Biochemical and Biophysical Research Communications 449 (2014) 338–343

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Selective stimulatory action of olfactory ensheathing glia-conditioned medium on oligodendroglial differentiation, with additional reference to signaling mechanisms

Litia A. Carvalho [a,b,c], Louise C. Vitorino [a,c], Roberta P.M. Guimarães [a,c], Silvana Allodi [a,b,c], Ricardo A. de Melo Reis [a,b,c,1], Leny A. Cavalcante [a,b,*,1]

[a] Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Ilha do Fundão, 21949-902 Rio de Janeiro, Brazil
[b] Programa de Pós-Graduação em Ciências Biológicas (Fisiologia), Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Ilha do Fundão, 21949-902 Rio de Janeiro, Brazil
[c] Programa de Pós-Graduação em Ciências Biológicas (Biofísica), Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Ilha do Fundão, 21949-902 Rio de Janeiro, Brazil

ARTICLE INFO

*Article history:*
Received 6 May 2014
Available online 20 May 2014

*Keywords:*
Oligodendrocytes
Hippocampus
ERK1
PI3K
p38-MAPK

ABSTRACT

We examined the effects of conditioned medium from olfactory ensheathing glia (OEGCM) on the differentiation of oligodendrocytes in mixed cultures of early postnatal hippocampi. Differentiation was judged from the numerical density (ND) of cells immunoreactive to 2′3′ cyclic nucleotide 3′phosphodiesterase (CNPase) and O4 antibodies. NDs increased according to inverted-U dose–response curves, particularly for CNPase+ cells (9-fold at optimal dilution) and these changes were blocked by inhibitors of ERK1, p38-MAPK, and PI3K. Our results raise the possibility that OEG secreted factor(s) may counteract demyelination induced by trauma, neurodegenerative diseases, and advanced age, and should stimulate novel methods to deliver these factors and/or potentiating chemicals.



## 1. Introduction

Olfactory axons may regenerate after sectioning of the fila olfactoria or within the olfactory fiber layer of the olfactory bulb [1,2]. Numerous studies on the potential of the olfactory ensheathing glia (OEG) for the promotion of axonal growth and/or myelination have been reported [3,4]. Although studies have provided evidence for and against the ability of the OEG themselves to myelinate central axons [5–9], few attempts have been made to test OEG as promoters of the differentiation and eventual myelination of oligodendroglial progenitor cells (OPC) (but see [10] for negative results).

The signaling pathways regulating OEG-neuronal and OEG-glial interactions are virtually unknown. This may be partially due to our poor understanding of OEG neurotrophins and other extracellular signals required for survival of neurons and/or oligodendrocytes [11,12]. Primary OEG are known to produce several trophic factors [13, 14, reviewed in 15], but only nerve growth factor (NGF), neurotrophin-4/5 (NT4/5) and neuregulin are considered to be secreted [13,16]. There is also some uncertainty regarding brain-derived neurotrophic factor (BDNF), which is thought to be produced in very small amounts [13,14] but seems to contribute significantly to axonal regeneration of cultured adult CNS neurons [17] and to be involved in OEG-enhanced axon regeneration, even on an unfavorable substrate such as myelin [18].

BDNF has also been described as involved in myelination by a direct action on oligodendrocytes [19]. Indeed, BDNF and its receptor, tropomyosin-related kinase B (TrkB), trigger several well-defined signaling cascades, including the Extracellular Signal-Regulated Kinase 1 (ERK1/MEK1) and the phosphatidylinositol-3 kinase (PI3K) [20,21]. There is also considerable work on p38 mitogen-activated protein kinases (p38MAPK) regulation of oligodendrocyte differentiation under the influence of insulin-like growth factor (IGF-1), or fibroblast growth factor 2 (FGF-2), but not BDNF [22,23].

In this study, we used a medium conditioned by cultured adult OEG (OEGCM) at different dilutions to investigate whether there is a monotonous (increasing or decreasing) change in the numerical density (ND) of cultured perinatal hippocampal cells expressing

* Corresponding author at: Programa de Neurobiologia, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Bloco G2-001, Ilha do Fundão, 21949-902 Rio de Janeiro, Brazil. Fax: +55 21 22808193.
*E-mail address:* leny@biof.ufrj.br (L.A. Cavalcante).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.05.051
0006-291X/

either of two markers of oligodendroglial differentiation: (a) 2′-3′ cyclic nucleotide 3′-phosphodiesterase, a protein mediating process outgrowth in OPCs [24,25], and (b) O4 antibody, a marker of gangliosides appearing at the pro-oligodendroblast stage as well as of the late-appearing glycolipids sulfatide and seminolipid [26,27]. To study signaling by OEG soluble factors, we used inhibitors for the ERK1, p38MAPK and PI3K signaling pathways.

## 2. Material and methods

### 2.1. Animals

Animal care was according with recommendations of the Sociedade Brasileira de Neurobiologia e Comportamento (Brazilian Society for Neurobiology and Behavior), and was approved by the Committee for the Use of Experimental Animals of our institution (CEUA IBCCF Protocol number 020).

### 2.2. Isolated cultures of olfactory ensheathing cells

OECs were collected and purified following our modification of a protocol previously described by [28]. Briefly, the olfactory nerve layer was removed and dissociated with a solution of 0.01% ethylene diamine tetraacetic acid (EDTA; Invitrogen, USA) [29,30]. After the removal of fibroblasts, astrocytes and oligodendrocytes by adhesion as in [28], the cells were plated onto laminin-coated (40 μg/mL, Sigma) 24-well cell plates in complete DMEM/F12 medium. The cells were maintained in 5% $CO_2$ at 37 °C, and the medium was changed every second day. After the cell culture had reached about 75% confluence (three weeks), the conditioned medium (OEGCM) was collected, filtered through a 0.2 mm membrane pore, aliquoted and stored at −70 °C until use.

### 2.3. Hippocampus mixed cell cultures

Hippocampus cell cultures were prepared following modifications of a protocol previously described by [31]. Briefly, four 0 to 2-day-old (postnatal; P0–P2) Wistar rats were decapitated, their brains removed and the hippocampus dissected. Dissociated filtered cells were plated on poly-L-lysine-coated (PLL, 100 μg/ml) glass coverslips at a final density of $10^4$ cells/coverslip. The cells were maintained in complete NB27 – Neurobasal medium A (Life Technologies) containing 2% B27 supplement (Life Biotechnology), termed (NB27, positive control) plus 2 mM L-glutamine, penicillin (50 mg/ml) and streptomycin (50 U/ml), or under either of the following conditions: DMEM/F12 + 10% FBS only (negative control) or NB27 containing OEGCM dilutions (1:1; 1:3; 1:5; 1:10, and 1:20), in a 5% $CO_2$ atmosphere at 37 °C for 72 h. After this period, the cultures were fixed with 4% PF, washed with PBS, and stored in this solution at 4 °C until processing for immunofluorescence.

### 2.4. Inhibition of survival signaling pathways in hippocampal cells

Once the optimal dilution of OEGCM (1:5) for maintenance of hippocampal cells was established, three well-known inhibitors

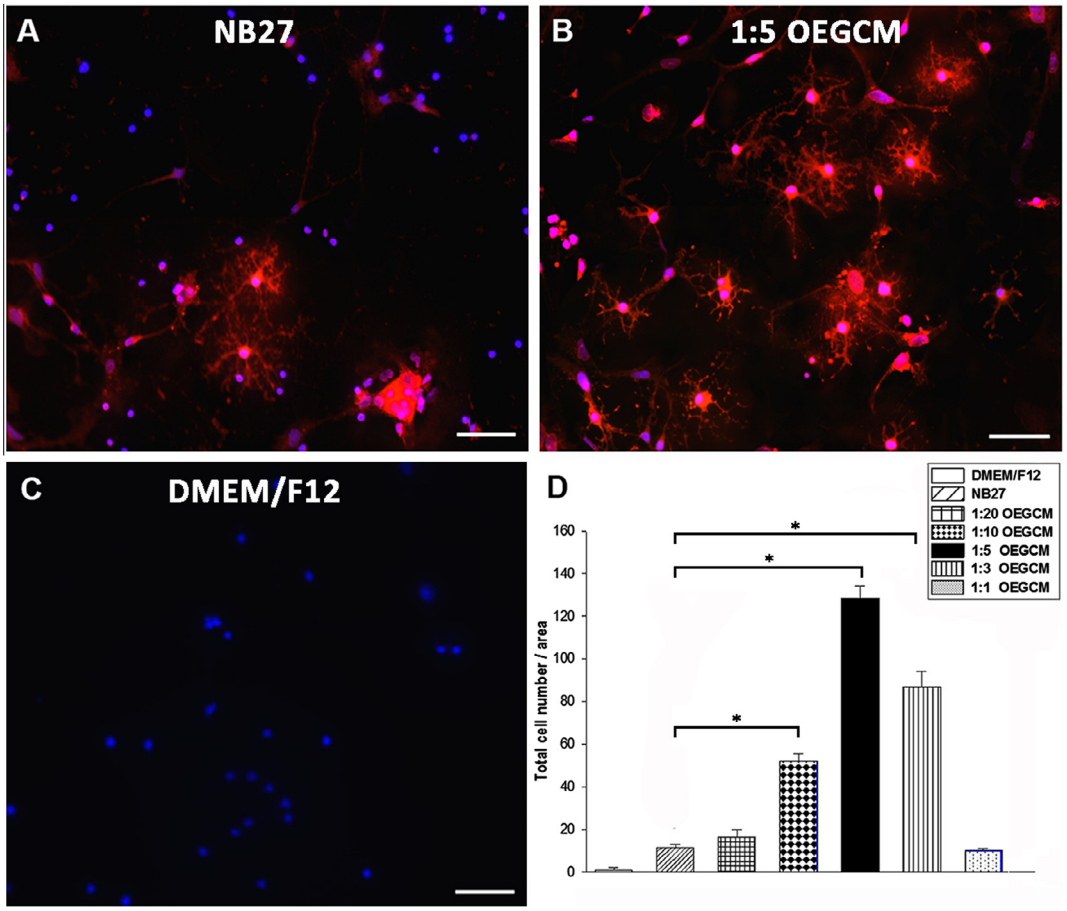


**Fig. 1.** OEG conditioned medium (OEGCM) increases the ND of CNPase-positive oligodendroglia in early postnatal hippocampal cultures, after 3 days of treatment. (A) Culture maintained in NB27; (B) treatment with 1:5 OEGCM; (C) culture in DMEM/F12; (D) quantification in all groups. Secondary antibody labeled with Alexa 594 (also in Fig. 2), nuclei stained with DAPI (all figures). Observe the inverted-U dose–response pattern, with maximal mean response (9× baseline) at 1:5 OEGCM. One-way ANOVA, Bonferroni test (treatment vs. culture in NB27), $^*p < 0.05$. ND = numerical density. Scale bar = 50 μm.

of signaling pathways related to cell survival were tested. These inhibitors (Cell Signaling, USA) were: LY294002, inhibitor of PI3K; SB203580, inhibitor of p38MAPK; and PD98059, inhibitor of ERK1, used at either 10 or 50 μM final concentrations. The cultures were maintained in 5% $CO_2$ at 37 °C for 48 h and fixed with 4% PF, washed with PBS and stored in this solution at 4 °C until processing for immunofluorescence.

### 2.5. Immunocytochemistry

Fixed cells on coverslips were processed for anti-CNPase (monoclonal mouse IgG, 1/100 ascitic fluid, Sigma–Aldrich, cat#C5922) according to [32], whereas for O4 (monoclonal mouse IgM, hybridoma, kindly donated by Dr. Adan Aguirre, SUNY – Stony Brook) we followed [33]. After that, the coverslips were washed with PBS/Triton or PBS and incubated with appropriate species- or subclass-specific secondary antibodies conjugated to Alexa Fluor 488 or 546 (1:500, Life Technologies) for 2 h at room temperature. Coverslips were mounted on glass slides with *n*-propyl gallate (Sigma–Aldrich).

### 2.6. Cell counts and statistical analysis

For cell counts, image mosaics containing 100 images/coverslip (14.33 $mm^2$ total area/coverslip), were obtained with an epifluorescence microscope (AxioImager M2, Carl Zeiss) and digital images were generated by AxioVision® Imaging software (Carl Zeiss). The graphs show the ND, i.e., the mean number in 3 experiments of cells positive for a specific marker in 10 fields (about 1.4 $mm^2$).

Data are expressed as mean ± standard error of the mean (SEM). Statistical evaluation was performed by One-way ANOVA followed by Bonferroni post-test for multiple comparisons (GraphPad Prism, version 5.0.0.288). Differences were considered statistically significant for $P < 0.05$.

## 3. Results

### 3.1. OEGCM increases the numerical density of CNPase-positive cells in neonatal hippocampal cultures

Previous studies employing cultures generated from the embryonic rat spinal cord plated on OEG showed that oligodendrocyte process extension was rather poor, perhaps due to a lack of secreted factors [10]. Since some growth factors, such as BDNF, have a regionally specific effect on oligodendrocytes [34], we approached this question by employing a different culture system and using conditioned medium to determine whether putative OEG-secreted trophic factors changed the differentiation potential of early postnatal hippocampal OPCs. First, we evaluated whether OEGCM was able to increase the number of cells reactive for CNPase, a marker of oligodendroglial lineage cells from the progenitor stage up to the mature oligodendrocyte [25,24,35,36]. As shown in Fig. 1, the addition of OEGCM from 1:10 to 1:3 increased significantly the ND of CNPase-positive cells, with a peak at 1:5 (9 times the ND of NB27 medium only).

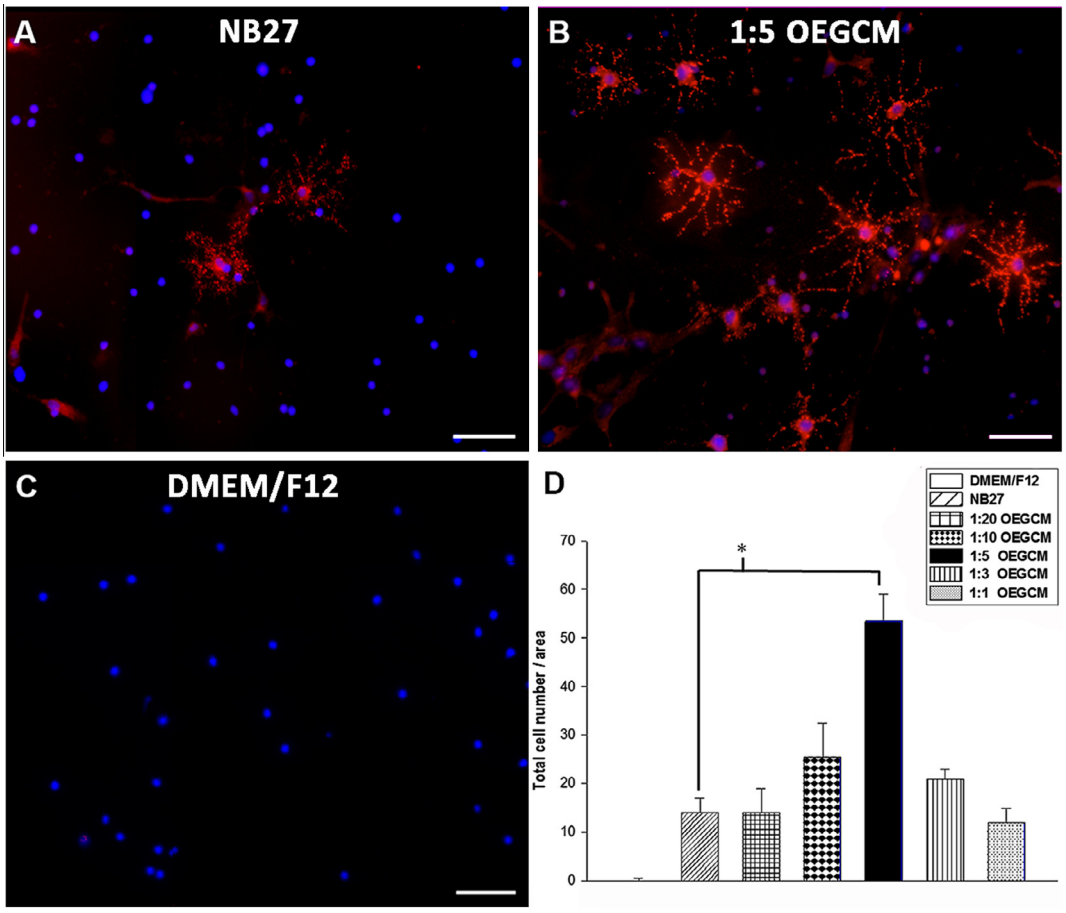


**Fig. 2.** OEGCM increases the ND of O4-positive oligodendroglia in cultures, as in Fig. 1. Culture conditions in A–C, fluorophores, and as in Fig. 1. Observe in D a similar trend of dose–response as in Fig. 1, but a less-marked mND increase (maximal mean response = 1.8× baseline) and a significance level reached only at 1:5 OEGCM. Statistics and abbreviations as in Fig. 1. Scale bar = 50 μm.

### 3.2. OEGCM increases the numerical density of O4-positive cells in neonatal hippocampal cultures

The majority of oligodendrocytes (OLs) are generated postnatally and pass through a series of phenotypic stages from immature to mature myelin-forming cells [36]. The progression along the OL-specific lineage can be characterized by the appearance of the O4 antigen [37]. Therefore, we investigated whether trophic activities secreted by OEG were able to increase the number of cells reactive for O4 antibody. As shown in Fig. 2, the addition of 1:5 OEGCM (in NB27 medium) increased the ND of O4-positive cells by 2.4 times (Fig. 2B and D); One-way ANOVA (Fig. 2A and D).

### 3.3. OEGCM effects on CNPase-positive OPCs/OLs are mediated through PI3K, ERK1 and p38MAPK signaling pathways in neonatal neural hippocampal cultures

Several trophic factors have been reported to induce differentiation and survival through activation of signaling transduction pathways, such as the PI3-K and/or the mitogen-activated protein

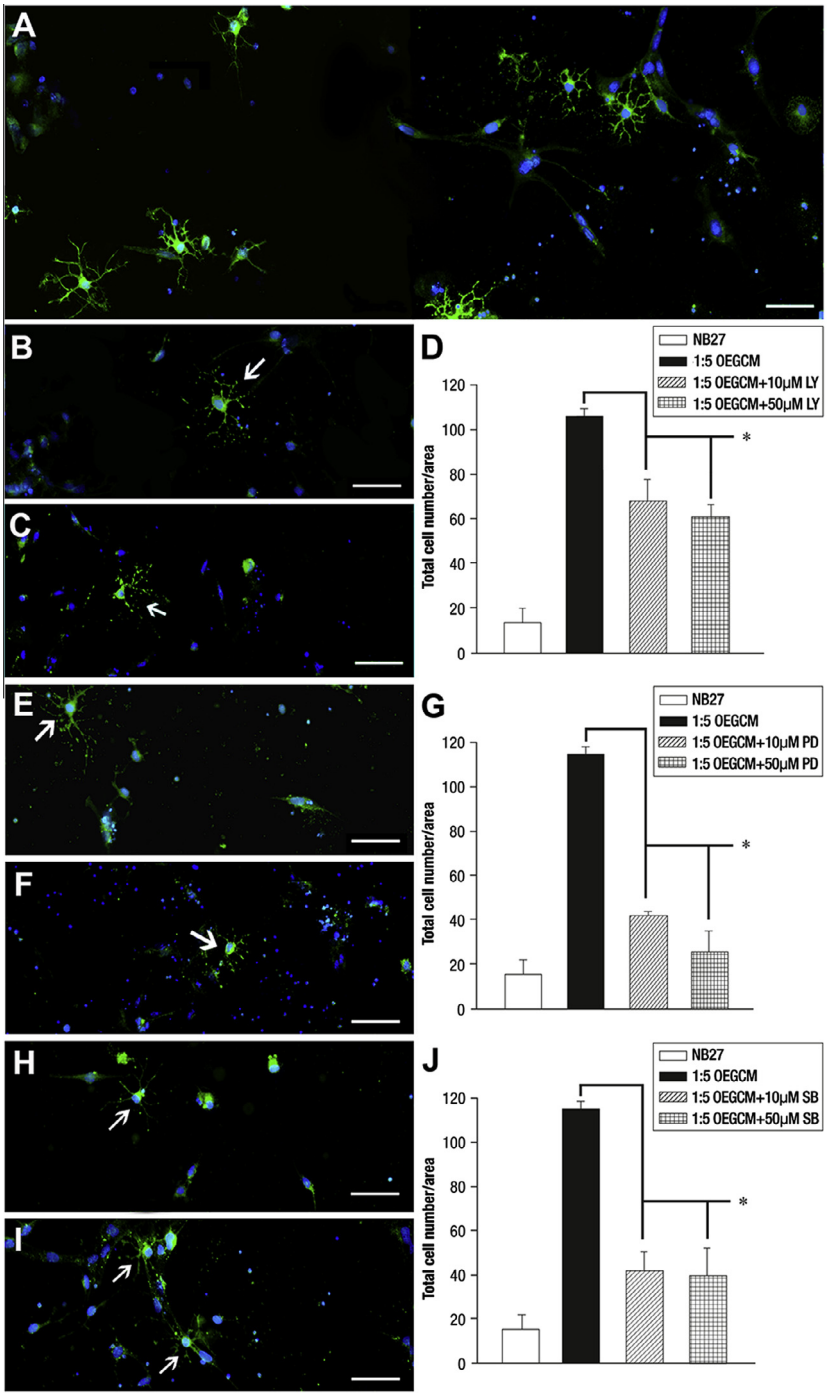


**Fig. 3.** The augmenting effect of OEGCM on CNPase + cells depends on the PI3K, and more markedly on the ERK1 and p38MAPK pathways. (A) Culture treated with 1:5 OEGCM for 2 days; (B and C) as in A plus 10 μM (B) or 50 μM (C) LY 294002; (E and F) as in A plus 10 μM (E) or 50 μM (F) PD98059; (H and I) as in A plus 10 μM (H) or 50 μM (I) SB203580; (D, G and J) quantification of ND after use of signaling inhibitors. Observe that cultures treated with inhibitors (small panels, arrows) show cells with apparently smaller processes than cells in the untreated culture. Statistics and abbreviations as in Fig. 1. Scale bar = 50 μm.

kinases, including the ERK1/MEK1 and the p38MAPK. In order to evaluate if these pathways are activated by OEGCM, the hippocampal cells were cultured for 2 days in the presence of LY294002, a PI3K inhibitor; or in the presence of PD98059, a known inhibitor for the ERK1/MEK1; or in the presence of SB203580, a p38MAPK inhibitor; and then reacted with an anti-CNPase antibody. Fig. 3 shows that addition of 10 μM (68 ± 9, Fig. 3B and D) or 50 μM LY294002 (61 ± 5, Fig. 3C and D) decreased the ND of CNPase-positive cells from differentiated hippocampal progenitors maintained in the presence of OEGCM for 48 h when compared to differentiated oligodendrocyte lineage cells in the absence of the inhibitor (105 ± 3; Fig. 3A). The same phenomena were observed after addition of 10 μM (38 ± 2, Fig. 3E and G) or 50 μM PD98059 (23 ± 9, Fig. 3F and G); or after the addition of 10 μM (38 ± 8, Fig. 3E and G) or 50 μM SB203580 (36 ± 11, Fig. 3F and G), showing a reduction of the ND of CNPase-positive cells from hippocampal progenitors after 48 h when compared to differentiated oligodendrocyte-lineage cells in NB27 + 1:5 OEG (105 ± 3; Fig. 3A).

## 4. Discussion

In this study we demonstrated for the first time that OEGCM can influence early postnatal hippocampal progenitor cells toward an oligodendroglial phenotype.

Although young neurons, as judged by βIII-tubulin immunoreactivity, were about 15 times more numerous in NB27 medium (not shown) than cells labeled with the general oligodendroglial marker, CNPase, the change in CNPase+ cells after OEGCM treatment at optimal dilution for only 2 days was marked.

This could not be attributed to exhaustion of neuron generation in early postnatal life in the hippocampus. Indeed, the behavior of progenitors in the CA1 and CA3 subfields changes with age. In fact, the percentages of neurons and oligodendrocytes differentiated from cultured neurospheres are about equal from both of these subfields at P4, although oligodendrocytes exceed neurons by 4 to 8-fold at P12 [38]. Additionally, our results from the use of specific inhibitors indicate that the effects of OEGCM on oligodendrocyte progenitors are mediated by the PI3K, ERK1, and p38MAPK pathways.

Several soluble factors affect oligodendrocyte differentiation, such as Neurotrophin 3 (NT3) [39], BDNF [40], and ciliary neurotrophic factor (CNTF) [41]. Other factors, implicated predominantly in myelination proper, are fibroblast growth factor (FGF) [42] and insulin-like growth factor 1 (IGF-1) [43]. However, this proposed specialization is not absolute, since, for instance, FGF-2 is a mitogen for OPCs in the pro-oligodendrocyte or late progenitor stage (O4+, galactocerebroside-negative) [44]. Furthermore, IGF-1 can promote proliferation, survival and differentiation of OPCs [45].

PI3K is a master regulator that can be activated by growth factors and cytokines, which have been extensively studied by Vanhaesebroech's group [46] in a variety of animal models and cell systems, but not including oligodendrocytes or involving their more important growth factors, with the possible exception of a PDGF. Our use of LY294002, an inhibitor of PI3K, resulted in reduction of the number of CNPase+ cells in vitro. There is evidence indicating that PI3K/Akt is a crucial pathway for the proliferation, differentiation and survival of OPCs [45,47]. Furthermore, inhibition of the lipid phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome 10), an inhibitor of the PI3K/Akt pathway, potentiates IGF-1 effects by increasing proliferation of OLPs in a concentration-dependent manner [48]. Nevertheless, we have no information on the presence of IGF-1 in OEGCM, although this factor has been reported to be present in OEG by immunocytochemistry [49].

The kinase p38MAPK was initially described as involved in the regulation of inflammatory cytokine biosynthesis [50]. However, its description as colocalized with CNPase in normal brain white matter provided a clue to its involvement in cell growth and survival [51]. The participation of p38 MAPK in Schwann myelination [52] and in morphological differentiation of OPCs [22,53] has been clearly demonstrated. Therefore, we can attribute the reduction of CNPase+ cells that we observed, and an apparent shrinkage of their processes, to the use of SB 203580, an inhibitor of p38MAPK α and β [54].

The role of the ERK1 signaling pathway in the oligodendrocyte lineage was described in proliferation, process extension [55,56], and cytokine-induced oligodendrocyte death [57]. Thus, it is reasonable to consider that the use of the ERK1 inhibitor may have contributed to the reduction of the number of CNPase+ cells, counteracting the beneficial effects of OEGCM addition. It has been shown that ERK1/2 are important regulators of oligodendroglial preprogenitor and early progenitor stages, and can mediate specific stimulatory effects of BDNF on myelin protein expression in the basal forebrain oligodendrocytes [20]. In addition, inhibition of ERK1 in cultures of OPC was accompanied by the inhibition of their differentiation [58] and process extension [59].

To conclude, the importance of this study resides in raising the possibility that a factor or, most probably, multiple factors secreted by OEG might be used in order to counteract demyelination induced by trauma, neurodegenerative diseases, and advanced age. Additionally, because OEG is located in relatively accessible route to the brain, our results may stimulate the development of novel methods to deliver these factors and/or potentiating chemicals.

## Acknowledgments

We thank Dr. Janet Reid for revising the language of the manuscript. This work was supported by grants from the Brazilian Research Council (CNPq), Rio de Janeiro State Foundation for Research Support (FAPERJ), Universidade Federal do Rio de Janeiro Program for Scientific Initiation (UFRJ – PIBIC), and Ministry of Education - Coordination of University Personnel (CAPES).

## References

[1] P.P. Graziadei, G.A. Monti Graziadei, Neurogenesis and neuron regeneration in the olfactory system of mammals. III. Deafferentation and reinnervation of the olfactory bulb following section of the fila olfactoria in rat, J. Neurocytol. 9 (1980) 145–162.
[2] J.R. Doucette, J.A. Kiernan, B.A. Flumerfelt, The re-innervation of olfactory glomeruli following transection of primary olfactory axons in the central or peripheral nervous system, J. Anat. 137 (Pt 1) (1983) 1–19.
[3] R. Devon, R. Doucette, Olfactory ensheathing cells myelinate dorsal root ganglion neurites, Brain Res. 589 (1992) 175–179.
[4] A. Ramon-Cueto, M. Nieto-Sampedro, Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants, Exp. Neurol. 127 (1994) 232–244.
[5] R.J. Franklin, J.M. Gilson, I.A. Franceschini, S.C. Barnett, Schwann cell-like myelination following transplantation of an olfactory bulb-ensheathing cell line into areas of demyelination in the adult CNS, Glia 17 (1996) 217–224.
[6] T. Imaizumi, K.L. Lankford, S.G. Waxman, C.A. Greer, J.D. Kocsis, Transplanted olfactory ensheathing cells remyelinate and enhance axonal conduction in the demyelinated dorsal columns of the rat spinal cord, J. Neurosci. 18 (1998) 6176–6185.
[7] G. Plant, P. Currier, E. Cuervo, M.L. Bates, Y. Pressman, M.B. Bunge, P.M. Wood, Purified adult ensheathing glia fail to myelinate axons under culture conditions that enable Schwann cells to form myelin, J. Neurosci. 22 (2002) 6083–6091.
[8] Y. Akiyama, K. Lankford, C. Radtke, C.A. Greer, J.D. Kocsis, Remyelination of spinal cord axons by olfactory ensheathing cells and Schwann cells derived from a transgenic rat expressing alkaline phosphatase marker gene, Neuron Glia Biol. 1 (2004) 47–55.
[9] J.G. Boyd, J. Lee, V. Skihar, R. Doucette, M.D. Kawaja, LacZ-expressing olfactory ensheathing cells do not associate with myelinated axons after implantation into the compressed spinal cord, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 2162–2166.

[10] A. Sorensen, K. Moffat, C. Thomson, S.C. Barnett, Astrocytes, but not olfactory ensheathing cells or Schwann cells, promote myelination of CNS axons in vitro, Glia 56 (2008) 750–763.
[11] B.A. Barres, R. Schmid, M. Sendnter, M.C. Raff, Multiple extracellular signals are required for long-term oligodendrocyte survival, Development 118 (1993) 283–295.
[12] M.V. Chao, Neurotrophins and their receptors. A convergence point for many signalling pathways, Nat. Rev. Neurosci. 4 (2003) 299–309.
[13] A.V. Boruch, J.J. Conners, M. Pipitone, G. Deadwyler, P.D. Storer, G.H. Devries, K.J. Jones, Neurotrophic and migratory properties of an olfactory ensheathing cell line, Glia 33 (2001) 225–229.
[14] E. Woodhall, A.K. West, M.I. Chuah, Cultured olfactory ensheathing cells express nerve growth factor, brain-derived neurotrophic factor, glia cell line-derived neurotrophic factor and their receptors, Brain Res. Mol. Brain Res. 88 (2001) 203–213.
[15] E.H.P. Franssen, F.M. de Bree, J.V. Verhaagen, Olfactory ensheathing glia: their contribution to primary olfactory nervous system regeneration and their regenerative potential following transplantation into the injured spinal cord, Brain Res. Rev. 56 (2007) 236–258.
[16] R.J. Thompson, B. Roberts, C.L. Alexander, S.K. Williams, S.C. Barnett, Comparison of neuregulin-1 expression in olfactory ensheathing cells, Schwann cells and astrocytes, J. Neurosci. Res. 61 (2000) 172–185.
[17] E. Pastrana, M.T. Moreno-Flores, J. Avila, F. Wandosell, L. Minichiello, J. Diaz-Nido, BDNF production by olfactory ensheathing cells contributes to axonal regeneration of cultured adult CNS neurons, Neurochem. Int. 50 (2007) 491–498.
[18] S.A. Runyan, P.E. Phelps, Mouse olfactory ensheathing glia enhance axon outgrowth on a myelin substrate in vitro, Exp. Neurol. 216 (2009) 95–104.
[19] J. Xiao, A.W. Wong, M.M. Willingham, M. van den Buuse, T.J. Kilpatrick, S.S. Murray, Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes, Neurosignals 18 (2010) 186–202.
[20] Y. Du, L.D. Lercher, R. Zhou, C.F. Dreyfus, Mitogen-activated protein kinase pathway mediates effects of brain-derived neurotrophic factor on differentiation of basal forebrain oligodendrocytes, J. Neurosci. Res. 84 (2006) 1692–1702.
[21] W.H. Zheng, R. Quirion, Comparative signaling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival, J. Neurochem. 89 (2004) 844–852.
[22] G. Fragoso, J.D. Haines, J. Robertson, L. Pedraza, W.E. Mushynski, G. Almazan, P38 mitogen-activated protein kinase is required for central nervous system myelination, Glia 55 (2007) 1531–1541.
[23] L.J. Chew, W. Coley, Y. Cheng, V. Gallo, Mechanisms of regulation of oligodendrocyte development by p38 mitogen-activated protein kinase, J. Neurosci. 30 (2010) 11011–11027.
[24] P.E. Braun, F. Sandillon, A. Edwards, J.M. Matthieu, A. Privat, Immunocytochemical localization by electron microscopy of 2′3′-cyclic nucleotide 3′-phosphodiesterase in developing oligodendrocytes of normal and mutant brain, J. Neurosci. 8 (1988) 3057–3066.
[25] J. Lee, M. Gravel, R. Zhang, P. Thibault, P.E. Braun, Process outgrowth in oligodendrocytes is mediated by CNP, a novel microtubule assembly myelin protein, J. Cell Biol. 170 (2005) 661–673.
[26] I. Sommer, M. Schachner, Cell that are O4 antigen-positive and O1 antigen-negative differentiate into O1 antigen-positive oligodendrocytes, Neurosci. Lett. 29 (1982) 183–188.
[27] R. Bansal, A.E. Warrington, A.L. Gard, B. Ranscht, S.E. Pfeiffer, Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development, J. Neurosci. Res. 24 (1989) 548–557.
[28] H.H. Nash, R.C. Borke, J.J. Anders, New method of purification for establishing primary cultures of ensheathing cells from the adult olfactory bulb, Glia 34 (2001) 81–87.
[29] H. Macedo-Ramos, F.S.O. Campos, L.A. Carvalho, I.B. Ramos, L.M. Teixeira, W. De Souza, L.A. Cavalcante, W. Baetas-da-Cruz, Olfactory ensheathing cells as putative host cells for *Streptococcus pneumoniae*: evidence of bacterial invasion via mannose receptor-mediated endocytosis, Neurosci. Res. 69 (2011) 308–313.
[30] L.A. Carvalho, A.F. Nobrega, I.D.P. Soares, S.L. Carvalho, S. Allodi, W. Baetas-da-Cruz, L.A. Cavalcante, The mannose receptor is expressed by olfactory ensheathing cells in the rat olfactory bulb, J. Neurosci. Res. 91 (2013) 1572–1580.
[31] G.J. Brewer, Isolation and culture of adult rat hippocampal neurons, J. Neurosci. Methods 71 (1997) 143–155.
[32] A. Santos-Silva, L.A. Cavalcante, Expression of the non-compact myelin protein 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) in olfactory bulb ensheathing glia from explant cultures, Neurosci. Res. 40 (2001) 189–193.
[33] X. Yuan, R. Chittajallu, S. Belachew, S. Anderson, C.J. McBain, V. Gallo, Expression of the green fluorescent protein in the oligodendrocyte lineage: a transgenic mouse for developmental and physiological studies, J. Neurosci. Res. 70 (2002) 529–545.
[34] Y. Du, T.Z. Fischer, L.N. Lee, L.D. Lercher, C.F. Dreyfus, Regionally specific effects of BDNF on oligodendrocytes, Dev. Neurosci. 25 (2003) 116–126.
[35] V. Younes-Rapozo, J. Berendonk, T. Savignon, A.C. Manhães, P.C. Barradas, Thyroid hormone deficiency changes the distribution of oligodendrocyte/myelinmarkers during oligodendroglial differentiation in vitro, Int. J. Dev. Neurosci. 24 (2006) 445–453.
[36] N. Baumann, D. Pham-Dinh, Biology of oligodendrocyte and myelin in the mammalian central nervous system, Physiol. Rev. 81 (2001) 871–927.
[37] A.L. Gard, S.E. Pfeiffer, Oligodendrocyte progenitors isolated directly from developing telencephalon at a specific phenotypic stage: myelinogenic potential in a defined environment, Development 106 (1989) 119–132.
[38] A.K. Shetty, B. Hattiangady, Postnatal age governs the extent of differentiation of hippocampal CA1 and CA3 subfield neural stem/progenitor cells into neurons and oligodendrocytes, Int. J. Dev. Neurosci. 31 (2013) 646–656.
[39] M.A. Kahn, S. Kumar, D. Liebl, R. Chang, L.F. Parada, J. de Vellis, Mice lacking NT-3, and its receptor TrkC, exhibit profound deficiencies in CNS glial cells, Glia 26 (1999) 153–165.
[40] M.W. Vondran, P. Clinton-Luke, J.Z. Honeywell, C.F. Dreyfus, BDNF+/− mice exhibit deficits in oligodendrocyte lineage cells of the basal forebrain, Glia 58 (2010) 848–856.
[41] B. Stankoff, M.S. Aigrot, F. Noël, A. Wattilliaux, B. Zalc, C. Lubetzki, Ciliary neurotrophic factor (CNTF) enhances myelin formation: a novel role for CNTF and CNTF-related molecules, J. Neurosci. 22 (2002) 9221–9227.
[42] M. Furusho, J.L. Dupree, K.A. Nave, R. Bansal, Fibroblast growth factor receptor signaling in oligodendrocytes regulates myelin sheath thickness, J. Neurosci. 32 (2012) 6631–6641.
[43] J. O'Kusky, P. Ye, Neurodevelopmental effects of insulin-like growth factor signaling, Front. Neuroendocrinol. 33 (2012) 230–251.
[44] W. Baron, B. Metz, R. Bansal, D. Hoekstra, H. de Vries, PDGF and FGF-2 signaling in oligodendrocyte progenitor cells: regulation of proliferation and differentiation by multiple intracellular signaling pathways, Mol. Cell Neurosci. 15 (2000) 314–329.
[45] Q.L. Cui, G. Almazan, IGF-I-induced oligodendrocyte progenitor proliferation requires PI3K/Akt, MEK/ERK, and Src-like tyrosine kinases, J. Neurochem. 100 (2007) 1480–1493.
[46] B. Vanhaesebroeck, S.J. Leevers, G. Panayotou, M.D. Waterfield, Phosphoinositide 3-kinases: a conserved family of signal transducers, Trends Biochem. Sci. 22 (1997) 267–272.
[47] G.S. Vemuri, F.A. McMorris, Oligodendrocytes and their precursors require phosphatidylinositol 3-kinase signaling for survival, Development 122 (1996) 2529–2537.
[48] M.L. De Paula, Q.L. Cui, S. Hossain, J. Antel, G. Almazan, The PTEN inhibitor bisperoxovanadium enhances myelination by amplifying IGF-1 signaling in rat and human oligodendrocyte progenitors, Glia 62 (2014) (2014) 64–77.
[49] G. Gudiño-Cabrera, M. Nieto-Sampedro, Schwann-like macroglia in adult rat brain, Glia 30 (2000) 49–63.
[50] J.C. Lee, J.T. Laydon, P.C. McDonnell, T.F. Gallagher, S. Kumar, D. Green, D. McNulty, M.J. Blumenthal, J.R. Heys, S.W. Landvatter, J.E. Strickler, M.M. McLaughlin, I.R. Siemens, S.M. Fisher, G.P. Livi, J.R. White, J.L. Adams, P. Young, A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, Nature 372 (1994) 739–746.
[51] M. Maruyama, T. Sudo, Y. Kasuya, T. Shiga, B. Hu, H. Osada, Immunolocalization of p38 MAP kinase in mouse brain, Brain Res. 887 (2000) 350–358.
[52] G. Fragoso, J. Robertson, E. Athlan, E. Tam, G. Almazan, W.E. Mushynski, Inhibition of p38 mitogen-activated protein kinase interferes with cell shape changes and gene expression associated with Schwann cell myelination, Exp. Neurol. 183 (2003) 34–46.
[53] N.R. Bhat, P. Zhang, S.B. Mohanty, P38 MAP kinase regulation of oligodendrocyte differentiation with CREB as a potential target, Neurochem. Res. 32 (2007) 293–302.
[54] K. Ono, J. Han, The p38 signal transduction pathway: activation and function, Cell Signal. 12 (2000) 1–13.
[55] R.L. Stariha, S.U. Kim, Protein kinase C and mitogen-activated protein kinase signalling in oligodendrocytes, Microsc. Res. Tech. 52 (2001) 680–688.
[56] R.L. Stariha, S. Kikuchi, Y.L. Siow, S.L. Pelech, M. Kim, S.U. Kim, Role of extracellular signal-regulated protein kinases 1 and 2 in oligodendroglial process extension, J. Neurochem. 68 (1997) 945–953.
[57] M. Horiuchi, A. Itoh, D. Pleasure, T. Itoh, MEK-ERK signaling is involved in interferon-gamma-induced death of oligodendroglial progenitor cells, J. Biol. Chem. 281 (2006) 20095–20106.
[58] S.L. Fyffe-Maricich, J.C. Karlo, G.E. Landreth, R.H. Miller, The ERK2 mitogen-activated protein kinase regulates the timing of oligodendrocyte differentiation, J. Neurosci. 31 (2011) 843–850.
[59] V. Younes-Rapozo, L.O. Felgueiras, N.L. Viana, I.M. Fierro, C. Barja-Fidalgo, A.C. Manhães, P.C. Barradas, A role for the MAPK/ERK pathway in oligodendroglial differentiation in vitro: stage specific effects on cell branching, Int. J. Dev. Neurosci. 27 (2009) 757–768.