Lysophosphatidic Acid Induces Clonal Generation of Mouse Neurospheres via Proliferation of Sca-1- and AC133-Positive Neural Progenitors

STANISLAV I. SVETLOV,1,2 TATYANA N. IGNATOVA,1 KEVIN K.W. WANG,1,2 RONALD L. HAYES,1,2 DENIS ENGLISH,3 and VALERY G. KUKEKOV 1

ABSTRACT

Neural stem/progenitor cells are clonogenic in vitro and produce neurospheres in serum-free medium containing epidermal growth factor (EGF) and fibroblast growth factor (FGF2). Here, we demonstrate that lysophosphatidic acid (LPA) instigated the clonal generation of neurospheres from dissociated mouse postnatal forebrain in the absence of EGF and FGF2. LPA induced proliferation of cells which co-expressed Sca-1 antigen and AC133, markers of primitive hematopoietic and neural stem/progenitor cells. Clonal expansion of these cells induced by LPA was inhibited by diacylglycerol-pyrophosphate (DGPP), an antagonist of the LPA receptor subtypes LPA1 and LPA3. Moreover, Sca-1- and AC133-positive cells of these neurospheres expressed LPA1, LPA2, and LPA3, suggesting important roles for these LPA receptors in proliferation of neural progenitors. LPA induced neurospheres to differentiate on an adherent laminin/poly-L-ornithine matrix. In differentiating neurospheres, LPA receptors co-localized with βIII-tubulin, nestin, and CNPase, but not with glial fibrillary acidic protein (GFAP), a marker of astrocyte lineage. Our results demonstrate for the first time that lysophosphatidic acid induces clonal neurosphere development via proliferation of AC133/Sca-1-positive stem cells by a receptor-dependent mechanism. This differentiation was characterized by the initial co-localization of neural specific antigens at sites of LPA receptor expression upon their interaction with the inducing agonist.

INTRODUCTION

Neural stem/progenitor cells are able to self-renew as well as differentiate into neuronal cells and glia in vitro. These cells differentiate clonally into neurospheres in serum-free medium containing epidermal growth factor (EGF) and fibroblast growth factor (FGF2) from single pluripotent stem cells. Subsequent differentiation of cells within the developing neurosphere results in a heterogeneous population of cells, including immature neurons and glia (1–5). Although propagation and differentiation of the cells in neurospheres can be achieved by EGF and FGF2, other factors as yet unidentified may play critical roles in neural development from stem cells.

In contrast to EGF and FGF2, the role of biologically active lipid growth factors such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) in stem and progenitor cell proliferation and differentiation is undefined. LPA is an endogenous lysophospholipid acting predominantly via three subtypes of G-protein-coupled LPA receptors: LPA1, LPA2, and LPA3 (6,7). Biological activities of LPA are related to cell survival and prolif-

1Department of Neuroscience, 2Center for Traumatic Brain Injury Studies, McKnight Brain Institute, University of Florida, Gainesville, FL 32610.
3The Experimental Cell Research Program, Methodist Research Institute, Indianapolis, IN 46202.
eration, cell motility, and morphogenetic differentiation, and are bidirectional. In postmitotic neurons, LPA induced growth cone collapse and cell death (8,9), whereas in oligodendrocytes, Schwann cells, and astrocytes, LPA promoted cell proliferation and survival (10–12). In the developing brain, the LPA₁ was abundantly expressed at sites of neurogenesis and the ventricular zone of cerebral cortex, and the receptor expression was restricted to the timeframe of neuroblast differentiation into mature neurons (13, 14). Similarly, oligodendrocytes expressed the LPA₁ only within a limited period of differentiation from the precursor to myelinated cells (15). Although these studies suggest an important role for LPA in neurogenesis, definitive information regarding the activity of LPA in proliferation or differentiation of primitive neural progenitors in vitro is lacking.

In this study, we show that LPA induces clonal generation of neurospheres from dissociated forebrain tissue in the absence of EGF and FGF2 via a receptor-dependent pathway. In these clones, LPA evoked proliferation of AC133 and Sca-1 antigen-positive early stem/progenitor cells, which co-expressed LPA₁, LPA₂, and LPA₃. LPA-generated clonal neurospheres were able to differentiate into cells of neuronal, oligodendrocyte, and astrocyte lineages. Taken together, these data reveal novel clonogenic activities for LPA toward neural stem cells and suggest an important role for the lipid in regulation of neural development.

MATERIALS AND METHODS

Brain tissue dissociation and cell preparation

Experiments were performed in C57BL/J6 mice at 7 day of postnatal age according to animal use protocol approved...
by IACUC at the University of Florida. Brain dissociation and cell isolation were performed essentially as described previously (1,16,17). Briefly, mouse forebrain tissue (typically from 5 mice for preparation) was transferred to a beaker containing 0.25% trypsin and EDTA and mixed on a magnetic stir plate for 15 min. Suspension was triturated with a plastic pipette, filtered through sterile gauze, collected in a 15-ml tube, and centrifuged for 5 min at 1200 rpm. The final pellet was resuspended in small volume of serum-free DMEM/F12 medium, verified visually to contain only single cells, and counted in a hemocytometer.

**Cell and neurosphere culture protocol**

The cells (10^5 cell/well) were placed in serum-free medium Dulbecco’s modified Eagle medium (DMEM)/F12, 1:1 supplemented with 0.8% methylcellulose (MC), insulin in the presence of 10 μM oleoyl-LPA (Avanti Polar Lipids, Inc) alone or in combination with 1 μM or 50 μM dioctanoylglycerol pyrophosphate (DGPP; C8:0, Avanti Polar Lipids, Inc.). Stock solutions of LPA were prepared as described in Materials and Methods in the presence of 10 μM LPA (1-oleoyl-2-hydroxy-sn-glycero-3-phosphate) or EGF + FGF2 (10 ng/ml each). Measurement of neurosphere size was performed at day 14 of growth in semisolid MC medium using phase-contrast microscope equipped with morphometry software. At least 200 spheres were measured in each experimental group; experiments were performed in triplicate. An unpaired t-test was used to assess significance of mean difference in experimental groups.

**Table 1. Quantitative Analysis of Clonal Neurospheres Generated by LPA as Compared to EGF + FGF2**

<table>
<thead>
<tr>
<th>Neurosphere parameter</th>
<th>LPA (10 μM)</th>
<th>EGF (10 ng/ml) + FGF2 (10 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (μm)</td>
<td>Number per field</td>
</tr>
<tr>
<td>Mean</td>
<td>79.5±</td>
<td>11.9±</td>
</tr>
<tr>
<td>Standard error (SEM)</td>
<td>2.76</td>
<td>4.4</td>
</tr>
<tr>
<td>Minimum</td>
<td>14.1</td>
<td>3</td>
</tr>
<tr>
<td>Maximum</td>
<td>204</td>
<td>17</td>
</tr>
</tbody>
</table>

a pn < 0.05.
bp p > 0.05 versus EGF + FGF2.

Neurospheres were produced as described in Materials and Methods in the presence of 10 μM LPA (1-oleoyl-2-hydroxy-sn-glycero-3-phosphate) or EGF + FGF2 (10 ng/ml each). Measurement of neurosphere size was performed at day 14 of growth in semisolid MC medium using phase-contrast microscope equipped with morphometry software. The neurospheres in six to eight visual fields per well were measured in a six-well plate under objective magnification of 20×. At least one plate per experimental condition was analyzed from one experimental preparation. The experiments were performed in triplicate. After 14 days of culture in MC medium, neurospheres were collected, washed, and plated onto glass cover slips sequentially coated with laminin and poly-L-ornithine in 12-well plates at a density of 10–20 neurospheres per cover slip in the presence of serum. After 4–6 h, the spheres are attached to the matrix. At this time, the medium was aspirated, and the cover slips were fixed for immunocytochemistry. In separate experiments, the medium was replaced with serum-free medium containing 10 μM LPA, and incubations were continued for additional 2 weeks to allow the cells to differentiate. The medium supplemented with 10 μM LPA was replaced every 3 days. The cover slips were fixed 14 days after culturing of adhered neurospheres, and then processed for immunocytochemistry.

**Immunocytochemistry of neurospheres**

Cover slips were fixed for 10–15 min with ice-cold 4% paraformaldehyde solution in phosphate-buffered saline (PBS) containing 2% sucrose, or with −20°C methanol, rinsed three times with PBS, and permeabilized with 0.5% Triton-X100 in PBS/2% sucrose for 5 min on ice. Cover slips were blocked with 20% goat serum (GS) in PBS for 30 min, and incubated with primary antibody in 20% of GS in PBS for 45 min. The following antibodies against LPA receptors were used at dilution of 1:100. Rabbit polyclonal antibody raised against the carboxy-terminal fragments of LPA1, LPA2, and LPA3 were purchased from Exalphan Biologials. Mouse monoclonal antibody against amino-terminal parts of LPA1 and LPA3 were gifts from Dr. Ken-
neuronal progenitors, whereas addition of LPA continuously supplemented with LPA (Fig. 1B). The cover slips were washed three times with PBS, and incubated with the secondary antibody labeled with fluorescent dye for 30 min. After extensive wash with PBS, the immunostaining procedure was repeated using secondary antibody coupled with different fluorescent dye. Finally, cover slips were mounted, and analyzed in laser confocal fluorescent microscope using appropriate filters.

Statistics

An unpaired t-test was used to assess significance of mean difference, and an F-test was employed to compare variances in neurosphere size and number in different experimental groups. p Values less than 0.05 were considered as statistically significant.

RESULTS

Lysophosphatidic acid induces clonal generation and supports growth of mouse neurospheres via LPA receptor-dependent pathways

The cells recovered after complete dissociation of mouse forebrain to single cells were plated at a density of 10⁵ cells/well in serum-free semisolid MC medium containing 10 μM oleoyl-LPA(C₁₈:1). Addition of MC to the medium prevented lateral diffusion of the cells and formation of cell aggregates within 1 h after plating as assessed by optical microscopy. A total 72–96 h after initiation of cultures, the cells maintained in the presence of LPA formed clones consisted of several cells (Fig. 1A). These cells continued to grow and formed distinguishable neurospheres during 1 week in culture. Cell aggregates and mostly tissue debris were found in the cultures without any growth factors, and they were discarded at this time point. Neurosphere growth persisted during the second week in semisolid medium continuously supplemented with LPA (Fig. 1B).

Neurosphere generation by LPA in the presence of DGPP, an antagonist of LPA₁/LPA₃ receptor, was examined by counting the size and number of neurospheres. DGPP at low concentrations preferentially inhibits LPA₃ but not LPA₁ activation by LPA, whereas, at high concentrations of DGPP, both LPA₁ and LPA₃ are inhibited (18,19). DGPP at 1 μM reduced significantly the size of neurospheres generated by LPA, whereas addition of 50 μM DGPP decreased considerably both the size and the number of LPA-generated clones formed (Fig. 1C,D).

These data suggest that the initial generation of clones by LPA requires predominantly the LPA₁ receptor, whereas the subsequent cell propagation and neurosphere growth are dependent on both LPA₁ and LPA₃ activation.

LPA alone generated the same number of neurospheres per field as did EGF + FGF2, whereas the diameter of neurospheres produced by LPA was significantly smaller than neurospheres generated by EGF + FGF2 (Table 1). There was no significant additive effect of combination of LPA and EGF + FGF2 on generated neurosphere size and number (data not shown).

LPA instigates growth of clonal neurospheres via proliferation of AC133- and Sca-1-positive cells, which co-express LPA receptors

Aliquots of LPA-generated neurospheres were placed onto glass cover slips coated with laminin/poly-L-ornithine and allowed to attach in presence of serum for 4–6 h. A characteristic neurosphere formed at this time point is shown in Fig. 2a (inset).

LPA-generated clonal neurospheres expressed both AC133 and Sca-1 antigens, markers of most primitive stem/progenitor cells, which markedly co-localized throughout the developing neurosphere (Fig. 2a–c). AC133 and Sca-1 co-localization was highly reproducible and consistent in neurospheres of different size. The most extensive expression and co-localization of AC133 and Sca-1 occurred in the neurosphere core (Fig. 2a–c). These cells exhibited LPA₁, LPA₂, and LPA₃, three subtypes of receptors for LPA (Fig. 2d–i). Strikingly, these receptors also co-localized in the neurosphere core (Fig. 2f,i, arrowheads). As shown in Fig. 2, the LPA₁, LPA₃, and, particularly LPA₂ receptor distribution was not uniform within the neurosphere. In addition, the magnitude of LPA receptor accumulation varied slightly in different neurospheres of the same preparation.

LPA receptors were expressed in cells harboring AC133 and Sca-1 antigen, as judged by a focal co-localization of LPA₁ and LPA₃ with both AC133 and Sca-1 within the neurosphere (Fig. 3). A majority of AC133- and Sca-1-positive cells exhibited comparable LPA₁ and LPA₃ expression levels (Fig. 3, yellow color, arrowheads), which was almost uniformly distributed in co-localization zones within the neurosphere.

LPA-generated clonal neurospheres differentiated into lineage-specific cells and co-expressed lineage markers and LPA receptors

Neurospheres were transferred onto cover slips coated with laminin/poly-L-ornithine, attached in the presence of serum, and then maintained in serum-free medium, sup-
FIG. 2. LPA induces expression of AC133, Sca-1 and LPA receptors in clonal neurospheres. The neurospheres were generated and maintained in cultures for 2 weeks in the presence of LPA. Then, neurospheres were transferred to cover slips coated with laminin/poly-L-ornithine and allowed to attach for 4–6 h in the presence of 10% serum. The cover slips were fixed and examined for the expression of AC133, Sca-1 (a–c), and LPA receptors (d–i) using double immunostaining with antibody against AC133 and Sca-1 antigens, LPA1, LPA2, and LPA3 as described in detail in Materials and Methods. Detection of the proteins was performed by fluorescent dye labeling (red, Texas Red; green, Alexa Green) followed by laser confocal microscopy. The images were taken at the same gain setting for each filter within one experimental set.

(a,b) AC133 (red) and Sca-1 (green) separate-wavelength visualization. (c) AC133/Sca-1 co-localization (yellow) at dual-wavelength visualization. Note the nearly 100% co-localization of AC133 and Sca-1 throughout the neurosphere, with the most extensive expression in the core of the neurosphere (arrowheads).

(d,g) LPA1 (red). (e) LPA3 (green) and (h) LPA2 (green) separate visualization. (f) LPA1/LPA3 co-localization. (i) LPA1/LPA2 co-localization assessed by dual visualization (yellow). Arrowheads indicate the accumulation of LPA receptors in the core of the neurosphere. Note the predominant expression of LPA1 over LPA3 and LPA2 (f,i, magenta color).

FIG. 3. Co-localization of AC133 and Sca-1 with LPA receptors in clonal neurospheres generated by LPA. The neurospheres were generated, grown, and fixed for immunocytochemistry as described in Fig. 2 and in Materials and Methods in detail. The fixed neurospheres were double immunostained with AC133 or Sca-1 and LPA1 or LPA3 antibody, followed by development of bound proteins with secondary antibody coupled with different fluorescent dyes. The images were taken using a laser confocal microscope and red, green, and dual filters. The images obtained using dual filters are shown.

(a) LPA1 (red)/Sca-1 (green), co-localization-yellow. (b) LPA1 (red)/AC133 (green). (c) LPA3 (red)/Sca1(green), co-localization-yellow. (d) LPA3 (red)/AC133 (green), co-localization-yellow. Arrowheads depict the areas of most extensive co-localization. The results shown are representative pictures of three independent experiments from two different neurosphere preparations.
plemented with LPA for additional 2 weeks. During this time, the cells migrated from the core of the neurosphere and differentiated, although the spheres retained a dense core containing several cellular layers (Fig. 4, inset).

The differentiated neurospheres expressed βIII-tubulin, a marker of immature neurons, which was co-localized with LPA1 and LPA3 predominantly in the residual core of the neurosphere (Fig. 3a,c, yellow color, arrowheads). A significant expression of nestin was also found that correlated with LPA1 and LPA accumulation (Fig. 3). A substantial prevalence of CNPase expression occurred in CNPase-positive cells as indicated by dual immunostaining (Fig. 5a,c, yellow color, arrowheads). In addition, expression of LPA3 receptors occurred in the cells, which did not exhibit significant amount of nestin (Fig. 4d, green color).

The cells of differentiated neurosphere expressed both CNPase and GFAP, markers of oligodendrocyte and astrocyte lineages (Fig. 5). Expression of LPA receptors occurred in CNPase-positive cells as indicated by dual immunostaining (Fig. 5a,c, yellow color, arrowheads). There was a substantial prevalence of CNPase expression over LPA1 and LPA3 (Fig. 5a,c, magenta color), whereas a separate expression of LPA1 was observed in several cells of neurosphere (Fig. 5a, green color). In contrast, the cells, which expressed the astrocyte lineage marker GFAP, did not accumulate significant amounts of LPA1 or LPA3 (Fig. 5b,d).

**DISCUSSION**

It has been shown previously that LPA stimulates proliferation of cortical neuroblasts (13,14,20), possibly via an LPA1-dependent mechanism (21). Potential roles for LPA and its receptors in proliferation and lineage differentiation of neural stem/progenitor cells have not been investigated.

In this study, we examined clonal generation of neurospheres by LPA from isolated cells of mouse forebrain. The clonogenic cells that form a neurosphere are pluripotent progenitors, which are able to differentiate to three types of neural cells—neurons, astrocytes, and oligodendrocytes—in the presence of EGF and FGF2 (1,22,23).

We found that LPA-generated neurospheres (Fig. 1A,B) from dissociates of 7-day-old postnatal mouse brain in the absence of EGF or FGF2. The size of the neurospheres after 2 weeks of growth was significantly lower than those produced by EGF + FGF2, but their number did not differ significantly (Table 1). DGPP, an antagonist of LPA1/LPA3, decreased the size of LPA-generated neurospheres, but did not affect their number at concentration of 1 μM, which is sufficient to inhibit LPA3 but not LPA1 (18,19). In contrast, 50 μM DGPP, which blocks LPA1 and LPA3, inhibited significantly both the number and the size of neurospheres (Fig. 1C,D).

These data suggest that the activation of the LPA1 is required for both the initiation of cell proliferation and growth of neurospheres, whereas LPA3 is necessary for propagation of the cells of already generated neurosphere.

Previously, Weiner and co-workers showed, using in situ hybridization, that the LPA1 mRNA transcript was undetectable in mouse forebrain sections at birth and reappeared at 12 days postnatal age (15). In our study, we isolated the cells from 7-day-old postnatal forebrain, and plated 10⁵ cells per well in six-well plates, which subsequently gave rise to no more than 200 clones per well (Fig. 1, Table 1). Hence, a maximum of one cell out from 500 initially obtained and plated was indeed pluripotent, expressed LPA receptor(s), responded to LPA, and generated neurospheres. In situ hybridization of LPA1 in mouse forebrain at day 6 of postnatal age might not detect these solitary cells and/or the sections examined might not include the particular niches containing pluripotent stem cells.

LPA induced proliferation of AC133 and Sca-1 antigen-positive cells, which were distributed throughout clonal neurosphere (Fig. 2a–c). The present paper is the first demonstration of Sca-1 expression in clones produced by LPA from neural progenitors derived from mouse postnatal brain. These findings are of particular importance, because AC133 and Sca-1 antigens have been considered as markers of most primitive hematopoietic cells in bone marrow (24). Recent reports have indicated that AC133 was also expressed in fetal brain stem/progenitor cells (25). Moreover, AC133-positive cell selection has been used for isolation of primitive progenitors from fetal brain (25,26). Sca-1-positive cells have been also found in normal mouse adult tissues including heart, kidney, and lung (27,28). Sca-1- and AC133-positive bone marrow stem cells have been shown to transdifferentiate into neural cells (29,30). Interestingly, adult cardiac Sca-1-positive cells could be differentiated into beating cardiomyocytes (31). It was reported previously that LPA and S1P triggered invasion of primitive hematopoietic cells into stromal cell layers, and expression of LPA1 correlated with immaturity and invasive activity of the primitive hematopoietic cells (32). More recently, we showed that hepatic ductular progenitor cells, which proliferated in mouse liver in response to hepatotoxin DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine), co-expressed Sca-1 and LPA receptors and that hepatic LPA levels were enhanced in these mice (33). These data suggested a functional link between proliferation of cells of hematopoietic origin and LPA system in the liver during injury/regeneration (33). In the present study, LPA-generated clonal neurospheres expressed all three types of LPA receptors (Fig. 2d–i). LPA1 and LPA3 were co-localized with both AC133 and Sca-1 antigens, further supporting the notion that LPA induces clonal growth through LPA receptor-dependent proliferation of...
FIG. 4. Expression and co-localization of LPA receptors with markers of neuronal lineage βIII-tubulin and nestin in differentiated neurospheres. The clonal neurospheres, produced and maintained for 2 weeks by LPA, attached to cover slips, and then cultured for an additional week in serum-free medium supplemented with LPA. The cover slips were fixed and double immunostained with antibody against LPA₁ or LPA₃ and βIII-tubulin or nestin. (a) LPA₁ (red)/βIII-tubulin (green), co-localization-yellow. (b) LPA₁ (green)/nestin (red), co-localization-yellow. (c) LPA₃ (red)/βIII-tubulin (green), co-localization (yellow). (d) LPA₃ (green)/nestin (red), co-localization-yellow. (a, inset) Phase-contrast microscopy of typical neurosphere differentiated for 1 week. Original magnification, 40X. Arrowheads indicate the most extensive co-localization; arrows point to separate expression of corresponding proteins. The results shown are representative pictures of three independent experiments from two different neurosphere preparations.

FIG. 5. LPA receptor co-localization with markers of oligodendrocyte and astrocyte lineages in differentiated neurospheres. Neurospheres were differentiated for 1 week in the presence of LPA, fixed, and stained with antibody against LPA₁ or LPA₃ and CNPase or GFAP using dual immunostaining techniques. (a) LPA₁ (green/CNPase (red), co-localization (yellow). (b) LPA₁ (red)/GFAP (green). (c) LPA₃ (green)/CNPase (red), co-localization (yellow). (d) LPA₃ (red)/GFAP (green). Arrowheads indicate the most extensive co-localization. The results shown are representative pictures of three independent experiments from two different neurosphere preparations.
most primitive stem/progenitor cells (Fig. 3). Thus, expression of LPA receptors and the ability of cells to respond to LPA by proliferation may represent a prominent, so far unrecognized characteristic of primitive neural progenitors.

LPA-produced clones differentiated to cell lineages, which exhibited phenotypical characteristics of neuronal cells and glia. These cells expressed nestin and βIII-tubulin, both markers of immature neurons/neuroblasts, which were co-localized with LPA1 and LPA3 (Fig. 4). These findings are in accordance with previously published data (14,15,34), which indicated expression of LPA1 in neuroblasts of subventricular zone (SVZ) in mouse embryonic brain in vitro and in vivo.

Clonal neurosphere developed into oligodendrocyte and astrocyte lineages as indicated by expression of CNPase and GFAP (Fig. 5). LPA1 and LPA3 were expressed in the cells of oligodendrocyte lineage, as shown by co-localization of LPA1 and LPA3 with CNPase (Fig. 5a,c). Previously, it was demonstrated that LPA1 mRNA was co-expressed with proteolipid protein (PLP), a marker of mature oligodendrocytes, but not GFAP (20). Moreover, a peak and subsequent down-regulation of LPA1 correlated with the myelinization process (15). Thus, our present data support these in vivo studies and further extend the evidence that LPA plays an important role in development of Schwann cells in CNS.

In contrast, the cells expressing GFAP did not accumulate detectable levels of LPA receptors. (Fig. 5b,d). Thus, the pathways of astrocyte differentiation in our system are not clear. One can suggest that nonreceptor-mediated effects of LPA might be involved in this process, or that LPA induced the release of growth factors from LPA-responsive cells, which can in turn stimulate differentiation of astrocyte lineage in autocrine and/or paracrine fashion. In addition, LPA receptors could be down-regulated in GFAP-expressing cells at 2 weeks of neurosphere maturation in culture.

In conclusion, LPA generated clonal neurospheres from postnatal mouse forebrain via proliferation of Sca-1- and AC133-positive primitive stem/progenitor cells in LPA receptor-dependent fashion. LPA also regulated differentiation of these cells into cells of neuronal and glial lineages. Taken together, these data provide important information on mechanisms of LPA receptor-dependent regulation of neural progenitors proliferation and development by lysophosphatidic acid in vitro.

ACKNOWLEDGMENTS

This work was supported in part by grant DK61649 to S.I.S and grant #HLOC1751 to D.E. The authors wish to thank Dr. Steindler for providing laboratory facility and Ms. Olga Tchigrinova for excellent technical assistance.

REFERENCES

16. Suslov ON, VG Kukkov, TN Ignatova and DA Steindler. (2002). Neural stem cell heterogeneity demonstrated by

SVETLOV ET AL.


Address reprint requests to:
Dr. Stanislav I. Svetlov
Department of Neuroscience
P.O. Box 100244
McKnight Brain Institute
University of Florida
100 South Newel Drive
Gainesville, FL 32610
E-mail: svetlov@mbi.ufl.edu

Received May 6, 2004; accepted August 24, 2004.