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RESEARCH ARTICLE

N.J. Kee · E. Preston · J.M. Wojtowicz

Enhanced neurogenesis after transient global ischemia in the dentate gyrus of the rat

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Abstract The dentate gyrus is one of the few areas of the mammalian brain where new neurons are continuously produced in adulthood. Certain insults such as epileptic seizures and ischemia are known to enhance the rate of neuronal production. We analyzed this phenomenon using the temporary occlusion of the two carotid arteries combined with arterial hypotension as a method to induce ischemia in rats. We measured the rate of cell production and their state of differentiation with a mitotic indicator, bromodeoxyuridine (BrdU), in combination with the immunohistochemical detection of neuronal markers. One week after the ischemic episode, the cell production in dentate gyrus was increased two- to threefold more than the basal level seen in control animals. Two weeks after ischemia, over 60% of these cells became young neurons as determined by colabeling with BrdU and a cytoplasmic protein (CRMP-4) involved in axonal guidance during development. Five weeks after the ischemia, over 60% of new neurons expressed calbindin, a calcium-binding protein normally expressed in mature granule neurons. In addition to more cells being generated, a greater proportion of all new cells remained in the differentiated but not fully mature state during the 2- to 5-week period after ischemia. The maturation rate of neurons as determined by the calbindin labeling and by the rate of migration from a proliferative zone into the granule cell layer was not changed when examined 5 weeks after ischemia. The results support the hypothesis that survival of dentate gyrus after ischemia is linked with enhanced neurogenesis. Additional physiological stimulation after ischemia may be exploited to stimulate maturation of new neurons and to offer new therapeutic strategies for promoting recovery of neuronal circuitry in the injured brain.

N.J. Kee · J.M. Wojtowicz (✉)
Department of Physiology, Medical Sciences Building,
University of Toronto, Toronto, ON, Canada, M5S 1A8
e-mail: martin.wojtowicz@utoronto.ca
Tel.: +1-416-9782899, Fax: +1-416-9784940

E. Preston
Institute for Biological Sciences, Building M54, Montreal Road,
National Research Council, Ottawa, ON, Canada, K1A 0R6

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Introduction

It is now well established that various types of insults such as artificially induced seizures, behavioral stress, or administration of glucocorticoids alter the production of new granule neurons in the dentate gyrus (DG) of the hippocampus (Cameron and Gould 1994; Gould et al. 1997; Parent et al. 1997). Liu et al. (1998) have reported that granule cell proliferation increases by about fivefold during 9–12 days following experimental transient ischemia in gerbils. Several weeks after the ischemia, a large proportion (approximately 60%) of the proliferating cells become neurons, as judged by coimmunolabeling with a mitotic indicator, bromodeoxyuridine (BrdU), and a neuronal marker, NeuN. This apparent addition of new neurons in the gerbil DG was in contrast to massive neuronal death and lack of neurogenesis in the CA1 field of the hippocampus. Similar results, but with less emphasis on fate of cells generated in DG, have been obtained in mice after transient forebrain ischemia (Takagi et al. 1999).

The process of neurogenesis in the adult DG has been studied in much greater detail in rats than in gerbils, and there is a critical period of 1–2 weeks after cell birth when the cell's fate is determined (Cameron et al. 1993; Gould et al. 1999a). While many cells die, some survive, and the rate of survival appears to depend on the selective types of physiological stimulation such as certain types of hippocampal-dependent learning (Gould et al. 1999a). Full maturation of new-born cells is believed to take approximately 4 weeks (Cameron et al. 1993).

In the present study, we asked whether enhancement of adult neurogenesis occurs in rats after the experimental bilateral occlusion of the carotid arteries combined with hypotension (two-vessel occlusion, 2VO model). This model of ischemic stroke consistently produces more damage in CA1 than in DG (Smith et al. 1984;

Sutherland et al. 1988), but the reason for this differential effect is not known. One possibility is that the relative resistance of DG to the ischemic insult is somehow related to the induction of new cell growth in this region. Interestingly, the CA3 region, which does not produce new neurons but receives the afferent input from the DG, shows intermediate vulnerability to ischemia (Smith et al. 1984; Sutherland et al. 1988). In this paper we also addressed the issue of a progression from immature to mature neurons after ischemia in the 2VO model and its consequences for functional survival of DG after stroke.

Materials and methods

2VO model

Twenty-two Sprague Dawley rats (3 months old, approximately 350 g; Charles River) were used in this study. Principles of laboratory animal care were followed and all procedures were approved by the University of Toronto Animal Care Committee. After a 2-week acclimation period in the animal facility, the 2VO procedure was performed according to the procedure used previously (MacManus et al. 1995). Animals were anesthetized with pentobarbital, intubated, and mechanically ventilated with 30% O_2 and 70% N_2. Colonic and tympanic temperatures were monitored and maintained at 37–38°C using a warming pad. The tail artery was cannulated and connected to a pressure transducer and a heparinized syringe, which was used to withdraw blood and to control pressure between 42 and 47 mmHg during blood withdrawal. Clips were placed on each common carotid artery for 12 min. Blood PO_2, PCO_2, pH, and glucose were monitored before and during ischemia. After 12 min, the clips were removed, the blood returned from the syringe, and wounds sutured. Normothermia was maintained until the rats recovered from anesthesia. Sham-operated (sham) animals underwent an identical procedure, with the exception that the carotid arteries were not clamped. This group served as a control to rule out possible factors other than ischemia that might alter the rate of neurogenesis, such as stress resulting from handling, surgery, and subsequent transportation to another laboratory for cell-labeling experiments.

BrdU administration

5-Bromo-2′-deoxyuridine (BrdU), obtained from Sigma, was dissolved in 0.9% phosphate-buffered saline (PBS) containing 10 mM NaOH. The solution (1 ml) was injected intraperitoneally three times (33.3 mg/kg) at 2-h intervals, starting at 9:00 a.m., for a total dosage of 100 mg BrdU/kg of body weight. All injections were performed 6 days after the 2VO procedure.

Tissue preparation and immunolabeling

Rats were anesthetized with pentobarbital sodium (Somnotol) at 3 days, 7 days, or 28 days after the BrdU injections. Animals were perfused intracardially with 100–150 ml of 0.9% NaCl, followed by perfusion with 100–150 ml of fixative solution (4% paraformaldehyde in PBS, pH 7.2). Brains were removed and postfixed overnight in the same solution. Serial hippocampal sections (20–30 µm) were made on a vibratome in a bath of PBS. For BrdU immunolabeling, DNA was denatured with 1 N HCl at 55°C for 30 min and sections were washed twice for 15 min in PBS at room temperature. Sections were incubated with blocking solution (0.3% Triton-X in PBS with normal horse serum) for 30 min and overnight with primary antibody solution for BrdU (1:100) containing 0.3% Triton-X/PBS. In double-labeling experiments, the incubating solution also contained anti-CRMP-4 antibody, Ab25 (1:10000; a gift from Dr. S. Hockfield, Yale University, New Haven, Conn.), calbindin antibody (CaBP, 1:500; Chemicon), or NeuN antibody (1:1000; Chemicon). All primary antibody incubations were done in multiwell plates at 4°C on a shaker, with at least 250 µl of solution per well. Sections were then washed with PBS for 15 min and incubated with secondary antibody (0.3% Triton-X/PBS with Alexa goat anti-rabbit or anti-mouse IgG at 1:500; Molecular Probes). Sections were washed in PBS, rinsed in distilled water, and mounted on slides with Permafluor anti-fade medium (Lipshaw Imunon).

Stereology

The whole left hippocampus was sectioned in each animal. BrdU-labeled nuclei were counted using a physical dissector method (Coggeshall and Lekan 1996). Ten pairs of adjacent 20-µm sections were selected from the whole length of the hippocampus following an unbiased procedure. The first pair was chosen randomly from the first 10 sections, and the subsequent 12–13 pairs were separated by a distance of 400–500 µm, depending on the tissue sample. The pairs were examined by digitizing one section with a Sensicam camera (Cooke) attached to an epifluorescence microscope and visual examination of the other section. Only nuclei that were present in the latter but not in the former section were counted as BrdU-positive. To determine the total number of immunopositive cells per hippocampus, the counts from ten sampled pairs were averaged and the mean count was multiplied by the total number of sections in the hippocampus. Separate counts were obtained for the hilus (not reported here) and for the combined areas of the subgranular zone (SGZ) and the granule cell layer (GCL). A small number of sections were lost during sectioning, so the total counts reported here are underestimated.

The absolute numbers of CRMP-4+ cells were estimated using an optical dissector method (Coggeshall 1992). Since the density of CRMP-4-labeled cells was very high in parts of the upper blade of the DG, precluding accurate counting, we limited our estimate to the lower blade. These measurements should be representative of the whole DG, since we observed that cell proliferation was enhanced almost equally in both blades (Fig. 1B). In this procedure the selection of the sections was made as described above for the physical dissector, but single sections instead of pairs were used. Each section was placed on the stage of a confocal microscope and examined at high magnification (63× objective, oil immersion). For each field of view, the focal plane was lowered 3 µm below the upper surface of the section and all cells below this plane were counted while focusing through the section. The cells from each field of view were added up to yield the total number of cells in the lower blade in this section. The cell numbers per each selected histological section were averaged and the mean value was multiplied by the total number of sections in the hippocampus. This was expected to provide an unbiased estimate of the total number of CRMP-4+ cells in the lower blade of DG.

Double-labeled cells (BrdU and CRMP-4, and BrdU and CaBP) were counted in the same sections as used for BrdU stereology above. To ensure that the label from two fluorochromes originates from the same cells, we confirmed the results using a confocal microscope. In each case the cells were scanned with the dual wavelengths at several focal planes and the resulting image examined.

Statistics

Statistical analysis was done using Sigmastat (Jandel) analysis software. Two-way ANOVA analysis was applied throughout, with the survival time and treatment (sham vs 2VO) as the variables. The power of the tests approximated 1.0 in all cases, making comparisons between the variables valid. The power for time × treatment comparisons between groups was low (less than 0.3), so the negative results of such comparisons were given little emphasis.

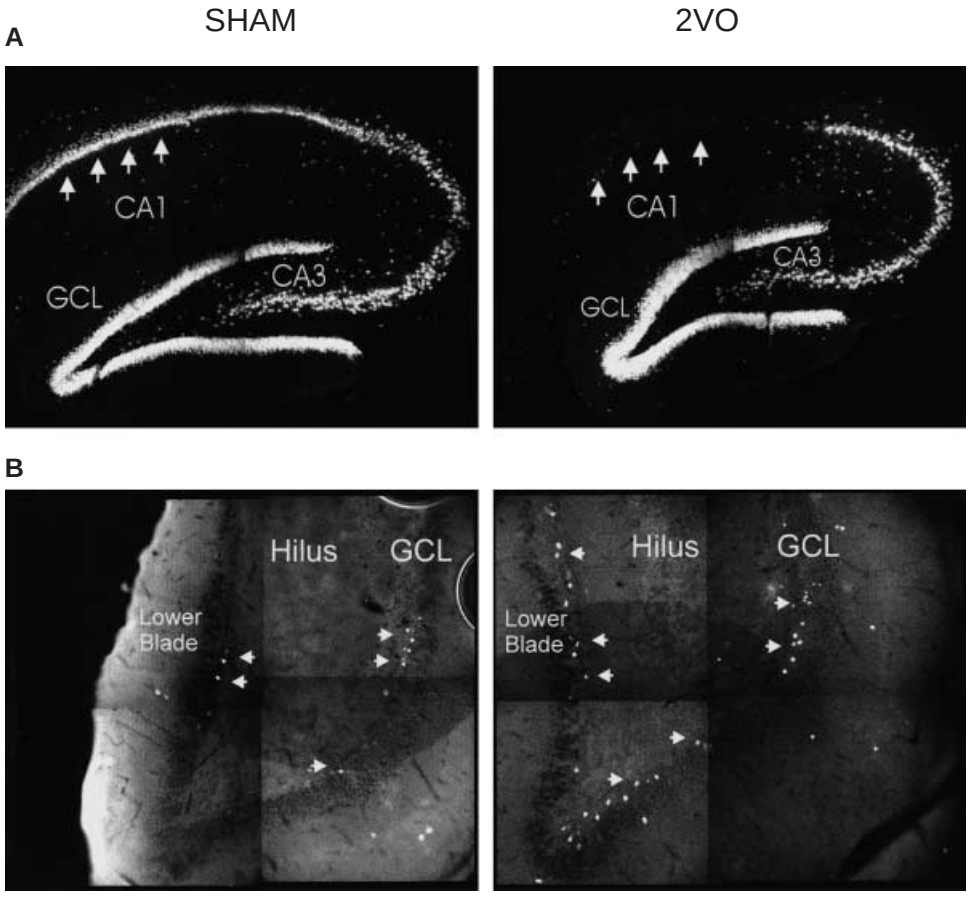

Fig. 1A, B Sample data showing cell loss in CA1 (**A**) and cell proliferation in dentate gyrus (*DG* in **B**) 13 days after ischemia. **A** Hippocampal section from a sham animal and a two-vessel occlusion (2VO) animal, showing pronounced cell loss in CA1(*arrows*) after ischemia. In contrast, DG was spared. Sections were stained for a neuronal marker, NeuN. **B** Bromodeoxyuridine-positive (BrdU+) cells in a representative section from a sham and a 2VO animal 1 week after BrdU injection. A large increase in the number of BrdU+ cells (*arrows*) is seen after ischemia. Granule cell layer (*GCL*), and hilus are indicated. The lower blade of the dentate gyrus is shown on the *left* and the upper blade on the *right* of each section

Results

Changes in cell proliferation after ischemia

Figure 1A shows typical effects of the ischemia on cell numbers in CA1 and DG regions of the hippocampus as defined by West et al. (1991) and illustrates the extent of prominent cell death in CA1 of a 2VO animal compared with sham animals. The neuronal loss in CA1 was dramatic. The density of NeuN+ cells in representative sections was 678±88 (SD; n=3) in control and 108±19 (SD; n=3) in 2VO animals at 13 days after ischemia. Thus confirming the effectiveness and reliability of the 2VO procedure. In contrast, the DG appeared undamaged.

Typical examples of BrdU labeling in dividing cells in DG of 2VO and sham rats are shown in Fig. 1B. Numerous labeled nuclei were present in the granule cell layer and a few within the hilus. Figure 2 summarizes counts of such cells taken 3 days, 7 days, or 28 days after BrdU injection (9 days,13 days, and 34 days postischemia) and shows that the number of proliferating cells was at least doubled in 2VO rats compared with sham rats at all time points. In sham and 2VO animals, there was a decrease in the number of labeled cells from 7 to 28 days after BrdU administration, which is probably a result of cell death as reported previously (Gould et al. 1999a). It is not likely that this drop is due to the label

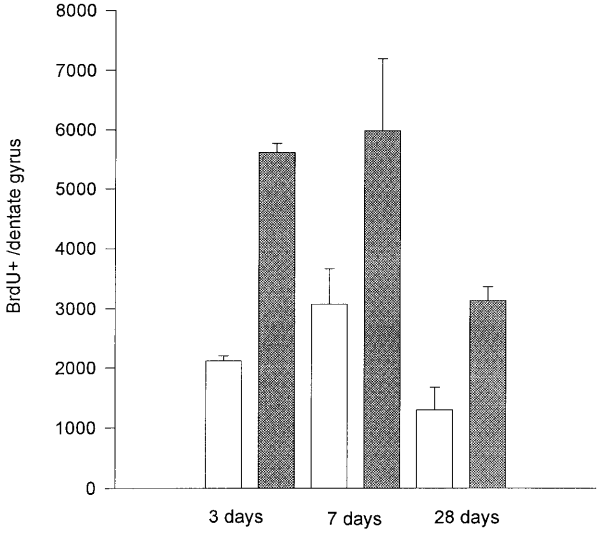

Fig. 2 Effects of ischemia on the combined number of BrdU+ cells in granule cell layer and subgranular zone 3 days,7 days, and 28 days after BrdU injection. BrdU was injected 6 days after the 2VO treatment. Two-way ANOVA showed a significant effect of treatment (P<0.005) and also a significant effect of time (P<0.001) after allowing for effects of differences in treatment. Sham group (n=3), *open bars*; 2VO group (n=3), *filled bars*. Pairwise multiple-comparison procedure (Student-Newman-Keuls method, P<0.05) indicated significant differences between the 3-day time point and the 28-day time point and between the 7-day and 28-day time points, but no difference between the 3- and 7-day time points

dilution, since such dilution would be expected to be most noticeable during the 1st week after the BrdU administration, yet there was no significant difference between numbers of BrdU+ cells between day 3 and day 7 in either group (Fig. 2). In contrast there was a significant difference between days 3 and 7 and day 28 ($P<0.05$). Thus only about half of the BrdU-labeled cells seen 1 week after BrdU injection survived after 4 weeks. When the data were analyzed statistically with two-way ANOVA using Ischemia and the Survival time as the two variables, the difference between sham and 2VO rats was found highly significant ($P<0.005$) with respect to treatment and with respect to time ($P<0.001$). We also observed an approximate doubling in the number of BrdU+ cells in the hilus of 2VO animals. These cells may be astrocytes or neurons but they were not studied in detail in the present report.

Effects of ischemia on cell differentiation

Colabeling of tissue sections with antibodies for BrdU and CRMP-4, a marker for immature neurons (Quinn et al. 1999), showed that ischemia increased the proportion of new cells that developed into neurons at all three survival periods (3 days, 7 days and 28 days post-BrdU) by about 50% (Fig. 3). This suggested that not only the cell proliferation but also the progression into the neuronal phenotype was enhanced by ischemia, since the undifferentiated cells and glia are not expected to express CRMP-4, a protein thought to be involved in axonal guidance during development (Quinn et al. 1999). The decrease in colabeling after 4 weeks was expected, since we had found previously that at 4–5 weeks CRMP-4 labeling was lost due to maturation of neurons and consequent loss of CRMP-4 protein (Kee et al. 1999). It should be noted that the decrease in CRMP-4 colabeling with time is an entirely different phenomenon than the decrease in the absolute number of BrdU+ cells shown above. Next, we verified the overall increase in CRMP-4 colabeling with treatment by measuring the absolute numbers of CRMP-4+ cells. Stereological measurements of CRMP-4+ cells in the lower blade of the DG (see Materials and methods) yielded an estimate of 35,435± 3,600 (SD; $n=3$) in the sham group and 44,140±4,100 (SD; $n=3$) in the 2VO group at 1 week. The additional 8,700 CRMP-4+ cells represents 38% increase in new cell number after ischemia. This effect was statistically significant (*t*-test, $P<0.05$). The absolute numbers of CRMP-4+ neurons are of course much higher than the numbers of CRMP-4+ cells colabeled with BrdU, since the BrdU application was brief and labeled only a subset of the young neurons generated during the period when BrdU was available.

Colabeling the tissue sections with antibodies for BrdU and a mature neuronal marker, CaBP, a calcium-binding protein normally present in mature granule neurons (Goodman et al. 1993), showed a dramatic increase in new neurons at 4 weeks in both groups of animals,

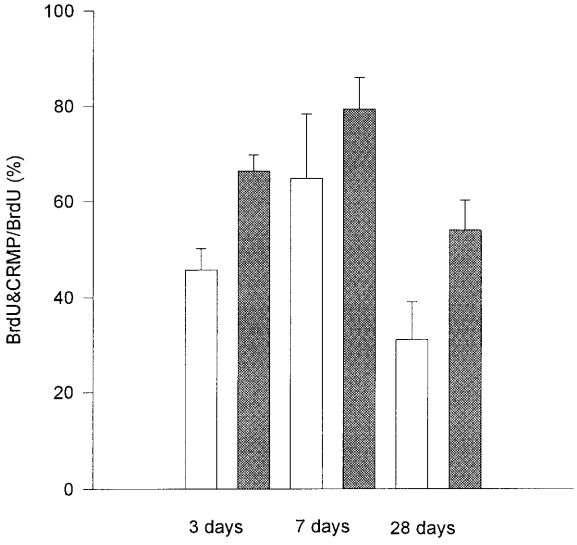

Fig. 3 Effects of ischemia on neuronal differentiation. The relative numbers of cells colabeled with BrdU and CRMP-4 are shown for each of the survival periods. Two-way ANOVA indicates a significant effect of treatment (2VO, *filled bars*; sham, *open bars*; $P<0.01$). The difference in the mean values among different levels of time (3 days, 7 days, and 28 days) are also greater than would be expected by chance after allowing for effects of differences in treatment ($P<0.01$)

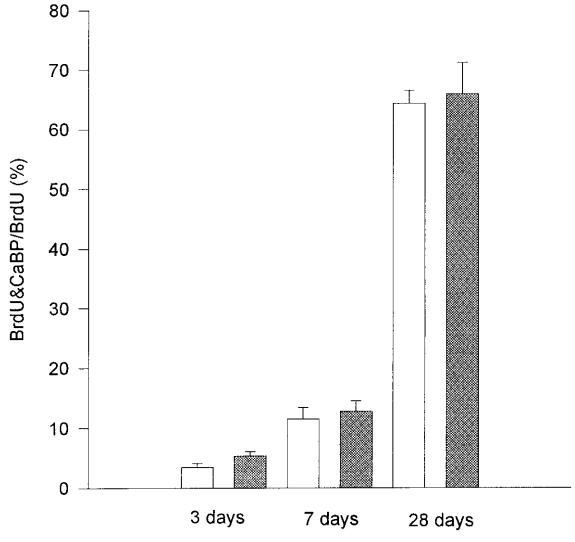

Fig. 4 Effects of ischemia on neuronal maturation. The relative numbers of cells colabeled with BrdU and calcium-binding protein (*CaBP*) are shown for each survival period. Two-way ANOVA indicates a significant effect of time ($P<0.01$), but no effect of treatment ($P=0.47$). 2VO, *filled bars*; sham, *open bars*

with about 65% of new cells being mature neurons at that time (Fig. 4). The relative numbers of colabeled cells differed with respect to time ($P<0.005$) but not with respect to treatment ($P=0.47$). Although the relative numbers of the CaBP- and BrdU-colabeled cells were not different between the sham and 2VO groups, the absolute numbers of the CaBP and BrdU+ cells were indeed much higher in 2VO animals. For example, at

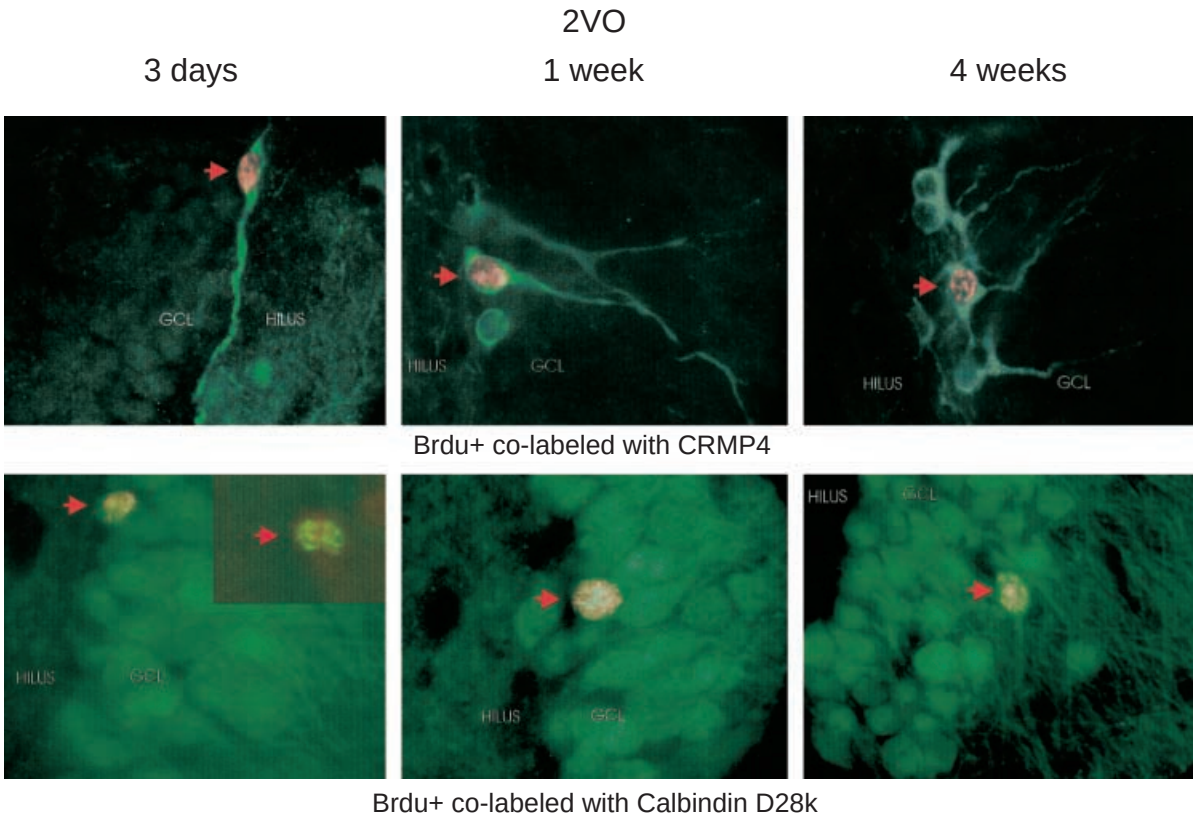

Fig. 5 Morphological features of newborn neurons produced in dentate gyrus after 2VO ischemia. A majority of neurons colabeled with CRMP-4 and BrdU (*red arrow*) were found in the proliferative zone located at the border between granule cell layer (*GCL*) and hilus. At 3 days, most cells had dendrites extended along the subgranular zone. At 1–4 weeks, cells developed a single dendrite projecting into the GCL. Neurons colabeled with BrdU and CaBP were found in progressively deeper regions of GCL as the survival time increased. Images were viewed under the fluorescent microscope using two different filters for the two markers and superimposed after the images were digitized with a Sensicam camera. The image inserted at the 3-day time point was obtained with a confocal microscope; it shows two recently divided, BrdU+ nuclei superimposed on a CaBP+ background. Very few (less than 5%) of such images were seen at this time point

4 weeks there were 824±425 (SD; n=3) such cells in the sham group and 2078±510 (SD; n=3) in the 2VO group. This large increase is in accordance with the overall increase in BrdU+ cells at 4 weeks in 2VO animals, shown in Fig. 2. Thus, neurogenesis was dramatically increased after ischemia, but the maturation as defined by expression of CaBP was not.

The estimates of CRMP-4- and BrdU-labeled neurons show that at all survival periods the numbers of labeled neurons was higher in 2VO animals (data in Fig. 3). The difference in the number of labeled cells at 3 days, 7 days, and 28 days was statistically significant according to a two-way ANOVA, taking the survival period and the ischemic treatment as two variables (P<0.05). The effect of the treatment was significant, but the effect of the survival period was not. In the case of the CaBP- and BrdU-labeled neurons, the result was the opposite. The number of colabeled cells increased progressively with time as expected, reflecting the significant effect of the survival time but not of the treatment. The reason for some of the values from Figs. 3 and 4 exceeding the 100% mark at 28 days is probably coexpression of the two markers in the same cells.

Morphology and location of surviving neurons

In order to assess the possibility that 2VO procedure accelerates the differentiation and maturation of neurons in more detail, we examined the morphology and topographical positions of labeled neurons in the SGZ and GCL. The rationale for this experiment was given by past studies showing that differentiation and maturation are associated with migration of the cells from SGZ to GCL (Cameron et al. 1993; Crespo et al. 1986).

In 2VO animals examined at 3 days with BrdU and CRMP-4 antibodies, most labeled cells were found in SGZ and showed distinctive morphology, characterized by an elongated, spindle-shaped cell body, oval nuclei, and dendritic processes spreading along the GCL/hilus border (Fig. 5). These, we believe, are the migrating, very immature granule neurons. They are similar in morphology to those seen in the white matter of the neocortex by Gould et al. (1999b). At 1 week, neurons were found at the lower edge of GCL, had round nuclei, and extended a single dendrite toward the molecular layer, in a classic granule cell manner (Wang et al. 2000). At 4 weeks, relatively few neurons were labeled with

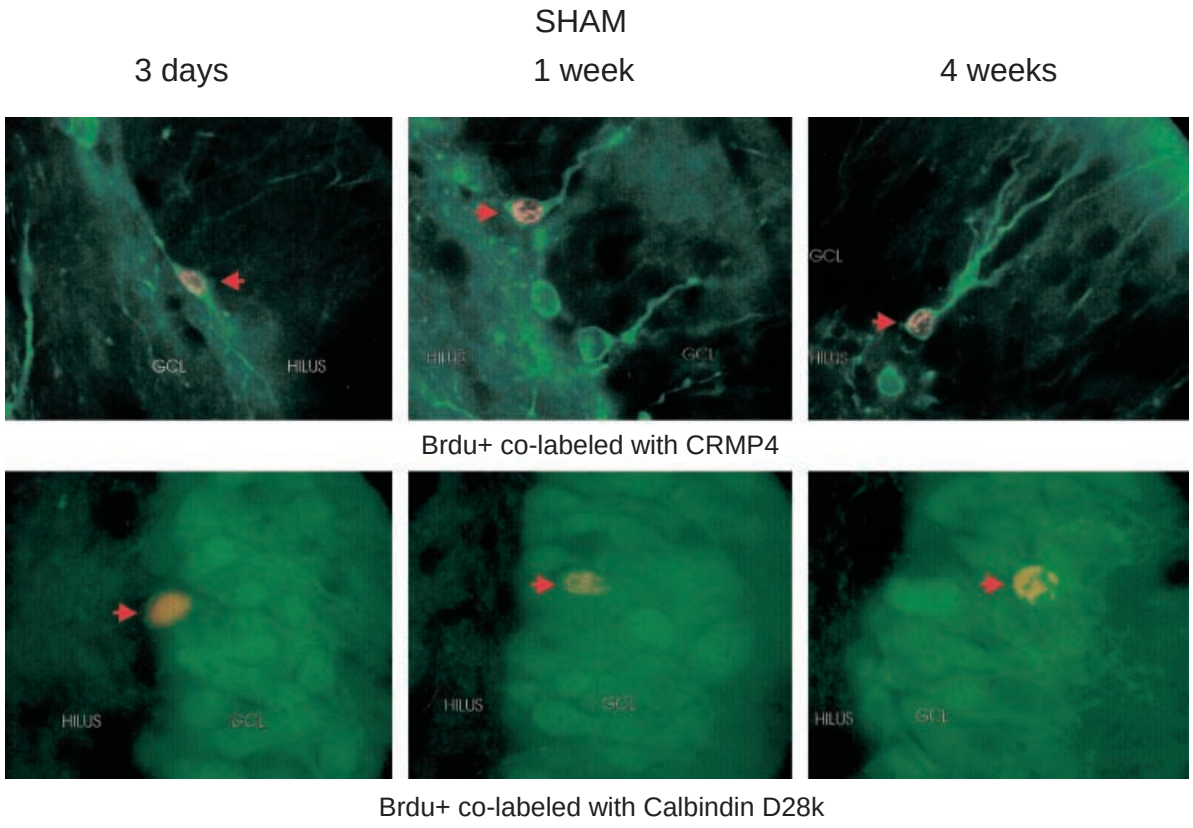

Fig. 6 Morphological features of new neurons produced in sham-operated control animals. The anatomical features and locations of the neurons were similar to those in the ischemic animals

CRMP-4, and those that did survive looked similar to those at 1 week (Fig. 5, upper right panel). Axons of these cells were difficult to visualize near the cell bodies but were clearly present in the hilus as they traversed toward the CA3 (not illustrated), confirming the previous observation that extension of axons is a rapid process in the young neurons (Hastings and Gould 1999). In sham animals the appearance of the CRMP-4- and BrdU-labeled neurons was similar to 2VO. No abnormal, collateral sprouting of the axons into the molecular layer was ever noted in either group.

The BrdU- and CaBP-labeled cells were always found in GCL, not in SGZ, indicating that this neuronal marker is acquired after the cells migrate into the GCL (Figs. 5, 6). At 3 days, a few cells were found in the first layer of GCL, near the hilus, where CRMP-4 labeling was most prominent. This suggests that the presence of CRMP-4 and CaBP can overlap, although it is not certain whether the two markers were present in the same or adjacent neurons. With longer survival times (7 days and 28 days), the cells were usually found deeper in the GCL. Only cell bodies and primary dendrites, characteristic of the mature morphology, were visible. The appearance of BrdU- and CaBP-labeled cells in the 2VO and sham groups was similar.

According to the data presented here and those of others (Seki and Arai 1993), cell migration from SGZ into the GCL correlates with neuronal maturation. We quantified the numbers of double-labeled cells as a proportion of the total BrdU+ cells in three regions: (1) SGZ, (2) inner region of GCL, and (3) middle-outer region of GCL. The border between GCL and SGZ was arbitrarily defined as the line delineating the innermost cell bodies within the GCL and the adjacent hilus. The data confirmed that the migration of cells occurs, since at 3 days a majority of double-labeled neurons were found in regions 1 and 2 and none in region 3. After 28 days, approximately 12% of CaBP and BrdU+ and 4% of CRMP-4 and BrdU neurons were found in region 3. The pattern of migration was similar in sham and 2VO animals, suggesting similar rates of neurogenesis.

Discussion

Our experiments confirmed the well-established relative resistance of DG to ischemia (Fig. 1A; Liu et al. 1998; Smith et al. 1984; Sutherland et al. 1988). The results of these studies show consistently that cell numbers in the DG are preserved, while those in CA1 are severely reduced.

A twofold increase in BrdU+ cells can be accounted for by a twofold increase in cell proliferation in 2VO animals (Figs. 1B, 2). This increase in BrdU+ cells persists for at least 4 weeks, which is sufficient for maturation of the neurons. Thus, it is essential to distinguish whether the persistent increase in the numbers of labeled neurons, i.e., their survival, is due to the enhanced proliferation,

maturation, or both. It should be noted that the magnitude of the increase is less than that observed by Liu et al. (1998) in gerbils after ischemia. The large, sixfold increase in that study occurred during a limited period of time after ischemia, which may be different in different species. We did not attempt to establish the time course of this effect in the rat, but instead concentrated our effort on the crucial issue of cell maturation. The study of Liu et al. (1998) used colabeling with BrdU and NeuN, a nuclear marker, which, when used alone, does not establish the degree of maturation of a neuron.

Cell proliferation and differentiation

At 3 days, approximately 50% of the sham and 70% of the 2VO cells were already differentiated into neurons as defined by labeling with BrdU and CRMP-4 and/or CaBP (data in Figs. 3 and 4 combined), with the majority of the cells labeled with CRMP-4 and fewer than 5% of cells labeled with CaBP. The remaining, unlabeled cells were presumably undifferentiated precursor cells, glia, or endothelium. Seven days after the BrdU injection, the number of BrdU-labeled cells was still approximately twofold higher in the 2VO group. Differentiation into neurons at this time point progressed to about 70% in the sham group and 80% in the 2VO group, but a majority of cells were still labeled with an immature marker, CRMP-4 (Fig. 3).

Four weeks after the injection, the number of the surviving cells declined significantly to about half the values present at 7 days, but the proportion of the cells labeled with CaBP increased dramatically. This confirms previous data showing that the maturation of the adult-generated cells takes approximately 4 weeks (Cameron et al. 1993).

Cell survival

Our data indicate that among the surviving cells, 4 weeks after BrdU injection and 5 weeks after 2VO-induced ischemia, nearly all cells in both groups differentiated into neurons, with a majority of them being morphologically mature as defined by expression of CaBP and migration into the GCL. However, it is likely that some neurons expressed both markers (CaBP and CRMP-4), since there is an overlap in the time course of their expression (see Figs. 3, 4). Those neurons expressing CRMP-4 at 28 days appeared to be arrested in a partially developed stage.

Interestingly, the 2VO procedure accelerated the production of the immature, CRMP-4-labeled cells but did not change the relative number of the mature neurons. Thus, ischemia appears to accelerate cell production and their differentiation into neurons but does not enhance their maturation. An alternative explanation of this oversupply of the young neurons is that their death, which is thought to prune the number of the newborn cells in the DG (Gould et al. 1999a), is somehow delayed in the 2VO group. This is not likely to be the case, since the total numbers of the cells are enhanced equally at all three time points (Fig. 2). This finding suggests the existence of a mechanism for a possible enhancement of maturation by brain stimulation, which is apparently missing in the 2VO animals. Gould et al. (1999a) reported that specific types of learning such as learning of spatial navigation in a water maze and trace eye-blink conditioning can enhance the net cell survival, possibly by reducing the rate of cell death. The developing consensus is that the novel stimuli rather than persistent stimuli are essential for promotion of adult neurogenesis (Barnea and Nottebohm 1996; Kempermann and Gage 1999; Nilsson et al. 1999). The nature of the stimulus responsible for this trophic effect is not known. The delay of the onset of neurogenesis is longer than most of the immediate effects of ischemia such as cell death in DG (Bengzon et al. 1997). Thus, the long-term changes in the chemical environment within the DG may be responsible for neurogenesis and the improved survival after ischemia. The novel finding in this study is that survival alone cannot be considered conclusive or beneficial without consideration of cell maturation. Perhaps the potential for cell maturation, apparently missing in the 2VO animals, could be increased further by elimination of cell death or by promotion of cell maturation with a therapeutic technique that could involve learning exercises or application of neurotrophic factors.

Is neurogenesis a key to survival?

The reason for selective sparing of DG after ischemia is still unknown. A number of factors have been implicated in this phenomenon, including early expression of genes involved in cell survival (Walton and Dragunow 2000). We propose that the enhanced neurogenesis could be a contributing factor and that the expression of some of the genes regulating cell survival could be either a reason for, or a consequence of, the appearance of new cells in DG. These new cells could promote cell survival of both new and old neurons by directly or indirectly increasing the levels of growth factors such as brain-derived neurotrophic factor (BDNF) in the region (Lindvall et al. 1994). The axons of new cells extend rapidly into the target CA3 region (Hastings and Gould 1999) and could supply a growth factor to promote the survival of neurons in this region as well. In contrast, the CA1 region lacks neurogenesis and is vulnerable to ischemia. The increase in neurogenesis is transient and lasts only several days after ischemia (Liu et al. 1998). Our data show that in each hippocampus a minimum of 2,000–3,000 new neurons are produced per day (Fig. 2). This is in keeping with the estimated 9,000 additional new neurons produced in 2VO animals in the lower blade of the DG, which appears to contain less than half of all neurons produced (see Materials and methods and Results). Thus the number of additional neurons is small in comparison

with the total of approximately 1 million already present in a young rat (West et al. 1991). Thus neurotrophins, probably associated with neurogenesis, and not the increase in the number of neurons per se, are likely to be important in the survival of DG after ischemia. The insulin-like growth factor (IGF-1) is one possible candidate for a growth-promoting substance involved in regulation of neurogenesis in DG (Aberg et al. 2000). The future discoveries of such substances will create possible therapeutic strategies for treatment of stroke damage even in those areas that do not support neurogenesis under normal circumstances.

In summary, the data suggest that the addition of new neurons in the adult DG after ischemia proceeds at an accelerated pace owing to an increased rate of proliferation and differentiation. However, the rate of full maturation is not enhanced, raising an opportunity for its stimulation by new, experimental therapeutic strategies.

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