Cellular and Molecular Events Underlying the Dysregulated Response of the Aged Brain to Stroke: A Mini-Review

Eugen Bogdan Petcu a, c  Veronica Sfredel e  Dieter Platt b  James G. Herndon d  Christof Kessler a  Aurel Popa-Wagner a

a Department of Neurology, University of Greifswald, Greifswald, and b Prof.-Dr.-D.-Platt-Stiftung für Alternsforschung, Nürnberg, Germany; c Griffith University School of Medicine, Southport, Australia; d Yerkes National Primate Research Center, Neuroscience Division, Emory University, Atlanta, Ga., USA; e University of Medicine and Pharmacy, Craiova, Romania

Key Words
Stroke-related mental disabilities • Stroke-related physical disabilities • Cytological response

Abstract
Background: Age-related brain injuries, including stroke, are a major cause of physical and mental disabilities. Objective: Therefore, studying the basic mechanism underlying functional recovery after brain stroke in aged subjects is of considerable clinical interest. Methods: This review summarizes the effects of age on recovery after stroke in an animal model, with emphasis on the underlying cellular mechanisms. Results: Data from our laboratory and elsewhere indicate that, behaviorally, aged rats were more severely impaired by stroke than young rats, and they also showed diminished functional recovery. Infarct volume did not differ significantly between young and aged animals, but critical differences were apparent in the cytological response to stroke, most notably an age-related acceleration in the development of the glial scar. Early infarct in older rats is associated with premature accumulation of BrdU-positive microglia and astrocytes, persistence of activated oligodendrocytes, a high incidence of neuronal degeneration and accelerated apoptosis. In aged rats, neuroepithelial-positive cells were rapidly incorporated into the glial scar, but these neuroepithelial-like cells did not make a significant contribution to neurogenesis in the infarcted cortex in young or aged animals. The response of plasticity-associated proteins like MAP1B, was delayed in aged rats. Tissue recovery was further delayed by an age-related increase in the amount of the neurotoxic C-terminal fragment of the β-amyloid precursor protein (Aβ) at 2 weeks poststroke. Conclusion: The available evidence indicates that the aged brain has the capability to mount a cytoproliferative response to injury, but the timing of the cellular and genetic response to cerebral insult is dysregulated in aged animals, thereby further compromising functional recovery. Elucidating the molecular basis for this phenomenon in the aging brain could yield novel approaches to neurorestoration in the elderly.

Introduction
Age-related brain injuries, including stroke, are a major cause of physical and mental disabilities. Therefore, studying the basic mechanism underlying functional recovery after brain stroke in aged subject is of considerable clinical interest.
Stroke Models Using Aged Animals Are Clinically More Relevant than Stroke Models in Young Animals

Aging is associated with declines in locomotor, sensory and cognitive performance in humans [1–4]. Many of these changes are due to an age-related functional decline of the brain.

Studies of stroke in experimental animals have demonstrated the neuroprotective efficacy of a variety of interventions, but most of the strategies that have been clinically tested failed to show benefit in aged humans. One possible explanation for this discrepancy between experimental and clinical studies may be the role that age plays in the recovery of the brain from insult. Indeed, age-dependent increase in the conversion of ischemic tissue into infarction suggests that age is a biological marker for the variability in tissue outcome in acute human stroke [5].

Although it is well known that aging is a risk factor for stroke [6–9], the majority of experimental studies of stroke have been performed on young animals, and therefore may not fully replicate the effects of ischemia on neural tissue in aged subjects [10–13]. In this light, the aged post-acute animal model is clinically most relevant to stroke rehabilitation and cellular studies, a recommendation made by the STAIR committee [14] and more recently by the Stroke Progress Review Group [15].

Stroke Models for Aged Rats

Over the past 10 years, several suitable models for stroke in aged rats have been established. All are based on the permanent [12, 16, 17] or transient occlusion of the middle cerebral artery (MCAO). Transient ischemia was accomplished for 30–120 min by means of a thrombus [18], by intraluminal filament occlusion [15, 19, 20] or by means of a hook attached to a micromanipulator [11]. Long-term hypoxia-ischemia could also be induced by unilateral common carotid artery occlusion [21].

Aged Rats Have Higher Mortality Rates but Not Necessarily Larger Infarcts

Generally, the mortality rate in aged rats is higher than that of young rats. The age difference in mortality is greatest if occlusion is produced by means of an embolus (47 vs. 9%) [18]. In comparison, the intraluminal filament method and photothrombosis produce lower (20–24%) poststroke mortality rates in aged rats [11, 15, 20].

In humans, there is no difference in infarct size with age [21, 22]. Some studies in rats found that cerebral infarct in aged rats was the same size as in young [11, 16–18, 23], while others found that the older rats had larger infarct areas [19, 20].

Aged Animals Recover More Slowly and Less Completely than Young Animals

Aging is associated with a declines in locomotor, sensory and cognitive performance in humans [1] and animals [2–4]. These declines are due in large part to an age-related functional decline of the brain.

Aged persons do not recover from stroke as well as younger persons do [24]. Rehabilitation aims at improving the physical and cognitive impairments and disabilities of patients with stroke. Therefore, studies on behavioral recuperation after stroke in aged animals are necessary and welcome. Various experimental settings have been used to assess the recovery of sensorimotor functions, spontaneous activity and memory after stroke in aged rats [13, 15, 16, 23]. Overall, the results indicate that aged rats have the capacity to recover behaviorally after cortical infarcts, albeit to a lesser extent than the young counterparts [12, 13, 15, 20, 23]. It should be kept in mind, however, that before stroke aged rats are already impaired compared to young animals and show significantly decreased performance in a variety of tests, such as spontaneous locomotor activity [23] and the Morris water maze [25].

As shown schematically in figure 1 (based upon work in our laboratory), all rats had diminished performance on the first day following MCAO, some of which was attributable to the surgery itself. Although recovery did occur in aged rats, its onset was delayed by up to 3 or 4 days depending on the difficulty of the testing [16, 24, 26, 27]. Similar findings have been reported recently for post-stroke recovery of mice prone to accelerated senescence [28].

The extent of recovery was also dependent on the complexity and difficulty of the test. For example, aged rats had difficulties in mastering complex tasks such as our neurological status test (which measures a complexity of motor, sensory, reflex and balance outcomes), the rotarod or the adhesive removal test (which are measures of somatosensory dysfunction) and the Morris water maze [18, 23, 25]. However, the recovery of aged rats on simpler tasks, such as the foot-fault test and the corner test is equivalent to that of young rats. Another factor influenc-
ing the performance level of aged rats is the infarct size, such that functional impairments in the group with the largest infarcts (20% tissue loss) were more severe than the functional impairments in the rats with 4% tissue loss [15]. Figure 1 summarizes all of these differences between young and aged rats in the timing and completeness of recovery following MCAO. The behavioral tests used to assess the recuperation after stroke are given, along with the biological significance of each test, in table 1.
Neurobiology of Tissue Recuperation after Stroke in Aged Animals

Poor recovery may reflect the combination of the more aggressive activation of factors leading to infarct progression (neuronal degeneration, apoptosis, phagocytosis), factors impeding tissue repair (astroglial scar, neurite inhibitory proteins) and neurotrophic factors.

At the same time, factors promoting brain plasticity and growth may be less responsive. Growth-promoting factors include growth-associated proteins GAP43 and CAP23, the growth-promoting transcription factor c-jun, the growth-promoting cell guidance molecule L1, the CDK5-inhibitor p21, microtubule-associated proteins MAP1B and MAP2, immature neurons marker double-cortin, and stem cell marker nestin [26, 29–31]. Pathogenesis of tissue damage is mainly due to inflammatory interactions involving cytokines, chemokines and leukocytes and neurotoxic factors like the C-terminal fragment of β-amyloid (Aβ) [11, 23, 26, 32–34]. One of the main findings is that both timing and magnitude of these factors is dysregulated in the postischemic aged rat brain (fig. 1).

Infarct Development Is Accelerated in Aged Animals

Functional imaging studies after stroke have shown that the reorganization in peri-infarct cortex or connected cortical regions correlates closely with functional recovery [35–37]. Therefore, these regions are mostly studied at cellular and molecular levels.

There are a number of studies on the evolution of infarct volume in aged rats. We recently found that aged rats usually develop an infarct within the first few days after ischemia [27].

In contrast to young animals where the infarct area represented 7% of the ipsilateral hemisphere (fig. 2A), on day 3, the necrotic zone of aged rats lacked NeuN immunopositivity in 28% of the ipsilateral cortical volume (fig. 2B). The infarcted area continued to expand, and by day 7 reached 35–41% of the ipsilateral cortical volume in both young (fig. 2C) and aged rats (fig. 2D). This suggests that the timing of neuronal loss in aged rats is accelerated, but the ultimate extent of brain cell loss is not significantly different from that in young rats. It should be noted, however, that the greater number of degenerating neurons in aged rats are seen only if the infarct area is relatively large; for small infarcts there is no age difference in the number of surviving neurons in the ischemic border zones [15, 17].

Neuronal Degeneration and Loss through Postischemic Apoptosis Are Accelerated in Aged Rats

Fluoro-Jade B staining showed that aged rats had an unusually high number of degenerating neurons in the infarct core as early as day 3 – 3.5-fold vs. young rats (fig. 3A–C). Interestingly, the number of degenerating neurons did not rise further in aged animals, even though the infarcted area continued to expand, so that by day 7 the numbers of degenerating neurons were almost the same in both age groups (fig. 3C) [23, 29].

A major cellular event that contributes to early infarct development in aged rats is augmented apoptosis [38]. Aging increases the susceptibility of the central nervous system to apoptotic events [39]. One possible mechanism

<table>
<thead>
<tr>
<th>Behavioral tests</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological status</td>
<td>rat is pulled gently by the tail and the presence or absence of circling is observed</td>
</tr>
<tr>
<td>Limb-placement symmetry</td>
<td>rat is held gently by the tail at the edge of a table; symmetry or asymmetry of forelimb placement is observed</td>
</tr>
<tr>
<td>Body proprioception</td>
<td>rat is touched lightly on each side of the body with a blunt probe; tests sensorimotor responsiveness</td>
</tr>
<tr>
<td>Response to vibrissae touch</td>
<td>a blunt stick is brushed against the vibrissae on each side, and presence or absence of response is noted; tests sensorimotor responsiveness</td>
</tr>
<tr>
<td>Beam-walking test (rotarod)</td>
<td>rat is tested for its ability to maintain balance while walking on a rotating rod; assesses fine vestibulomotor function</td>
</tr>
<tr>
<td>Inclined plane</td>
<td>the ability of each animal to maintain its position at a given angle on an inclined plane is determined</td>
</tr>
<tr>
<td>Spontaneous activity</td>
<td>rat is placed in a large cage and the number of crossings of a bisecting line is determined; assesses interest in exploration of a novel environment</td>
</tr>
<tr>
<td>T-mazes</td>
<td>rat is placed in a t-maze in which one of the arms of the maze is baited with a reward; tests working and reference memory</td>
</tr>
<tr>
<td>Radial-arm maze</td>
<td>rat is placed in an 8-arm radial maze, elevated 60 cm above the floor; tests spatial working memory</td>
</tr>
</tbody>
</table>
of increased expression of pro-apoptotic proteins in aged animals is via increased NO production by constitutive NO synthase isoforms in a model of transient global ischemia [40]. The particular vulnerability of the aged brain to apoptosis is confirmed by our finding that aged rats had considerably more apoptotic cells 3 days after ischemia (fig. 3E) than young rats (fig. 3D) (2-fold increase over young rats, p < 0.02) (fig. 3H) [27]. On day 7, the ratio was unexpectedly reversed such that aged rats (fig. 3G) now had a smaller number of apoptotic cells than young rats (fig. 3F) (1.7-fold difference, p < 0.05, fig. 3H). However, if the damage to the cerebral cortex is extensive, there is no difference in infarct size or the number of cells undergoing apoptosis between aged and young adults [17].
Postischemic Cellular Proliferation Is Prematurely Increased in Aged Rats and Contributes to an Early Scar Buildup

Our data not only show greater cell death in the infarct zone of aged rats on day 3 poststroke, but also that there are more newly generated cells at this time.

Pulse labeling with BrdU shortly before sacrifice revealed a dramatic increase in proliferating cells in the infarcted area of aged rats on day 3 (fig. 4B), which significantly exceeded the number in young rats at the same time point (fig. 4A). By day 7, the number of BrdU-positive cells had increased substantially in the infarcted area of young rats, too (fig. 4C). Nevertheless, the difference in the number of BrdU-positive cells remained at day 7 poststroke (fig. 4D), at which point the number of BrdU-positive cells peaked in both age groups before abruptly declining to control levels by days 14–28 (not shown).

After multiple BrdU treatments of post-stroke rats, the colocalization of monocytic (ED1) and proliferation (BrdU) markers was maximal on day 7 post-stroke for both young (fig. 4E) and aged (fig. 4F) rats. Although the young rats had a slightly higher cumulative rate of co-expression, i.e. about 45% of the BrdU-positive cells also were ED1 positive, compared to a 37% co-expression rate in aged rats, although this difference is not statistically significant.

The reason for the early accumulation of BrdU-positive cells in the lesioned hemisphere of aged rats remains unknown. We speculate that 2 age-associated factors could be important: (1) a decreased plasticity of the cerebral vascular wall [41] and (2) an early, precipitous inflammatory reaction to injury [23]. The increased fragil-
ity of aged blood vessels due to decreases in distensible components of the microvessels such as elastin [42] may lead, upon ischemic stress, to the fragmentation of cerebral capillaries that would promote the leakage of hematogenous cells into the infarct area [43, 44].

With double-labeling techniques, the proliferating cells in the aged rats brain after stroke were identified either as reactive microglia (45%), oligodendrocyte progenitors (17%), astrocytes (23%), CD8+ lymphocytes (4%), or apoptotic cells (<1%) [27].

**Early, Fulminant Phagocytic Activity of Brain Macrophages in the Postischemic Aged Rat Brain**

Pathogenesis of tissue damage is mainly due to inflammatory interactions involving cytokines, chemokines and leukocytes, and to accumulation of neurotoxic factors like the C-terminal fragment of Aβ [11, 23, 26, 32–34]. Unfortunately, there are very few studies done on such factors in post-stroke aged animals.

Upon examining the phenotype, we found that many proliferating BrdU-positive cells also express markers of brain macrophages, such as ED1. The phagocytic activity of brain macrophages may contribute to the early, rapid development of the infarct in aged animals, and is part of the more general inflammatory reaction occurring after stroke [11, 23, 45, 46]. Markedly increased activity of activated microglia/monocytes has also been reported in senescence-accelerated mice following intracerebral hemorrhage [28].

Activated macrophages generate free radicals, the production of which is augmented in aged subjects following cerebral ischemia [47, 48]. A related consideration is that the vulnerability of brain tissue to traumatic injury [49], especially to DNA damage and oxidative stress, also in-
creases with age [17, 50]. Consistent with such observations is our finding of pronounced microglia activation 3 days poststroke in aged (fig. 5B) but not young (fig. 5A) rats [23].

Rapid Delimitation of the Infarct Area by Scar-Forming Nestin- and GFAP-Positive Cells

In aged animals the infarcted area was already visible at day 3 and was circumscribed by a rim of activated astrocytes (fig. 5D). At this time point there was no accumulation of activated astrocytes in the perinfarcted area of young rats (fig. 5C).

The proliferating astrocytes lead to a premature formation of the scar in aged rats, a phenomenon that limits the recovery of function in aged animals. It should be noted that there are at least 3 cell types contributing the formation of the astroglial scar: nestin-positive cells that are the first to delineate the scar in the brains of aged rats (3 days), followed by GFAP-positive astrocytes (7 days) and finally by cells expressing the N-terminal fragment of β-APP (14 days) [23, 26, 51].

Precipitous and Persistent Expression of the Neurotoxic C-Terminal Fragment of β-APP in the Infarcted Area of Aged Rats

Cerebral ischemia promotes conditions that are favorable to the focal accumulation of neurotoxic factors such as Aβ, especially in the aged brain [11]. In aged rats, the
neurotoxic C-terminal fragment of β-APP steadily accumulated over time and reached a maximum on day 14 in aged rats (fig. 5F) compared to young rats (fig. 5E).

Evidence derived from mice expressing the 100-aminoacid carboxy-terminal fragment of β-APP indicates that this fragment may promote synaptic degeneration and neuronal death [51] and impair learning [52]. Notably, the neurodegeneration is accelerated with increasing age [51]. It seems that, in general, an overexpression of β-APP695 in postmitotic neurons results in neuronal degeneration due to intracellular accumulation of this isoform [53].

**Regenerative Potential of the Brain Appears to Be Competent up to 20 Months of Age**

To explore the potential of older animals to initiate regenerative processes following cerebral ischemia, we studied the expression of the juvenile-specific cytoskeletal protein, microtubule-associated protein 1B (MAP1B); the adult-specific protein, microtubule-associated protein 2 (MAP2); the axonal growth marker, βIII-tubulin, in male Sprague-Dawley rats at 3 months and 20 months of age.

Focal cerebral ischemia, produced by reversible MCAO, resulted in vigorous expression of both MAP1B penumbra of 3-month-old (fig. 6A) and, to a lesser extent, 20-month-old rats (fig. 6B) at 14 days following the stroke [26, 29]. Similarly, MAP2 protein and mRNA were upregulated in the perinfarcted area at almost the same levels both in young (fig. 6C) and aged (fig. 6D). Somewhat lower levels of expression were noted for the axonal growth marker, βIII-tubulin, in the perinfarcted area of aged rats (fig. 6F) compared to young rats (fig. 6E). Collectively, these results suggest that the regenerative potential of the brain at the structural level is competent up to 20 months of age.

Recent studies confirm that mechanisms for self-repair in the young brain also operate in the aged brain. For example, stroke causes increased numbers of new striatal neurons despite lower basal cell proliferation in the subventricular zone in the aged brain [54, 55]. However, despite conserved proliferative activity in the subventricular zone, the number of neurons that reach the injury site is quite modest, as was shown recently for doublecortin-positive neurons in the infarcted area of aged rats [27]. One possible explanation is that lateral ventricle-derived nestin-positive cells do not pass the corpus callosum barrier, and therefore cannot contribute to generation of neurons in the neocortex. Indeed, current evidence indicates that the great majority of newly formed cells in the adult brain are non-neuronal [56–58].

Recent studies also indicate that the molecular profile of growth-promoting genes is very different between aged and young adult groups during the sprouting response to lesions in the CNS. Aged individuals activate most growth-promoting genes at later time-points following stroke than young adults. This includes a delayed induction of GAP43, CAP23 and the growth-promoting transcription factor c-jun. The growth-promoting cell guidance molecule L1 and the CDK5 inhibitor p21 are actually downregulated during the axonal sprouting process in aged individuals compared with a robust and early upregulation of these 2 molecules in young adults [30, 31].

Few neuroprotectants are effective in aged rodents. A major goal of clinical research is to limit the infarct size and one major line of investigation has involved the hypothesis that infarct size is determined by the degree of excitotoxicity. This line of reasoning is based on the observation that excessive concentrations of glutamate can lead to neuronal death.

The failure of multiple clinical trials to demonstrate any neuroprotective efficacy of several glutamate or N-methyl-D-aspartate receptor antagonists has led investigators to search for other potential causative mechanisms. Good candidates are antagonists to the N-methyl-D-aspartate receptor antagonist, e.g. MK-801, and the AMPA receptor antagonist, e.g. NBQX. However, both MK-801 and NBQX were found to be less-effective neuroprotectants in aged than in young rats [59]. Nevertheless, a more recent study showed that treatment of aged rats with sildenafil, a phosphodiesterase type 5 inhibitor used to enhance cGMP-mediated relaxation of pulmonary vasculature, improves functional recovery following stroke in both young and aged rats. This treatment may exert its effects by promoting brain plasticity through enhancement of angiogenesis and synaptogenesis [18].

A more general method of neuroprotection that is efficacious in young rats is ischemic preconditioning. However, the degree of protection was reduced in aged compared to young rats [60]. A likely explanation is that the brains of aged animals showed a reduced stress response that is likely to act neuroprotectively to stroke [17].

Neurosteroids have been recently shown to be effective as neuroprotective agents for ischemic stroke. Treatment with physiological concentrations of estradiol decreases ischemic injury by almost 50% compared to sham-operated controls, in both young and aging rats.

---

Gerontology 2008;54:6–17

Petcu/Sfredel/Platt/Herndon/Kessler/
Popa-Wagner
[61, 62]. It is possible that the protective function of estradiol in this model is the suppression of apoptosis in the infarct area, resulting in enhanced neuronal survival in the penumbral region of the infarct [61, 62].

The use of stem cells to replace neurons lost after stroke potentially offers a novel approach to treatments aimed at improving recovery of tissue and function [63, 64]. Such a treatment might utilize the endogenous reserves of stem cells located in the subventricular zone or the subgranular zone of the hippocampus. One major concern, however, with any therapy designed to boost neurogenesis following stroke is that the capacity to produce new neurons may be diminished in the hippocampus and olfactory bulb of aged animals [65–71]. Despite this, a cause for optimism is that a variety of treatments, such as environment enrichment [72], administration of growth factors [66, 73] and induction of epileptic seizures [74] can increase the production of new neurons in aged animals, although at a lower level than in young animals. Even more encouraging is a recent study demonstrating the same degree of neurogenesis in the striatum of old and young animals [55]. Even though this study reported lower levels of neuron production by aged animals in the subgranular and subventricular zones, the report of equivalent levels in the striatum indicates that the potential for self-repair following stroke persists in the aged brain. While the use of the organism’s own stem cells has many advantages, this technique is in its infancy, and the field still awaits an unambiguous proof of principle.

Another experimental approach that has received considerably more attention is the use of external sources of stem cells. One important question is the type of cells that should be used. Both fetal [75] and murine stem cell lines [76, 77] have been used successfully as grafts to improve functional deficits after experimental stroke in rats. Adult stem cells, such as those derived from human umbilical cord blood, have also proven efficacious [78–81].

The appropriate route of stem cell administration must also be determined. One approach is transplantation either into the lesioned hemisphere, the contralateral hemisphere or both. Other possible targets for stem cell administration are the striatum [55, 77], the cortical parenchyma or the cerebral ventricles [76]. Following unilateral stroke, the grafted stem cells appear to be attracted both to the site of damage and to the corresponding contralateral region, suggesting the existence of both local repair processes and those involved in plastic changes in contralateral motor pathways [76].

An additional second route of administration of stem cells is via the circulation, either intravenously [78, 82–84] or by injection into the carotid artery [85]. The field of stroke therapy using stem cells is a new but promising area, and it is hoped that studies to be carried out in the near future may validate a general therapeutic approach.

Conclusions

These results show that: (1) compared to young rats, aged rats develop a larger infarct area, as well as a necrotic zone characterized by a higher rate of cellular degeneration, and a larger number of apoptotic cells; (2) in both aged and young rats, the early, intense, proliferative activity following stroke leads to a precipitous formation of growth-inhibiting scar tissue, a phenomenon amplified by the persistent expression of neurotoxic factors, and (3) the regenerative potential of the rat brain is largely preserved up to 20 months of age but gene expression, temporally displaced, has a lower amplitude and is sometimes of relatively short duration.

References


52 Nalbantoglu J, Santiago-Tirado G, Lahsaini
48 Floyd RA, Hensley K: Nitrone inhibition of
50 Aliev G, Smith MA, Seyidov D, Neal ML,
53 Nishimura I, Uetsuki T, Dani SU, Ohsawa Y,
55 Darsalia V, Heldmann U, Lindvall O, Kokaia
54.70.40.11 - 1/6/2018 11:51:42 AM
55. Darsalia V, Heldmann U, Lindvall O, Kokaia
58 Hess DC, Hill WD, Carroll JE, Borlongan
59 Suzuki Y, Takagi Y, Nakamura R, Hashimoto-
60 He Z, Crook JE, Meschia JF, Brett TG, Dick-
61 Wise PM: Estrogen therapy: Does it help or
62 Dubal DB, Rau SW, Shaghruje PJ, Zhu H, Yu
63 Haas S, Weidner N, Winkler J: Adult stem
cell therapy in stroke. Curr Opin Neurol
64 Bliss T, Guzman R, Daadi M, Steinberg GK:
65 Mirich JM, Williams NC, Berlau DJ, Brunjes
66 Jin K, Minami M, Xie L, Sun Y, Mao XO,
Wang Y, Simon RP, Greenberg DA: Ischemia-induced neurogenesis is preserved but
67 Kempermann G, Gast D, Gage FH: Neuro-
plasticity in old age: sustained fivefold in-
duction of hippocampal neurogenesis by
long-term environmental enrichment. Ann
Neurol 2002;52:135–143.
68 Tropepe V, Craig CG, Morshhead CM, van der
Koooy D: Transforming growth factor-alpha
null and senescent mice show decreased neu-
ral progenitor cell proliferation in the fore-
brain subependyma. J Neurosci 1997;17:
7850–7859.
69 Bondolfi L, Ermini F, Long JM, Ingram DK,
Jucker M: Impact of age and caloric restric-
tion on the dentate gyrus of mice. Neuro-
70 Heine VM, Maslam S, Joels M, Lucassen PJ:
71 Cameron HA, McKay RD: Restoring pro-
duction of hippocampal neurons in old age.
72 Kempermann G, Kuhn HG, Gage FH: Experi-
ence-induced neurogenesis in the senes-
73 Decker L, Picard-Riera N, Lachapelle F, Ba-
ron-Van Evercooren A: Growth factor treat-
ment promotes mobilization of young but
not aged adult subventricular zone precur-
sors in response to demyelination. J Neurosci
74 Gray WP, May K, Sundstrom LE: Seizure in-
duced dentate neurogenesis does not dimin-
ish with age in rats. Neurosci Lett 2002;330:
335–338.
75 Sorensen JC, Grabowski M, Zimmer J, Jo-
hansson BF: Fetal neocortical tissue blocks
implanted in brain infarcts of adult rats in-
terconnect with the host brain. Exp Neurol
76 Modo M, Stroemer RP, Tang E, Patel S,
Hodges E: Effects of implantation site of
stem cell grafts on behavioral recovery from
77 Wong AM, Hodges H, Horsburgh K: Neural stem cell grafts reduce the extent of neuronal
damage in a mouse model of global ischaem.
Brain Res 2005;1063:140–150.
78 Lu D, Sanberg PR, Mahmood A, Li Y, Wang
L, Sanchez-Ramos J, Chopp M: Intravenous
administration of human umbilical cord
blood reduces neurological deficit in the rat
after traumatic brain injury. Cell Transplant
79 Saporta S, Kim JJ, Willing AE, Fu ES, Davis
CD, Sanberg PR: Human umbilical cord
blood stem cells infusion in spinal cord in-
jury: engraftment and beneficial influence on
behavior. Hematother Stem Cell Res 2003;
12:271–278.
80 Xiao J, Nan Z, Motooka Y, Low WC: Trans-
plantation of a novel cell line population of
umbilical cord blood stem cells ameliorates
neurological deficits associated with ischemic
brain injury. Stem Cells Dev 2005;14:
722–733.
81 Nan Z, Grande A, Sanberg CD, Sanberg PR,
Low WC: Infusion of human umbilical cord
blood ameliorates neurologic deficits in rats
with hemorrhagic brain injury. Ann NY
82 Willing AE, Vendrame M, Mallery J, Cas-
sady CJ, Davis CD, Sanchez-Ramos J, San-
berg PR: Mobilized peripheral blood cells
administered intravenously produce functional
recovery in stroke. Cell Transplant 2003;
83 Liu H, Homouo O, Harada K, Nakamura K,
Houkin K, Hamada H, Kocsis JD: Neuropro-	ection by PlGF gene-modified human mes-
enchymal stem cells after cerebral ischaemia.
Brain 2006;129:2734–2745.
84 Homna T, Homnou O, Iihoshi S, Harada K,
Houkin K, Hamada H, Kocsis JD: Intravenous
infusion of immortalized human mes-
enchymal stem cells prevents against injury in
a cerebral ischemia model in adult rat. Exp
85 Shen LI, Li Y, Chen J, Zhang J, Vanguri P,
Borjeman N, Chopp M: Intracarotid trans-
plantation of bone marrow stromal cells in-
creases axon-myelin remodeling after stroke.
Neuroscience 2006;137:393–399.