

# Brain Plasticity Following Ischemia: Effect of Estrogen and Other Cerebroprotective Drugs

Edina A. Wappler<sup>1,2</sup>, Klára Felszeghy<sup>3</sup>, Mukesh Varshney<sup>4</sup>,  
Raj D. Mehra<sup>4</sup>, Csaba Nyakas<sup>3</sup> and Zoltán Nagy<sup>5</sup>

<sup>1</sup>*Department of Pharmacology, Tulane University, New Orleans*

<sup>2</sup>*Department of Anaesthesiology and Intensive Therapy,  
Semmelweis University, Budapest*

<sup>3</sup>*Neuropsychopharmacology Research Unit of Semmelweis University and  
Hungarian Academy of Sciences, Budapest*

<sup>4</sup>*Department of Anatomy, All India Institute of Medical Sciences, New Delhi*

<sup>5</sup>*Cardiovascular Center, Department Section of Vascular Neurology,  
Semmelweis University, Budapest*

<sup>1</sup>USA

<sup>4</sup>India

<sup>2,3,5</sup>Hungary

## 1. Introduction

Cytoprotection and brain regeneration are both potential future therapies in the treatment of cerebral ischemia, both based on animal research data. Since Kaas and colleagues first described the reorganization of the sensory cortex after peripheral nerve injury it has become clear that “we can no longer consider the injured brain as a normally wired brain with a missing puzzle piece” (Kaas et al., 1983 as cited in Nudo, 2006) and thus there has been intense research subsequently focused on understanding the regeneration processes following different brain injuries.

In rodent models, permanent and transient middle cerebral artery occlusions (MCAO) are the most relevant for representing human ischemic stroke. The lesioned brain areas in these experimental cases are well comparable to those found in humans. Global cerebral ischemia has been investigated using several models including (1) hypotension with bilateral carotid occlusion, or (2) four vessel (2 carotid arteries + 2 vertebral arteries) occlusion with normotension, (3) hypoxia, and (4) cardiac arrest models in rats or mice, or (5) the transient carotid occlusion in gerbil. These global brain ischemia models mimic severe hypotension, cardiac arrest, hypoxia, cardiac surgery, among other conditions in the human, however, while in rodent models the most vulnerable region is the hippocampus, in human both cortical and subcortical lesions are common. This is one reason why these models are difficult to translate into human studies in global cerebral ischemia, however, longer ischemic periods in rodents also result in patchy cortical and subcortical damage together with the hippocampal damage (Back et al., 2004; Erdő and Hossmann, 2007; Wappler et al.,

2009 and 2010). Though, these models are useful to investigate the pathophysiological mechanisms of brain ischemia, but it is important to note that in human stroke patients there are usually several other factors to consider, such as obesity, hypertension, diabetes, age and medications a patient may be using (Li and Carmichael, 2006; Popa-Wagner et al., 2007; Wappler et al., 2010) which may also determine the size of the lesion together with the regenerative potential of the tissue. This emphasizes the importance of experiments that utilize disease model and not just young, healthy male subjects to investigate brain injury, which will hopefully bring us closer to an understanding of the complex clinical conditions that result in stroke (Nudo, 2007; Popa-Wagner et al., 2007; Wappler et al., 2010). In addition to understanding the process of ischemic brain injury, examining the potential effects of the drugs that afford protection against ischemia is just as crucial under different conditions.

In this chapter we present an overview of the studies describing the regenerative potential of the brain due to ischemic damage. Furthermore, we discuss drugs that increase brain plasticity after ischemic insult in animal models, focusing on estrogen. In addition, we describe a brief study examining acute  $17\beta$ -estradiol treatment on synaptic plasticity in the brain with a short (4 days) and a long term (25 days) follow up in young (4 months old) and old (18 months old) gerbils after global cerebral ischemia. The aim of this study was to investigate whether changes in synaptic density can be maintained after a longer period of single dose estrogen treatment, as we have demonstrated an early induction of neuronal plasticity using this model (Wappler et al., 2011b). Maintenance of synaptic density may be an important factor underlying the previously described better functional outcome using this model that was investigated up to the 2<sup>nd</sup> week after brain ischemia (Wappler et al., 2010). In our current study, we examined two different age groups because synaptic reorganization is known to decrease with advancing age (Kim and Jones, 2010). Our results can help elucidate how synaptic reorganization progresses in the brain after global cerebral ischemia due to a single treatment of a cerebroprotective drug and how brain plasticity is influenced by age.

## **2. Brain regeneration after cerebral ischemia**

Cerebral plasticity is the ability of the brain to change its structure and function during maturation, learning, environmental challenges or pathology (Di Filippo et al., 2008; Lledo et al., 2006). The exact mechanism of spontaneous brain regeneration after brain ischemia is not fully understood; however, there is a remarkable number of publications that describe several mechanisms participating in this process.

Here we discuss the following features in brain regeneration: 1. neural plasticity 2. vascular plasticity and 3. glial plasticity.

### **2.1 Neuronal plasticity**

For almost one hundred years neuroscientists have believed that the adult primate brain, and therefore the human brain, is structurally stable and does not form new synapses or grow new cells (Gould et al., 1999). It is clear by now that certain brain regions generate new cells, and that the continuous “rewiring” of the brain is an important physiological function.

Cortical interneurons but not pyramidal cells have been described to have intense arborization as axonal sprouting, dendritic growth and branching under physiological conditions (Lee et al., 2006) throughout adulthood. The “baseline” cerebral plasticity; however, is much more limited in the mature brain, compared to the developing brain, where high activity takes place. This phenomenon is generated by those structural and functional “brakes”, such as myelin, and several neuromodulators, that actively suppress plasticity in the adulthood (Bavelier et al., 2010).

Following cerebral ischemia, or other types of brain insult; however, neuronal plasticity is reactivated in the surviving cells in order to compensate for cell death and to preserve functionality of the damaged but not dead areas (Blizzard et al. 2011; Carmichael, 2006). The possible mechanisms of neural plasticity include dendritic reorganization, axonal sprouting, and activation of endogenous pluripotent cells that can differentiate into neurons. While it has been shown that interneurons undergo structural remodeling in post-traumatic cortical lesions, signs of neural plasticity have not been detected in pyramidal neurons (Blizzard et al., 2011).

### 2.1.1 Dendritic, axonal and synaptic plasticity

Early studies demonstrated that following entorhinal lesion, as the result of neuronal projections from the contralateral hippocampus, new synapses are formed in the damaged cortex (Lynch et al., 1973). In addition, several subsequent studies have shown dendritic and axonal reorganization after experimental brain ischemia with dynamic changes of synaptic density in the injured brain region (Benowitz and Carmichael, 2010; Brown et al., 2010; Lu et al., 2004; Mostany et al., 2010; Scheff et al., 2005; Sulkowski et al., 2006; Takatsuru et al., 2009; etc.). The most active neuronal regeneration occurs up to 2-3 weeks after brain injury (Blizzard et al. 2011; Jones and Schallert, 1992), which provides a wide therapeutic window in cerebral ischemia.

Cerebral ischemia induces axonal sprouting within the peri -infarct zone and contralateral side. This post-ischemic axonal sprouting establishes new neuronal connection pattern for the damaged brain areas (Carmichael, 2003). Axonal sprouting after different central nervous system injuries can be detected by using growth-associated protein-43 (GAP-43) as it is a marker of regeneration in the adulthood (Benowitz and Routtenberg, 1987; Benowitz and Perrone-Bizzozero, 1991; Simon et al., 2001; Wappler et al., 2011b). During brain development GAP-43 is highly associated with the elongating axons (Benowitz and Routtenberg, 1987; Benowitz and Perrone-Bizzozero, 1991), which becomes less concentrated proximal to the cell soma while the axon is growing, but stays in the growth cone (Benowitz and Perrone-Bizzozero, 1991). GAP-43 is also thought to be involved in neurotransmitter release. The highest GAP-43 immunoreactivity was detected on the 4<sup>th</sup> postnatal day, when the most active synaptogenesis takes place. This is followed by a rapid decrease in the immunoreactivity with only a few brain regions expressing GAP-43 later in the adulthood. These regions are the cerebral cortex, the hippocampus, the hypothalamus, the amygdala, the striatum, the medial substantia nigra, raphe nuclei, locus coeruleus, olfactory bulb, olfactory tubercule, preoptic area, and stria terminalis (Benowitz and Perrone-Bizzozero, 1991; Yao et al., 1993). Each of these regions has a different level of constant reorganization. In the hippocampus, this reorganization is related to synaptic remodeling during memory formation (Benowitz and Perrone-Bizzozero, 1991; Holahan et

al., 2007). Astrocytes and perivascular extracellular matrix are involved in guiding axonal growth and provide a scaffolding surface for this growth (Nedergaard and Dirnagl, 2005).

Shortly after focal cerebral ischemia many neurons in the penumbra lose their dendritic spines in an attempt to survive (Brown et al., 2008; Benowitz and Carmichael, 2010). Nevertheless up to two weeks after stroke dendritic density and turnover increases in the peri-infarct cortex and also in the contralateral hemisphere, which plays a major role in brain regeneration (Brown et al., 2010; Mostany et al., 2010; Takatsuru et al., 2009). In addition, following cardiac arrest decreased microtubule-associated protein 2 (MAP-2) expression was detected in the rat reflecting lower dendritic density (Sulkowski et al., 2006). Recent studies have shown dendritic arbor shortening in the ischemic penumbra in the first weeks following stroke with further loss of dendritic branches after the first month in cortical pyramidal cells (Mostany and Portera-Cailliau, 2011). However, dendritic changes in the basilar tree of these cells or in other neuronal cell types in the cortex could not be ruled out. In contrast, enhanced dendritic arborization has been described in cortical pyramidal neurons following photothrombotic brain ischemia (Brown et al., 2010). Thus, the changes in dendrite vary among different cerebral ischemia models.

In different central nervous system injuries synaptogenesis (formation of new synapses) is critical because new connections restore the cell communication and signaling. Following injury, surviving neurons have been described to form new synapses to compensate for the lost contact surfaces even if pre-traumatic synapse number is not achieved in the traumatized area (Lu et al., 2004; Scheff et al., 2005). Therefore examining synaptic density is a widely used technique to track neuronal plasticity following brain lesions. Both synaptophysin (SYP) and synapsin-I are widely used synaptic markers to assess synaptic density. The vesicular transmembrane protein, SYP, is expressed in the presynaptic terminal and its expression seems to be dispensable in neurotransmission (Becher et al., 1996; Eshkind and Leube, 1995; McMahon et al., 1996) but may be involved in fine-tuning of synaptic activity and in vesicle biogenesis (Becher et al., 1996; Janz and Sudhof, 1998). Synapsin-I is a phosphoprotein located on the small synaptic vesicles in the presynaptic terminal (De Camilli et al., 1983; Schiebler et al., 1986), and participates in regulating plasticity (Roshal et al., 1993; Wei et al., 2011). Following axonal sprouting and dendritic reorganization, sometimes just 21 days after the ischemia, synaptic density increases suggesting the development of mature synapses (Stroemer et al., 1995 as cited in Carmichael, 2003). In addition it has been shown in a rodent model that synaptic density steadily decreased up to one week following global cerebral ischemia (Sulkowski et al., 2006).

### 2.1.2 Endogenous neurogenesis

While it was not believed that the adult human brain was able to form new neurons, Altman and Das provided the first evidence that neurogenesis occurred in the mature rodent brain (Altman and Das 1965 and 1967). These data opened a new chapter in neuroscience research. Besides the physiological functions, such as memory formation and learning, endogenous neurogenesis has become a major focus of research in different brain lesions (Gao et al., 2009; Kokaia and Lindvall, 2003; Shen et al., 2010; etc.) and their potential therapies (Kim et al., 2009; Leker et al., 2009; Xiong et al., 2011). The majority of the endogenous cerebral stem cells are located in the subventricular zone and hippocampal

subgranular zone and can generate either neuronal or glial cells (Zhao et al., 2008) in the lesion site using signals from the damaged cells for their activation and migration released.

These pluripotent cells express certain proteins that are typically present during brain development, and therefore are useful markers to track neurogenesis in adulthood following brain injury, such as ischemia. One of the markers is nestin, an intermediate filament protein, which is expressed in the astrocytes and radial glia cells in the developing brain and disappears after the 11<sup>th</sup> postnatal day in the rat (Kalman and Ajtai, 2001). Although previous data suggest that nestin immunopositivity in the adult brain is associated with immature cells that are involved in neurogenesis (von Bohlen und Halbach, 2007; Yue et al. 2006), it also a marker of reactive gliosis following various brain lesions (see e.g. Duggal et al. 1997). It also have been reported that following focal ischemia in the rat nestin positive cells from the ipsilateral subventricular zone can differentiate into glial cells (Holmin et al., 1997; Nakagomi et al., 2009; Shen et al., 2010). Therefore, in the adult brain, nestin expression recurs in both proliferating cells and in reactive astrocytes.

## 2.2 Vascular plasticity

Vascular plasticity includes processes such as angiogenesis and arteriogenesis. Angiogenesis is related to hypoxia and results in new capillaries from the pre-existing vessels, whereas arteriogenesis is induced most importantly by increased shear stress that results in newly formed blood vessels (Heil and Sharper, 2004; Heil et al., 2006; Schierling et al., 2009; Xiong et al., 2010); however, the differences in the cause and the result is usually are not this clear cut. Angiogenesis has a major role in brain regeneration after ischemia as increased blood supply directly enhances cell survival and regenerative processes (Font et al., 2010). Blood vessels not only provide metabolic support but also participate in neurogenesis by leading progenitor cells to the site of injury (Jin et al., 2002; Kojima et al., 2010 as cited in Font et al., 2010; Sun et al., 2010; Udo et al., 2008; Xiong et al., 2010; Yang et al., 2010). There is extensive evidence that neovascularization (both angiogenesis and arteriogenesis) is induced following acute (del Zoppo and Mabuchi, 2003; Issa et al., 2005) and chronic (Busch et al., 2003; Wappler et al., 2011a) brain ischemia.

Hypoxia induced hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) are the most common angiogenesis stimulators (Busch et al., 2003; Carmeliet and Collen, 1997; Liu et al., 1995; Levy et al., 1995) and are involved in capillary sprouting rather than in larger collateral vessel remodeling (Busch et al., 2003; Carmeliet and Collen, 1997) because they act on endothelial cells without inducing smooth muscle proliferation (Busch et al., 2003). In addition, several other factors, such as fibroblast growth factor (FGF) (Issa et al., 2005), angiopoetins (Lin et al., 2000), transforming growth factor  $\beta$  (TGF $\beta$ ) (Haggani et al., 2005; Krupinski et al., 1996), platelet derived growth factor (PDGF) (Issa et al., 2005; Krupinski et al., 1997), tissue-type plasminogen activator (Carmeliet and Collen, 1997), etc. are just as critical in new vessel formation after brain injury. Most of these molecules have separate effects on cerebroprotection and regeneration, such as the TGF $\beta$ -s (Vincze et al., 2010). In addition, nitric oxide derived from endothelial nitric oxide synthetase (eNOS) also induces endothelial cell proliferation and migration, smooth muscle cell differentiation, and other angiogenic processes, where ischemia and shear stress are triggering mechanisms (Amano et al., 2003; Cui et al., 2009; Murohara et al., 1998; Papapetropoulos et al., 1997; Prior et al., 2003; Rudic et al., 1998). Angiogenesis inhibitors, such as endostatin, angiostatin,

thrombospondin-1 and thrombospondin-2 have also been detected following brain ischemia (Issa et al., 2005), which provide another avenue for therapeutic interventions.

In addition to these growth factors extracellular matrix proteins, such as laminin and dystroglycan complex (DGC) proteins, are also involved in vascular remodeling in the brain (Wappler et al., 2011a). The DGC proteins may have an important function in signal transduction connecting the extracellular signals and laminin itself with a wide variety of intracellular proteins, such as nitric oxide synthase, ion channels, kinases, and actin (Culligan and Ohlenieck, 2002; Wappler et al., 2011a). Thus, certain DGC proteins make good immunohistochemical markers of vascular reorganization (Wappler et al., 2011a) in addition to the more frequently used laminin.

Neuroregenerative agents that increase angiogenesis, such as estrogen (Ardelt et al., 2005), have been described to improve functional outcome in models of cerebral ischemia (Hermann and Zechariah, 2009; Goldstein, 2009). These experimental data correlate with clinical data where higher microvessel density in the brain after ischemia was accompanied by shorter recovery time and longer survival (Christoforidis et al., 2005; Font et al., 2010; Krupinski et al., 1994; Navarro-Sobrinio et al., 2011; Wei et al., 2001).

### 2.3 Glial plasticity

Glial cells in the brain include astrocytes, microcytes, and oligodendrocytes, of which astrocytes are the most numerous. In the last decade glial cells have been recognized not just for structural but for metabolic and for trophic support. By secreting nerve growth factor [NGF], basic fibroblast growth factor [bFGF], transforming growth factor  $\beta$  [TGF- $\beta$ ], platelet-derived growth factor [PDGF], brain-derived neurotrophic factor [BDNF], ciliary neurotrophic factor, Neuropilin-1, vascular endothelial growth factor [VEGF], etc., they modulate the function of neurons and other cell types. Glial cells are active participants of synaptic interactions and higher level of cerebral function; and key elements of cerebral blood flow regulation (Araque and Navarrete, 2010; Attwell et al., 2010; Iadecola and Nedergaard, 2007; Metea and Newman, 2006; Nedergaard and Dirnagl, 2005). Glial cells also play a key role in regulating neuronal survival and regeneration by regulating the extracellular ion homeostasis, supporting other cells` energy metabolism, reducing glutamate toxicity, promoting neurogenesis, synaptogenesis and angiogenesis, activating endothelial cells, disrupting blood brain barrier (BBB), increasing inflammation, etc. (Himeda et al., 2007; Nedergaard and Dirnagl, 2005; Trendelenburg and Dirnagl, 2005). Generating new astrocytes is also an important feature in brain regeneration that has been mentioned previously in this chapter.

The formation of glial scar and its beneficial and non-beneficial properties are also of great interest when investigating astrocytic reaction following focal brain ischemia. Unlike other tissues where injury repair results in a fibrous scar, brain injury is followed by a special scar formed by the activated astorocytes and the extracellular matrix molecules of proteoglycans. These include heparan sulfate, dermatan keratan sulfate, sulfate proteoglycans, and chondroitin sulfate, which are released by reactive astrocytes to compose a barrier of axonal growth. The role glial scar formation following brain ischemia is still unknown and intense research is ongoing to understand if it is harmful or supportive (Rolls et al., 2009; Silver and Miller, 2004). Besides its support on the injured tissue (Rolls et al., 2009), its inhibitory effect

on axonal growth is equally important (Cole and McCabe, 1991; Filder et al., 1999; Katoh-Semba et al., 1995; Rudge and Silver, 1990; Smith-Thomas et al., 1994; Snow et al., 1990). Nevertheless, sulphated proteoglycans have also been described as supporting axonal growth (Hikino et al., 2003; Nakanishi et al., 2006), making glial cell based therapeutic strategies more difficult to design. On In contrast, myelin-associated inhibitors from oligodendrocytes and myelin debris, namely myelin-associated glycoprotein (MAG), Nogo-A and oligodendrocyte myelin glycoprotein (OMgp) have clearly inhibitory effect on neurite outgrowth (for review see Yiu and He, 2006) and are under investigation because blocking their function results in enhanced brain regeneration.

### 3. Inducing cerebral plasticity following brain ischemia

A therapeutic window for drugs that increase neural plasticity is wider than those that target cytoprotection following cerebral ischemia (Zhang and Chopp, 2009) giving hope for improved functional outcomes in more stroke patients. A cerebroprotective drug can increase synaptic density in several ways. Cytoprotection, a process where cells can utilize more energy to maintain features that are not necessary in cell survival, can contribute to increased neuronal survival and therefore plasticity in the injured area. Presumably for the same reason increased oxygen and metabolic support improve cellular plasticity after different ischemic events in the brain as already mentioned above.

Whether anti-apoptotic genes are able to induce neuronal plasticity by themselves other than by improving metabolic status is important to understand the pathophysiology of brain ischemia. In order to investigate this question we used Bcl-Xl or Bcl-2 gene construct transfections in an *in vitro* hypoxia model and we observed increased expression of synaptophysin-I and nestin mRNAs and proteins under normoxic conditions. Following hypoxia only nestin expression was significantly different from the untreated hypoxic group (Gal et al., 2009). These data indicate increased that anti-apoptotic gene expression itself can contribute to the amelioration of brain plasticity and its effect might be modified under different stress conditions. Several drugs that are known to be cytoprotective against cerebral ischemia, such as (-)-D-Deprenyl (Simon et al, 2001), and 17 $\beta$ -estradiol (Wappler et al., 2011), also participate in brain regeneration. Although both have anti-apoptotic effect, (-)-D-Deprenyl increases GAP-43 expression whereas 17 $\beta$ -estradiol treatment does not, suggesting that similar pathways may mediate enhanced regeneration through different intracellular signaling (Simon et al, 2001; Szilagyi et al., 2009; Wappler et al., 2011) both *in vitro* and *in vivo*. This is supported by btudies on other cerebroprotective drugs, such as erythropoietin (EPO) (Iwai et al., 2010), statins (Céspedes-Rubio et al., 2010), amphetamine (Liu et al., 2011), melatonin (Chen et al., 2009; González-Burgos et al., 2007), and different spices (Kannappan et al., 2011), where different ways of imporoved brain plasticity was described.

#### 3.1 Estrogen

##### 3.1.1 Estrogen in the brain, estrogen receptors

Corpechot and colleges described the first time that the cerebral sex steroid concentration is much higher than the circulating estrogen level both in men and women (Corpechot et al.,

1981). Subsequently, estrogen synthesis (Le Gascone et al., 1987) and the essential enzymes (Hojo et al., 2004) involved were detected in the brain.

The majority of the investigated intracellular effects of estrogen are related to two estrogen receptors (ERs) in the brain, ER- $\alpha$  and ER- $\beta$ . Both of these receptors are expressed in the central nervous system; however, their distribution show different pattern. While ER- $\alpha$  is highly expressed in the hippocampus, hypothalamus, and preoptic area accompanied by a low expression in the cortex, ER- $\beta$  is densely expressed in the cortex together with a high receptor density in the hippocampus, amygdala, cerebellum, etc. (Brann et al., 2011; Merchenthaler et al., 2003; Shughrue et al., 1997, Shughrue and Merchenthaler, 2000). Both of these ERs form homo- or hetero dimers after binding an estrogen molecule, such as estrone, estriol, or the most effective 17 $\beta$ -estradiol (E2). These dimers can bind to the estrogen responsive elements of the DNA and regulate the expression of several genes, such as bcl-2, IGF-1 (insulin like growth factor-1), NGF, (BDNF (McKenna and O'Malley, 2002; Merchenthaler et al., 2003; Nilsson et al., 2001; Sharma and Mehra, 2008). However, besides this "classical" signaling pathway, which requires hours to days to take place, estrogen can induce rapid changes via its non-genomic pathways. These non-genomic responses are mediated through extranuclear ERs, and occur within minutes of estrogen exposure through activation of several signaling cascades, such as phosphatidylinositol-3-kinase (PI3K), extracellular signal regulated kinase (ERK), mitogen-activated protein kinase (MAPK) or protein kinase C (PKC) (Bourque et al., 2009; Brann et al., 2011; Koszegi et al., 2011; Lebesgue et al., 2009; Rebas et al., 2005). Extranuclear receptors have been detected in the cytoplasm of the cell body, but also in the dendrites and axons of the neurons, while ER immunoreactivity was also seen in the organelle membranes, and synaptic vesicles (Milner et al., 2005). In addition, other brain cells, such as glial cells, have also been shown to express ERs (Milner et al., 2005; Woolley, 2007).

A third estrogen receptor, the G-protein-coupled ER (GPR30), has also been detected in the brain (Funakoshi et al., 2006); however, limited data is available regarding its role under physiological and pathological conditions. One of its reported functions in the hippocampus is to increase synaptic transmission (Filardo et al., 2002; Lebesgue et al., 2009). This receptor is more likely to be associated with the ERK/CREB intracellular signaling pathway (Lebesgue et al., 2009), and presumably activates other intracellular signaling cascades as well. GPR30 protein expression was described in the neuronal plasma membrane and endoplasmic reticulum in several brain regions, such as the hippocampus (Funakoshi et al., 2006; Matsuda et al., 2008).

There is strong evidence that the three different estrogen receptors can crosstalk, for example regulating gene expression through ERK and Src signaling via transcription factor, and histone phosphorylation (Brann et al., 2011; Madak-Erdogan et al., 2008). GPR30 pathway also can crosstalk with other extranuclear pathways through Akt activation (Lebesgue et al., 2009).

The effect of age on ERs is worth to mention here as the incidence of cerebral ischemia is higher in the elderly and the old, which can modify the effect of estrogen therapy. Both ER- $\alpha$  and ER- $\beta$  expressing cell number decreased significantly in the hippocampus of the aged rats; however, optical density of immunoreactivity per cell showed a significant increase for both ER- $\alpha$  and ER- $\beta$  immunoreactivity in the CA1 neurons, whereas in CA3 neurons, it was

significantly reduced (Mehra et al., 2005). Increased expression of ERs per cell is supposedly a compensatory phenomenon. ER- $\beta$  immunoreactivity was, however, found decreased in the CA1 dendritic synapses in old female rats in another study (Waters et al., 2011).

### 3.1.2 Estrogen: Afforded protection and plasticity following brain ischemia

Estrogens are known to increase synaptic density in the intact brain (McEwen, 2002; Merchenthaler et al., 2003; Rune et al., 2006; Sá et al., 2009; Sharma et al., 2007; Woolley, 2007) even following administration of a single dose of this hormone (Sá et al., 2009; Wappler et al., 2011b). Even during oestrus cycle there is an intense fluctuation in dendritic density in rodents. Furthermore, ovariectomy or menopause itself results in a significant decrease of synaptic and dendritic density (Ojo et al., 2011; Woolley and McEwen, 1992). Data on estrogen effect also suggest that it acts directly at synapses by activating second messenger signaling, resulting in a rapid altering in neuronal excitability, synaptic transmission, and/or synaptic plasticity (Woolley, 2007). There is; however, limited data on neuronal plasticity following brain ischemia (Wappler et al., 2011b).

Several studies have shown that E2 therapy is neuroprotective in cerebral ischemia. Estrogen increases the number of surviving cells following ischemia (Liu et al., 2009; Merchenthaler et al., 2003; Platha et al., 2004; Wappler et al., 2010), reduces excitotoxicity (Connell et al., 2007; Herson et al., 2009; Weaver et al., 1997), inflammation (Herson et al., 2009; Stein, 2001; Suzuki et al., 2007), moderates blood-brain barrier dysfunction (Liu et al., 2005), is antioxidant (Connell et al., 2007), increases cerebral blood flow (Herson et al., 2009; Hurn et al., 1995; Pelligrino et al., 1998), reduces spontaneous postischemic hyperthermia (Platha et al., 2004), etc. Cerebral ischemia studies in ER- $\alpha$  and ER- $\beta$  KO mice models, and pharmacological receptor inhibition have shown that ER- $\alpha$  is the primary mediator of neuroprotection. (Brann et al., 2011; Dubal et al., 2001; Liu et al., 2009; Merchenthaler et al., 2003; Miller et al., 2005). Both genomic and non-genomic effects seem to be involved in estrogen afforded neuroprotection (Merchenthaler et al., 2003). Selective GPR30 agonists have also been found neuroprotective in *in vitro* and *in vivo* models of brain ischemia (Gingerich et al., 2010; Lebesque et al., 2010; Zhang et al., 2010), however, its specific role in the pathophysiology of ischemic attack is still unknown.

Genomic effects of estrogen includes the inhibition of apoptosis (through bcl-2, bax, caspase-3); the diminution of inflammation (e.g. through tumor necrosis factor- $\alpha$ ; interleukin 1, and 6); the induction of growth factor, structural protein, and neuropeptide expression; etc. (Merchenthaler et al., 2003). High dose, acute estrogen treatment in global cerebral ischemia also induces cerebral plasticity by increasing synapsin-I and nestin gene expression in gerbils as we described previously (Wappler et al., 2011b). GAP-43 expression was however not elevated further due to the treatment compared to the already increased level after brain ischemia in our model.

Most of the data on estrogen's effect in brain ischemia were observed following chronic rather than acute treatment in rodents, which is a postmenopausal estrogen supplementation model as opposed to a model of acute therapy. There have also been a small number of studies that used older, or diseased animals, or females, especially investigating long term outcome (Wappler et al., 2010).

## **4. Estrogen modulates synaptic density, age, and subregion dependently in the gerbil hippocampus after global brain ischemia**

Our previous reports have demonstrated protective effects of E2 pre-treatment in gerbils following ischemia by increased cell survival, memory function and attention (Wappler et al., 2010). We also showed that increased cerebral plasticity takes place 4 days after the ischemia in the same model (Wappler et al., 2011b). Therefore, we hypothesized that E2 pre-treatment increase the hippocampal synaptic plasticity both in shorter (4 days) and longer (25 days) time points in gerbils at different ages.

### **4.1 Materials and methods**

#### **4.1.1 Animals**

Ovariectomized gerbils of 4 (young), and 18 (old) months of age were used in our experiments. The animals were housed in an air-conditioned room at  $22\pm 1$  °C with a 12 h light/dark cycle, and had free access to water and food. All the procedures on animals were approved by the Animal Examination Ethical Council of the Animal Protection Advisory Board at the Semmelweis University, Budapest, Hungary.

#### **4.1.2 Surgery and 17 $\beta$ -estradiol treatment**

The gerbils were anaesthetized with halothane (induction: 4%, maintaining: 1.5-2.5%) in a 30% O<sub>2</sub>/70% N<sub>2</sub>O mixture, breathing spontaneously via a face mask. Bilateral ovariectomy, and 10 min bilateral carotid occlusion or sham neck surgery were performed as previously described (Wappler et al. 2010). Briefly, bilateral ovariectomy was performed through lateral incisions in each animal. Two weeks later transient bilateral carotid artery occlusion (10 min) was established through a midline cervical incision using atraumatic aneurysm clips (Codman, Johnson and Johnson, Le Locle, NE, Switzerland). The neck tissue was reunited in two layers with non-absorbable, 4.0 silk sutures (Ethicon, Johnson and Johnson). Sham surgery consisted of the midline cervical incision and carotid preparation followed by 10 min period, after which the incision was closed. Thirty minutes prior to surgery, estradiol treated group was given 17 $\beta$ -estradiol (Sigma Chemical Co. St Louis, MO, USA) 0.4 ml/100 g (4 mg/kg) body weight. On the other hand, sham-operated and untreated ischemic animals were injected vehicle solution (50% alcohol in normal saline) in a dose of 0.4 ml/100 g body weight intraperitoneally.

#### **4.1.3 Immunohistochemistry**

On the post-operative day (POD) 4 or 25 (n=5 in each groups) animals were sacrificed under deep halothane anesthesia, and brains were isolated and immersion fixed first in 10% buffered paraformaldehyde for 2 days, then in 4% buffered paraformaldehyde for another 5 days. The brain tissues were then embedded into paraffin. From each animal five 20 $\mu$ m-thick coronal sections, 100  $\mu$ m apart from each other were prepared as previously described (Mehra et al., 2005; Mehra et al., 2007). Goat anti-polyvalent IHC Staining Kit (Labvision, Neomaker lab, USA) was used according to manufacturer protocol for the immunohistochemical localization. SYP specific rabbit polyclonal antibody (Santa Cruz Biotechnology, USA) in a 1:200 dilution was used for 48 – 72 hours at 4 °C for the incubation. Sections were then incubated with biotinylated secondary antibody for 24 hours at 4 °C

followed by streptavidin-HRP complex for 2 hours at RT. For proper maintenance of the cytoarchitectural integrity including preventing undesirable background staining, the sections were thoroughly rinsed with wash buffer (0.1M PBS, pH 7.4) between each incubation steps. Localization of the antigen-antibody site was done with the substrate-chromogen reaction using DAB. Immunoreactive sites became brownish under the bright field microscope. Adjacent sections were stained with cresyl violet (CV) to facilitate demarcation of various layers and subfields of the hippocampus. Intermittently some IHC stained sections were also counterstained with CV for the same purpose.

To eliminate non-specific staining, negative controls were processed by incubating the sections with species-specific normal serum, whereas human breast cancer tissue served as the positive controls (data not shown). Sections from all the groups and the immunohistochemical controls were processed simultaneously and repeatedly to rule out any procedural variations.

#### **4.1.4 Image analysis**

Semi-quantitative estimation of synaptophysin immunoreactivity (SYP-ir) was carried out on every layer (such as the stratum oriens, stratum pyramidale, and stratum radiatum) in the CA1 and CA2-3 subfields of the dorsal hippocampus in all animals. For the semi-quantitative analysis, integrated optical density (IOD) of SYP-ir was measured using image from five hippocampal sections of each animal, 100  $\mu\text{m}$  apart from each other as previously described (Mehra et al., 2005). These images were viewed under the Nikon Microphot -Fx microscope mounted with a Cool Snap Digital camera (Roper Scientific, USA) and attached to the image analysis system driven by Image Pro-Plus software (v 6.2, Media Cybernetics, USA). The size of the sampling field was 5000  $\mu\text{m}^2$ , where 7-9 non-overlapping digital photomicrographs per section were taken. The quantitative analysis was the same as previously described (Mehra et al., 2005). In brief, photomicrographs were first converted to gray scale with proper background correction, and a standard optical density curve was generated for each image prior to analysis (density of corpus callosum devoid of any pre-or postsynaptic protein was measured as background, and subtracted from the image). IOD was measured as cumulative sum of the optical density of immunodense areas. Mathematical values of IOD for comparison between the groups were obtained as arbitrary units (mathematical algorithm) by the analysis software. Data from individual animals of each group were pooled together and the results were expressed as mean IOD  $\pm$  SD.

#### **4.1.5 Statistics**

Statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego California USA). One-way ANOVA with post-hoc Newman-Keuls Multiple Comparison Test was done to compare mean IOD between groups. The level of significance was set at  $p < 0.05$ .

### **4.2 Results**

#### **4.2.1 Synaptophysin immunoreactivity: Aspect of age**

Cell loss in this model of cerebral brain ischemia can be detected both in CA1, and CA3 regions following 10 minutes occlusion, in contrast to the short occlusion times, where only

the CA1 region is affected (Wappler et al., 2010), however, there are surviving cells that gives positive staining to synaptic markers. In the present study, the observed changes in SYP-ir were not limited to just one layer (stratum oriens, stratum pyramidale, or stratum radiatum), but the whole hippocampal region in each case, therefore we discuss our data using the CA1 or the CA3 hippocampal regions.

Young animals showed a significantly lower SYP-ir compared to the old animals after ovariectomy, which resulted in a significant difference between the baseline levels ( $p < 0.01$  young control vs. old control) (Fig.1., panel A). This might have been caused by the more pronounced change in the circulating estrogen level after ovariectomy in the young than in the old, where the estrogen production of the ovaries is low or there is no estrogen production at all. Due to this difference between the baseline values, age-comparisons were more difficult to make between age groups. Changes are therefore presented as percentages of the age-matched controls (Fig.1., panel B). Old gerbils had more severe synaptic loss in the CA1 area than young gerbils where no significant change following the injury was detected (see 4.2.2. for more details and significant differences among each age group). Estrogen treatment had, however, a positive impact on the synaptic density following ischemia in young animals in the CA3 region (see 4.2.2. for more details and significances between treated and non-treated groups), whereas, the same treatment was less effective in the old gerbils, but still helped improved SYP-ir to a certain extent (Fig.1., panel B).

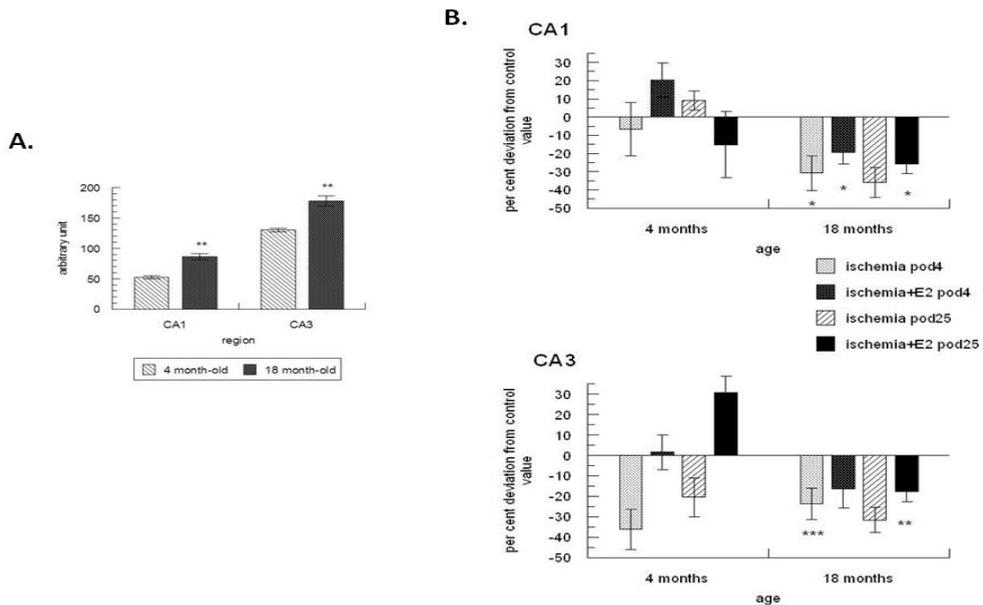


Fig. 1. SYP-ir in the gerbil hippocampus after global cerebral ischemia and estrogen treatment: age comparison. Panel A: control (OVX) groups. Data are expressed as means $\pm$ SEM. See detailed description in the text. \*\* $p < 0.01$  vs. young group same hippocampal region. Panel B: effect of ischemia and ischemia+E2 treatment. Data are expressed as percentages of the age-matched controls $\pm$ SEM. See detailed description in the text. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. the same treatment group of the 4 months old animals.

Please note that not every significant change is marked on this figure that you can find in the text.

#### 4.2.2 Synaptophysin immunoreactivity: Aspect of time and synaptic regeneration

Cerebral ischemia itself decreased SYP-ir only in the CA2-3 region in the young, but decreased in both CA1 and CA2-3 regions in the old animals. This can be explained by the fact that ovariectomy itself decreases synaptic density in the young animals (Woolley and McEwen, 1992), and not every area is affected the same way. Areas that are more dependent on E2 to preserve their synapses might show a relatively lower decrease after ischemia, as their baseline synaptic density is very low, and another stress that is not severe enough, can not cause a significant decrease. In contrast, ovariectomy does not make a significant difference in the circulating E2 levels in the old gerbils, however, there is a slight, but progressive loss of synaptic density and an increased vulnerability to ischemia with age (Ojo et al., 2011; Popa-Wagner et al., 2007), the latter causing a significant decrease even compared to a slightly lower control level. In addition, in the young, at the early time point there was an improvement due to E2 pre-treatment in the CA1 region ( $p < 0.05$  young ischemia POD4 vs. young ischemia+E2 POD4; significance not shown on figure), but at the later time point there was a decrement in synaptic density in the E2-treated group compared to the ischemic group ( $p < 0.05$  young ischemia POD25 vs. young ischemia+E2 POD25; significance not shown on figure). This can be explained by a higher estrogen-dependency and vulnerability in the CA1 region compared to the CA2-3 region in gerbils. In the old animals ischemia significantly decreased synaptic density in the CA1 region, however, we did not observe significant improvement with the estrogen pre-treatment in POD4. It only was observed at the late time point, which was close to be significant ( $p = 0.054$  old ischemia POD25 vs. old ischemia+E2 POD25). This is probably because of the decreasing tendency in SYP-ir following ischemia itself by POD25 that made a more prominent difference between the treated and non-treated group, as E2 treatment seemed to preserve the POD4 synaptic density level. We would like to note that there was no signs of recovery in the old animals following ischemia itself as SYP-ir did not increase by POD25 compared to the POD4 value in the same group.

Moreover, in this study, estrogen pre-treatment increased synaptic density in the hippocampal CA2-3 region in both age groups, however, the increment was more pronounced in the young. The young group showed further increment in synaptic density following ischemia (Fig.2.). In the CA2-3 region at the early and late time point synaptic density increased following estrogen pre-treatment in the young group ( $p < 0.01$  young ischemia POD4 vs. young ischemia+E2 POD4; and  $p < 0.001$  young ischemia POD25 vs. young ischemia+E2 POD25; significances not shown on figure), in addition, following ischemia itself SYP-ir also increased in the young in the CA2-3 area ( $p < 0.05$  young ischemia POD4 vs. young ischemia POD25; significance not shown on figure). However, no significant changes were detected in the old group with the estrogen pre-treatment, and no regenerative changes were observed after ischemia itself either.

#### 4.2.3 Summarizing our results

Even a single dose of E2 treatment can induce long term changes in synaptic density in the injured hippocampus in our gerbil model of cerebral ischemia. At the same time, differences

in age, as well as differences in the investigated brain regions, modulate the degree and the permanence of this E2 effect.

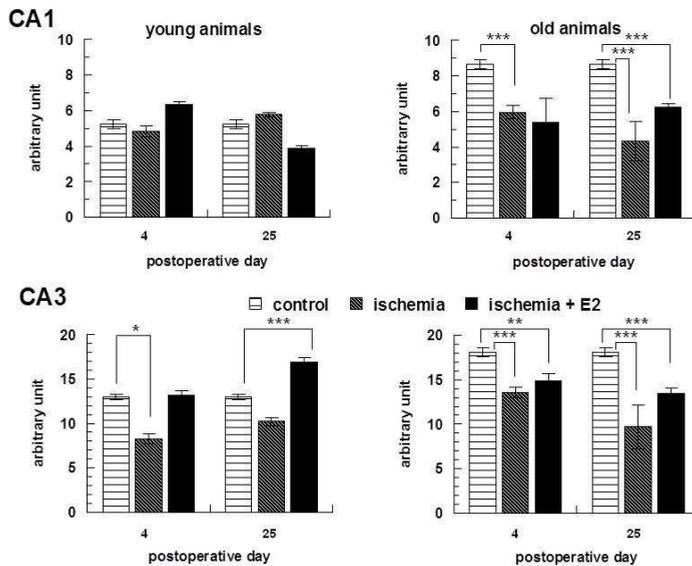


Fig. 2. SYP-ir in the gerbil hippocampus after global cerebral ischemia and estrogen treatment: dynamic changes with time. See detailed description in the text. Data are expressed as means $\pm$ SEM. \* $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\* $p < 0.001$  vs. age-matched control. Please note that only significant changes vs. control groups are shown on the figure, differences between ischemia and ischemia+E2, etc. can be found in the text.

It was an unexpected result for us, that the CA3 region was more vulnerable to ischemia, in terms of synaptic loss, than the CA1 region in the young animals. This result, however, was probably due to the previous ovariectomy that might have had a bigger impact on the CA1 region. This is consistent with our results in the aged animals, where the ischemia decreased synaptic density more in the CA1 region, as expected in this model. It is also possible that aging may predispose to a tendency of diminished synaptogenesis and ability to improve synaptic density, especially in the hippocampal CA3 subfield. Our results emphasize the importance of investigating cerebral regenerative potential in older, female animals as well as at later time points following ischemic injury.

## 5. Conclusion

Post-ischemic brain regeneration is well documented at the tissue, cellular, and subcellular levels that offer further opportunities for drug development to improve functional outcome. In addition, estrogen, a well known regulator of synaptic density, has a long term impact on regeneration after global cerebral ischemia even as a single, high-dose treatment. Age, however, has influence on its effects, which highlights the importance of using old animals in this field.

## 6. Acknowledgement

This work was supported by OTKA-NKTH-K-69240. The authors would like to thank Paige Katz, PhD and Nancy Busija, MA for critical reading and editing this paper.

## 7. References

- Altman, J; Das, G.D. (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol*, Vol. 124, No. 3 (1965 Jun), pp. 319-335.
- Altman, J.; Das, G.D. (1967) Postnatal neurogenesis in the guinea-pig. *Nature*, Vol. 214, No. 5093 (1967 Jun), pp. 1098-1101.
- Amano, K.; Matsubara, H.; Iba, O.; Okigaki, M.; Fujiyama, S.; Imada, T.; Kojima, H.; Nozawa, Y.; Kawashima, S.; Yokoyama, M; Iwasaka, T. (2003) Enhancement of ischemia-induced angiogenesis by eNOS overexpression. *Hypertension*, Vol. 41, No. 1 (2003 Jan), pp. 156-162.
- Araque, A.; Navarrete, M. (2010) Glial cells in neuronal network function. *Philos Trans R Soc Lond B Biol Sci*, Vol. 365, No. 1551 (2010 Aug), pp. 2375-2381.
- Ardelt, A.A.; McCullough, L.D.; Korach, K.S.; Wang, M.M.; Munzenmaier, D.H.; Hurn, P.D. (2005) Estradiol regulates angiopoietin-1 mRNA expression through estrogen receptor-alpha in a rodent experimental stroke model. *Stroke*, Vol. 36, No. 2 (2005 Feb), pp. 337-341.
- Attwell, D.; Buchan, A.M.; Charpak, S.; Lauritzen, M.; Macvicar, B.A.; Newman, E.A. Glial and neuronal control of brain blood flow. *Nature*, Vol. 468, No. 7321 (2010 Nov), pp. 232-243.
- Back, T.; Hemmen, T.; Schüler, O.G. (2004) Lesion evolution in cerebral ischemia. *J Neurol*, Vol. 251, No. 4 (2004 Apr), pp. 388-397.
- Bavelier, D.; Levi, D.M.; Li, R.W.; Dan, Y.; Hensch, T.K. (2010) Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *J Neurosci*, Vol. 30, No. 45 (2010 Nov), pp. 14964-14971.
- Becher, A; Drenckhahn, A; Pahner, I; Margittai, M; Jahn, R; Ahnert-Hilger, G. (1996) The synaptophysin-synaptobrevin complex: a hallmark of synaptic vesicle maturation. *J Neurosci*, Vol. 19, No. 6 (1996 Mar), pp. 1922-1931.
- Benowitz, L.I., Routtenberg, A. (1987) A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism, and synaptic plasticity. *Trends Neurosci*, Vol. 10, No. 12 (1987 Dec), pp. 527-532.
- Benowitz, L.I.; Perrone-Bizzozero, N.I. (1991) The relationship of GAP-43 to the development and plasticity of synaptic connections. *Ann N Y Acad Sci*, Vol. 627 (1991), pp. 58-74.
- Benowitz, L.I.; Carmichael, S.T. (2010) Promoting axonal rewiring to improve outcome after stroke. *Neurobiol Dis*, Vol. 37, No. 2 (2010 Feb), pp. 259-266.
- Blizzard, C.A.; Chuckowree, J.A.; King, A.E.; Hosie, K.A.; McCormack, G.H.; Chapman, J.A.; Vickers, J.C.; Dickson, T.C. (2011) Focal damage to the adult rat neocortex induces wound healing accompanied by axonal sprouting and dendritic structural plasticity. *Cereb Cortex*, Vol. 21, No. 2 (2011 Feb), pp.281-291.
- Bourque, M.; Dluzen, D.E.; Di Paolo, T. (2009) Neuroprotective actions of sex steroids in Parkinson's disease. *Front Neuroendocrinol*, Vol. 30, No. 2 (2009 Jul), pp. 142-157.

- Brann, D.; Raz, L.; Wang, R.; Vadlamudi, R.; Zhang, Q. (2011) Estrogen signaling and neuroprotection in cerebral ischemia. *J Neuroendocrinol*, in press
- Brown, C.E.; Wong, C.; Murphy, T.H. (2008) Rapid morphologic plasticity of peri-infarct dendritic spines after focal ischemic stroke. *Stroke*, Vol. 39, No. 4 (2008 Apr), pp. 1286-1291.
- Brown, C.E.; Boyd, J.D.; Murphy, T.H. (2010) Longitudinal in vivo imaging reveals balanced and branch-specific remodeling of mature cortical pyramidal dendritic arbors after stroke. *J Cereb Blood Flow Metab*, Vol. 30, No. 4 (2010 Apr), pp. 783-91.
- Busch, H.J.; Buschmann, I.R.; Mies, G.; Bode, C.; Hossmann, K.A. (2003) Arteriogenesis in hypoperfused rat brain. *J Cereb Blood Flow Metab*, Vol. 23, No. 5 (2003 May), pp.621-628.
- Carmeliet, P.; Collen, D. (1997) Molecular analysis of blood vessel formation and disease. *Am J Physiol*, Vol. 273, No. 5 (1997 Nov), pp. H2091-104.
- Carmichael, S.T. (2003) Plasticity of cortical projections after stroke. *Neuroscientist*, Vol. 9, No. 1 (2003 Feb), pp. 64-75.
- Carmichael, S.T. (2006) Cellular and molecular mechanisms of neural repair after stroke: making waves. *Ann Neurol*, Vol. 59, No. 5 (2006 May), pp. 735-742.
- Céspedes-Rubio, A.; Jurado, F.W.; Cardona-Gómez, G.P. (2010) p120 catenin/ $\alpha$ N-catenin are molecular targets in the neuroprotection and neuronal plasticity mediated by atorvastatin after focal cerebral ischemia. *J Neurosci Res*, Vol. 88, No. 16 (2010 Dec), pp. 3621-3634.
- Chen, H.Y.; Hung, Y.C.; Chen, T.Y.; Huang, S.Y.; Wang, Y.H.; Lee, W.T.; Wu, T.S.; Lee, E.J. (2009) Melatonin improves presynaptic protein, SNAP-25, expression and dendritic spine density and enhances functional and electrophysiological recovery following transient focal cerebral ischemia in rats. *J Pineal Res*, Vol. 47, no. 3 (2009 Oct), pp. 260-270.
- Christoforidis, G.A.; Mohammad, Y.; Kehagias, D.; Avutu, B.; Slivka, A.P. (2005) Angiographic assessment of pial collaterals as a prognostic indicator following intra-arterial thrombolysis for acute ischemic stroke. *AJNR Am J Neuroradiol*, Vol. 26, No. 7 (2005 Aug), pp. 1789-1797.
- Cole, G.J.; McCabe, C.F. (1991) Identification of a developmentally regulated keratin sulfate proteoglycan that inhibits cell adhesion and neurite outgrowth. *Neuron*, Vol. 7, No. 6 (1991 Dec), pp. 1007-1018.
- Connell, B.J.; Crosby, K.M.; Richard, M.J.; Mayne, M.B.; Saleh, T.M. (2007) Estrogen-mediated neuroprotection in the cortex may require NMDA receptor activation. *Neuroscience*, Vol. 146, No. 1 (2007 Apr), pp. 160-169.
- Corpéchet, C.; Robel, P.; Axelson, M.; Sjövall, J.; Baulieu, E.E. (1981) Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc Natl Acad Sci USA*, Vol. 78, No. 8 (1981 Aug), pp. 4704-4707.
- Cui, X.; Chopp, M.; Zacharek, A.; Zhang, C.; Roberts, C.; Chen, J. (2009) Role of endothelial nitric oxide synthetase in arteriogenesis after stroke in mice. *Neuroscience*, Vol. 159, No. 2 (2009 Mar), pp. 744-750.
- Culligan, K. and Ohlenieck, K. (2002) Diversity of the brain dystrophin-glycoprotein complex. *J. Biomed. Biotechnol.*, Vol. 2, No. 1 (2002), pp. 31-36.
- De Camilli, P.; Harris, S.M. Jr.; Huttner, W.B.; Greengard, P. (1983) Synapsin I (Protein I), a nerve terminal-specific phosphoprotein. II. Its specific association with synaptic

- vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes. *J Cell Biol.* Vol. 96, No. 5 (1983 May), pp. 1355-1373.
- del Zoppo, G.J.; Mabuchi, T. (2003) Cerebral microvessel responses to focal ischemia. *J Cerebr Blood Flow Metab.* Vol. 23, No. 8 (2003 Aug), pp. 879-894.
- Di Filippo, M.; Tozzi, A.; Costa, C.; Belcastro, V.; Tantucci, M.; Picconi, B; Calabresi, P. (2008) Plasticity and repair in the post-ischemic brain. *Neuropharmacology*, Vol. 55, No. 3 (2008 Sep), pp. 353-362.
- Dubal, D.B.; Zhu, H.; Yu, J.; Rau, S.W.; Shughrue, P.J.; Merchenthaler, I.; Kindy, M.S.; Wise, P.M. (2001) Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. *Proc Natl Acad Sci USA*, Vol. 98, No. 4 (2001 Feb), pp. 1952-1957.
- Duggal, N.; Schmidt-Kastner, R.; Hakim, A.M. (1997) Nestin expression in reactive astrocytes following focal cerebral ischemia in rats. *Brain Res.* Vol. 768, No. 1-2 (1997 Sep), pp. 1-9.
- Erdő, F.; Hossmann, K.A. (2007) Animal models of cerebral ischemia--testing therapeutic strategies in vivo. *Ideggyogy Sz.* Vol. 60, No. 9-10 (2007 Sep), pp. 356-369.
- Eshkind, L.G.; Leube, R.E. (1995) Mice lacking synaptophysin reproduce and form typical synaptic vesicles. *Cell Issue Res.* Vol. 282, No. 3 (1995 Dec), pp. 423-433.
- Fidler, P.S.; Schuette, K.; Asher, R.A.; Dobbertin, A.; Thornton, S.R.; Calle-Patino, Y.; Muir, E.; Levine, J.M.; Geller, H.M.; Rogers, J.H.; Faissner, A.; Fawcett, J.W. (1999) Comparing astrocytic cell lines that are inhibitory or permissive for axon growth: the major axon-inhibitory proteoglycan is NG2. *J Neurosci.* Vol. 19, No. 20 (1999 Oct), pp. 8778-8788.
- Filardo, E.J.; Quinn, J.A.; Frackelton, A.R. Jr; Bland, K.I. (2002) Estrogen Action Via the G Protein-Coupled Receptor, GPR30: Stimulation of Adenylyl Cyclase and cAMP-Mediated Attenuation of the Epidermal Growth Factor Receptor-to-MAPK Signaling Axis. *Mol Endocrinol.* Vol. 16, No. 1 (2002 Jan), pp. 70-84.
- Font, A.M.; Arboix A.; Krupinski J. (2010) Angiogenesis, neurogenesis and neuroplasticity in ischemic stroke. *Curr Cardiol Rev.* Vol. 6, No 3 (2010 Aug), pp. 238-244.
- Funakoshi, T.; Yanai, A.; Shinoda, K.; Kawano, M.M.; Mizukami, Y. (2006) G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. *Biochem Biophys Res Commun.* Vol. 346, No. 3 (2006 Aug), pp. 904-910.
- Gál, A.; Pentélenyi, K.; Reményi, V.; Wappler E.A.; Sáfrány G.; Skopál J.; Nagy Z. (2009) Bcl-2 or bcl-XL gene therapy increases neural plasticity proteins nestin and c-fos expression in PC12 cells. *Neurochem Int.* Vol. 55, No. 5 (2009 Sept), pp.349-353.
- Gao, X.; Enikolopov, G.; Chen, J. (2009) Moderate traumatic brain injury promotes proliferation of quiescent neural progenitors in the adult hippocampus. *Exp Neurol.* Vol. 219, No. 2 (2009 Oct), pp. 516-523.
- Gingerich, S.; Kim, G.L.; Chalmers, J.A.; Koletar, M.M.; Wang, X.; Wang, Y.; Belsham, D.D. (2010) Estrogen receptor  $\alpha$  and G-protein coupled receptor 30 mediate the neuroprotective effects of 17 $\beta$ -estradiol in novel murine hippocampal cell models. *Neuroscience*, Vol. 170, No. 1 (2010 Sep), pp. 54-66.
- Goldstein, L.B. (2009) Statins and ischemic stroke severity: cytoprotection. *Curr Atheroscler Rep.* Vol. 11, No. 4 (2009 Jul), pp. 296-300.
- González-Burgos, I.; Letechipía-Vallejo, G.; López-Loeza, E.; Morali, G.; Cervantes, M. (2007) Long-term study of dendritic spines from hippocampal CA1 pyramidal cells, after

- neuroprotective melatonin treatment following global cerebral ischemia in rats. *Neurosci Lett*, Vol. 423, No. 2 (2007 Aug), pp. 162-166.
- Gould, E.; Reeves, A.J.; Graziano, M.S.; Gross, C.G. (1999) Neurogenesis in the neocortex of adult primates. *Science*, Vol. 286, No. 5439 (1999 Oct), pp. 548-552.
- Haggani, A.S.; Nesic, M.; Preston, E.; Baumann, E.; Kelly, J.; Stanimirovic, D. (2005) Characterization of vascular protein expression patterns in cerebral ischemia/reperfusion using laser capture microdissection and ICAT-nanoLC-MS/MS. *FASEB J*, Vol. 19, No. 13 (2005 Nov), pp. 1809-1821.
- Heil, M.; Schaper, W. (2004) Influence of mechanical, and molecular factors on collateral artery growth (arteriogenesis). *Circ. Res.*, 95 (2004), pp. 449-458.
- Heil, M.; Eitenmüller, I.; Schmitz-Rixen, T. and Schaper, W. (2006) Arteriogenesis versus angiogenesis: similarities and differences. *J. Cell. Mol. Med.*, Vol. 10, No. 1 (2006 Jan-Mar), pp. 45-55.
- Hermann, D.M.; Zechariah, A. (2009) Implications of vascular endothelial growth factor for postischemic neurovascular remodeling. *J Cereb Blood Flow Metab*, Vol. 29, No. 10 (2009 Oct), pp. 1620-1643.
- Herson, P.S.; Koerner, I.P.; Hurn, P.D. (2009) Sex, sex steroids and brain injury. *Semin Reprod Med*, Vol. 27, No. 3 (2009 May), pp. 229-239.
- Hikino, M.; Mikami, T.; Faissner, A.; Vilela-Silva, A.C.; Pavão, M.S.; Sugahara, K. (2003) Oversulfated dermatan sulfate exhibits neurite outgrowth-promoting activity toward embryonic mouse hippocampal neurons: implications of dermatan sulfate in neuritogenesis in the brain. *J Biol Chem*, Vol. 278, No 44 (2003 Oct), pp. 43744-43754.
- Himeda, T.; Tounai, H.; Hayakawa, N.; Araki, T. (2007) Postischemic alterations of BDNF, NGF, HSP 70 and ubiquitin immunoreactivity in the gerbil hippocampus: pharmacological approach. *Cell Mol Neurobiol*, Vol. 27, No. 2 (2007 Mar), pp. 229-250.
- Holahan, M.R.; Honegger, K.S.; Tabatadze, N.; Routtenberg, A. (2007) GAP-43 gene expression regulates information storage. *Learn Mem*, Vol. 14, No. 6 (2007 Jun), pp. 407-415.
- Hojo, Y.; Hattori, T.A.; Enami, T.; Furukawa, A.; Suzuki, K.; Ishii, H.T.; Mukai, H.; Morrison, J.H.; Janssen, W.G.; Kominami, S.; Harada, N.; Kimoto, T.; Kawato, S. (2004) Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons. *Proc Natl Acad Sci USA*, Vol. 101, No. 3 (2004 Jan), pp. 865-870.
- Holmin, S.; Almqvist, P.; Lendahl, U.; Mathiesen, T. (1997) Adult nestin-expressing subependymal cells differentiate to astrocytes in response to brain injury. *Eur J Neurosci*, Vol. 9, No. 1 (1997 Jan), pp. 65-75.
- Hurn, P.D.; Littleton-Kearney, M.T.; Kirsch, J.R.; Dharmarajan, A.M.; Traystman, R.J. (1995) Postischemic cerebral blood flow recovery in the female: effect of 17 betaestradiol. *J Cereb Blood Flow Metab*, Vol. 15, No. 4 (1995 Jul), pp. 666-672.
- Iadecola, C. & Nedergaard, M. (2007) Glial regulation of the cerebral microvasculature. *Nat. Neurosci*, Vol. 10, No. 11 (2007 Nov), pp. 1369-1376.
- Issa, R.; AlQteishat, A.; Mitsios, N.; Saka, M.; Krupinski, J.; Tarkowski, E.; Gaffney, J.; Slevin, M.; Kumar, S.; Kumar P. (2005) Expression of basic fibroblast growth factor mRNA

- and protein in the human brain following ischaemic stroke. *Angiogenesis*, Vol. 8, No. 1 (2005), p. 53-62.
- Iwai, M.; Stetler, R.A.; Xing, J.; Hu, X.; Gao, Y.; Zhang, W.; Chen, J.; Cao, G. (2010) Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. *Stroke*, Vol. 41, No. 5 (2010 May), pp. 1032-1037.
- Janz, R.; Sudhof, T.C. (1998) Cellugyrin, a novel ubiquitous form of synaptogyrin that is phosphorylated by pp60c-src. *J Biol Chem*, Vol. 273, No. 5 (1998 Jan), pp. 2851-2857.
- Jin, K.; Zhu, Y.; Sun, Y.; Mao, X.O.; Xie, L.; Greenberg, D.A. (2002) Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc Natl Acad Sci USA*, Vol. 99, No. 18 (2002 Sep), pp. 11946-11950.
- Jones, T.A.; Schallert, T. (1992) Overgrowth and pruning of dendrites in adult rats recovering from neocortical damage. *Brain Res*, Vol. 581, No. 1 (1992 May), pp. 156-60.
- Kaas, J.H.; Merzenich, M.M.; Killackey, H.P. (1983) The reorganization of somatosensory cortex following peripheral nerve damage in adult and developing mammals. *Annu Rev Neurosci*. Vol. 6 (1983), pp.325-356.
- Kálmán, M.; Ajtai, B.M. (2001) A comparison of intermediate filament markers for presumptive astroglia in the developing rat neocortex: immunostaining against nestin reveals more detail, than GFAP or vimentin. *Int J Dev Neurosci*, Vol. 19, No. (2001 Feb), pp. 101-108.
- Kannappan, R.; Gupta, S.C.; Kim, J.H.; Reuter, S.; Aggarwal, B.B. (2011) Neuroprotection by spice-derived nutraceuticals: you are what you eat! *Mol Neurobiol*, Vol. 44, No. 2 (2011 Oct), pp. 142-59.
- Katoh-Semba, R.; Matsuda, M.; Kato, K.; Oohira, A. (1995) Chondroitin sulphate proteoglycans in the rat brain: candidates for axon barriers of sensory neurons and the possible modification by laminin of their actions. *Eur J Neurosci*, Vol. 7, No. 4 (1995 Apr), pp. 613-621.
- Kim, H.J.; Leeds, P.; Chuang, D.M. (2009) The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. *J Neurochem*, Vol. 110, No. 4 (2009 Aug), pp. 1226-1240.
- Kim, S.Y.; Jones, T.A. (2010) Lesion size-dependent synaptic and astrocytic responses in cortex contralateral to infarcts in middle-aged rats. *Synapse*. Vol. 64, No. 9 (2010 Sep), pp. 659-671.
- Kojima, T.; Hirota, Y.; Ema, M.; Takahashi, S.; Miyoshi, I.; Okano, H.; Sawamoto, K. (2010) Subventricular zone-derived neural progenitor cells migrate along a blood vessel scaffold toward the post-stroke striatum. *Stem Cells*, Vol. 28, No 3 (2010 Mar), pp. 545-554.
- Kokaia, Z.; Lindvall, O. (2003) Neurogenesis after ischemic brain insults. *Curr Opin Neurobiol*, 2003, Vol. 13, No. 1 (2003 Feb), pp. 127-132.
- Koszegi Z, Szego EM, Cheong RY, Tolod-Kemp E, Abrahám IM. (2011) Postlesion Estradiol Treatment Increases Cortical Cholinergic Innervations via Estrogen Receptor- $\alpha$  Dependent Nonclassical Estrogen Signaling in Vivo. *Endocrinology*, Vol. 152, No. 9 (2011 Sep), pp. 3471-3482.

- Krupinski, J.; Kaluza, J.; Kumar, P.; Kumar, S.; Wang, J.M. (1994) Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke*, Vol. 25, No. 9 (1994 Sep), pp. 1794-1798.
- Krupinski, J.; Kumar, P.; Kumar, S.; Kaluza, J. (1996) Increased expression of TGF-beta 1 in brain tissue after ischemic stroke in humans. *Stroke*, Vol. 27, No. 5 (1996 May), pp. 852-857.
- Krupinski, J.; Issa, R.; Bujny, T.; Slevin, M.; Kumar, P.; Kumar, S.; Kaluza, J. (1997). A putative role for platelet-derived growth factor in angiogenesis and neuroprotection after ischemic stroke in humans. *Stroke*, Vol. 28, No. 3 (1997 Mar), pp. 564-573.
- Lebesgue, D.; Chevalleyre, V.; Zukin, R.S.; Etgen, A.M. (2009) Estradiol rescues neurons from global ischemia-induced cell death: multiple cellular pathways of neuroprotection. *Steroids*, Vol. 74, No. 7 (2009 Jul), pp. 555-561.
- Lebesgue, D.; Traub, M.; De Butte-Smith, M.; Chen, C.; Zukin, R.S.; Kelly, M.J.; Etgen, A.M. (2010) Acute administration of non-classical estrogen receptor agonists attenuates ischemia-induced hippocampal neuron loss in middle-aged female rats. *PLoS One*, Vol. 5, No. 1 (2010 Jan), pp. e8642.
- Le Goascogne, C.; Robel, P.; Gouézou, M.; Sananès, N.; Baulieu, E.E.; Waterman, M. (1987) Neurosteroids: cytochrome P-450 $\alpha$  in rat brain. *Science*, Vol. 237, No. 4819 (1987 Sep), pp. 1212-1215.
- Lee, W.C.; Huang, H.; Feng, G.; Sanes, J.R.; Brown, E.N.; So, P.T.; Nedivi, E. (2006) Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. *PLoS Biol*, Vol. 4, No. 2 (2006 Feb), pp. e29. Erratum in: *PLoS Biol*, Vol. 4, No 5 (2006 May), e126.
- Leker, R.R.; Toth, Z.E.; Shahar, T.; Cassani-Ingoni, R.; Szalayova, I.; Key, S.; Bratnicsak, A.; Mezey, E. (2009) Transforming growth factor alpha induces angiogenesis and neurogenesis following stroke. *Neuroscience*, Vol. 163, No. 1 (2009 Sep), pp. 233-243.
- Levy, A.P.; Levy, N.S.; Wegner, S.; Goldberg, M.A. (1995) Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J Biol Chem*, Vol. 270, No. 22 (1995 Jun), pp. 13333-13340.
- Li, S.; Carmichael, S.T. (2006) Growth-associated gene and protein expression in the region of axonal sprouting in the aged brain after stroke. *Neurobiol Dis*, Vol. 23, No. 2 (2006 Aug), pp. 362-373.
- Lin, T.N.; Wang, C.K.; Cheung, W.M.; Hsu, C.Y. (2000) Induction of angiopoietin and Tie receptor mRNA expression after cerebral ischemia-reperfusion. *J Cereb Blood Flow Metab*, Vol. 20, No. 2 (2000 Feb), pp. 387-395.
- Liu, H.S.; Shen, H.; Harvey, B.K.; Castillo, P.; Lu, H.; Yang, Y.; Wang, Y. (2011) Post-treatment with amphetamine enhances reinnervation of the ipsilateral side cortex in stroke rats. *Neuroimage*, Vol. 56, No. 1 (2011 May), pp. 280-289.
- Liu, M.; Dziennis, S.; Hurn, P.D.; Alkayed, N.J. (2009) Mechanisms of gender-linked ischemic brain injury. *Restor Neurol Neurosci*, Vol. 27, No. 3 (2009), pp. 163-179.
- Liu, R.; Wen, Y.; Perez, E.; Wang, X.; Day, A.L.; Simpkins, J.W.; Yang, S.H. (2005) 17 $\beta$ -estradiol attenuates blood-brain barrier disruption induced by cerebral ischemia-reperfusion injury in female rats. *Brain Res*, Vol. 1060, No. 1-2 (2005 Oct), pp. 55-61

- Liu, Y.; Cox, S.R.; Morita, T.; Kourembanas, S. (1995) Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. *Circ Res*, Vol. 77, No. 3 (1995 Sep), pp. 638-643.
- Lledo, P.M.; Alonso, M.; Grubb, M.S. (2006) Adult neurogenesis and functional plasticity in neuronal circuits. *Nature Reviews Neuroscience*, Vol. 7, No. 3 (2006 Mar), pp. 179-193.
- Lu, D.; Goussev, A.; Chen, J.; Pannu, P.; Li, Y.; Mahmood, A.; Chopp, M. (2004) Atorvastatin reduces neurological deficit and increases synaptogenesis, angiogenesis, and neuronal survival in rats subjected to traumatic brain injury. *J Neurotrauma*, Vol. 21, No. 1 (2004 Jan), pp. 21-32.
- Lynch, G.; Deadwyler, S.; Cotman, G. (1973) Postlesion axonal growth produces permanent functional connections. *Science*, Vol. 180, No. 4093 (1973 Jun), pp. 1364-1366.
- Madak-Erdogan, Z.; Kieser, K.J.; Kim, S.H.; Komm, B.; Katzenellenbogen, J.A.; Katzenellenbogen, B.S. (2008) Nuclear and extranuclear pathway inputs in the regulation of global gene expression by estrogen receptors. *Mol Endocrinol*, Vol. 22, No. 9 (2008 Sep), pp. 2116-2127.
- Matsuda, K.; Sakamoto, H.; Mori, H.; Hosokawa, K.; Kawamura, A.; Itose, M.; Nishi, M.; Prossnitz, E.R.; Kawata, M. (2008) Expression and intracellular distribution of the G protein-coupled receptor 30 in rat hippocampal formation. *Neurosci Lett*, Vol. 441, No. 1 (2008 Aug), pp. 94-99.
- McEwen, B. (2002) Estrogen actions throughout the brain. *Recent Prog Horm Res*, Vol. 57 (2002), pp. 357-384.
- McKenna, N.J.; O'Malley, B.W. (2002) Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell*, Vol. 108, No. 4 (2002 Feb), pp. 465-74.
- McMahon, H.T.; Boshakov, V.Y.; Janz, R.; Hammer, R.E.; Siegelbaum, S.A.; Sudhof, T.C. (1996) Synaptophysin, a major vesicle protein, is not essential for neurotransmitter release. *Proc Natl Acad Sci USA*, Vol. 93, No. 10 (1996 May), pp. 4760-4764.
- Mehra, R.D.; Sharma, K.; Nyakas, C.; Vij, U. (2005) Estrogen receptor alpha and beta immunoreactive neurons in normal adult and aged female rat hippocampus: a qualitative and quantitative study. *Brain Res*, Vol. 1056, No. 1 (2005 Sep), pp. 22-35.
- Merchenthaler, I.; Dellovade, T.L.; Shughrue, P.J. (2003) Neuroprotection by estrogen in animal models of global and focal ischemia. *Ann N Y Acad Sci*, Vol. 1007 (2003 Dec), pp. 89-100.
- Metea, M. R. & Newman, E. A. (2006) Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. *J Neurosci*, Vol. 26, No. 11 (2008 Mar), pp. 2862-2870.
- Miller, N.R.; Jover, T.; Cohen, H.W.; Zukin, R.S.; Etgen, A.M. (2005) Estrogen can act via estrogen receptor alpha and beta to protect hippocampal neurons against global ischemia-induced cell death. *Endocrinology*, Vol. 146, No. 7 (2005 Jul), pp. 3070-3079.
- Milner, T.A.; Ayoola, K.; Drake, C.T.; Herrick, S.P.; Tabori, N.E.; McEwen, B.S.; Warriar, S.; Alves, S.E. (2005) Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. *J Comp Neurol*, Vol. 491, No. 2 (2005 Oct), pp. 81-95.
- Mostany, R.; Chowdhury, T.G.; Johnston, D.G.; Portonovo, S.A.; Carmichael, S.T.; Portera-Cailliau, C. (2010) Local hemodynamics dictate long-term dendritic plasticity in peri-infarct cortex. *J Neurosci*, Vol. 30, No. 42 (2010 Oct), pp. 14116-14126.

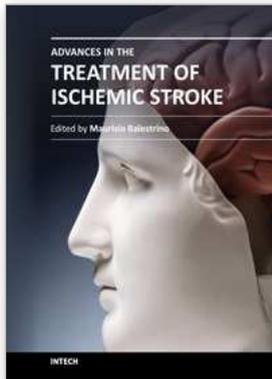
- Mostany, R.; Portera-Cailliau, C. (2011) Absence of large-scale dendritic plasticity of layer 5 pyramidal neurons in peri-infarct cortex. *J Neurosci*, Vol. 31, No. 5 (2011 Feb), pp. 1734-1738.
- Murohara, T.; Asahara, T.; Silver, M.; Bauters, C.; Masuda, H.; Kalka, C.; Kearney, M.; Chen, D.; Symes, J.F.; Fishman, M.C.; Huang, P.L.; Isner, J.M. (1998) Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest*, Vol. 101, No. 11 (1998 Jun), pp. 2567-2578.
- Nakagomi, T.; Taguchi, A.; Fujimori, Y.; Saino, O.; Nakano-Doi, A.; Kubo, S.; Gotoh, A.; Soma, T.; Yoshikawa, H.; Nishizaki, T.; Nakagomi, N.; Stern, D.M.; Matsuyama, T. (2009) Isolation and characterization of neural stem/progenitor cells from post-stroke cerebral cortex in mice. *Eur J Neurosci*, Vol. 29, No. 9 (2009 Apr), pp. 1842-1852.
- Nakanishi, K.; Aono, S.; Hirano, K.; Kuroda, Y.; Ida, M.; Tokita, Y.; Matsui, F.; Oohira, A. (2006) Identification of neurite outgrowth-promoting domains of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan, and involvement of phosphatidylinositol 3-kinase and protein kinase C signaling pathways in neuritogenesis. *J Biol Chem*, Vol. 281, No. 34 (2006 Aug), pp. 24970-24978.
- Navarro-Sobrinho, M.; Rosell, A.; Hernández-Guillamon, M.; Penalba, A.; Boada, C.; Domingues-Montanari, S.; Ribó, M.; Alvarez-Sabín, J.; Montaner, J. (2011) A large screening of angiogenesis biomarkers and their association with neurological outcome after ischemic stroke. *Atherosclerosis*, Vol. 216, No. 1 (2011 May), pp. 205-211.
- Nedergaard, M.; Dirnagl, U. (2005) Role of glial cells in cerebral ischemia. *Glia*. Vol. 50, No. 4 (2005 Jun), pp. 281-286.
- Nilsson, S.; Mäkelä, S.; Treuter, E.; Tujague, M.; Thomsen, J.; Andersson, G.; Enmark, E.; Pettersson, K.; Warner, M.; Gustafsson, J.A. (2001) Mechanisms of Estrogen Action. *Physiol Rev*, Vol. 81, No. 4 (2001 Oct), pp. 1535-1565.
- Nudo, R.J. (2006) Mechanisms for recovery of motor function following cortical damage. *Curr Opin Neurobiol*, Vol. 16, No. 6 (2006 Dec), pp. 638-464.
- Nudo, R.J. (2007) Postinfarct cortical plasticity and behavioral recovery. *Stroke*. (February 2007); Vol. 38, No. 2 Suppl, pp. 840-845.
- Ojo, B.; Rezaie, P.; Gabbott, P.L.; Davies, H.; Colyer, F.; Cowley, T.R.; Lynch, M.; Stewart, M.G. (2011) Age-related changes in the hippocampus (loss of synaptophysin and glial-synaptic interaction) are modified by systemic treatment with an NCAM-derived peptide, FGL. *Brain Behav Immun*, 2011 Oct, in press.
- Papapetropoulos, A.; Garcia-Cardena, G.; Madri, J.A.; Sessa, W.C. (1997) Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *J Clin Invest*, Vol. 100, No. 12 (1997 Dec), pp. 3131-3139.
- Pelligrino, D.A.; Santizo, R.; Baughman, V.L.; Wang, Q. (1998) Cerebral vasodilating capacity during forebrain ischemia: effects of chronic estrogen depletion and repletion and the role of neuronal nitric oxide synthase. *Neuroreport*, Vol. 9, No. 14 (1998 Oct), pp. 3285-3291.
- Plahta, W.C.; Clark, D. L.; Colbourne, F. (2004) 17beta-estradiol pretreatment reduces CA1 sector cell death and the spontaneous hyperthermia that follows forebrain ischemia in the gerbil. *Neuroscience*, Vol. 129, No. 1 (2004 Sep), pp. 187-193.

- Prior, B.M.; Lloyd, P.G.; Ren, J.; Li, Z.; Yang, H.T.; Laughlin, M.H.; Terjung, R.L. (2003) Arteriogenesis: role of nitric oxide. *Endothelium*, Vol. 10, No. 4-5 (2003), pp. 207-216.
- Popa-Wagner, A.; Carmichael, S.T.; Kokaia, Z.; Kessler, C.; Walker, L.C. (2007) The response of the aged brain to stroke: too much, too soon? *Curr Neurovasc Res*, Vol. 4, No. 3 (2007 Aug), pp. 216-227.
- Rolls, A.; Shechter, R.; Schwartz, M. (2009) The bright side of the glial scar in CNS repair. *Nat Rev Neurosci*, Vol. 10, No. 3 (2009 Mar), pp. 235-241.
- Rebas, E.; Lachowicz, L.; Lachowicz, A. (2005) Estradiol modulates the synapsins phosphorylation by various protein kinases in the rat brain under in vitro and in vivo conditions. *J Physiol Pharmacol*, Vol. 56, No. 1 (2005 Mar), pp. 39-48.
- Rosahl, T.W.; Geppert, M.; Spillane, D.; Herz, J.; Hammer, R.E.; Malenka, R.C.; Südhof, T.C. (1993) Short-term synaptic plasticity is altered in mice lacking synapsin I. *Cell*, Vol. 75, No. 4 (1993 Nov), pp. 661-670.
- Rudic, R.D.; Shesely, E.G.; Maeda, N.; Smithies, O.; Segal, S.S.; Sessa, W.C. (1998) Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest*, Vol. 101, No. 4 (1998 Feb), pp. 731-736.
- Rudge, J.S.; Silver, J. (1990) Inhibition of neurite outgrowth on astroglial scars in vitro. *J Neurosci*, Vol. 10, No. 11 (1990 Nov), pp. 3594-3603.
- Rune, G.M.; Lohse, C.; Prange-Kiel, J.; Fester, L.; Frotscher, M. (2006) Synaptic plasticity in the hippocampus: effects of estrogen from the gonads or hippocampus? *Neurochem Res*, Vol. 31, No. 2 (2006 Feb), pp. 145-155.
- Sá, S.I.; Lukoyanova, E.; Madeira, M.D. (2009) Effects of estrogens and progesterone on the synaptic organization of the hypothalamic ventromedial nucleus. *Neuroscience*, Vol. 162, No. 2 (2009 Aug), pp. 307-316.
- Sharma, K.; Mehra, R.D.; Dhar, P.; Vij, U. (2007) Chronic exposure to estrogen and tamoxifen regulates synaptophysin and phosphorylated cAMP response element-binding (CREB) protein expression in CA1 of ovariectomized rat hippocampus. *Brain Res*, Vol. 1132, No. 1 (2007 Feb), pp. 10-19.
- Sharma, K.; Mehra, R.D. (2008) Long-term administration of estrogen or tamoxifen to ovariectomized rats affords neuroprotection to hippocampal neurons by modulating the expression of Bcl-2 and Bax. *Brain Res*, Vol. 1204 (2008 Apr), pp. 1-15.
- Scheff, S.W.; Price, D.A.; Hicks, R.R.; Baldwin, S.A.; Robinson, S.; Brackney, C. (2005) Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. *J Neurotrauma*, Vol. 22, No. 7 (2005 Jul), pp. 719-732.
- Schiebler, W.; Jahn, R.; Doucet, J.P.; Rothlein, J.; Greengard, P. (1986) Characterization of synapsin I binding to small synaptic vesicles. *J Biol Chem*, Vol. 261, No. 26 (1986 Sept), pp. 8383-8390.
- Schierling, W.; Troidl, K.; Mueller, C.; Troidl, C.; Wustrack, H.; Bachmann, G.; Kasprzak, P.M.; Schaper, W.; Schmitz-Rixen, T. (2009) Increased intravascular flow rate triggers cerebral arteriogenesis. *J Cereb Blood Flow Metab*, Vol. 29, No. 4 (2009 Apr), pp. 726-737.
- Shen, C.C.; Yang, Y.C.; Chiao, M.T.; Cheng, W.Y.; Tsuei, Y.S.; Ko, J.L. (2010) Characterization of endogenous neural progenitor cells after experimental ischemic stroke. *Curr Neurovasc Res*, Vol. 7, No. 1 (2010 Feb), pp. 6-14.

- Shughrue, P.J.; Lane, M.V.; Merchenthaler, I. (1997) Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol*, Vol. 388, No. 4 (1997 Dec), pp. 507-525.
- Shughrue, P.J.; Merchenthaler, I. (2000) Estrogen is more than just a "sex hormone": novel sites for estrogen action in the hippocampus and cerebral cortex. *Front Neuroendocrinol*, Vol. 21, No. 1 (2000 Jan), pp. 95-101.
- Silver, J.; Miller, J.H. (2004) Regeneration beyond the glial scar. *Nat Rev Neurosci*, Vol. 5, No. 2 (2004 Feb), pp. 146-156.
- Simon, L.; Szilágyi, G.; Bori, Z.; Orbay, P.; Nagy, Z. (2001) (-)-D-Deprenyl attenuates apoptosis in experimental brain ischaemia. *Eur J Pharmacol*, Vol. 430, No. 2-3 (2001 Nov), pp. 235-241.
- Smith-Thomas, L.C.; Fok-Seang, J.; Stevens, J.; Du, J.S.; Muir, E.; Faissner, A.; Geller, H.M.; Rogers, J.H.; Fawcett, J.W. (1994) An inhibitor of neurite outgrowth produced by astrocytes. *J Cell Sci*, Vol. 107, Pt 6 (1994 Jun), pp. 1687-1695.
- Snow, D.M.; Steindler, D.A.; Silver, J. (1990) Molecular and cellular characterization of the glial roof plate of the spinal cord and optic tectum: a possible role for a proteoglycan in the development of an axon barrier. *Dev Biol*, Vol. 138, No. 2 (1990 Apr), pp. 359-376.
- Stein, D.G. (2001) Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? *Trends Neurosci*, Vol. 24, No. 7 (2001 Jul), pp. 386-391.
- Stroemer, R.P.; Kent, T.A.; Hulsebosch, C.E. (1995) Neocortical neural sprouting, synaptogenesis, and behavioral recovery after neocortical infarction in rats. *Stroke*, Vol. 26, No. 11 (1995 Nov), pp. 2135-2144.
- Sulkowski, G.; Strużyńska, L.; Lenkiewicz, A.; Rafałowska, U. (2006) Changes of cytoskeletal proteins in ischaemic brain under cardiac arrest and reperfusion conditions. *Folia Neuropathol*, Vol. 44, No. 2 (2006), pp. 133-139.
- Sun, J.; Zhou, W.; Ma, D.; Yang, Y. (2010) Endothelial cells promote neural stem cell proliferation and differentiation associated with VEGF activated Notch and Pten signaling. *Dev Dyn*, Vol. 239, No. 9 (2010 Sep), pp. 2345-2353.
- Suzuki, S.; Brown, C.M.; Wise, P.M. (2009) Neuroprotective effects of estrogens following ischemic stroke. *Front Neuroendocrinol*, Vol. 30, No. 2 (2009 Jul), pp. 201-211.
- Szilágyi, G.; Simon, L.; Wappler, E.; Magyar, K.; Nagy, Z. (2009) (-)-Deprenyl-N-oxide, a (-)-deprenyl metabolite, is cytoprotective after hypoxic injury in PC12 cells, or after transient brain ischemia in gerbils. *J Neurol Sci*, Vol. 283, No. 1-2 (2009 Aug), pp. 182-186.
- Takatsuru, Y.; Fukumoto, D.; Yoshitomo, M.; Nemoto, T.; Tsukada, H.; Nabekura, J. (2009) Neuronal circuit remodeling in the contralateral cortical hemisphere during functional recovery from cerebral infarction. *J Neurosci*, Vol. 29, No. 32 (2009 Aug), pp. 10081-10086.
- Trendelenburg, G.; Dirnagl, U. (2005) Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. *Glia*, Vol. 50, No. 4 (2005 Jun), pp. 307-320.
- Udo, H.; Yoshida, Y.; Kino, T.; Ohnuki, K.; Mizunoya, W.; Mukuda, T.; Sugiyama, H. (2008) Enhanced adult neurogenesis and angiogenesis and altered affective behaviors in mice overexpressing vascular endothelial growth factor 120. *J Neurosci*, Vol. 28, No. 53 (2008 Dec), pp. 14522-14536.

- Vincze, C.; Pál, G.; Wappler, E.A.; Szabó, E.R.; Nagy, Z.G.; Lovas, G.; Dobolyi, A. (2010) Distribution of mRNAs encoding transforming growth factors-beta1, -2, and -3 in the intact rat brain and after experimentally induced focal ischemia. *J Comp Neurol*, Vol. 518, No. 18 (2010 Sep), pp. 3752-3770.
- von Bohlen Und Halbach, O. (2007) Immunohistological markers for staging neurogenesis in adult hippocampus. *Cell Tissue Res*, Vol. 329, No. 3 (2007 Sep), pp. 409-420.
- Wappler, E.A.; Szilágyi, G.; Gál, A.; Skopál, J.; Nyakas, C.; Nagy, Z.; Felszeghy, K. (2009) Adopted cognitive tests for gerbils: validation by studying ageing and ischemia. *Physiol Behav*, Vol. 97, No. 1 (2009 Apr), pp. 107-114.
- Wappler, E.A.; Felszeghy, K.; Szilágyi, G.; Gál, A.; Skopál, J.; Mehra, R.D.; Nyakas, C.; Nagy, Z. (2010) Neuroprotective effects of estrogen treatment on ischemia-induced behavioural deficits in ovariectomized gerbils at different ages. *Behav Brain Res*, Vol. 209, No. 1 (2010 May), pp. 42-48.
- Wappler, E.A.; Adorján, I.; Gál, A.; Galgóczy, P.; Bindics, K.; Nagy, Z. (2011a) Dynamics of dystroglycan complex proteins and laminin changes due to angiogenesis in rat cerebral hypoperfusion. *Microvasc Res*, Vol. 81, No. 2 (2011 Mar), pp. 153-159.
- Wappler, E.A.; Gál, A.; Skopál, J.; Nagy, Z. (2011b) Single, high-dose 17 $\beta$ -estradiol therapy has anti-apoptotic effect and induces cerebral plasticity following transient forebrain ischemia in gerbils (Short communication). *Acta Physiol Hung*, Vol. 98, No. 2 (2011 Jun), pp. 189-194.
- Waters, E.M.; Yildirim, M.; Janssen, W.G.; Lou, W.Y.; McEwen, B.S.; Morrison, J.H.; Milner, T.A. (2011) Estrogen and aging affect the synaptic distribution of estrogen receptor beta-immunoreactivity in the CA1 region of female rat hippocampus. *Brain Res*, Vol. 1379 (2011 Mar), pp. 86-97.
- Weaver, C.E. Jr; Marek, P.; Park-Chung, M.; Tam S.W.; Farb, D.H. (1997) Neuroprotective activity of a new class of steroidal inhibitors of the N-methyl-d-aspartate receptor. *Proc Natl Acad Sci USA*, Vol. 94, No. 19 (1997 Sep), pp. 10450-10454.
- Wei, H.; Masterson, S.P.; Petry, H.M.; Bickford, M.E. (2011) Diffuse and specific tectopulvinar terminals in the tree shrew: synapses, synapsins, and synaptic potentials. *PLoS One*, Vol. 6, No 8 (2011 Aug), pp. e23781.
- Wei, L.; Erinjeri, J.P.; Rovainen, C.M.; Woolsey, T.A. (2001) Collateral growth and angiogenesis around cortical stroke. *Stroke*, Vol. 32, No. 9 (2001 Sep), pp. 2179-2184.
- Woolley, C.S.; McEwen, B.S. (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J Neurosci*, Vol. 12, No. 7 (1992 Jul), pp. 2549-54. Erratum in *J Neurosci* 1992 Oct, Vol. 12, No. 10.
- Woolley, C.S. (2007) Acute effects of estrogen on neuronal physiology. *Annu Rev Pharmacol Toxicol*, Vol. 47 (2007), pp. 657-680.
- Xiong, M.; Cheng, G.Q.; Ma, S.M.; Yang, Y.; Shao, X.M.; Zhou, W.H. (2011) Post-ischemic hypothermia promotes generation of neural cells and reduces apoptosis by Bcl-2 in the striatum of neonatal rat brain. *Neurochem Int*, Vol. 58, No. 6 (2011 May), pp. 625-33.
- Xiong, Y; Mahmood, A.; Chopp, M. (2010) Neurorestorative treatments for traumatic brain injury. *Discov Med*, Vol. 10, No. 54 (2010 Nov), pp. 434-442.
- Yang, X.T.; Bi, Y.Y.; Feng, D.F. (2011) From the vascular microenvironment to neurogenesis. *Brain Res Bull*, Vol. 84, No. 1 (2011 Jan), pp. 1-7.

- Yao, G.L.; Kiyama, H.; Tohyama, M. (1993) Distribution of GAP-43 (B50/F1) mRNA in the adult rat brain by in situ hybridization using an alkaline phosphatase labeled probe. *Brain Res Mol Brain Res*, Vol. 18, No. 1-2 (1993 Apr), pp. 1-16.
- Yiu, G.; He, Z. (2006) Glial inhibition of CNS axon regeneration. *Nat Rev Neurosci*, Vol. 7, No. 8 (2006 Aug), pp. 617-627.
- Yue, F.; Chen, B.; Wu, D.; Dong, K.; Zeng, S.E.; Zhang, Y. (2006) Biological properties of neural progenitor cells isolated from the hippocampus of adult cynomolgus monkeys. *Chin Med J*, Vol. 119, No. 2 (2006 Jan), pp. 110-116.
- Zhang, B.; Subramanian, S.; Dziennis, S.; Jia, J.; Uchida, M.; Akiyoshi, K.; Migliati, E.; Lewis, A.D.; Vandenbark, A.A.; Offner, H.; Hurn, P.D. (2010) Estradiol and G1 reduce infarct size and improve immunosuppression after experimental stroke. *J Immunol*, Vol. 184, No. 8 (2010 Apr), pp. 4087-4094.
- Zhang, Z.G.; Chopp, M. (2009) Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. *Lancet Neurol*, Vol. 8, No. 5 (2009 May), pp. 491-500.
- Zhao, C.; Deng, W.; Gage, F.H. (2008) Mechanisms and functional implications of adult neurogenesis. *Cell*, Vol. 132, No. 4 (2008 Feb), pp. 645-660.



## **Advances in the Treatment of Ischemic Stroke**

Edited by Dr. Maurizio Balestrino

ISBN 978-953-51-0136-9

Hard cover, 246 pages

**Publisher** InTech

**Published online** 02, March, 2012

**Published in print edition** March, 2012

In recent years research on ischemic stroke has developed powerful therapeutic tools. The novel frontiers of stem cells therapy and of hypothermia have been explored, and novel brain repair mechanisms have been discovered. Limits to intravenous thrombolysis have been advanced and powerful endovascular tools have been put at the clinicians' disposal. Surgical decompression in malignant stroke has significantly improved the prognosis of this often fatal condition. This book includes contributions from scientists active in this innovative research. Stroke physicians, students, nurses and technicians will hopefully use it as a tool of continuing medical education to update their knowledge in this rapidly changing field.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Edina A. Wappler, Klára Felszeghy, Mukesh Varshney, Raj D. Mehra, Csaba Nyakas and Zoltán Nagy (2012). Brain Plasticity Following Ischemia: Effect of Estrogen and Other Cerebroprotective Drugs, *Advances in the Treatment of Ischemic Stroke*, Dr. Maurizio Balestrino (Ed.), ISBN: 978-953-51-0136-9, InTech, Available from: <http://www.intechopen.com/books/advances-in-the-treatment-of-ischemic-stroke/brain-regeneration-following-ischemia-effect-of-estrogen-and-other-cerebroprotective-drugs>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.