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Original Article

Electrophysiological Properties of Mouse Cortical Neuron Progenitors Differentiated *In Vitro* and *In Vivo*

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Abstract: Central neurons are highly vulnerable to injury and have limited ability to regenerate. Therefore, transplantation of exogenous neuronal progenitor cells has been considered a potential therapy for the restoration of lost neurons and associated brain function. In a previous study, we found that when injected into rat brain following focal ischemia, cortical neuronal progenitor cells cultured from mouse brain can migrate into ischemic areas and differentiate into cells with morphological and biochemical features of neurons. However, no direct electrophysiological evidence was provided to indicate that these cells become functional neurons *in vivo*. In this study, we measured the electrophysiological properties of neuronal progenitor cells from embryonic mouse cerebral cortex, both in cell culture and in rat brain slices following intracerebral injection. We demonstrate that some of these cells differentiate to express electrophysiological properties expected of mature neurons, including tetrodotoxin-sensitive Na^+ channels and N-methyl-D-aspartate receptor channels. These results support the feasibility of cell-replacement therapy for stroke using exogenous neuronal progenitors.

Key Words: Progenitor cell, differentiation, neuron, patch-clamp, ion channel, ischemia

Introduction

The central nervous system is vulnerable to a range of injuries, but has limited capacity for self-repair, and it has been widely accepted that differentiated neurons cannot regenerate. Recent studies, however, have demonstrated that endogenous neurogenesis occurs in some areas of the adult brain following trauma, ischemia, or neurodegenerative diseases [1-5]. Therefore, stimulating endogenous neurogenesis may be a promising way to replace lost neurons and their function [6-8]. One obstacle to this approach is that the limited number of new neurons produced by endogenous neurogenesis may be insufficient to repair large brain lesions. In this regard, exogenous cell replacement therapy may be a promising alternative method [9-11]. A critical challenge inherent in this approach is the need to induce transplanted cells to differentiate into mature neurons.

We showed previously that, when injected into rat brain following focal ischemia, neuronal progenitor cells (NPCs) cultured from mouse cerebral cortex can migrate into injured brain areas and differentiate into cells bearing neurochemical markers of neurons and glia [10]. However, no direct electrophysiological evidence indicates whether these cells can differentiate into functional mature neurons *in vivo*. In this study, we measured the electrophysiological properties of NPCs from embryonic mouse cerebral cortex, both in cell culture, and in rat brain slices following intracerebral injection. We demonstrate that these NPCs can indeed differentiate into cells bearing electrophysiological characteristics of mature neurons, including the presence of functional voltage-gated Na^+ channels and N-methyl-D-aspartate (NMDA) receptor-gated channels. These findings further suggest that transplantation of exogenously derived NPCs following brain ischemia may be a useful

therapeutic strategy for stroke treatment.

Materials and methods

Animals

The use of animals for cell culture, brain slice recording, and *in vivo* experiments was approved by the Institutional Animal Care and Use Committees of Legacy Clinical Research Center and the Buck Institute.

Preparation of cortical neural progenitor cells

E16 Swiss mice were anesthetized with halothane followed by cervical dislocation. Fetuses were rapidly removed and placed in Dulbecco's Phosphate Buffered Saline (Gibco). Cerebral cortices were dissected and incubated with 0.05% trypsin-EDTA (Gibco) for 10 min at 37°C, followed by trituration with fire-polished glass pipettes, and plated on poly-L-ornithine-coated (Sigma, St. Louis, MO) cover slips at a density of 1 x 106 cells per cover slip. Cells were maintained in Neurobasal medium (Gibco) supplemented with B27 (Gibco). Fibroblast growth factor-2 (FGF-2, 20 ng/ml, Sigma) was also added to the culture medium to promote proliferation of progenitor cells [12-14]. Cells were cultured at 37°C in a humidified 5 % CO2 atmosphere incubator. For experiments with undifferentiated cells, cultures were fed three times per week with the above medium and used for electrophysiological recording 5-6 days following the initial plating, or after various passages. For differentiation of NPCs, cultures were fed with FGF-2-free medium for 6-15 days before recording.

Cell culture of GFP-expressing cortical neuronal progenitor cells

Mating pairs of transgenic mice expressing enhanced green fluorescent protein (GFP) under the control of chicken β-actin promoter and cytomegalovirus enhancer (C57BL/6-TgN(ACTbEGFP)1Osb) [15] were purchased from the Jackson Laboratory (Bar Harbor, ME) for breeding. E15 C57BL/6-TgN(ACTbEGFP)1Osb embryos were then used for culturing cortical NPCs as described above. Cells were collected at first passage for intracerebral injection.

Focal ischemia in rat and injection of GFP-positive NPCs

Transient focal ischemia was induced in male SD rats (280 - 310 g) by middle cerebral artery occlusion (MCAO), using the suture technique described previously [10]. Rats were anesthetized with 4% isoflurane in 70% N2O/30%O2 using a mask. The right external carotid artery was surgically exposed and dissected, and a monofilament nylon suture (3-0, 19 mm long) was inserted from the external carotid artery into the right internal carotid artery to occlude the origin of the right middle cerebral artery. The suture was removed 1 h later to allow reperfusion.

Cell transplantation

Twenty-fours hours after the onset of ischemia, rats were re-anesthetized and GFP-positive cells (1.2x105 cells/μl in PBS, 3 μl in total volume) were injected into the ipsilateral striatum, as detailed previously [10].

Preparation of brain slices

Two to six weeks after injection of GFP-positive progenitor cells, rats were anesthetized with halothane and decapitated. Brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 1.25 KH2PO4, 26 NaHCO3, 3 KCl, 10 glucose, 2 CaCl2, and 1 MgCl2, and saturated with 95% O2/5% CO2. Coronal brain slices containing striatum were cut at 400-μm thickness by a vibratome (VT1000s, Leica) and maintained in oxygenated ACSF at room temperature. Following ~60 min of incubation, individual slices were transferred to a submerged slice chamber with a volume of 0.5 ml (Warner Instruments, Hamden, CT) and perfused with oxygenated ACSF. GFP-positive cells were selected for patch-clamp recording under an upright microscope (Eclipse E600-FN) equipped with a combination of infrared differential interference contrast (IR-DIC) and epifluorescence attachment.

Patch-clamp recording in brain slices and in cultured neuronal progenitor cells

Whole-cell recording in brain slices and in cultured neuronal progenitor cells was performed as described previously [16,17]. Patch electrodes were constructed from thin-walled borosilicate glass (1.5 mm diameter, WPI, Sarasota, FL) on a two-stage puller (Model PP83, Narishige, Tokyo). The tips of

Table 1. Expression of voltage-gated Na^+ currents and NMDA-mediated currents in differentiated and undifferentiated cortical progenitor cells *in vitro*

Treatment	Passage No.	Cm (pF)	Expression of Na^+ Currents	Expression of NMDA currents
FGF 20 μM	P0	8.16 ± 0.35 (n = 18)	0/11	0/8
	P1		0/3	0/3
	P2		0/3	0/2
FGF withdrawn	P0	14.98 ± 1.37 (n = 28)	4/13	4/13
	P1		3/13	N/A
	P2		7/11	N/A

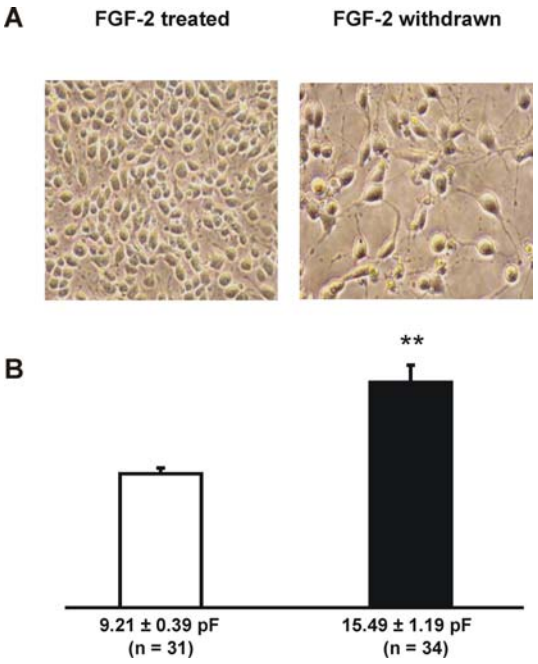

Figure 1. NPCs before and after differentiation *in vitro*. A, Morphology of cortical NPCs before (left) and after (right) FGF-2 withdrawal. In the presence of FGF-2, cells remained small with rounded shapes and few processes. After switching to FGF-2-free medium for >5 days, the majority of cells showed neuron-like morphology, with larger cell bodies and multiple processes. B, Summary data shows increased cell capacitance after (black bar) compared to before (white bar) FGF-2 withdrawal. **p<0.01.

the electrodes were heat-polished on a Narishige microforge (Model MF-83) to a final diameter of 1-2 μm. The patch electrodes had resistance between 3 to 5 MΩ when filled with intracellular solution. Whole-cell currents were recorded using Axopatch 1-D or 200B amplifiers (Axon Instruments, Foster City, CA) in the voltage-clamp mode. Data were filtered at 2 kHz and digitized on-line using Digidata 1320A DAC units (Axon Instruments). The on-line acquisition was done using pClamp software (version 8, Axon Instruments).

For brain slice recording, ACSF with 5 mM tetraethylammonium (TEA) was used as the extracellular solution. The pipette solution (300-310 mOsm, pH 7.2) contained (in mM): 130 CsMeSO4, 5 EGTA, 2 MgCl2, 1 CaCl2, 10 HEPES, and 2 ATP-Na2. For cultured neuronal progenitor cells, extracellular solution (320-335 mOsm, pH 7.4) contained (in mM): 140 NaCl, 5.4 KCl, 2 CaCl2, 25 HEPES, and 10 glucose. Patch electrodes contained (in mM): 140 CsF, 35 CsOH, 10 HEPES, 2 MgCl2, 11 EGTA, 2 TEA and 1 CaCl2 (300 mOsm, pH 7.3). Na^+ current was activated with depolarizing pulses from a holding potential of -90 mV. NMDA current was elicited by fast perfusion of cells with 100 μM NMDA in the presence of co-agonist glycine (5 μM). For brain slice recording, a flow rate of 2-3 ml/min was controlled by a ValveLink 8 perfusion system (AutoMate Scientific, Inc., Oakland, CA), whereas for cultured progenitor cells, a multi-barrel fast perfusion system (SF-77, Warner Instrument Co., Hamden, CT) was employed to achieve rapid exchange of extracellular solutions.

Results

Proliferation and differentiation of cortical neuronal progenitor cells in culture

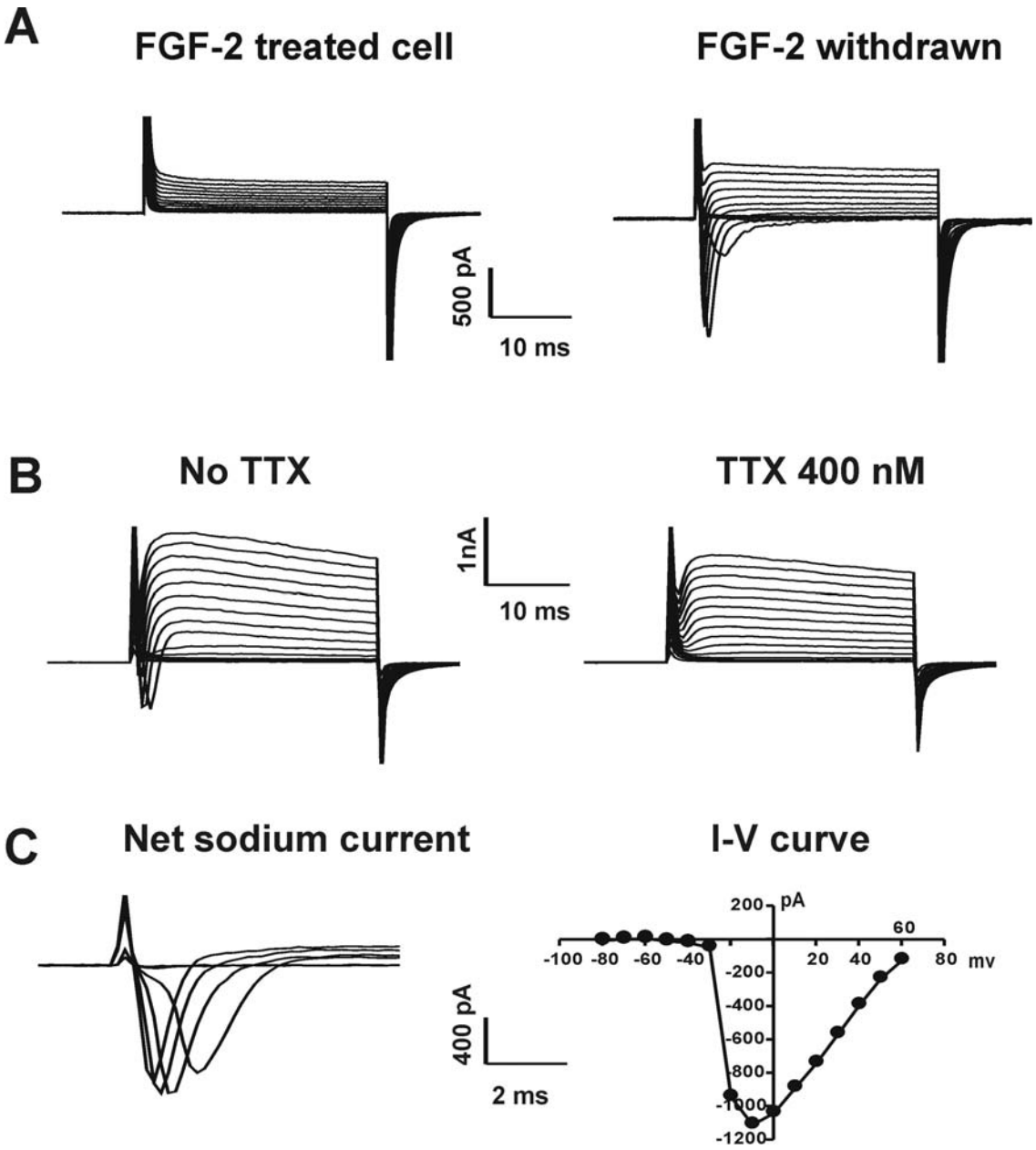

Figure 2. Expression of voltage-gated Na^+ current in differentiated NPCs *in vitro*. A, Representative traces showing voltage-gated currents in cultured cortical NPCs before (left) and after (right) withdrawal of FGF-2 from the culture medium. B, Representative traces showing voltage-gated Na^+ currents recorded from a differentiated (FGF-2-withdrawn) NPC in the absence (left panel) and presence (right panel) of tetrodotoxin (TTX, 400 nM). C, Net voltage-gated Na^+ current (left) and its I-V relationship (right) from a differentiated NPC. The reversal potential of this current is around +60 mV, consistent with the equilibrium potential for Na^+ ions.

In cultures fed with Neurobasal medium containing fresh FGF-2 (20 ng/ml) every 2-3 days, cells started to proliferate about 3 days after plating, and became confluent at 7-10 days. However, they had a very immature appearance: small and round with few processes (**Figure 1A**, left). These cells could be passaged at least 5 times without losing the ability to proliferate. After switching to FGF-2-free medium for more than 5 days, the majority of the cells differentiated to assume a neuron-like morphology, with a larger cell body

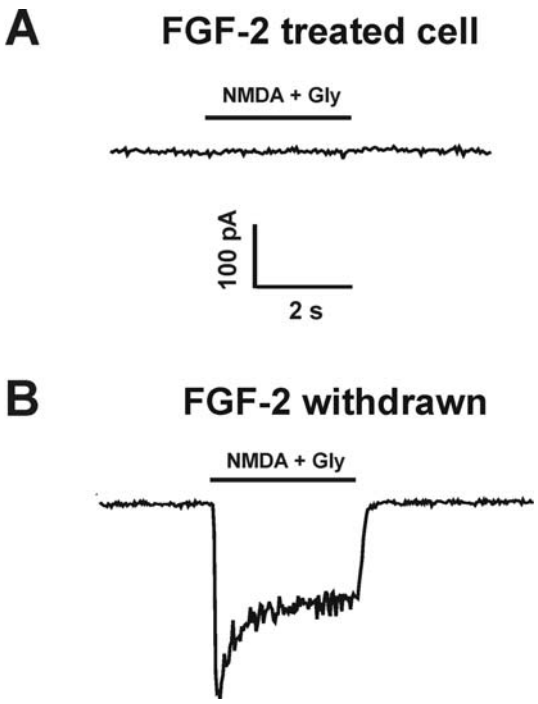

Figure 3. Expression of NMDA current in differentiated NPCs in vitro. A, Representative current trace showing the lack of NMDA (100 μM)- and glycine (5 μM)-stimulated current in cortical NPCs cultured in the presence of FGF-2. B, Representative current trace showing NMDA (100 μM)- and glycine (5 μM)-stimulated current in a differentiated NPC following withdrawal of FGF-2.

and multiple processes (**Figure 1A**, right). The apparent increase in cell size following differentiation was confirmed by measurement of cell capacitance (Cm) after 5-8 days in culture (**Figure 1B** and **Table 1**). Cultures from which FGF-2 was withdrawn comprised primarily cells expressing markers for immature cells of neuronal lineage (≥40% each immunopositive for nestin, doublecortin and Hu), with ≤1% of cells reactive for the astroglial marker, glial fibrillary acidic protein.

Expression of voltage-gated Na^+ channel and NMDA channel currents following cell differentiation in culture

Expression of voltage-gated Na^+ channel currents and NMDA receptor-gated channel currents is an important electrophysiological feature of mature functional neurons. Therefore, we tested for the presence of voltage-gated Na^+ currents and NMDA-evoked currents before and after FGF withdrawal from cultures. For Na^+ current, membrane potential was held initially at -90 mV and cells were then depolarized to +70 mV by a series of voltage pulses at increments of 10 mV. In cells cultured with FGF, membrane depolarization did not activate detectable Na^+ current, recorded 5-8 days after plating at passage 0, 1, or 2 (**Figure 2A**, left panel & **Table 1**). However, 5-8 days following FGF withdrawal, membrane depolarization induced typical voltage-gated Na^+ currents (**Table 1**, and **Figure 2A**, right panel). These inward currents could be blocked by bath perfusion of terodotoxin (TTX, 400 nM), indicating activation of voltage-gated Na^+ channels (**Figure 2B**). In addition, the reversal potential of this current was around +60 mV, consistent with the equilibrium potential for Na^+ ions (**Figure** 2C).

NMDA channels were activated by perfusing cells with Mg^{2+}-free extracellular solution containing 100 μM NMDA and 5 μM glycine. As was the case for Na^+ currents, no NMDA currents could be recorded in cells cultured with FGF (**Figure 3A**). However, 5-14 days following FGF-withdrawal, 4/13 cells demonstrated NMDA-evoked currents (**Table 1 and Figure 3B**).

Differentiation and maturation of cortical progenitor cells in vivo

To determine whether transplanted NPCs can also differentiate into functional mature neurons in the ischemic brain *in vivo*, cultured NPCs from transgenic GFP-positive mice were injected into rat striatum, 24 hr after MCAO. Rats were then killed at different time points and electrophysiological recordings were performed on GFP-positive cells in brain slices. GFP-positive cells were visualized under an upright microscope (Eclipse E600-FN) equipped with a combination of infrared differential interference contrast (IR-DIC) and epifluorescence attachments (**Figure 4A**).

One to two weeks following injection, GFP-positive cells could be observed throughout all coronal sections, with increased density in peri-infarct striatum and cortex [10]. Patch-clamp recordings were performed in GFP-positive cells with neuron-like morphology under IR-DIC microscopy. As shown in **Table 2**, within 2 weeks following injection, none of the

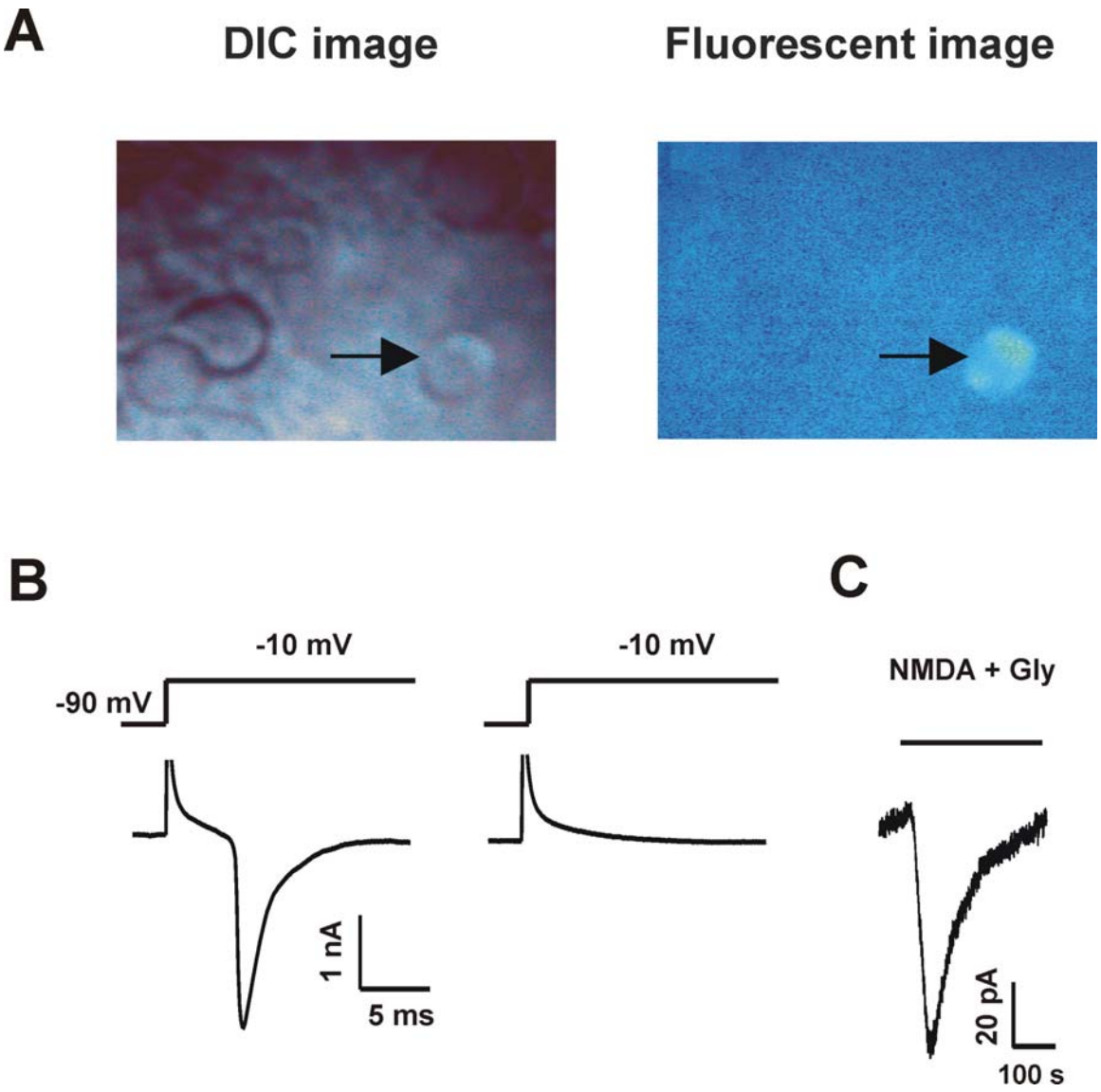

Figure 4. Expression of voltage-gated Na+ currents and NMDA currents in differentiated NPCs, transplanted in vivo and detected in brain slices. A, (Left) IR-DIC image of NPC, 5 weeks following MCAO and intrastriatal transplantation. (Right) Fluorescent image of the same GFP-positive cell. B, Sample trace showing Na+ current induced by membrane depolarization in a GFP-positive cell in a brain slice (Left). The current was completely blocked by 500 nM TTX (Right). C, NMDA (100 μM)- and glycine (5 μM)-induced current recorded from a GFP-positive cell in a brain slice.

GFP-positive cells demonstrated TTX-sensitive Na^+ or NMDA currents (n = 9 cells from 4 rats). However, 5-6 weeks following injection, 2/9 GFP-positive cells had inward TTX-sensitive Na^+ currents, consistent with differentiation toward a mature neuronal phenotype (**Table 2** and **Figure** 4B). In the same cell, perfusion of extracellular solution with 100 μM NMDA and 5 μM glycine also activated an inward NMDA current (**Figure 4C**).

Discussion

Generation of fully differentiated and specialized neurons from exogenous stem cells has been proposed as a promising approach for the treatment of acute and chronic brain disorders [18]. Due to the high degree of complexity of the nervous system, however, few examples of successful differentiation into mature neurons, as

confirmed by electrophysiological recordings, have been described. Ideally, once transplanted into the injured or diseased brain, exogenous stem cells should be able to migrate to affected brain regions, differentiate and replace dead neurons, damaged neural circuits, and lost functions.

Our previous studies showed that, following post-ischemic injection into the lateral ventricle of rat brain, mouse cortical neuronal progenitor cells can migrate to ischemic striatum and nearby cortex within one week [10]. Although the majority of surviving cells expressed markers for astroglia (glial fibrillary acidic protein) or immature neurons (doublecortin), a substantial number of cells displayed the mature neuronal marker, NeuN [10]. However, no electrophysiological evidence was obtained to further characterize the functional fate of these cells. In this regard, we performed patch-clamp recording in brain slices and studied the electrophysiological properties of the transplanted GFP-positive cells at different times following their intrastriatal administration. We focused on the expression of voltage-gated Na^+ channels and NMDA channels, two important electrophysiological features of mature neurons. We found that 5 weeks after implantation of NPCs, some of these cells had developed TTX-sensitive voltage-gated Na^+ channel and NMDA channel currents, consistent with differentiation toward mature, functional neurons. Future studies will be required to determine whether these cells also form synaptic connections with other neurons and integrate into neuronal networks.

The cells used for injection were collected from cultures of embryonic cerebral cortex, which were enriched with cells of neuronal lineage, as indicated by immunohistochemical staining. In the presence of FGF, a growth factor that enhances the proliferation of NPCs [19], these cultures can undergo many passages without losing their capacity for proliferation, thus providing a convenient and abundant source of NPCs for *in vivo* transplantation. Upon withdrawal of FGF, nearly 50% of the cultured cells demonstrated the presence of voltage-gated Na^+ current within 1- 2 weeks *in vitro* (**Table 1**), a strong indication of differentiation toward mature neurons. The mechanisms that control neuronal differentiation in the adult brain *in vivo* are not fully understood and are likely more complex than is reflected in cell culture conditions. Factors that either stimulate or inhibit neuronal differentiation may co-exist *in vivo*, and the local environment and neuronal activity may strongly influence the fate of transplanted cells. It is therefore not surprising that only limited number of injected NPCs could differentiate into mature neurons *in vivo*.

Thought the mechanism is unclear, focal cerebral ischemia may modify the fate and behavior of transplanted neural precursor cells. The toxic substance released by ischemic tissue may limit the survival of transplanted cells. This might also account for fewer mature neurons detected after implanted *in vivo* as compared with those in the *in vitro* preparation. On the other hand, ischemia has been shown to increase the entry of neural precursors from the ventricles and bloodstream into the brain and enhance their migration within the brain [10]. The factors that account for enhanced migration of transplanted precursor cells are unknown. One possibility is that ischemic brain tissue releases growth factors including stem cell factor, heparin binding epidermal growth factor-like growth factor, and vascular endothelial growth factor that can stimulate neurogenesis [13]. Because some of these factors also have chemotactic effects [20], they may have a role in ischemia-induced migration of neuronal precursor cells.

Previous studies have demonstrated that endogenous neurogenesis is enhanced in pathological conditions such as brain ischemia [5,21-24] and trauma [1,24-26]. Nevertheless, the capacity of endogenous neurogenesis may be too limited to serve as a basis for functional brain repair. Interestingly, Nakatomi and colleagues showed that endogenous neurogenesis in the ischemic hippocampus can be dramatically enhanced by intracerebroventricular infusion of growth factors [23]. Our findings that transplanted NPCs can migrate to ischemic brain regions and differentiate into cells with biochemical [10] and electrophysiological (this study) properties of mature neurons, suggest that transplantation of exogenous NPCs may have potential value in the treatment of stroke.

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References

[1] Gould, E. and Tanapat, P. (1997). Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience 80, 427-436.

[2] Snyder, E. Y., Yoon, C., Flax, J. D., and Macklis, J. D. (1997). Multipotent neural precursors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. Proc Natl Acad Sci USA 94, 11663-11668.

[3] Song, H. J., Stevens, C. F., and Gage, F. H. (2002). Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. Nat Neurosci 5, 438-445.

[4] van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., and Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. Nature 415, 1030-1034.

[5] Zhang, R. L., Zhang, Z. G., Zhang, L., and Chopp, M. (2001). Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 105, 33-41.

[6] Abe, K. (2000). Therapeutic potential of neurotrophic factors and neural stem cells against ischemic brain injury. J Cereb Blood Flow Metab. 20, 1393-1408.

[7] Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., and Lindvall, O. (2002). Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 8, 963-970.

[8] Zhang, R. L., Zhang, Z. G., and Chopp, M. (2005). Neurogenesis in the adult ischemic brain: generation, migration, survival, and restorative therapy. Neuroscientist+- 11, 408-416.

[9] Bjorklund, A. and Lindvall, O. (2000). Cell replacement therapies for central nervous system disorders. Nat Neurosci 3, 537-544.

[10] Jin, K., Sun, Y., Xie, L., Mao, X. O., Childs, J., Peel, A., Logvinova, A., Banwait, S., and Greenberg, D. A. (2005). Comparison of ischemia-directed migration of neural precursor cells after intrastriatal, intraventricular, or intravenous transplantation in the rat. Neurobiol Dis 18, 366-374.

[11] Rossi, F. and Cattaneo, E. (2002). Opinion: neural stem cell therapy for neurological diseases: dreams and reality. Nat Rev Neurosci 3, 401-409.

[12] Gritti, A., Parati, E. A., Cova, L., Frolichsthal, P., Galli, R., Wanke, E., Faravelli, L., Morassutti, D. J., Roisen, F., Nickel, D. D., and Vescovi, A. L. (1996). Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J Neurosci 16, 1091-1100.

[13] Jin, K., Mao, X. O., Sun, Y., Xie, L., and Greenberg, D. A. (2002). Stem cell factor stimulates neurogenesis *in vitro* and *in vivo*. J Clin Invest 110, 311-319.

[14] Ray, J., Peterson, D. A., Schinstine, M., and Gage, F. H. (1993). Proliferation, differentiation, and long-term culture of primary hippocampal neurons. Proc Natl Acad Sci USA. 90, 3602-3606.

[15] Okabe, M., Ikawa, M., Kominami, K., Nakanishi, T., and Nishimune, Y. (1997). 'Green mice' as a source of ubiquitous green cells. FEBS Lett 407, 313-319.

[16] Jia, Z., Agopyan, N., Miu, P., Xiong, Z., Henderson, J., Gerlai, R., Taverna, F. A., Velumian, A., MacDonald, J., Carlen, P., Abramow-Newerly, W., and Roder, J. (1996). Enhanced LTP in mice deficient in the AMPA receptor GluR2. Neuron 17, 945-956.

[17] Xiong, Z., Lu, W., and MacDonald, J. F. (1997). Extracellular calcium sensed by a novel cation channel in hippocampal neurons. Proc Natl Acad Sci U SA 94, 7012-7017.

[18] Lindvall, O. and Kokaia, Z. (2006). Stem cells for the treatment of neurological disorders. Nature 441, 1094-1096.

[19] Gage, F. H., Coates, P. W., Palmer, T. D., Kuhn, H. G., Fisher, L. J., Suhonen, J. O., Peterson, D. A., Suhr, S. T., and Ray, J. (1995). Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci USA 92, 11879-11883.

[20] Faber-Elman, A., Solomon, A., Abraham, J. A., Marikovsky, M., and Schwartz, M. (1996). Involvement of wound-associated factors in rat brain astrocyte migratory response to axonal injury: *in vitro* simulation. J Clin Invest 97, 162-171.

[21] Jin, K., Minami, M., Lan, J. Q., Mao, X. O., Batteur, S., Simon, R. P., and Greenberg, D. A. (2001). Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA 98, 4710-4715.

[22] Liu, J., Solway, K., Messing, R. O., and Sharp, F. R. (1998). Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. J Neurosci 18, 7768-7778.

[23] Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T., and Nakafuku, M. (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by

recruitment of endogenous neural progenitors. Cell 110, 429-441.

[24] Yoshimura, S., Takagi, Y., Harada, J., Teramoto, T., Thomas, S. S., Waeber, C., Bakowska, J. C., Breakefield, X. O., and Moskowitz, M. A. (2001). FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. Proc Natl Acad Sci USA 98, 5874-5879.

[25] Johansson, C. B., Momma, S., Clarke, D. L., Risling, M., Lendahl, U., and Frisen, J. (1999). Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96, 25-34.

[26] Yamamoto, S., Yamamoto, N., Kitamura, T., Nakamura, K., and Nakafuku, M. (2001). Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. Exp Neurol 172, 115-127.