migrate and to survive (Franklin et al., 1996; Franklin and Blakemore, 1997). Given the multifocality of MS, this could be a major limitation since therapeutic approaches would require multiple local injections to deliver OPCs in a number of lesion areas. On the other hand, SVZ cells are endowed with a remarkable capacity to migrate and homing into demyelinated parenchyma even when implanted systemically (intravenous or intrathecal). In fact, systemic administration of SVZ-derived NPCs exerts anti-inflammatory action in models of multifocal CNS disorders, including in experimental autoimmune encephalomyelitis (EAE) (Pluchino et al., 2003; Pluchino et al., 2005).

A major requirement for MS cell therapy regards proper cell differentiation of the transplanted cells. Upon grating, cells need to be highly migratory in order to reach the multiple lesion sites, a characteristic of the round and bipolar early OPCs. Nonetheless, the engrafted reparative cells should then be able to further differentiate and mature in the host tissue, ultimately giving rise to myelinating oligodendrocytes. Importantly, SVZ-derived oligodendrocytes undergo all the developmental stages from the early OPCs to the mature myelinating oligodendrocytes recapitulating the developmental process (Levison and Goldman, 1993; Menn et al., 2006) and are able to remyelinate axons in animal models of demyelinating injuries (Akiyama et al., 2001; Cayre et al., 2006; Keirstead et al., 1999; Pluchino et al., 2003; Smith and Blakemore, 2000). Progressive restriction of the cell fate in SVZ cells is specified by extrinsic and intrinsic factors. Accordingly, extracellular soluble factors or genetic manipulation may

be used to drive differentiation of SVZ cells in culture towards a certain phenotype. Efforts are being brought together to optimize the *in vitro* fate specification for induction into the oligodendrocyte cell lineage, a strategy that may substantially increase the outcome from SVZ cells transplantation in MS. Likewise, treatment of the reparative cells with factors that promote their survival can be an adjuvant approach to achieve better results, since the cells are engrafted in a highly hostile environment. As aforementioned it is advantageous to graft cells in early stages of differentiation, when they are migratory and plastic enough to accommodate in the host tissue. Treatment of the engrafted cells *in locus*, even if the cells are pre-specified *in vitro*, can be critical to sustain survival and promote the full differentiation along the oligodendrocytic lineage, namely the expression of myelin proteins and potentiation of remyelination.

Functional improvements due to transplantation may derive from a neuroprotective effect of the grafted cells over the host tissue. Accordingly, transplants of SVZ-derived NPCs improved the outcome in the EAE model of MS by releasing a plethora of factors with immunomodulatory or neuroprotective properties (Martino and Pluchino, 2006).

Additionally, it is important to note that, in humans, cell transplantation therapies using adult NPCs should rely on an autologous transplant, requiring firstly extraction of NPCs from the patient, and a second surgery for the transplantation of the cells, after an intermediate step of cell type conditioning in culture. Alternatively, transplantable NPCs from *post-mortem* human CNS tissue may be an option (Laywell et al., 1999), although graft rejection may occur. Also, Schwann cells may be a source for autologous transplantation following peripheral nerve biopsies, nevertheless these cells poorly contribute to remyelination (Lavdas et al., 2008). Moreover, NSCs from embryonic origin (eNSCs), or even pluripotent embryonic stem cells (ESCs) obtained from the inner cell mass of the early-stage human blastocyst may be used as a source of myelinating oligodendrocyte. However, the use of ESCs/eNSCs comports ethical issues and safety concerns, including possible teratoma formation and possible graft rejection. Also, skin fibroblasts can be reprogrammed to a pluripotent state by retroviral expression of certain transcription factors, generating inducible pluripotent stem cells (iPSCs) (Han et al., 2012; Takahashi and Yamanaka, 2006). This approach offers minimal ethical concerns and allows unlimited expandability of the cells, broad patterning potential and patient DNA match (autologous transplantation). Nevertheless,

it entails some concerns related with tumor formation and also genetic and epigenetic instability.

In conclusion, to envisage a successful cell transplantation therapy for MS, it is mandatory to: 1) harbor a detailed knowledge on the pathology; 2) conclude from studies on animal models that faithfully mimic the human disease; 3) optimize the differentiation and functional integration of grafted cells; 4) and probably develop adjuvant therapies to help sustaining axons and recovery from the disease (for instance, by treatment of the cells with pro-survival and pro-myelinating factors, or genetic engineering to induce expression of such factors, many of them which are lost in chronic stages of the disease).

#### **4. Enhancing oligodendrocyte differentiation: a bottleneck in MS cell therapy**

As previously mentioned, failure to differentiate and myelinate the vulnerable axons is a limiting factor to adequate remyelination. On the other hand, induction of the oligodendroglial phenotype and differentiation are crucial for effective transplantation strategies based on the use of SVZ-derived NPCs. Thus, efforts are being brought together to screen soluble factors or to develop genetic tools able to instruct these cells into the oligodendrocyte lineage and foster their differentiation. Such a treatment could be used to assist endogenous remyelination or to guide transplanted cells differentiation. In this context, our group developed a method that rapidly evaluates cell differentiation in SVZ cell cultures. This tool may be applied to screen multiple factors in order to enrich the progeny of SVZ cells in a given phenotype (Agasse et al., 2008; Grade et al., 2010; Grade et al., 2012). The method consists in measuring the intracellular  $\text{Ca}^{2+}$  changes evoked by KCl, histamine, and thrombin on single cells in SVZ cultures, and is based on the observation that each cell type displays a distinguishable profile of  $[\text{Ca}^{2+}]_i$  oscillations during the stimulation protocol: neurons respond to KCl, precursor cells to histamine, oligodendrocytes to thrombin, and astrocytes are non-responsive to the three compounds (Fig. 1).

##### **4.1. Oligodendrogenesis by triiodothyronine (T3) hormone**

Several factors have been pinpointed as inducers of oligodendrocyte specification and differentiation from rodent NSCs. Among those, fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF) and the thyroid hormone T3 seem to be central. Indeed, intraventricular infusion of FGF-2 stimulates the

proliferation of OPCs from SVZ cells and their terminal differentiation in oligodendrocytes, in the postnatal and adult brain (Azim et al., 2012). Nevertheless, the growth factor inhibits myelination in the intact (Azim et al., 2012) or demyelinated brain (Butt and Dinsdale, 2005) therefore attenuating repair capacity, likely via FGFR1 (Zhou et al., 2012). The soluble factor PDGF has a potent mitogenic effect on the OPCs population, regulating their numbers and oligodendrocyte generation during development and in the adulthood (Calver et al., 1998; Woodruff et al., 2004). However, excessive PDGFR $\alpha$  activation in the rodent SVZ leads to the appearance of glioma-like hyperplasias (Jackson et al., 2006). In agreement, PDGF has been implicated in tumour initiation and tumorigenicity (Jiang et al., 2011; Varela et al., 2004) therefore cancelling any prospect of usage in a cell therapy for demyelinating diseases by direct application. On the other hand, a cohort of evidences supports a role for thyroid hormone T3 in inducing oligodendrocyte differentiation and no apparent drawbacks. Consistent evidences were obtained in cultures, from human embryonic and fetal stem/progenitor cells (Fritsche et al., 2005; Kang et al., 2007; Murray and Dubois-Dalcq, 1997), in rodent embryonic and adult NSCs (Johe et al., 1996; Whittemore et al., 1999), but also *in vivo* in demyelinated SVZ of young adult rats (Franco et al., 2008). Moreover, the hormone accelerates oligodendrocyte development from the early stage of OPCs into more differentiated stages, an effect observed both *in vitro*, in OPC cultures (Barres et al., 1994; Billon et al., 2002; Tokumoto et al., 1999) and *in vivo*, in resident OPCs that are activated upon demyelination (Baas et al., 2002; Calza et al., 2002). Besides, T3 has been shown to promote the synthesis of myelin-specific proteins (Jeannin et al., 1998; Strait et al., 1997) by a direct action on oligodendrocytes, which express T3 receptors (Puymirat, 1992). In agreement, T3 administration enhances remyelination in animal models of demyelinating disease (Fernandez et al., 2004; Franco et al., 2008).

Remyelination may be a recapitulation of the developmental myelination. In fact, thyroid hormones have a crucial role in the neurodevelopment of vertebrates. Thyroid hormones deficiency during development, leads to structural abnormalities of the brain by affecting cell migration, differentiation, synaptogenesis and myelination. Hypothyroidism delays the deposition of myelin, causing hypomyelination, whereas hyperthyroidism accelerates it (Dussault and Ruel, 1987; Legrand, 1986; Walters and Morell, 1981). Thus, T3 acts on several stages of the oligodendrocyte development, since cell-cycle exit and terminal differentiation until myelinogenesis. T3 acts directly at

the transcription level by binding to nuclear thyroid hormone receptors (TRs: TR $\alpha$ 1, TR $\alpha$ 2, TR $\beta$ 1 and TR $\beta$ 2) encoded by TR $\alpha$  and TR $\beta$  genes (Rogister et al., 1999). As a result, the hormone activates or represses the transcription of specific target genes, which include genes encoding: the major myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG) and 2'3'-cyclic nucleotide-3'-phosphohydrolase (CNP) (Tosic et al., 1992), neurotrophins and their receptors, components of the cytoskeleton and co-activators of TR (Konig and Moura Neto, 2002).

In addition to the oligodendrogenic effect, T3 may exert a protective action on oligodendrocytes via nerve growth factor (NGF). In fact, the gene encoding NGF is among the target genes of T3 and, under physiological conditions, T3 regulates the endogenous synthesis of NGF in CNS (Calza et al., 1997). A study in marmosets has shown that NGF administration protects oligodendrocytes from cell death and ameliorates the pathological scenario in EAE model of MS, likely through immunomodulatory activity (Villoslada et al., 2000). Furthermore, neurotrophins can improve remyelination by directly influencing proliferation, differentiation, survival and turnover of oligodendrocytes in the demyelinated lesions (Althaus, 2004). In conclusion, T3 hormone may be a physiologically relevant promoter of oligodendrocyte cell specification, maturation and myelinogenesis of SVZ cells to be grafted in demyelinated lesions.

## **5. Effect of demyelinated environment in oligodendrogenesis by transplanted NPCs**

*In vitro* pre-commitment of SVZ cells to differentiate into the appropriate phenotype has been seen as a pivotal step in the development of cell-based transplantation therapies for CNS disorders. Nevertheless, cell differentiation triggered by oligodendrogenic factors treatment *in vitro* might be affected by experimental conditions and not recapitulate the same differentiation program when cells are engrafted in the injured tissue. To address this issue, we used an *ex vivo* model of cuprizone (CPZ)-induced toxicity in organotypic hippocampal slices and monitored the differentiation of grafted eGFP-labeled SVZ cells (Fig. 2). Administration of CPZ in the diet is widely used as a demyelinating protocol *in vivo* studies, causing demyelination of the fiber tracts in the *corpus callosum* (Matsushima and Morell, 2001), cortex (Skripuletz et al., 2008) and hippocampus (Hoffmann et al., 2008; Koutsoudaki et al.,

2009; Norkute et al., 2009). Interestingly, *in vitro* studies by Cammer (1999) have shown a detrimental effect of 1 h of CPZ treatment (25  $\mu$ M) in oligodendrocytes, and reported the presence of swollen or enlarged mitochondria in these cells, recalling the mitochondrial hyperplasia observed in the brains of CPZ-treated mice. Mitochondrial dysfunction is believed to be an early event leading to apoptotic collapse of oligodendrocytes and consequent myelin sheath degeneration (reviewed in Matsushima and Morell, 2001). Moreover, although *in vivo* studies show that the peak of myelination in rodents occurs between P10 and P60, Haber et al. (2009) have shown that hippocampal slice cultures obtained from P6-7 mice are able to develop myelin. The authors observed a progressive increase of MBP immunoreactivity over the course of 60 days *in vitro* (DIV), being that, at 10 DIV many MBP fibers were present in Ammon's Horn subregions. In addition, using electron microscopy the same authors detected myelinated axons as early as at 7 DIV. Herein, we used hippocampal slices obtained from P6-8 mice and further grown in culture for 15 DIV, thus harboring many myelinated axons. Experimentally, we incubated the 15 DIV-hippocampal slices with 25  $\mu$ M CPZ, for 24 h. After this step, we expose the cultures to fresh medium and then grafted eGFP-SVZ neurospheres, into the intact or injured slices, nearby dentate gyrus (DG) (Fig. 3A; one neurosphere per slice). Following 1 week we investigated oligodendrocytic differentiation emerging from SVZ cells exposed to the host environment.

We observed that grafted SVZ cells maintain the capability to generate oligodendrocytes, including cells with NG2 and O4 immunoreactivity and a typical ramified morphology (Fig. 3B). Interestingly, quantification of the percentage of NG2<sup>+</sup> and O4<sup>+</sup> cells among the total of eGFP cells, revealed that differentiation of SVZ grafted cells in O4<sup>+</sup> or NG2<sup>+</sup> cells is promoted by the injured environment (NG2<sup>+</sup> cells: CTRL, 13.60  $\pm$  2.99% vs. CPZ, 33.09  $\pm$  1.44%; O4<sup>+</sup> cells: CTRL, 16.40  $\pm$  0.74% vs. CPZ, 29.72  $\pm$  1.67%; n=9-12 slices and more than 3000 cells per condition; 2 independent cultures; Fig. 3C,D). Indeed, we observed a higher number of oligodendrocytes generated by neurospheres grafted into demyelinated organotypic hippocampal slices, as compared to those grafted in intact slices. These findings indicate that the environment provided by the demyelinated host tissue instructs or favors SVZ cells differentiation towards the phenotype of the lost cells. Thus, this finding highlights the importance of the disease environment dictating the fate choice of the transplanted uncommitted SVZ cells.

We detected an instructive role of the demyelinated tissue environment on the differentiation of SVZ cells toward the oligodendrocytic phenotype. This effect may result from a number of secreted or cell-contact cues that are present in the demyelinated but not in the intact host tissue. CPZ toxicity leads to the degeneration of oligodendrocytes, and consequent disruption of myelin, followed by microgliosis and astrogliosis in the demyelinated tracts (Hiremath et al., 1998; Koutsoudaki et al., 2009; Matsushima and Morell, 2001; Skripuletz et al., 2008). Typically, activated glial cells secrete pro-inflammatory molecules, like cytokines and chemokines, which may guide the differentiation of SVZ cells. One cannot exclude, however, a possible effect of other players in the toxicity microenvironment, like cell debris and death signals, on the differentiation of SVZ cells. Rivera et al. (2009) have shown a robust oligodendroglial differentiation from NPCs when these were co-transplanted with mesenchymal stem cells (MSCs) in postnatal intact organotypic hippocampal slices, but extremely weak if NPCs were transplanted alone. In our model, even intact slices supported some degree of oligodendrogenesis from transplanted NPCs. Nevertheless, we used NPCs obtained from postnatal mouse SVZ while in Rivera et al. (2009) the authors transplanted NPCs derived from adult rat SVZ and DG, therefore with different properties. More importantly, we treated the cells with T3 hormone during the period of differentiation which, as aforementioned, is known to foster oligodendrogenesis. Indeed, while an injured environment may likely support small levels of oligodendrogenesis from non-treated NPCs, an intact slice would probably display non-appreciable levels.

Importantly, hippocampal demyelination has been observed in the brains of MS patients, in parallel with microglial accumulation and cognitive deficits (Geurts et al., 2007). Our *ex vivo* model of demyelination allowed us to detect an important effect of the demyelinated host tissue favoring the differentiation of grafted SVZ cells into oligodendroglia. In agreement, other studies reported that SVZ cells transplanted into demyelinated areas show a tendency to undergo differentiation in oligodendrocytes, and often myelinate nude axons (Akiyama et al., 2001; Cayre et al., 2006; Keirstead et al., 1999; Smith and Blakemore, 2000). However these studies were based on transplantations approaches in the large white matter tracts of the *corpus callosum* and spinal cord, where the intact local environment *per se* is known to trigger oligodendrogenesis of transplanted SVZ cells (Cayre et al., 2006).

Although *in vivo* studies provide the ultimate integrative scenario devoid of cell culture artifacts, many aspects of the biology of remyelination can be addressed in *ex*

*in vivo* models. Accordingly, grafting of myelinogenic cells and/or pharmacological treatments, aiming at the amelioration of oligodendrocyte differentiation or remyelination, can be tested in slice cultures. Recently, others have been developing various *ex vivo* models to study remyelination of endogenous or transplanted NPCs. Indeed, transplantation of OPCs into organotypic cerebellar slice cultures, derived from demyelinated shiverer mice (Mbp mutant), resulted in the enwrapping of compact myelin sheaths with proper formation of the specialized domains, including nodes, internodes, juxtaparanodes and paranodes (Bin et al., 2012). Moreover, using lysolecithin-induced demyelination in cerebellar, brain stem or spinal cord slices, Zhang et al. (2011) have shown that endogenous OPCs proliferate and the axons re-acquire a myelin sheath, which is thinner and shorter, partially recapitulating the mechanism of remyelination *in vivo*. Interestingly, a recent study monitored the dynamics of myelination by using time-lapse imaging of GFP-labelled murine NPCs transplanted in spinal cord explants derived from shiverer mice (Ioannidou et al., 2012). Evaluation of survival, differentiation, dynamics of remyelination, and impulse conduction in *ex vivo* models of demyelination may offer a powerful strategy to test a wide range of potentially protective or instructive compounds, envisioning efficient remyelination in future cell therapy for MS.

## 6. Conclusion

In early phases of MS, the CNS displays a remarkable regenerative capacity upon transient demyelinating episodes. Disease progression is, however, accompanied by a gradual change in multiple and synergistic factors that overturn the outcome of this scenario, from a permissive to an inhibitory remyelination environment. A major roadblock found in chronic MS lesions is the impairment of differentiation of OPCs and remyelination. Therefore, identification of key molecules that dictate differentiation of OPCs may be important to help sustaining endogenous remyelination in the chronic lesions. Alternatively, an efficient cocktail of oligodendrogenic factors is pivotal for the success of SVZ transplantation therapies in MS. We believe that new tools to screen oligodendrogenic factors or to study and sculpt the processes of differentiation and remyelination upon injury, in a shorter time window provided by *ex vivo* models, are required for a comprehensive strategy to repair brain lesions in MS. Although research on the field has been witnessing great expansion, still translation to the clinical arenas requires many hurdles to be overcome.



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**References**

- Agasse F, Bernardino L, Silva B, Ferreira R, Grade S, Malva JO (2008) Response to histamine allows the functional identification of neuronal progenitors, neurons, astrocytes, and immature cells in subventricular zone cell cultures. *Rejuvenation Res* 11:187-200.
- Akiyama Y, Honmou O, Kato T, Uede T, Hashi K, Kocsis JD (2001) Transplantation of clonal neural precursor cells derived from adult human brain establishes functional peripheral myelin in the rat spinal cord. *Exp Neurol* 167:27-39.
- Albrecht PJ, Murtie JC, Ness JK, Redwine JM, Enterline JR, Armstrong RC, Levison SW (2003) Astrocytes produce CNTF during the remyelination phase of viral-induced spinal cord demyelination to stimulate FGF-2 production. *Neurobiol Dis* 13:89-101.
- Althaus HH (2004) Remyelination in multiple sclerosis: a new role for neurotrophins? *Prog Brain Res* 146:415-432.
- Armstrong RC, Le TQ, Flint NC, Vana AC, Zhou YX (2006) Endogenous cell repair of chronic demyelination. *J Neuropathol Exp Neurol* 65:245-256.
- Azim K, Raineteau O, Butt AM (2012) Intraventricular injection of FGF-2 promotes generation of oligodendrocyte-lineage cells in the postnatal and adult forebrain. *Glia* 60:1977-1990.
- Baas D, Legrand C, Samarut J, Flamant F (2002) Persistence of oligodendrocyte precursor cells and altered myelination in optic nerve associated to retina degeneration in mice devoid of all thyroid hormone receptors. *Proc Natl Acad Sci U S A* 99:2907-2911.
- Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol* 55:458-468.
- Barres BA, Lazar MA, Raff MC (1994) A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. *Development* 120:1097-1108.

- Billon N, Jolicoeur C, Tokumoto Y, Vennstrom B, Raff M (2002) Normal timing of oligodendrocyte development depends on thyroid hormone receptor alpha 1 (TRalpha1). *Embo J* 21:6452-6460.
- Bin JM, Leong SY, Bull SJ, Antel JP, Kennedy TE (2012) Oligodendrocyte precursor cell transplantation into organotypic cerebellar shiverer slices: a model to study myelination and myelin maintenance. *PLoS One* 7:e41237.
- Blakemore WF, Crang AJ (1985) The use of cultured autologous Schwann cells to remyelinate areas of persistent demyelination in the central nervous system. *J Neurol Sci* 70:207-223.
- Blakemore WF, Crang AJ (1988) Extensive oligodendrocyte remyelination following injection of cultured central nervous system cells into demyelinating lesions in adult central nervous system. *Dev Neurosci* 10:1-11.
- Butt AM, Dinsdale J (2005) Fibroblast growth factor 2 induces loss of adult oligodendrocytes and myelin in vivo. *Exp Neurol* 192:125-133.
- Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD (1998) Oligodendrocyte population dynamics and the role of PDGF in vivo. *Neuron* 20:869-882.
- Calza L, Giardino L, Aloe L (1997) Thyroid hormone regulates NGF content and p75LNGFR expression in the basal forebrain of adult rats. *Exp Neurol* 143:196-206.
- Calza L, Fernandez M, Giuliani A, Aloe L, Giardino L (2002) Thyroid hormone activates oligodendrocyte precursors and increases a myelin-forming protein and NGF content in the spinal cord during experimental allergic encephalomyelitis. *Proc Natl Acad Sci U S A* 99:3258-3263.
- Cammer W (1999) The neurotoxicant, cuprizone, retards the differentiation of oligodendrocytes in vitro. *J Neurol Sci* 168:116-120.
- Cayre M, Bancila M, Virard I, Borges A, Durbec P (2006) Migrating and myelinating potential of subventricular zone neural progenitor cells in white matter tracts of the adult rodent brain. *Mol Cell Neurosci* 31:748-758.
- Chang A, Tourtellotte WW, Rudick R, Trapp BD (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. *N Engl J Med* 346:165-173.
- Chari DM, Blakemore WF (2002) Efficient recolonisation of progenitor-depleted areas of the CNS by adult oligodendrocyte progenitor cells. *Glia* 37:307-313.

- Coman I, Barbin G, Charles P, Zalc B, Lubetzki C (2005) Axonal signals in central nervous system myelination, demyelination and remyelination. *J Neurol Sci* 233:67-71.
- Crang AJ, Blakemore WF (1989) The effect of the number of oligodendrocytes transplanted into X-irradiated, glial-free lesions on the extent of oligodendrocyte remyelination. *Neurosci Lett* 103:269-274.
- Dussault JH, Ruel J (1987) Thyroid hormones and brain development. *Annu Rev Physiol* 49:321-334.
- Fernandez M, Giuliani A, Pirondi S, D'Intino G, Giardino L, Aloe L, Levi-Montalcini R, Calza L (2004) Thyroid hormone administration enhances remyelination in chronic demyelinating inflammatory disease. *Proc Natl Acad Sci U S A* 101:16363-16368.
- Flachenecker P, Stuke K (2008) National MS registries. *J Neurol* 255 Suppl 6:102-108.
- Franco PG, Silvestroff L, Soto EF, Pasquini JM (2008) Thyroid hormones promote differentiation of oligodendrocyte progenitor cells and improve remyelination after cuprizone-induced demyelination. *Exp Neurol* 212:458-467.
- Franklin RJ, Blakemore WF (1997) To what extent is oligodendrocyte progenitor migration a limiting factor in the remyelination of multiple sclerosis lesions? *Mult Scler* 3:84-87.
- Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. *Nat Rev Neurosci* 9:839-855.
- Franklin RJ, Bayley SA, Blakemore WF (1996) Transplanted CG4 cells (an oligodendrocyte progenitor cell line) survive, migrate, and contribute to repair of areas of demyelination in X-irradiated and damaged spinal cord but not in normal spinal cord. *Exp Neurol* 137:263-276.
- Fritsche E, Cline JE, Nguyen NH, Scanlan TS, Abel J (2005) Polychlorinated biphenyls disturb differentiation of normal human neural progenitor cells: clue for involvement of thyroid hormone receptors. *Environ Health Perspect* 113:871-876.
- Geurts JJ, Bo L, Roosendaal SD, Hazes T, Daniels R, Barkhof F, Witter MP, Huitinga I, van der Valk P (2007) Extensive hippocampal demyelination in multiple sclerosis. *J Neuropathol Exp Neurol* 66:819-827.
- Grade S, Agasse F, Bernardino L, Malva JO (2012) Functional identification of neural stem cell-derived oligodendrocytes. *Methods Mol Biol* 879:165-178.

- Grade S, Agasse F, Bernardino L, Silva CG, Cortes L, Malva JO (2010) Functional identification of neural stem cell-derived oligodendrocytes by means of calcium transients elicited by thrombin. *Rejuvenation Res* 13:27-37.
- Groves AK, Barnett SC, Franklin RJ, Crang AJ, Mayer M, Blakemore WF, Noble M (1993) Repair of demyelinated lesions by transplantation of purified O-2A progenitor cells. *Nature* 362:453-455.
- Haber M, Vautrin S, Fry EJ, Murai KK (2009) Subtype-specific oligodendrocyte dynamics in organotypic culture. *Glia* 57:1000-1013.
- Han DW, Tapia N, Hermann A, Hemmer K, Hoing S, Arauzo-Bravo MJ, Zaehres H, Wu G, Frank S, Moritz S, Greber B, Yang JH, Lee HT, Schwamborn JC, Storch A, Scholer HR (2012) Direct Reprogramming of Fibroblasts into Neural Stem Cells by Defined Factors. *Cell Stem Cell* 10:465-472.
- Hiremath MM, Saito Y, Knapp GW, Ting JP, Suzuki K, Matsushima GK (1998) Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *J Neuroimmunol* 92:38-49.
- Hoffmann K, Lindner M, Groticke I, Stangel M, Loscher W (2008) Epileptic seizures and hippocampal damage after cuprizone-induced demyelination in C57BL/6 mice. *Exp Neurol* 210:308-321.
- Ioannidou K, Anderson KI, Strachan D, Edgar JM, Barnett SC (2012) Time-lapse imaging of the dynamics of CNS glial-axonal interactions in vitro and ex vivo. *PLoS One* 7:e30775.
- Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, Vandenberg S, Alvarez-Buylla A (2006) PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron* 51:187-199.
- Jeannin E, Robyr D, Desvergne B (1998) Transcriptional regulatory patterns of the myelin basic protein and malic enzyme genes by the thyroid hormone receptors alpha1 and beta1. *J Biol Chem* 273:24239-24248.
- Jiang Y, Boije M, Westermarck B, Uhrbom L (2011) PDGF-B can sustain self-renewal and tumorigenicity of experimental glioma-derived cancer-initiating cells by preventing oligodendrocyte differentiation. *Neoplasia* 13:492-503.
- Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD (1996) Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. *Genes Dev* 10:3129-3140.

- Kang SM, Cho MS, Seo H, Yoon CJ, Oh SK, Choi YM, Kim DW (2007) Efficient induction of oligodendrocytes from human embryonic stem cells. *Stem Cells* 25:419-424.
- Keirstead HS, Ben-Hur T, Rogister B, O'Leary MT, Dubois-Dalcq M, Blakemore WF (1999) Polysialylated neural cell adhesion molecule-positive CNS precursors generate both oligodendrocytes and Schwann cells to remyelinate the CNS after transplantation. *J Neurosci* 19:7529-7536.
- Konig S, Moura Neto V (2002) Thyroid hormone actions on neural cells. *Cell Mol Neurobiol* 22:517-544.
- Koutsoudaki PN, Skripuletz T, Gudi V, Moharreggh-Khiabani D, Hildebrandt H, Trebst C, Stangel M (2009) Demyelination of the hippocampus is prominent in the cuprizone model. *Neurosci Lett* 451:83-88.
- Lachapelle F, Gumpel M, Baulac M, Jacque C, Duc P, Baumann N (1983) Transplantation of CNS fragments into the brain of shiverer mutant mice: extensive myelination by implanted oligodendrocytes. I. Immunohistochemical studies. *Dev Neurosci* 6:325-334.
- Lavdas AA, Papastefanaki F, Thomaidou D, Matsas R (2008) Schwann cell transplantation for CNS repair. *Curr Med Chem* 15:151-160.
- Laywell ED, Kukekov VG, Steindler DA (1999) Multipotent neurospheres can be derived from forebrain subependymal zone and spinal cord of adult mice after protracted postmortem intervals. *Exp Neurol* 156:430-433.
- Legrand J (1986) Thyroid hormone effects on growth and development. In: *Thyroid Hormone Metabolism*, pp 503-534, Hennemann G, ed. New York: M Dekker, Inc.
- Levison SW, Goldman JE (1993) Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. *Neuron* 10:201-212.
- Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 47:707-717.
- Martino G, Pluchino S (2006) The therapeutic potential of neural stem cells. *Nat Rev Neurosci* 7:395-406.

- Mason JL, Toews A, Hostettler JD, Morell P, Suzuki K, Goldman JE, Matsushima GK (2004) Oligodendrocytes and progenitors become progressively depleted within chronically demyelinated lesions. *Am J Pathol* 164:1673-1682.
- Matsushima GK, Morell P (2001) The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol* 11:107-116.
- Murray K, Dubois-Dalcq M (1997) Emergence of oligodendrocytes from human neural spheres. *J Neurosci Res* 50:146-156.
- Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Van Evercooren AB (1999) Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci* 11:4357-4366.
- Nait-Oumesmar B, Picard-Riera N, Kerninon C, Decker L, Seilhean D, Hoglinger GU, Hirsch EC, Reynolds R, Baron-Van Evercooren A (2007) Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. *Proc Natl Acad Sci U S A* 104:4694-4699.
- Norkute A, Hieble A, Braun A, Johann S, Clarner T, Baumgartner W, Beyer C, Kipp M (2009) Cuprizone treatment induces demyelination and astrocytosis in the mouse hippocampus. *J Neurosci Res* 87:1343-1355.
- Penderis J, Shields SA, Franklin RJ (2003) Impaired remyelination and depletion of oligodendrocyte progenitors does not occur following repeated episodes of focal demyelination in the rat central nervous system. *Brain* 126:1382-1391.
- Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Evercooren AB (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc Natl Acad Sci U S A* 99:13211-13216.
- Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, Martinello M, Cattalini A, Bergami A, Furlan R, Comi G, Constantin G, Martino G (2005) Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature* 436:266-271.
- Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U, Amadio S, Bergami A, Furlan R, Comi G, Vescovi AL, Martino G (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* 422:688-694.

- Puymirat J (1992) Thyroid receptors in the rat brain. *Prog Neurobiol* 39:281-294.
- Rivera FJ, Siebzehnubel FA, Kandasamy M, Couillard-Despres S, Caioni M, Poehler AM, Berninger B, Sandner B, Bogdahn U, Goetz M, Bluemcke I, Weidner N, Aigner L (2009) Mesenchymal stem cells promote oligodendroglial differentiation in hippocampal slice cultures. *Cell Physiol Biochem* 24:317-324.
- Rogister B, Ben-Hur T, Dubois-Dalcq M (1999) From neural stem cells to myelinating oligodendrocytes. *Mol Cell Neurosci* 14:287-300.
- Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnet P (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. *Nat Neurosci* 11:1024-1034.
- Sim FJ, Zhao C, Penderis J, Franklin RJ (2002) The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J Neurosci* 22:2451-2459.
- Skripuletz T, Lindner M, Kotsiari A, Garde N, Fokuhl J, Linsmeier F, Trebst C, Stangel M (2008) Cortical demyelination is prominent in the murine cuprizone model and is strain-dependent. *Am J Pathol* 172:1053-1061.
- Smith KJ, Blakemore WF, McDonald WI (1979) Central remyelination restores secure conduction. *Nature* 280:395-396.
- Smith KJ, Blakemore WF, McDonald WI (1981) The restoration of conduction by central remyelination. *Brain* 104:383-404.
- Smith PM, Blakemore WF (2000) Porcine neural progenitors require commitment to the oligodendrocyte lineage prior to transplantation in order to achieve significant remyelination of demyelinated lesions in the adult CNS. *Eur J Neurosci* 12:2414-2424.
- Strait KA, Carlson DJ, Schwartz HL, Oppenheimer JH (1997) Transient stimulation of myelin basic protein gene expression in differentiating cultured oligodendrocytes: a model for 3,5,3'-triiodothyronine-induced brain development. *Endocrinology* 138:635-641.
- Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663-676.
- Taveggia C, Feltri ML, Wrabetz L (2010) Signals to promote myelin formation and repair. *Nat Rev Neurol* 6:276-287.

- Tokumoto YM, Durand B, Raff MC (1999) An analysis of the early events when oligodendrocyte precursor cells are triggered to differentiate by thyroid hormone, retinoic acid, or PDGF withdrawal. *Dev Biol* 213:327-339.
- Tosic M, Torch S, Comte V, Dolivo M, Honegger P, Matthieu JM (1992) Triiodothyronine has diverse and multiple stimulating effects on expression of the major myelin protein genes. *J Neurochem* 59:1770-1777.
- Utzschneider DA, Archer DR, Kocsis JD, Waxman SG, Duncan ID (1994) Transplantation of glial cells enhances action potential conduction of amyelinated spinal cord axons in the myelin-deficient rat. *Proc Natl Acad Sci U S A* 91:53-57.
- Varela M, Ranuncolo SM, Morand A, Lastiri J, De Kier Joffé EB, Puricelli LI, Pallotta MG (2004) EGF-R and PDGF-R, but not bcl-2, overexpression predict overall survival in patients with low-grade astrocytomas. *J Surg Oncol* 86:34-40.
- Villoslada P, Hauser SL, Bartke I, Unger J, Heald N, Rosenberg D, Cheung SW, Mobley WC, Fisher S, Genain CP (2000) Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system. *J Exp Med* 191:1799-1806.
- Walters SN, Morell P (1981) Effects of altered thyroid states on myelinogenesis. *J Neurochem* 36:1792-1801.
- Whittemore SR, Morassutti DJ, Walters WM, Liu RH, Magnuson DS (1999) Mitogen and substrate differentially affect the lineage restriction of adult rat subventricular zone neural precursor cell populations. *Exp Cell Res* 252:75-95.
- Williams A, Piaton G, Lubetzki C (2007a) Astrocytes--friends or foes in multiple sclerosis? *Glia* 55:1300-1312.
- Williams A, Piaton G, Aigrot MS, Belhadi A, Theaudin M, Petermann F, Thomas JL, Zalc B, Lubetzki C (2007b) Semaphorin 3A and 3F: key players in myelin repair in multiple sclerosis? *Brain* 130:2554-2565.
- Wolswijk G (1998) Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. *J Neurosci* 18:601-609.
- Woodruff RH, Fruttiger M, Richardson WD, Franklin RJ (2004) Platelet-derived growth factor regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination. *Mol Cell Neurosci* 25:252-262.



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- Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessler N, Suter U, Richardson WD, Franklin RJ (2010) CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell Stem Cell* 6:578-590.
- Zhang H, Jarjour AA, Boyd A, Williams A (2011) Central nervous system remyelination in culture--a tool for multiple sclerosis research. *Exp Neurol* 230:138-148.
- Zhou YX, Pannu R, Le TQ, Armstrong RC (2012) Fibroblast growth factor 1 (FGFR1) modulation regulates repair capacity of oligodendrocyte progenitor cells following chronic demyelination. *Neurobiol Dis* 45:196-205.

**Figure legends**

**Figure 1 – Single-cell calcium imaging (SCCI) as a method to assess functional cell differentiation in SVZ cultures.** Experimental protocol implemented to functionally identify SVZ-derived cells. SVZ cells loaded with the calcium probe Fura-2 AM are continuously perfused in Krebs solution and stimulated at different time intervals as shown by the time sequences. Cell-specific responses are observed when applying the sequence depicted on top: neurons respond to KCl, immature cells respond to histamine, oligodendrocytes respond to thrombin and astrocytes are non-responsive to any of these compounds.

**Figure 2 – Schematic representation of the co-cultures protocol developed to explore whether the *ex vivo* grafting of SVZ cells in a injured tissue alters the SVZ cells fate.** Organotypic hippocampal slices were prepared from wild type (WT) mice brains and injured by exposure to 25  $\mu$ M CPZ, for 24 h (black dashed line). The toxin was then removed and eGFP-labelled SVZ neurospheres were individually grafted in the intact or injured hippocampal slices (one sphere per slice). Following 1 week under, 30 nM T3 treatment (blue dashed line), evaluation of SVZ cell differentiation was performed.

**Figure 3 – Differentiation of eGFP-SVZ grafted cells in CPZ-treated organotypic hippocampal slices.** A, Representative image of an organotypic hippocampal slice grafted with an eGFP-SVZ neurosphere. The co-culture was allowed to develop during 1 week, under T3 treatment (BF, bright field). B, 1 week upon grafting, NG2<sup>+</sup> and O4<sup>+</sup> cells were found among the progeny of SVZ transplanted cells, either implanted in intact or injured slices. C, Quantification of the number of NG2<sup>+</sup> and O4<sup>+</sup> cells among the grafted eGFP-SVZ cells in intact or injured paradigm. D, Representative images of NG2 (left panel) or O4 (right panel) immunostaining on the co-cultures, counterstained with Hoechst 33342 (blue nuclei). Arrows indicate cells where colocalization of eGFP signal with the cell type markers was found. Scale bars: A, 500  $\mu$ m; B, 20  $\mu$ m; D, 50  $\mu$ m.

Figure 1

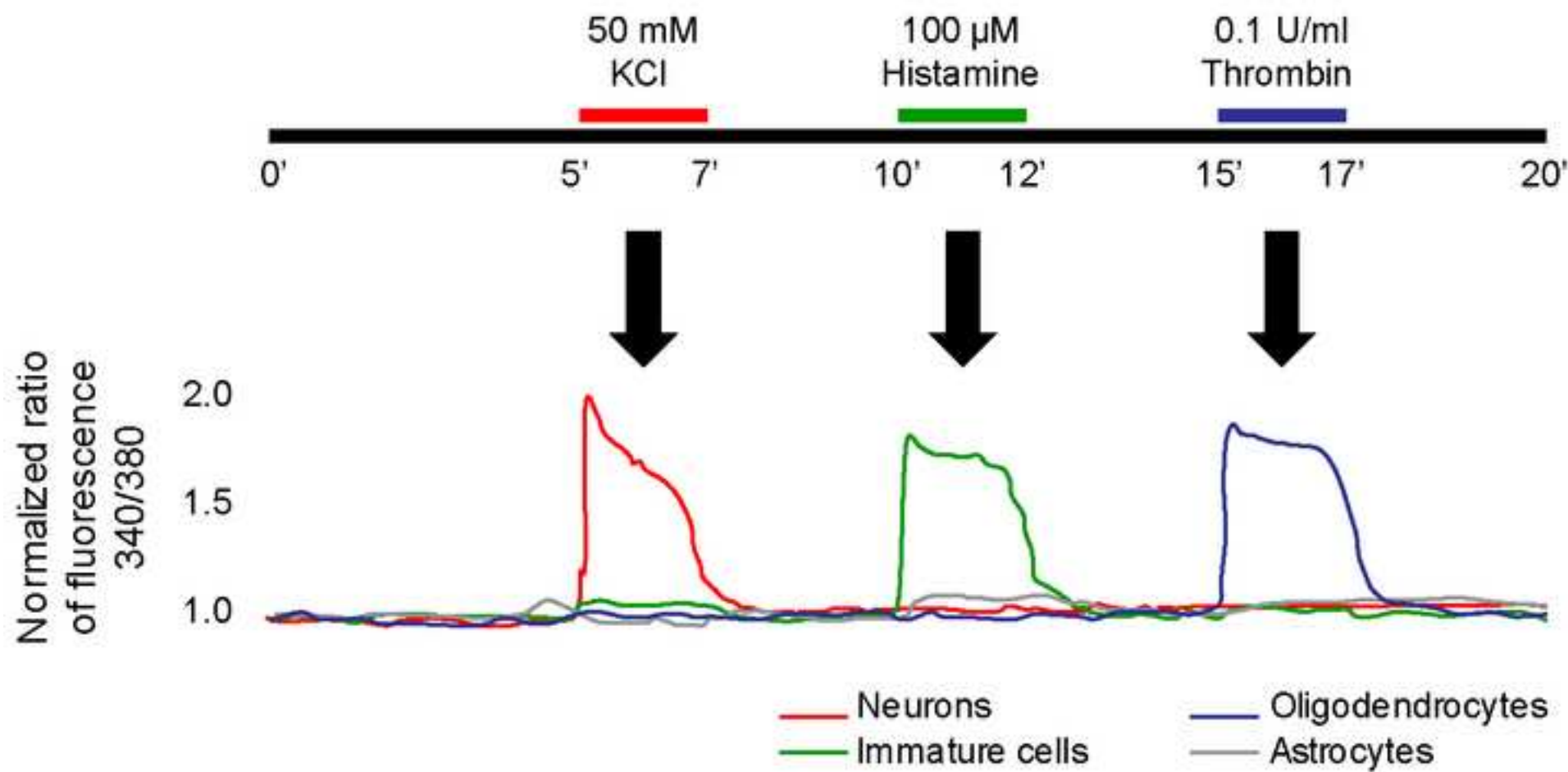


Figure 2

