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Review

# Perturbed cellular response to brain injury during aging

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ABSTRACT

Old age is associated with an enhanced susceptibility to stroke and poor recovery from brain injury, but the cellular processes underlying these phenomena are only partly understood. Therefore, studying the basic mechanisms underlying structural and functional recovery after brain injury in aged subjects is of considerable clinical interest. Behavioral and cytological analyses of rodents that have undergone experimental injury show that: (a) behaviorally, aged rodents are more severely impaired by ischemia than are young animals, and older rodents also show diminished functional recovery; (b) compared to young animals, aged animals 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; (c) both astrocytes and macrophages are activated strongly and early following stroke in aged rodents; (d) in older animals, the premature, intense cytoproliferative activity following brain injury leads to the precipitous formation of growth-inhibiting scar tissue, a phenomenon amplified by the persistent expression of neurotoxic factors; (e) though the timing is altered, the regenerative capability of the brain is largely preserved in rats, at least into early old age. Whether endogenous neurogenesis contributes to spontaneous recovery after stroke has not yet been established. If neurogenesis from endogenous neuronal stem cells is to be used therapeutically, an individual approach will be required to assess the possible extent of neurogenic response as well as the possibilities to alter this response for functional improvement or prevention of further loss of brain function.



## 1. Stroke in aged animals

Age-related brain injuries are a major cause of physical and mental disabilities for which no satisfactory treatment exists. The most prevalent form of brain injury in the elderly results from stroke (Donnan et al., 2008), which can be defined as a disruption of cerebral blood flow owing to vascular blockage or hemorrhage. Understanding the basic mechanisms underlying functional recovery after cerebral stroke in aging subjects is likely to yield new insights into the treatment of brain injury in the clinic.

### *1.1. Stroke in aged animals is clinically more relevant than is stroke in young animals*

Aging is associated with a diminution of locomotor, sensory and cognitive performance in humans (Grady and Craik, 2000) and animals (Clayton et al., 2002; Mesches et al., 2004; Navarro et al., 2005), part of which is due to age-related functional decline of the brain. Studies of cerebral ischemia 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 humans. One possible explanation for this discrepancy between experimental and clinical studies could be the role that age plays in the recovery of the brain from insult. Indeed, the age-dependent increase in conversion of ischemic tissue into infarction suggests that age is a biological marker for the variability in tissue outcome in acute human stroke (Ay et al., 2005).

Although it is well known that aging is a risk factor for stroke (Barnett, 2002; Seshadri et al., 2006), most 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 (Wang et al., 1995; Popa-Wagner et al., 1998; Brown et al., 2003; Markus et al., 2005). In this light, the aged post-acute animal model is clinically most pertinent to stroke rehabilitation and dementia studies, as recommended both by the STAIR committee (Subramanyam et al., 1999) and the Stroke Progress Review Group (Lindner et al., 2003).

Recent data indicate that the aged brain is more vulnerable to acute neurodegeneration than is the young adult brain, and that

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neurological function is more severely impaired by brain injury in aged rodents than in young rodents (Yager et al., 2006; Onyszchuk et al., 2008;). This is equally true for elderly human patients (Tokutomi et al., 2008). Furthermore, aged rats show an accelerated development of the infarct in the first week post-stroke as compared to their young counterparts (Popa-Wagner et al., 2007a,b).

All of these factors together complicate the rational development of therapeutic strategies in the elderly, who are most likely to experience stroke. Indeed, two recent studies have shown that co-administration of a plasminogen activator inhibitor type 1-derived peptide, EEIIMD, with tissue plasminogen activator (tPA), a drug currently used for thrombolysis in stroke units, showed no improvement in total infarct volume, edema formation, or functional outcome in aged rats (Tan et al., 2009). Moreover, attenuation of oxidative stress with apocynin, which is effective in young rats in improving structural and functional restoration, actually exacerbated stroke injury and diminished functional outcome in aged rats (Kelly et al., 2009).

### *1.2. Recuperation from brain injury in the aged subjects is governed by a complex cytological response*

Poor recovery from brain injuries such as stroke and traumatic brain injury (TBI) in aged subjects reflects a balance of factors leading to infarct progression (neuronal degeneration, oxidative stress, glutamate excitotoxicity, apoptosis, phagocytosis), factors impeding tissue repair (astroglial scar, neurite inhibitory proteins), and factors promoting angiogenesis and brain plasticity and repair (neuronal survival factors, insulin-like growth factor 1, vascular growth factors, bone morphogenetic proteins).

Both the timing and the magnitude of these phenomena are dysregulated in the post-ischemic, aged rat brain. Following infarction, sensorimotor function is impaired in most animals, but young rats begin to recover after a brief period of only 1–2 days. In contrast, behavioral recovery in the aged animals does not commence until 2–4 days post-infarct. Furthermore, young animals fully recover after 10–15 days, whereas the aged rats only recover to about 70% of pre-stroke sensorimotor functionality during the same period. No further recovery was noted in aged rats after day 15 post-stroke (Rosen et al., 2005; Buchhold et al., 2008). Similar observations have been reported recently in two murine models of brain injury, a model of moderate controlled impact injury to the somatosensory cortex, and a model of intracerebral hemorrhage. In both models, motor function recovery was delayed and significantly attenuated in aged mice (Gong et al., 2004; Lee et al., 2005; Onyszchuk et al., 2008).

Among the therapeutic options to improve functional recovery in aged subjects, it should be mentioned that housing of post-stroke animals in an enriched environment may help to improve behavioral performance (Buchhold et al., 2008; Paban et al., in press). Enhanced functional recovery and brain reorganization in the aged rat brain could also be achieved by neutralization of the neurite inhibitory protein Nogo when given after ischemic injury.

Both stroke and TBI result in increased oxidative stress, impaired cellular energy metabolism, overactivation of glutamate receptors (resulting in cellular $Ca^{2+}$ overload), and exaggerated neuroinflammation. Accordingly, glutamate receptor antagonists and nicotinamide, which prevents $NAD^{+}$ depletion, have been reported to limit the extent of neuronal damage and to protect neurons against excitotoxicity in animal models of TBI and focal ischemic stroke, respectively (Mattson, 2008; Liu et al., 2009).

The mechanisms involved in poor outcomes following brain injury in aged subjects are not well understood. One factor appears to be the reduced expression of genes implicated in neuroprotective pathways (Anderson et al., 2009). There is considerable evidence that activation of neurotrophic factor signaling pathways can reduce neuronal damage and improve functional outcome in animal models of traumatic brain and spinal cord injury (Mattson, 2008). These findings implicate neuronal plasticity as a general mechanism for recovery in the central nervous system (Markus et al., 2005).

## 2. Perturbed cellular response to stroke in aging

### *2.1. Infarct development and neuronal degeneration are accelerated in aged rats*

Several interconnected factors may contribute to the rapid development of the infarct in aged animals: (i) early neuronal death; (ii) apoptosis; (iii) an early, fulminant phagocytic activation of brain macrophages that remove the remnants of dead neurons and other cellular debris (Badan et al., 2003; Doré et al., 2003). A related consideration is that increased permeability of the blood–brain barrier may contribute to exacerbated neuronal damage and prolonged functional recovery following stroke in aged rats (DiNapoli et al., 2007; Onyszchuk et al., 2008). It should be noted that the vulnerability of brain tissue to traumatic injury (Hoane et al., 2004), and to oxidative stress in particular, also increases with age (Aliev et al., 2002; Floyd and Hensley, 2002; Onyszchuk et al., 2008).

In one study, measurement of the infarct volume at day 3 post-stroke has indicated that 15% of the total ipsilateral cortical volume was devoid of NeuN-immunoreactive neurons in young animals (Popa-Wagner et al., 2007a,b). NeuN immunoreactivity is a marker of neuronal viability, and thus sharply delineates the infarct border (Badan et al., 2003; Popa-Wagner et al., 2007a,b; Buchhold et al., 2008). The degeneration of neurons in young rats continued to progress such that, at 1 week, the infarcted area had stabilized at approximately 37% of the total volume of the ipsilateral hemisphere. In contrast to young animals, 28% of the ipsilateral cortical volume lacked NeuN immunopositivity in aged rats on day 3 post-stroke, and the infarcted area continued to expand to 41% of the cortical volume by day 7. Thus, the development of the infarct was more rapid in aged rats, but by day 7, the cortical infarcts were not significantly different in size in the two age groups (Popa-Wagner et al., 2007a,b). A similar observation has been reported for neuronal density and volume of tissue lost at 28 days after intracerebral hemorrhage in young vs. aged rats (Wasserman et al., 2008).

The age difference in infarct development after stroke is made evident by a mild episode of cerebral ischemia that causes moderate neuronal degeneration in young animals; in contrast, aged, ischemic rats showed a high degree of degeneration already at day 3 (Badan et al., 2003). However, housing of post-stroke animals in an enriched environment may help to reduce the infarct volume. The effect is well established for very young (10 days of age) and young (90 days of age) animals. Conflicting results have been reported for older rats. One study reports that rats receiving the insult at 6 months and housed in environmentally enriched cages actually showed an increased volume of brain damage compared to controls (Saucier et al., 2007) while another study reported a beneficial effect of an enriched environment on infarct volume recovery, albeit with less efficiency than in young counterparts (Buchhold et al., 2008).

Fluoro Jade B-staining confirmed that, 3 days after cerebral ischemia, young rats had few obviously degenerating neurons in the infarcted area (Popa-Wagner et al., 2007a,b). The number of degenerating neurons then increased rapidly through day 7 and reached a maximum at days 7–14. Fluoro JadeB-staining showed that aged rats had an unusually high number of degenerating neurons in the infarct core already on day 3 (3.5-fold vs. young