
# $K_{ATP}$ Channel blockade instructs microglia to foster brain repair and neurogenesis after stroke

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# $K_{ATP}$ CHANNEL BLOCKADE INSTRUCTS MICROGLIA TO FOSTER BRAIN REPAIR AND NEUROGENESIS AFTER STROKE

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# Chapter 7.

# DISCUSSION

Ischemic stroke is a result of a transient or permanent reduction in cerebral blood flow restricted to a certain and delimited region. In humans, ischemic stroke occurs most often in the area perfused by the MCA (Mhairi Macrae 1992). It is noteworthy that while in animal models the onset of ischemia and reperfusion can be precisely defined, in humans this is not always possible. For example, the onset of symptoms might not coincide with the onset of cerebral ischemia, or there might be a delay before the patients become aware of these symptoms as in stroke at night or stroke syndromes characterized by unawareness of deficits. Thus, it is difficult to define accurately the time window in which a certain drug might be effective in each patient (Dirnagl et al., 1999). However, in the last years, studies using animal models of stroke have provided remarkable contribution to our understanding of the pathophysiology of ischemic stroke (Lo 2008). One of the most extended stroke models involves transient or permanent MCAO in the rats and mice. When the pathobiology of human stroke is compared with these experimental models, emerging data indicates that the rat tMCAO model may be the best mimicking human ischemic stroke (Yamori et al., 1976). Experimental tMCAO is equivalent to human stroke because cerebral vessel occlusion is seldom permanent, as are most cases human ischemic stroke that have spontaneous or thrombolytic rt-PA therapy-induced reperfusion (Jin et al., 2010).

Tissue damage following cerebral ischemia results from the interaction of complex pathophysiological processes such as excitotoxicity, peri-infarct depolarizations, inflammation and apoptosis (Amantea et al., 2009). Even though the cellular and molecular changes characteristic for this area have been thoroughly studied in animal models, therapies based on these searches have resulted unsuccessful (Arsene et al., 2011; Donnan 2008). Then, given that stroke is caused, at least initially, by a disorder of blood flow in the brain, favorable attempts to establish early reperfusion can reduce the magnitude and extent of tissue injury. Actually, the early blood flow recovery by rt-PA (thrombolytic) is the only current pharmacological treatment approved in humans that has given good results so far (Ekholm et al., 1993; The National Institute of Neurological Disorders 1995). On the other hand, the standard view of neuroprotection, which has long been exclusively neurocentric, is no longer accepted. Instead it has been replaced by a more integrative approach that recognizes

the importance of dynamic interactions between cells that form the neurovascular unit to be committed to brain repair (Ohab and Carmichael 2008; Yang and Rosenberg 2011). Therefore, in the future, it is very likely that interventions will combine strategies that enhance both early reperfusion and neuroprotection.

The most noticeable finding of this study is that Gbc administered between 6 and 24 hours after reperfusion to tMCAO rats significantly reduces the lesion severity, as it improves motor and neurological outcome. When we designed the study we were aware that functional motor recovery after stroke is difficult to assess within the first 3 days, because the period of major brain tissue destruction is not finished and the animals are still recovering from anesthesia and surgery. Thus, in our experimental conditions we expected a discrete functional improvement, if any. Even so, we found a significant 25% improvement in the neuroscore of rats treated with a low dose of Gbc, which means an important neuroprotective effect of the drug that is maintained at long term. At cellular and molecular levels, brain motor and behavior functions are a consequence of the activity of several neuron types from different brain areas that coordinate their functions through a crosstalk of complex signaling pathways. Preservation of these functions after a stroke must involve, multiple actions to both retail cell death and potentiate neuroprotective and restorative processes. According to that, Gbc may present multiple brain targets with several biological functions, which would have significant advantages over individual target drugs or a cocktail of drugs.

Previous preclinical and clinical data suggest a neuroprotective role of Gbc to treat stroke (Kunte et al., 2007; Simard et al., 2009). Sulfonylureas such as Gbc close the $K_{ATP}$ channel by interaction with two drug-binding sites on SUR subunits (Mikhailov et al., 2001) and are widely used to treat diabetes. Simard and colleagues proposed that the astroglial $NC_{Ca\text{-}ATP}$ channel mediates the Gbc-induced prevention of edema after cerebral ischemia (Simard et al., 2006), while in their studies the function of the $K_{ATP}$ channel remained unclear. $K_{ATP}$ channels have been found in neurons, astrocytes, oligodendrocytes and capillaries under ischemic conditions (Simard et al., 2006), whereas our group and others have also suggested the microglial expression of these channels (McLarnon et al., 2001; Ramonet et al., 2004). Therefore, as Gbc may bind to constitute functional $K_{ATP}$ channels after ischemic stroke, other possible

effects of Gbc might explain the effectiveness of this drug in the treatment of stroke.

We herein demonstrated that $K_{ATP}$ channel subunit expression is upregulated in BV2 microglia cell line after LPS+IFNγ activation. Given that the SUR1 subunit has particularly high affinity for Gbc and ATP (Dörschner et al., 1999; Matsuo et al., 2000), we used Gbc to characterize the cellular response of reactive microglia to $K_{ATP}$ channel blockade. Thus, we observed that Gbc increased BV2 reactive morphology, TNFα release and phagocytic capacity. We also confirmed $K_{ATP}$ channel expression in primary rat microglial cultures. When microglial cells became activated after exposure to LPS+IFNγ, they upregulated SUR1, Kir6.1 and Kir6.2 subunits, showing thus higher specific Gbc-labeling extended to the plasmalemmal membrane. These results suggest that microglia activation involves $K_{ATP}$-channel overexpression, and the subsequent protein translocation to the cell surface to regulate phagocytic activity and release of cytokines/chemokines (Ortega et al., 2012a; Virgili et al., 2011). Gbc possibly also blocks the SUR1 subunits in $K_{ATP}$- or $NC_{Ca\text{-}ATP}$- channels expressed in neurons (Toulorge et al., 2010), astrocytes (Simard et al., 2009) and capillary endothelial cells (Simard et al., 2010), however, our results also suggest a crucial role for the $K_{ATP}$ channel in the control of microglial neuroprotective activity after stroke.

In ischemic stroke we can difference two temporal phases. First, the acute phase (minutes to hours) is characterized by a rapid release from the injured tissue of ROS and proinflammatory mediators (cytokines/chemokines). These mediators induce the expression of the adhesion molecules on cerebral endothelial cells and on leukocytes promoting homing of circulating leukocytes and neutrophils (Jin et al., 2010). Secondly, in the subacute phase (hours to days), infiltrating cells participate in the inflammatory response by release of cytokines and chemokines, especially excessive production of ROS and induction/activation of MMP-9. This causes a more extensive activation of resident cells and infiltration of peripheral cells, eventually leading to disruption of the BBB (Yang and Rosenberg 2011), brain edema, neuronal death, and hemorrhagic transformation (Amantea et al., 2009). Regardless of their origin, many of these proinflammatory factors have a dual role at early and late stages of stroke, where microglia has a key role in the pathobiology of the process. Microglia in normal conditions normally monitor the brain environment sensing and eliminating defunct synapses (Tremblay et al., 2011; Wake et al., 2009), controlling

developmental synaptogenesis (Bessis et al., 2006) and clearing newborn adult hippocampal neuroprogenitors (Sierra et al., 2010). After an ischemic insult, microglia but also neurons, astrocytes and oligodendrocytes upregulate a wide panel of cytokines (Mabuchi et al., 2000). While it has been accepted for many years that proinflammatory cytokines, such as IL-1β or TNFα, appear to exacerbate cerebral injury, others microglia-released molecules (e.g. IL-6, IL-10 and transforming growth factor-beta) appears to provide neuroprotection (Iadecola and Anrather 2011). This dogma is now challenged by recent studies, which reflect complex roles of microglia activity with conflicting effects. For instance, notwithstanding the well-known negative effect of TNFα, the effect of this cytokine in general, and on neuronal survival in particular, is largely dependent on the context, timing, and dosage of its activity (Hallenbeck 2002; Lenzlinger et al., 2001). TNFα secretion is crucial for autocrine fast microglial activation with cytotoxic effects, however, neuronal death or survival are TNFα dose dependent (Bernardino et al., 2008), since it activates two specific receptors: TNFR1, with an intracellular death domain, and TNFR2 with higher affinity and mainly involved in neuroprotection (Fontaine et al., 2002). These two receptors are key elements in modulating neuronal sensitivity to ischemia, with microglial-derived TNFα being crucial to determine the survival of endangered neurons in the acute phase of focal cerebral ischemia (Lambertsen et al., 2009). In addition, TNFα is proposed as the molecular mediator of the microglial-mediated synapse removal, likely to be important in remodeling of neuronal circuits and remyelination processes in the ischemic brain to ensure function (Arnett et al., 2003; Wake et al., 2009). Thus, often, a clear distinction between cytokines that are either harmful or beneficial cannot be established, since the cytotoxic proinflammatory cytokines IL-1β and TNFα released from activated microglia may evoke a neuroprotective or pro-myelin regenerative response. This also applies for neurodegenerative processes, since TNFα protects neurons against amyloid-beta-peptide-mediated toxicity under pathological conditions (Barger et al., 1995), and has a role in homoeostatic synaptic scaling under physiological conditions (Stellwagen and Malenka 2006).

Neumann and colleagues (Neumann et al., 2006) have established that after acute injury such as trauma or stroke, appropriately activated microglia and its pro-

inflammatory mediators, may primarily have a neuroprotective role, and therefore, anti-inflammatory treatment within the protective time window of microglia would be counterintuitive. Consequently, *in vivo* identification of a drug target able to prompt the beneficial effects of the primary cellular response, as well as, of the inflammatory reaction in the early phases of stroke would be beneficial for neuroprotection and brain repair; with the subsequent better patient outcome.

In our *in vivo* study we showed that reactive microglia from tMCAo animals upregulated SUR1, SUR2B and Kir6.2 subunits of $K_{ATP}$ channels in the infarct core. Thus, $K_{ATP}$ channels should directly participate in the control of microglial reactivity *in vivo*, with Gbc treatment resulting in a strengthening of the neuroprotective role of microglia/macrophages in these early stages of stroke. First we assessed whether Gbc was able to reach the lesion in case of BBB disruption. We found that Gbc administered after MCAO reached the ischemic hemisphere with a 3-fold concentration increase. In addition, although the volume of lesion measured by $T_2$-MRI was not significantly reduced, Gbc administered at 6, 12 and 24 hours after triggered 50% neuronal preservation in the peri-infarcted region, increased the peri-infarct volume and improved motor neurological outcome. It is noteworthy to mention that, most rodent models of stroke focus on the outcome measurement of lesion size in terms of infarct volume and brain edema. Within the first 3 days of lesion, the methodological approach and accuracy in these measurements are essential, since brain edema development may lead to overestimation of infarct volume (Lin et al., 1993). This accuracy reaches crucial importance in the assessment of drugs activity, as their neuroprotective effects may not be reflected by these two parameters (Walberer et al., 2010). Under our conditions, we used MRI to assess changes in the size of the lesion volume, but this approach cannot give a precise indication of the severity of the injury (Simard et al., 2010) and is not accurate enough to measure the necrotic core of the lesion. In this line, we agree other authors that have described an effect of Gbc in reducing infarct volume, edema and disability in different stroke models (Simard et al., 2006; Simard et al., 2010; Simard et al., 2009). However in those experiments the procedure of Gbc treatment is different to our approach. In Simard's experiments, Gbc dose presenting morphological effects (33 µg/kg) is at least 5 times higher than the highest dose we used herein (6 µg/Kg). At

more similar treatment conditions (Glibenclamide loading dose = 3.3 μg/kg 4 or 6 h after reperfusion versus 6 μg/Kg 6 h after reperfusion), Simard's experiments presented no reduction of edema as well. As this lack of morphological changes does not correlate with the preservation of function, we considered the stereological counting of neurons as more accurate assessment of Gbc-derived neuroprotection. Thereby, when we included histological methods, measurement of the necrotic and peri-infarct volumes (Lin et al., 1993) and neuronal counts defined the morphological and cellular bases of the motor function improvement induced by $K_{ATP}$ channel blockade. Our results strongly support the idea that the fate of the ischemic penumbra during the early steadies of the injury is considered the crucial element for stroke recovery (Furlan et al., 1996).

Stroke causes irreversible tissue damaged in the infarct core, whereas other hypoperfused areas may be at risk but are potentially salvageable. A few minutes after the onset of ischemia, tissue damage occurs in the centre of ischemic injury, where cerebral blood flow is reduced by more than 80%. In this core region, cell death rapidly develops as a consequence of the acute energy failure and loss of ionic gradients associated with permanent and anoxic depolarization, known as excitotoxicity (Dirnagl et al., 1999; Hossmann 1994; Mitsios et al., 2006). The degree of ischemia decreases with distance from the infarct core because collateral vessels maintain sufficient blood flow to allow the potential survival of cells in areas adjacent to the core of the infarct. Cells in this penumbra area have impaired function but remain viable for a period of time (Ginsberg 1997). The ischemic penumbra is a dynamic target that evolves over time and the infarct core evolves rapidly in the first few hours, therefore supporting the concept that "time is brain" (Saver 2006). The fate of this ischemic penumbra during the early steadies of the injury is considered the crucial element for stroke recovery (Furlan et al., 1996) since in the necrotic zone astrocytes and neurons are determined to die. Transcriptional upregulation of SUR1 in the ischemic brain has been related to $NC_{Ca\text{-}ATP}$ channels of neurons, astrocytes and capillary endothelial cells of the peri-infarct region (Simard et al., 2006). As activation of $NC_{Ca\text{-}ATP}$ channels in astrocytes causes cell blebbing characteristic of cytotoxic edema, the Gbc-mediated reduction of infarct volume in permanent MCAO animals has been linked with blockade of these channels (Simard et al., 2009),

although this finding is still a hot topic of debate (Favilla et al., 2011; Simard et al., 2011), and Sala-Rabanal and cols. (2012) have recently refuted this hypothesis. Using recombinant cell line co-transfected with TRMP4, the pore-forming subunit of $NC_{Ca\text{-}ATP}$ channels, and SUR1 genes these authors observed that the coupling between TRPM4 and SUR1 is unlikely to happen (Sala-Rabanal et al., 2012). Despite all this challenges, Simard et al ((Simard et al., 2009)) ruled out the involvement of $K_{ATP}$ channels in the process. Interestingly, they did not assess the ischemia-induced expression changes of the trpm4 gene, (Simard et al., 2010) and, despite the massive neuronal loss, immunoblots revealed no concentration changes of Kir6.1 and Kir6.2 proteins in the ischemic core (Simard et al., 2006). Our findings complete Simard's results and argue for a contribution of $K_{ATP}$ channels to the neuroprotective effects of Gbc by reducing the lesion's severity. Thus, we found that reactive microglia enhances SUR1, Kir6.1 and Kir6.2 protein expression *in vitro*, and that amoeboid microglia express $K_{ATP}$ channels in the necrotic core of the lesion *in vivo*. This upregulation contributed to the enhancement of SUR1 found by Simard and cols. (Simard et al., 2006) and helped to compensate for a putative decrease in Kir6.1 and Kir6.2 subunits due to the massive neuronal loss in the necrotic zone. Moreover, our results also explains the increased Kir6.1 and Kir6.2 expression in absence of the further neuronal damage in brain hypoxia described by other authors (Yamada et al., 2001).

We herein also found the cerebral area occupied by CD3-immunopositive cells for lymphocytes was of small size, which might be related with little infiltration of blood cells 3 days after tMCAO. Infiltrated macrophages and granulocytes are proposed to not play major roles in the early progression of ischemic neuronal damage, whereas reactive microglia are already detected in the zone (Mabuchi et al., 2000). Furthermore, a transition of monocyte-derived cells into microglia is a very rare event that only occurs under highly defined host conditions (Mildner et al., 2007). Thus, microglial activity in the necrotic core would be crucial in determining the fate of the ischemic tissue. Microglia phagocytic abilities are essential for the clearance of cell debris and toxic compounds of the lesioned tissue, which underlies neuroprotection (Polazzi and Monti 2010). In addition, dying PMNs infiltrated in the site of lesion, mediate neurotoxicity by the release of toxic intracellular compounds

(Denes et al., 2007; Weston et al., 2007). Consequently, prompt phagocytosis of apoptotic PMNs by microglia might prevent the secretion of toxic compounds, thus, might be an effective strategy to protect neurons from PMNs neurotoxicity (Napoli and Neumann 2009; Neumann et al., 2008). In this line, we here provide evidence that Gbc increases the reactive morphology and phagocytic capacity of BV2 microglia activated with LPS+IFNγ. Also, *in vivo* the $K_{ATP}$ channel blockade slightly enhances neuronal loss in the necrotic core, thus microglial cells in presence of Gbc are likely to increase their efficiency to clear generated apoptotic cell debris. This activity is beneficial since it reduces the secretion of proinflammatory cytokines (Magnus et al., 2001), chemoattractants and the migration of T lymphocytes (Chan et al., 2006). Also, in hypoxia-ischemia, acute brain damage or excitotoxicity, microglia neuroprotective reaction includes a phagocytic response to calcium deposit formation (Herrmann et al., 1998). The extension of calcification after hypoxia-ischemia in a brain area depends on the intensity of the acute phase and on the characteristics of each area of pathology (Lievens et al., 2000; Nonoda et al., 2009; Rodriguez et al., 2001). We here found a Gbc-induced decrease in ischemic brain calcification with a reduced size and number of deposits, which represents a reduction in tMCAO-induced neuronal suffering and may be related to boosted microglial phagocytic activity. Overall, these data suggest that cell debris clearing from the lesion core will provide an optimal environment for neuroprotection in the surrounding tissue. In this scenario the Gbc-induced neuronal preservation observed in the peri-infarct region may result from the interaction of all the processes explained above. If true, the functional recovery here found will reflect an enhancement of the neuroprotective microglial activity in the necrotic core. This control of microglia activity fully deals with the idea that, in stroke, targeting a single point or a single pathway does not yield sufficient protection, and that the emphasis should be on the targets that mediate cross-talk between multiple cell death mechanisms (Sun and Hu 2009).

Continuous crosstalk mediated by several signaling molecules, takes place between neurons, microglia and astrocytes, differently regulating their relationships in both health and disease (Polazzi and Monti 2010). In this regard, we here found a Gbc-induced increase of S100β in reactive astrocytes of the white mater, probably due to the $NC_{Ca\text{-}ATP}$ channel blockade (Simard et al., 2009), which may influence

microglial-mediated neuroprotection. Released S100β modifies astrocytic, neuronal and microglial activities, whose effects depend on its extracellular concentration and the expression of the specific receptor RAGE. At micromolar concentrations, S100β upregulates TNFα expression in activated microglia (Bianchi et al., 2010), and also stimulates microglia migration via RAGE-dependent up-regulation of chemokine expression and release. In addition, factors that modulate microglial reactivity, such as intracellular calcium concentration or TNFα, modify the RAGE response to S100β (Edwards and Robinson 2006) in a crosstalk that integrates these signaling systems. Thus, microglia reaction directly interacts with the concomitant astroglial reaction and the factors that determine this interaction, such as TNFα and S100β participate in the regulation of the activated phenotype of microglia after injury.

Likewise, among others chemokines, increased mRNA expression for MCP-1, also known as CCL2, and macrophage inflammatory protein-1 alpha has been described in the rat brain after focal cerebral ischemia (Hinojosa et al., 2011; Jin et al., 2010; Liberto et al., 2004). MCP-1 is express by neurons at 12 h after focal brain ischemia, but has also been found in astrocytes and microglia at later stages following the insult. We here found that MCP-1 released by microglia may modify ischemia-induced neurogenesis by promoting NPs neuronal differentiation. There is little doubt that ischemic stroke influence precursor cells and adult neurogenesis (Arvidsson et al., 2002; Kokaia and Lindvall 2003; Yamashita et al., 2006). Nonetheless, whether microglial cells influence neurogenesis and the fate of the newborn neurons is still controversial. In the present study, ischemia increased neuroblasts proliferation and migration towards the lesion 3 days after reperfusion and this persisted up to one month. Inhibition of the microglial $K_{ATP}$ channel with Gbc led to a further increase in the number of migrating neuroblasts, thereby indicating that Gbc modifies the cell lineage choice or enhances progenitor cell proliferation and migration. Our results also showed that the number of newly generated neurons increased in the cortex 30 days after reperfusion and that Gbc potentiated this effect. Although we cannot rule out the possibility that neural progenitors migrate from the SVZ and establish themselves in the ipsilateral cortex network, we found no co-localization of BrdU-positive cells with the classical rostral migratory stream derived neuronal markers (i.e., calbindin, calretinin, tyrosine

hydroxylase, and parvalbumin). These newborn cortical neurons may be originated from potential resident neural stem cells within the cortex (Shimada et al., 2010). Several authors have proposed the presence of these endogenous quiescent neural stem cells in the cerebral cortex, and that their proliferation and differentiation to mature neurons is induced by ischemic insults (Cameron and Dayer 2008; Gu et al., 2000; Jiang et al., 2001; Kuge et al., 2009; Magavi et al., 2000; Nakagomi et al., 2009; Nakayama et al., 2010; Ohira et al., 2009). Although the number of these cells is very small, strategies to foster the intrinsic neurogenesis would be highly relevant for clinical approaches to facilitate neural repair and functional recovery.

Microglial cells participate in modifications of stem cell proliferation (Aarum et al., 2003; Walton et al., 2006), migration and/or differentiation into neurons after stroke and status epilepticus through producing trophic factors and inflammatory cytokines/chemokines (Butovsky et al., 2006; Ekdahl et al., 2009). In order to explain whether blockade of the microglial $K_{ATP}$ channel has the capacity to promote neurogenesis *in vivo*, we moved to an *in vitro* model using the neurosphere assay. We here showed that after inhibition of NPs proliferation in presence of LPS+IFNγ, the specific blockade of the microglial $K_{ATP}$ channel cause a release of a soluble factor that enhance the activation of these NPs. Moreover, progenitor cells cultured with non-challenged microglia and treated with Gbc gave rise to a higher number of beta-III-tubulin-positive cells. Further characterization of releasing cytokines determined that the $K_{ATP}$ channel blockade boost the production of MCP-1, which does not appear to directly activate an inflammatory response in microglia or cause neuronal damage (Hinojosa et al., 2011). MCP-1 and stromal cell-derived factor-1alpha trigger migration of newly generated neuroblasts from neurogenic regions to ischemic damaged areas (Amantea et al., 2009; Ekdahl et al., 2009; Mantovani et al., 2004; Tran et al., 2006). In addition, we found that MCP-1 promotes NPs neuronal differentiation, as reported previously elsewhere (Liu et al., 2007b; Turbic et al., 2011). It is noteworthy that this chemokine also protects neurons against inflammatory damage caused by NMDA-mediated excitotoxicity (Eugenin et al., 2003), being very useful after brain damage. However, whether MCP-1 plays a role in the survival of NPs remains to be determined. Our results suggest that the microglial $K_{ATP}$ channel blockade after brain injury may cause an increase on the production of

the MCP-1 *in vivo*, enhancing the migration of precursors to the site of injury to repair the lesioned region and protect neurons. Thus, potentiation of microglial proneurogenic phenotype could represent a new strategy to enforce endogenous brain regenerative processes, which in turn could have a significant impact in the outcome of the patient.

The pro- or anti-neurogenic niche would depend on the degree of microglia activation and the balance between the pro- and anti-inflammatory cytokines produced (Battista et al., 2006). For instance, acutely activated microglia induces an inflammatory response detrimental for neurogenesis, while chronically activated microglia is permissive to neuronal differentiation and cell survival (Cacci et al., 2008; Ekdahl et al., 2003). This raises the possibility that in a chronically altered environment, persistently activated microglia could display protective functions that foster rather than attenuate brain repair processes. Interestingly, microglial $K_{ATP}$-channel has been described previously as a drug target to regulate cell reactive state, controlling the release of a diversity of inflammatory mediators, such as NO, IL-6 or TNFα, or even modifying microglial phagocytic activity (Ortega et al., 2012a; Virgili et al., 2011). Therefore, the blockage of the microglial $K_{ATP}$ channel endows microglia to a new distinct phenotype, similar to the chronic proinflammatory phenotype described above and able to enhance NPs activation and differentiation.

Angiogenesis is also activated after cerebral ischemia and plays an important role for striatal neurogenesis after stroke (Thored et al., 2007). Molecules such as vascular endothelial growth factor (Jin et al., 2003) and erythropoietin (Zhang et al., 2005) stimulate both angiogenesis and neurogenesis and lead to improved functional recovery alter stroke in the early postischemic phase. Intriguingly, NPs use neurovasculature as a scaffold for their migration towards the lesion (Kojima et al., 2010). In our study Gbc further increased the diameter of microvessels in the non-lesioned cortex and RECA-1 immunoreactivity in the hippocampus, and we found stimulated glial proliferation in both regions. These observations relate tissue macrophages/microglia with brain angiogenesis as propose for other developing organs (Fantin et al., 2010). Vasculature attracts microglial cells and stimulates them to release angiogenic factors (Rymo et al., 2011), with subsequent growth stimulation of neural stem cells (Androutsellis-Theotokis et al., 2010). Therefore, optimizing

vascularization appears as an important strategy to promote neurogenesis and repair in the stroke-damaged brain (Thored et al., 2007).

Overall, in this study we demonstrate that Gbc improves functional neurological outcome in stroke, accompanied by neuron preservation in the core of the ischemic brain. These Gbc effects identify $K_{ATP}$ channels as a key target for strengthening the neuroprotective role of microglia in the acute phase after focal cerebral ischemia, enhance long-term neurogenesis and brain repair processes. This also provides new therapeutic avenues for the treatment of other neurological disorders that involve microglia.

# Chapter 8.

# CONCLUSIONS

From the present work we have obtained the following conclusions:

1. BV2 microglia cell line enhances the expression of functional $K_{ATP}$ channels after a pro-inflammatory stimulation. When $K_{ATP}$ channel was blocked with glibenclamide, BV2 microglia increased their reactive morphology, TNFα release and early phagocytic capacity. Isolated cultured rat microglia also express functional $K_{ATP}$ channels.

2. Glibenclamide reaches the ischemic brain hemisphere after rat MCAO, probably as a result of BBB disruption. Moreover reactive microglia from tMCAO rats' upregulate the $K_{ATP}$ channel expression in the necrotic core, which makes microglia/macrophages a target to glibenclamide actions in the early stages of stroke.

3. Glibenclamide administered at 6, 12 and 24 hours after reperfusion significantly improved sensorimotor and memory recovery at long term. This functional recovery is based in a glibenclamide-induced neuroprotection since this drug also increased both the peri-infarct volume of the ischemic brain and the neuronal preservation into this region.

4. Although glibenclamide did not modify gliosis or glial cell density; early $K_{ATP}$ channel blockade increased clearance of the debris and decreased calcification in the ischemic brain. This represents a boosted microglial phagocytic activity that in turn reduces tMCAO-induced neuronal suffering in the boundary of the necrotic core.

5. Glibenclamide increased both the number of migrating neuroblasts three days after ischemia and the number of newly generated neurons in the lesioned cerebral cortex thirty days after reperfusion. Thereby early microglial $K_{ATP}$ channel blockade modifies the cell lineage choice and/or enhances progenitor cell proliferation and migration in the ischemic brain.

6. $K_{ATP}$ channel blockade increased angiogenesis in cerebral cortex and hippocampus of ischemic rats, which is tightly associated with glial

proliferation and may contribute to the glibenclamide-mediated enhancement of neurogenesis.

7. Blockade of microglial $K_{ATP}$ channel *in vitro* increased the number of immature neurons on neurosphere cultures, and blockade of the channel after a pro-inflammatory stimulus enhanced the activation of neural precursor cells and boosted the microglia production of MCP-1.

8. $K_{ATP}$ channels constitute a key target for the control of neuroprotective and inflammatory microglia activity in the acute phase of focal cerebral ischemia. Therefore, sulfonylureas may offer clinical therapeutics for stroke through short-term inflammation-related beneficial effects that include potentiation of neurogenesis and repair processes and lead to sensorimotor and memory recovery.

# Chapter 9.

# GENERAL BIBLIOGRAPHY

# A

Aarum J, Sandberg K, Haeberlein SL, Persson MA. 2003. Migration and differentiation of neural precursor cells can be directed by microglia. Proc Natl Acad Sci USA 100(26):15983-15988.

Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Small JE, Herrlinger U, Ourednik V, Black PM and others. 2000. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci USA 97(23):12846-51.

Acarin L, Gonzalez B, Castellano B. 2000. Neuronal, astroglial and microglial cytokine expression after an excitotoxic lesion in the immature rat brain. Euro J Neurosc 12:3505-3520.

Aguado T, Monory K, Palazuelos J, Stella N, Cravatt B, Lutz B, Marsicano G, Kokaia Z, Guzman M, Galve-Roperh I. 2005. The endocannabinoid system drives neural progenitor proliferation. FASEB Journal 19(12):1704-1706.

Aguilar-Bryan L, Bryan J. 1999. Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr Rev 20(2):101-35.

Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd AE, González G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA. 1995. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268(5209):423-6.

Akrouh A, Halcomb SE, Nichols CG, Sala-Rabanal M. 2009. Molecular biology of K(ATP) channels and implications for health and disease. IUBMB Life 61(10):971-8.

Aloisi F. 2001. Immune function of microglia. Glia 36(2):165-179.

Amantea D, Nappi G, Bernardi G, Bagetta G, Corasaniti MT. 2009. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. FEBS Journal 276(1):13-26.

Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. 2009. Classification of stroke subtypes. Cerebrovasc Dis 27(5):493-501.

Androutsellis-Theotokis A, Rueger MA, Park DM, Boyd JD, Padmanabhan R, Campanati L, Stewart CV, LeFranc Y, Plenz D, Walbridge S and others. 2010. Angiogenic factors stimulate growth of adult neural stem cells. PLoS ONE 5(2):e9414.

Arboix A. 2007. Potential impact of sulfonylureas in the outcome of type 2 diabetic patients with ischemic stroke. Stroke 38(9):2413-4.

Arnett HA, Wang Y, Matsushima GK, Suzuki K, Ting JP-Y. 2003. Functional genomic analysis of remyelination reveals importance of inflammation in oligodendrocyte regeneration. J Neurosci 23(30):9824-32.

Arsene D, Vasilescu F, Toader C, Bălan A, Popa C, Ardeleanu C. 2011. Clinico-pathological correlations in fatal ischemic stroke. An immunohistochemical study of human brain penumbra. Rom J Morphol Embryol 52(1):29-38.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nature Medicine 8(9):963-970.

Arvidsson A, Kokaia Z, Lindvall O. 2001. N-methyl-D-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. Eur J Neurosci 14(1):10-8.

Ashcroft FM. 2005. ATP-sensitive potassium channelopathies: focus on insulin secretion. J Clin Invest 115(8):2047-58.

Ashcroft FM, Gribble FM. 1998. Correlating structure and function in ATP-sensitive K+ channels. Trends Neurosci 21(7):288-94.

Ashcroft FM, Harrison DE, Ashcroft SJ. 1984. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 312(5993):446-8.

Ashcroft FM, Kakei M, Kelly RP, Sutton R. 1987. ATP-sensitive K+ channels in human isolated pancreatic β-cells. FEBS Lett 215(1):9-12.

Ashford ML, Boden PR, Treherne JM. 1990. Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. Pflugers Arch 415(4):479-83.

Astrup J, Siesjö BK, Symon L. 1981. Thresholds in cerebral ischemia - the ischemic penumbra. Stroke 12(6):723-5.

Avshalumov MV, Rice ME. 2003. Activation of ATP-sensitive K+ (K(ATP)) channels by H2O2 underlies glutamate-dependent inhibition of striatal dopamine release. Proc Natl Acad Sci USA 100(20):11729-34.

# B

Bailey SL, Carpentier PA, McMahon EJ, Begolka WS, Miller SD. 2006. Innate and adaptive immune responses of the central nervous system. Crit Rev Immunol 26(2):149-88.

Ballanyi K. 2004. Protective role of neuronal KATP channels in brain hypoxia. J Exp Biol 207(Pt 18):3201-3212.

Barger SW, Van Eldik LJ, Mattson MP. 1995. S100β protects hippocampal neurons from damage induced by glucose deprivation. Brain Res 677(1):167-170.

Battista D, Ferrari CC, Gage FH, Pitossi FJ. 2006. Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. Eur J Neurosci 23(1):83-93.

Bejot Y, Caillier M, Rouaud O, Benatru I, Maugras C, Osseby G-V, Giroud M. 2007. [Epidemiology of strokes. Impact on the treatment decision]. Presse Med 36(1 Pt 2):117-27.

Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N. 2003. Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol Cell Neurosci 24(3):623-31.

Bengzon J, Kokaia Z, Elmér E, Nanobashvili A, Kokaia M, Lindvall O. 1997. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc Natl Acad Sci USA 94(19):10432-7.

Bernardino L, Agasse F, Silva B, Ferreira R, Grade S, Malva JO. 2008. Tumor necrosis factor-α modulates survival, proliferation, and neuronal differentiation in neonatal subventricular zone cell cultures. Stem Cells 26(9):2361-2371.

Bessis A, Béchade C, Bernard D, Roumier A. 2006. Microglial control of neuronal death and synaptic properties. Glia 55(3):233-238.

Bianchi R, Giambanco I, Donato R. 2010. S100B/RAGE-dependent activation of microglia via NF-kappaB and AP-1 Co-regulation of COX-2 expression by S100B, IL-1beta and TNF-alpha. Neurobiol Aging 31(4):665-77.

Blais V, Rivest S. 2004. Effects of TNF-α and IFN-γ on nitric oxide-induced neurotoxicity in the mouse brain. J Immunol 172(11):7043-52.

Block ML, Zecca L, Hong J-S. 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57-69.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-54.

Brown H, Prescott R. 1999. Applied mixed models in medicine. Chichester: John Wiley & Sons.

Butovsky O, Talpalar AE, Ben-Yaakov K, Schwartz M. 2005. Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. Mol Cell Neurosci 29(3):381-93.

Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, Martino G, Schwartz M. 2006. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis

and oligodendrogenesis from adult stem/progenitor cells. MolCell Neurosci 31(1):149-160.

# C

Cacci E, Ajmone-Cat MA, Anelli T, Biagioni S, Minghetti L. 2008. In vitro neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. Glia 56(4):412-425.

Cameron HA, Dayer AG. 2008. New Interneurons in the Adult Neocortex: Small, Sparse, but Significant? Biological Psychiatry 63(7):650-655.

Campbell JD, Sansom MSP, Ashcroft FM. 2003. Potassium channel regulation. EMBO Rep 4(11):1038-42.

Carmichael ST. 2006. Cellular and molecular mechanisms of neural repair after stroke: making waves. Annals of Neurology 59(5):735-742.

Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. 2006. CNS immune privilege: hiding in plain sight. Immunol Rev 213:48-65.

Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, Melchiorre C. 2008. Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem 51(3):347-72.

Chan A, Hummel V, Weilbach FX, Kieseier BC, Gold R. 2006. Phagocytosis of apoptotic inflammatory cells downregulates microglial chemoattractive function and migration of encephalitogenic T cells. J Neurosci Res 84(6):1217-24.

Chen M, Dong Y, Simard JM. 2003. Functional coupling between sulfonylurea receptor type 1 and a nonselective cation channel in reactive astrocytes from adult rat brain. J Neurosci 23(24):8568-8577.

Chen M, Simard JM. 2001. Cell swelling and a nonselective cation channel regulated by internal Ca2+ and ATP in native reactive astrocytes from adult rat brain. J Neurosci. p 6512-21.

Chuquet J, Hollender L, Nimchinsky EA. 2007. High-resolution in vivo imaging of the neurovascular unit during spreading depression. J Neurosci 27(15):4036-44.

Clement JP, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar-Bryan L, Bryan J. 1997. Association and stoichiometry of K(ATP) channel subunits. Neuron 18(5):827-38.

Culmsee C, Krieglstein J. 2007. Ischaemic brain damage after stroke: new insights into efficient therapeutic strategies. International Symposium on Neurodegeneration and Neuroprotection. EMBO Rep 8(2):129-33.

# D

Das S, Basu A. 2008. Inflammation: a new candidate in modulating adult neurogenesis. J Neurosci Res. p 1199-208.

Das S, Dutta K, Kumawat KL, Ghoshal A, Adhya D, Basu A. 2011. Abrogated Inflammatory Response Promotes Neurogenesis in a Murine Model of Japanese Encephalitis. PLoS ONE 6(3):e17225.

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan W-B. 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8(6):752-8.

Deierborg T, Roybon L, Inacio AR, Pesic J, Brundin P. 2010. Brain injury activates microglia that induce neural stem cell proliferation ex vivo and promote differentiation of neurosphere-derived cells into neurons and oligodendrocytes. Neuroscience 171(4):1386-1396.

del Zoppo GJ, Becker KJ, Hallenbeck JM. 2001. Inflammation after stroke: is it harmful? Arch Neurol 58(4):669-72.

Denes A, Vidyasagar R, Feng J, Narvainen J, McColl BW, Kauppinen RA, Allan SM. 2007. Proliferating resident microglia after focal cerebral ischaemia in mice. J Cereb Blood Flow Metab 27(12):1941-53.

Dirnagl U, Iadecola C, Moskowitz MA. 1999. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22(9):391-7.

Dirscherl K, Karlstetter M, Ebert S, Kraus D, Hlawatsch J, Walczak Y, Moehle C, Fuchshofer R, Langmann T. 2010. Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. J Neuroinflammation 7:3.

Donnan GA. 2008. The 2007 Feinberg lecture: a new road map for neuroprotection. Stroke 39(1):242.

Dörschner H, Brekardin E, Uhde I, Schwanstecher C, Schwanstecher M. 1999. Stoichiometry of sulfonylurea-induced ATP-sensitive potassium channel closure. Mol Pharmacol 55(6):1060-6.

# E

Ebinger M, De Silva DA, Christensen S, Parsons MW, Markus R, Donnan GA, Davis SM. 2009. Imaging the penumbra - strategies to detect tissue at risk after ischemic stroke. J Clin Neurosci 16(2):178-87.

Eder C. 1998. Ion channels in microglia (brain macrophages). Am J Physiol 275:C327-C342.

Edwards D, Berry JJ. 1987. The efficiency of simulation-based multiple comparisons. Biometrics 43(4):913-28.

Edwards MM, Robinson SR. 2006. TNF alpha affects the expression of GFAP and S100B: implications for Alzheimer's disease. J Neural Transm 113(11):1709-15.

Ekdahl CT, Claasen J-H, Bonde S, Kokaia Z, Lindvall O. 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci USA 100(23):13632-7.

Ekdahl CT, Kokaia Z, Lindvall O. 2009. Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158(3):1021-1029.

Ekholm A, Katsura K, Kristián T, Liu M, Folbergrová J, Siesjö BK. 1993. Coupling of cellular energy state and ion homeostasis during recovery following brain ischemia. Brain Res 604(1-2):185-91.

Enkvetchakul D, Nichols CG. 2003. Gating mechanism of KATP channels: function fits form. J Gen Physiol 122(5):471-80.

Eugenin EA, D'Aversa TG, Lopez L, Calderon TM, Berman JW. 2003. MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. J Neurochem 85(5):1299-311.

# F

Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C. 2010. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 116(5):829-40.

Favilla CG, Mullen MT, Ali M, Higgins P, Kasner SE, Collaboration VISTAV. 2011. Sulfonylurea use before stroke does not influence outcome. Stroke 42(3):710-5.

Feigin VL. 2005. Stroke epidemiology in the developing world. Lancet 365(9478):2160-1.

Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U. 2002. Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. J Neurosci 22(7):RC216.

Frijns CJM. 2002. Inflammatory Cell Adhesion Molecules in Ischemic Cerebrovascular Disease. Stroke 33(8):2115-2122.

Furlan M, Marchal G, Viader F, Derlon JM, Baron JC. 1996. Spontaneous neurological recovery after stroke and the fate of the ischemic penumbra. Ann Neurol 40(2):216-26.

# G

Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Rovira MB, Forette F and others. 2005. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 64(9):1553-1562.

Ginsberg MD. 1997. The new language of cerebral ischemia. AJNR Am J Neuroradiol 18(8):1435-45.

Glezer I, Simard AR, Rivest S. 2007. Neuroprotective role of the innate immune system by microglia. Neuroscience 147(4):867-883.

Graeber MB, Streit WJ. 2010. Microglia: biology and pathology. Acta Neuropathol 119(1):89-105.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. 1982. Analysis of nitrate, nitrite, and [$^{15}$N]nitrate in biological fluids. Anal Biochem 126(1):131-8.

Gribble FM, Reimann F. 2003. Sulphonylurea action revisited: the post-cloning era. Diabetologia 46(7):875-891.

Gribble FM, Tucker SJ, Ashcroft FM. 1997. The interaction of nucleotides with the tolbutamide block of cloned ATP-sensitive K+ channel currents expressed in Xenopus oocytes: a reinterpretation. J Physiol (Lond) 504 ( Pt 1):35-45.

Grysiewicz RA, Thomas K, Pandey DK. 2008. Epidemiology of ischemic and hemorrhagic stroke: incidence, prevalence, mortality, and risk factors. Neurol Clin 26(4):871-95, vii.

Gu W, Brännström T, Wester P. 2000. Cortical neurogenesis in adult rats after reversible photothrombotic stroke. J Cereb Blood Flow Metab 20(8):1166-73.

# H

Halestrap AP. 2006. Calcium, mitochondria and reperfusion injury: a pore way to die. BiochemSocTrans 34(Pt 2):232-237.

Haley EC, Kassell NF, Alves WM, Weir BK, Hansen CA. 1995. Phase II trial of tirilazad in aneurysmal subarachnoid hemorrhage. A report of the Cooperative Aneurysm Study. J Neurosurg 82(5):786-90.

Hallenbeck JM. 2002. The many faces of tumor necrosis factor in stroke. Nat Med 8(12):1363-8.

Hanisch U, Kettenmann H. 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387-94.

Hayashi T, Abe K. 2004. Ischemic neuronal cell death and organellae damage. Neurol Res 26(8):827-34.

Herrmann G, Stünitz H, Nitsch C. 1998. Composition of ibotenic acid-induced calcifications in rat substantia nigra. Brain Res 786:205-214.

Hertz L, Peng L, Dienel GA. 2007. Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. J Cereb Blood Flow Metab 27(2):219-49.

Hinojosa AE, Garcia-Bueno B, Leza JC, Madrigal JLM. 2011. CCL2/MCP-1 modulation of microglial activation and proliferation. J Neuroinflammation 8:77.

Hossmann K-A. 2006. Pathophysiology and therapy of experimental stroke. Cell Mol Neurobiol 26(7-8):1057-83.

Hossmann KA. 1994. Viability thresholds and the penumbra of focal ischemia. Ann Neurol 36(4):557-65.

# I

Iadecola C, Anrather J. 2011. The immunology of stroke: from mechanisms to translation. Nat Med 17(7):796-808.

Ingall T. 2004. Stroke--incidence, mortality, morbidity and risk. J Insur Med 36(2):143-52.

# J

Jaskolski F, Mulle C, Manzoni OJ. 2005. An automated method to quantify and visualize colocalized fluorescent signals. J Neurosci Methods 146(1):42-9.

Jellinger KA, Attems J. 2003. Incidence of cerebrovascular lesions in Alzheimer's disease: a postmortem study. Acta Neuropathol 105(1):14-17.

Ji JF, He BP, Dheen ST, Tay SSW. 2004. Expression of chemokine receptors CXCR4, CCR2, CCR5 and CX3CR1 in neural progenitor cells isolated from the subventricular zone of the adult rat brain. Neurosci Lett 355(3):236-40.

Jiang W, Gu W, Brannstrom T, Rosqvist R, Wester P. 2001. Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. Stroke 32(5):1201-1207.

Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA. 2001. Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA 98(8):4710-4715.

Jin K, Sun Y, Xie L, Peel A, Mao XO, Batteur S, Greenberg DA. 2003. Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. MolCell Neurosci 24(1):171-189.

Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, Shen J, Mao Y, Banwait S, Greenberg DA. 2006. Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci USA 103(35):13198-202.

Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. 2002. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci USA 99(18):11946-11950.

Jin R, Yang G, Li G. 2010. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol 87(5):779-789.

Johnston KC, Li JY, Lyden PD, Hanson SK, Feasby TE, Adams RJ, Faught RE, Haley EC. 1998. Medical and neurological complications of ischemic stroke: experience from the RANTTAS trial. RANTTAS Investigators. Stroke 29(2):447-53.

Johnston SC, Mendis S, Mathers CD. 2009. Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. Lancet Neurol 8(4):345-54.

# K

Karhunen H, Pitkänen A, Virtanen T, Gureviciene I, Pussinen R, Ylinen A, Sivenius J, Nissinen J, Jolkkonen J. 2003. Long-term functional consequences of transient occlusion of the middle cerebral artery in rats: a 1-year follow-up of the development of epileptogenesis and memory impairment in relation to sensorimotor deficits. Epilepsy Res. p 1-10.

Kempermann G. 2006. Adult neurogenesis: stem cells and neuronal development in the ... New York Oxford University Press.

Kettenmann H, Hanisch U-K, Noda M, Verkhratsky A. 2011. Physiology of Microglia. Physiol Rev 91(2):461-553.

Khanna R, Roy L, Zhu X, Schlichter LC. 2001. $K^+$ channels and the microglial respiratory burst. Am J Physiol 280(4):C796-C806.

Kim BJ, Kim M-J, Park J-M, Lee S-H, Kim Y-J, Ryu S, Kim YH, Yoon B-W. 2009. Reduced neurogenesis after suppressed inflammation by minocycline in transient cerebral ischemia in rat. J Neurol Sci 279(1-2):70-5.

Kim SU, de Vellis J. 2005. Microglia in health and disease. J Neurosci Res 81(3):302-13.

Kitamura Y, Takata K, Inden M, Tsuchiya D, Yanagisawa D, Nakata J, Taniguchi T. 2004. Intracerebroventricular injection of microglia protects against focal brain ischemia. J Pharmacol Sci 94(2):203-6.

Kline CF, Hund TJ, Mohler PJ. 2010. Ankyrin regulates KATP channel membrane trafficking and gating in excitable cells. Channels 4(1):55-7.

Kline CF, Kurata HT, Hund TJ, Cunha SR, Koval OM, Wright PJ, Christensen M, Anderson ME, Nichols CG, Mohler PJ. 2009. Dual role of K ATP channel C-terminal motif in membrane targeting and metabolic regulation. Proc Natl Acad Sci USA 106(39):16669-74.

Knudson CM, Tung KS, Tourtellotte WG, Brown GA, Korsmeyer SJ. 1995. Bax-deficient mice with lymphoid hyperplasia and male germ cell death. Science 270(5233):96-9.

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 28(3):545-54.

Kokaia Z, Lindvall O. 2003. Neurogenesis after ischaemic brain insults. Current Opinion in Neurobiology 13(1):127-132.

Kriz J. 2006. Inflammation in ischemic brain injury: timing is important. Crit Rev Neurobiol 18(1-2):145-57.

Kuge A, Takemura S, Kokubo Y, Sato S, Goto K, Kayama T. 2009. Temporal profile of neurogenesis in the subventricular zone, dentate gyrus and cerebral cortex following transient focal cerebral ischemia. Neurol Res 31(9):969-76.

Kunte H, Schmidt S, Eliasziw M, del Zoppo GJ, Simard JM, Masuhr F, Weih M, Dirnagl U. 2007. Sulfonylureas improve outcome in patients with type 2 diabetes and acute ischemic stroke. Stroke 38(9):2526-2530.

# L

Lalancette-Hébert M, Gowing G, Simard A, Weng YC, Kriz J. 2007. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 27(10):2596-605.

Lambertsen KL, Clausen BH, Babcock AA, Gregersen R, Fenger C, Nielsen HH, Haugaard LS, Wirenfeldt M, Nielsen M, Dagnaes-Hansen F and others. 2009. Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. J Neurosci 29(5):1319-30.

Lawson K. 2000. Potassium channel openers as potential therapeutic weapons in ion channel disease. Kidney Int. p 838-45.

Lenzlinger PM, Morganti-Kossmann MC, Laurer HL, McIntosh TK. 2001. The duality of the inflammatory response to traumatic brain injury. Mol Neurobiol 24(1-3):169-81.

Levin BE. 2001. Glucosensing neurons do more than just sense glucose. Int J Obes Relat Metab Dis 25(Supp 5):568-572.

Levin BE, Dunn-Meynell AA, Routh VH. 2001. Brain glucosensing and the K(ATP) channel. Nat Neurosci 4(5):459-60.

Liberto CM, Albrecht PJ, Herx LM, Yong VW, Levison SW. 2004. Pro-regenerative properties of cytokine-activated astrocytes. J Neurochem. p 1092-100.

Lievens JC, Bernal F, Forni C, Mahy N, Kerkerian-Le Goff L. 2000. Characterization of striatal lesions produced by glutamate uptake alteration: cell death, reactive gliosis, and changes in GLT1 and GADD45 mRNA expression. Glia 29(3):222-32.

Lin TN, He YY, Wu G, Khan M, Hsu CY. 1993. Effect of brain edema on infarct volume in a focal cerebral ischemia model in rats. Stroke 24(1):117-21.

Liss B, Bruns R, Roeper J. 1999. Alternative sulfonylurea receptor expression defines metabolic sensitivity of K-ATP channels in dopaminergic midbrain neurons. EMBO J. p 833-46.

Liu S, Levine SR. 2008. The Continued Promise of Neuroprotection for Acute Stroke Treatment. J Exp Stroke Transl Med 1(1):1-8.

Liu S, Levine SR, Winn HR. 2010. Targeting ischemic penumbra: part I - from pathophysiology to therapeutic strategy. J Exp Stroke Transl Med 3(1):47-55.

Liu S, Qu Y, Stewart TJ, Howard MJ, Chakrabortty S, Holekamp TF, McDonald JW. 2000. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 97(11):6126-6131.

Liu X, Wang Q, Haydar TF, Bordey A. 2005. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. Nature Neuroscience 8(9):1179-1187.

Liu XS, Zhang ZG, Zhang RL, Gregg SR, Meng H, Chopp M. 2007a. Comparison of in vivo and in vitro gene expression profiles in subventricular zone neural progenitor cells from the adult mouse after middle cerebral artery occlusion. Neuroscience 146(3):1053-61.

Liu XS, Zhang ZG, Zhang RL, Gregg SR, Wang L, Yier T, Chopp M. 2007b. Chemokine ligand 2 (CCL2) induces migration and differentiation of subventricular zone cells after stroke. J Neurosci Res 85(10):2120-5.

Lo EH. 2008. Experimental models, neurovascular mechanisms and translational issues in stroke research. Br J Pharmacol 153 Suppl 1:S396-405.

Lo EH, Moskowitz MA, Jacobs TP. 2005. Exciting, radical, suicidal: how brain cells die after stroke. Stroke 36(2):189-92.

# M

Mabuchi T, Kitagawa K, Ohtsuki T, Kuwabara K, Yagita Y, Yanagihara T, Hori M, Matsumoto M. 2000. Contribution of microglia/macrophages to expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. Stroke 31(7):1735-43.

Macas J, Nern C, Plate KH, Momma S. 2006. Increased generation of neuronal progenitors after ischemic injury in the aged adult human forebrain. J Neurosci 26(50):13114-9.

Magavi SS, Leavitt BR, Macklis JD. 2000. Induction of neurogenesis in the neocortex of adult mice. Nature 405(6789):951-955.

Magnus T, Chan A, Grauer O, Toyka KV, Gold R. 2001. Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation. J Immunol 167(9):5004-10.

Mahad DJ, Ransohoff RM. 2003. The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Semin Immunol 15(1):23-32.

Mahy N, Prats A, Riveros A, Andrés N, Bernal F. 1999. Basal ganglia calcification induced by excitotoxicity: an experimental model characterised by electron microscopy and X-ray microanalysis. Acta Neuropathol 98(3):217-225.

Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. 2004. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 25(12):677-86.

Masia R, Caputa G, Nichols CG. 2007. Regulation of KATP channel expression and activity by the SUR1 nucleotide binding fold 1. Channels 1(4):315-23.

Matsuo M, Tanabe K, Kioka N, Amachi T, Ueda K. 2000. Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. J Biol Chem 275(37):28757-63.

McLarnon JG, Franciosi S, Wang X, Bae JH, Choi HB, Kim SU. 2001. Acute actions of tumor necrosis factor-α on intracellular $Ca^{2+}$ and $K^{+}$ currents in human microglia. Neuroscience 104(4):1175-84.

Melander A, Lebovitz HE, Faber OK. 1990. Sulfonylureas. Why, which, and how? Diabetes Care 13 Suppl 3:18-25.

Merkle FT, Mirzadeh Z, Alvarez-Buylla A. 2007. Mosaic organization of neural stem cells in the adult brain. Science 317(5836):381-384.

Mhairi Macrae I. 1992. New models of focal cerebral ischaemia. Br J Clin Pharmacol 34(4):302-8.

Mikhailov MV, Campbell JD, De Wet H, Shimomura K, Zadek B, Collins RF, Sansom MSP, Ford RC, Ashcroft FM. 2005. 3-D structural and functional characterization of the purified $K_{ATP}$ channel complex Kir6.2-SUR1. EMBO J 24(23):4166-75.

Mikhailov MV, Mikhailova EA, Ashcroft SJ. 2001. Molecular structure of the glibenclamide binding site of the β-cell $K_{ATP}$ channel. FEBS Lett 499(1-2):154-60.

Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch U-K, Mack M, Heikenwalder M, Brück W, Priller J, Prinz M. 2007. Microglia in the adult brain arise from Ly-6ChiCCR2$^{+}$ monocytes only under defined host conditions. Nat Neurosci 10(12):1544-53.

Milligan ED, Watkins LR. 2009. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci. p 23-36.

Minger SL, Ekonomou A, Carta EM, Chinoy A, Perry RH, Ballard CG. 2007. Endogenous neurogenesis in the human brain following cerebral infarction. Regen Med 2(1):69-74.

Mitsios N, Gaffney J, Kumar P, Krupinski J, Kumar S, Slevin M. 2006. Pathophysiology of acute ischaemic stroke: an analysis of common signalling mechanisms and identification of new molecular targets. Pathobiology 73(4):159-75.

Mohammadi MT, Shid-Moosavi SM, Dehghani GA. 2011. Contribution of nitric oxide synthase (NOS) in blood-brain barrier disruption during acute focal cerebral ischemia in normal rat. Pathophysiology 19(1):13-20.

Molina-Holgado E, Molina-Holgado F. 2010. Mending the broken brain: neuroimmune interactions in neurogenesis. J Neurochem 114(5):1277-90.

Monje ML, Toda H, Palmer TD. 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760-1765.

Moro MA, Cárdenas A, Hurtado O, Leza JC, Lizasoain I. 2004. Role of nitric oxide after brain ischaemia. Cell Calcium 36(3-4):265-75.

Morrissey A, Parachuru L, Leung M, Lopez G, Nakamura TY, Tong X, Yoshida H, Srivastiva S, Chowdhury PD, Artman M and others. 2005. Expression of ATP-sensitive K+ channel subunits during perinatal maturation in the mouse heart. Pediatric Research 58(2):185-92.

Moss DW, Bates TE. 2001. Activation of murine microglial cell lines by lipopolysaccharide and interferon-gamma causes NO-mediated decreases in mitochondrial and cellular function. European Journal of Neuroscience 13(3):529-538.

# N

Nakagomi T, Taguchi A, Fujimori Y, Saino O, Nakano-Doi A, Kubo S, Gotoh A, Soma T, Yoshikawa H, Nishizaki T and others. 2009. Isolation and characterization of neural stem/progenitor cells from post-stroke cerebral cortex in mice. European Journal of Neuroscience 29(9):1842-1852.

Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, Tamura A, Kirino T, Nakafuku M. 2002. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110(4):429-441.

Nakayama D, Matsuyama T, Ishibashi-Ueda H, Nakagomi T, Kasahara Y, Hirose H, Kikuchi-Taura A, Stern DM, Mori H, Taguchi A. 2010. Injury-induced neural stem/progenitor cells in post-stroke human cerebral cortex. European Journal of Neuroscience 31(1):90-98.

Napoli I, Neumann H. 2009. Microglial clearance function in health and disease. Neuroscience 158(3):1030-8.

Nedergaard M, Kraig RP, Tanabe J, Pulsinelli WA. 1991. Dynamics of interstitial and intracellular pH in evolving brain infarct. Am J Physiol 260(3 Pt 2):R581-8.

Neumann J, Gunzer M, Gutzeit HO, Ullrich O, Reymann KG, Dinkel K. 2006. Microglia provide neuroprotection after ischemia. FASEB J 20(6):714-6.

Neumann J, Sauerzweig S, Rönicke R, Gunzer F, Dinkel K, Ullrich O, Gunzer M, Reymann KG. 2008. Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. J Neurosci 28(23):5965-75.

Nicholas RS, Wing MG, Compston A. 2001. Nonactivated microglia promote oligodendrocyte precursor survival and maturation through the transcription factor NF-kappa B. Eur J Neurosci 13(5):959-67.

Nichols CG. 2006. $K_{ATP}$ channels as molecular sensors of cellular metabolism. Nature 440(7083):470-476.

Nimmerjahn A, Kirchhoff F, Helmchen F. 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314-1318.

Nonoda Y, Saito Y, Itoh M, Nakagawa E, Sugai K, Takahashi A, Otsuki T, Saito Y, Arima K, Mizuguchi M and others. 2009. Activation of microglia/macrophages expressing phosphorylated S6 ribosomal protein in a case of hemimegalencephaly with progressive calcification and atrophy. J Neurol Sci 287(1-2):52-9.

# O

Ohab JJ, Carmichael ST. 2008. Poststroke neurogenesis: emerging principles of migration and localization of immature neurons. The Neuroscientist. p 369-80.

Ohira K, Furuta T, Hioki H, Nakamura KC, Kuramoto E, Tanaka Y, Funatsu N, Shimizu K, Oishi T, Hayashi M and others. 2009. Ischemia-induced neurogenesis of neocortical layer 1 progenitor cells. Nat Neurosci 13(2):173-179.

Ohno-Shosaku T, Yamamoto C. 1992. Identification of an ATP-sensitive K+ channel in rat cultured cortical neurons. Pflugers Arch 422(3):260-6.

Orrenius S, Zhivotovsky B, Nicotera P. 2003. Regulation of cell death: the calcium-apoptosis link. NatRevMolCell Biol 4(7):552-565.

Ortega FJ, Gimeno-Bayon J, Espinosa-Parrilla JF, Carrasco JL, Batlle M, Pugliese M, Mahy N, Rodríguez MJ. 2012a. ATP-dependent potassium channel blockade strengthens microglial neuroprotection after hypoxia-ischemia in rats. Exp Neurol Feb 24 (*Epub ahead of print*). doi: 10.1016/j.expneurol.2012.02.010

Ortega FJ, Vidal-Taboada JM, Mahy N, Rodriguez MJ. 2012b. Molecular Mechanisms of Acute Brain Injury and Ensuing Neurodegeneration; Brain Damage: InTech.

Ortega-Perez I, Murray K, Lledo P-M. 2007. The how and why of adult neurogenesis. J Mol Hist 38(6):555-62.

# P

Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. 2002. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Annals of Neurology 52(6):802-813.

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. 1997. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. Journal of Neuroscience 17(10):3727-3738.

Paul SL, Srikanth VK, Thrift AG. 2007. The large and growing burden of stroke. Curr Drug Targets 8(7):786-93.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates. San Diego: Academic Press, Inc. U.S.A.

Perry VH, Nicoll JAR, Holmes C. 2010. Microglia in neurodegenerative disease. Nat Rev Neurol 6(4):193-201.

Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Evercooren AB-V. 2002. Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proc Natl Acad Sci USA 99(20):13211-6.

Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U, Amadio S, Bergami A and others. 2003. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 422(6933):688-94.

Polazzi E, Monti B. 2010. Microglia and neuroprotection: from in vitro studies to therapeutic applications. Prog Neurobiol 92(3):293-315.

Price CJS, Menon DK, Peters AM, Ballinger JR, Barber RW, Balan KK, Lynch A, Xuereb JH, Fryer T, Guadagno JV and others. 2004. Cerebral neutrophil recruitment, histology, and outcome in acute ischemic stroke: an imaging-based study. Stroke 35(7):1659-64.

Proks P, Ashcroft FM. 2009. Modeling $K_{ATP}$ channel gating and its regulation. Prog Biophys Mol Biol 99(1):7-19.

Prüss H, Prass K, Ghaeni L, Milosevic M, Muselmann C, Freyer D, Royl G, Reuter U, Baeva N, Dirnagl U and others. 2008. Inducible nitric oxide synthase does not mediate brain damage after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 28(3):526-39.

Puurunen K, Jolkkonen J, Sirviö J, Haapalinna A, Sivenius J. 2001. An alpha(2)-adrenergic antagonist, atipamezole, facilitates behavioral recovery after focal cerebral ischemia in rats. Neuropharmacology 40(4):597-606.

# R

Ramonet D, Rodriguez MJ, Pugliese M, Mahy N. 2004. Putative glucosensing property in rat and human activated microglia. Neurobiol Dis 17:1-9.

Reynolds BA, Weiss S. 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255(5052):1707-10.

Rodriguez MJ, Martínez-Sánchez M, Bernal F, Mahy N. 2004. Heterogeneity between hippocampal and septal astroglia as a contributing factor to differential in vivo AMPA excitotoxicity. J Neurosci Res 77(3):344-53.

Rodriguez MJ, Prats A, Malpesa Y, Andres N, Pugliese M, Batlle M, Mahy N. 2009. Pattern of injury with a graded excitotoxic insult and ensuing chronic medial septal damage in the rat brain. J Neurotrauma 26(10):1823-34.

Rodriguez MJ, Robledo P, Andrade C, Mahy N. 2005. In vivo co-ordinated interactions between inhibitory systems to control glutamate-mediated hippocampal excitability. J Neurochem 95(3):651-661.

Rodriguez MJ, Ursu G, Bernal F, Cusí V, Mahy N. 2001. Perinatal human hypoxia-ischemia vulnerability correlates with brain calcification. Neurobiol Dis 8:59-68.

Rolls A, Shechter R, London A, Segev Y, Jacob-Hirsch J, Amariglio N, Rechavi G, Schwartz M. 2008. Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation. PLoS Med 5(8):e171.

Rolls A, Shechter R, London A, Ziv Y, Ronen A, Levy R, Schwartz M. 2007. Toll-like receptors modulate adult hippocampal neurogenesis. Nat Cell Biol 9(9):1081-8.

Rymo SF, Gerhardt H, Wolfhagen Sand F, Lang R, Uv A, Betsholtz C. 2011. A Two-Way Communication between Microglial Cells and Angiogenic Sprouts Regulates Angiogenesis in Aortic Ring Cultures. PLoS ONE 6(1):e15846.

# S

Sahathevan R, Brodtmann A, Donnan GA. 2012. Dementia, stroke, and vascular risk factors; a review. Int J Stroke 7(1):61-73.

Sala-Rabanal M, Wang S, Nichols CG. 2012. On the potential interactions between Non-Selective cation channel TRPM4 and the sulfonylurea receptor SUR1. J Biol Chem.

Sapolsky RM. 2001. Cellular defenses against excitotoxic insults. Journal of Neurochemistry 76:1601-1611.

Sasmono RT, Oceandy D, Pollard JW, Tong W, Pavli P, Wainwright BJ, Ostrowski MC, Himes SR, Hume DA. 2003. A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. Blood 101(3):1155-63.

Saura J, Tusell JM, Serratosa J. 2003. High-yield isolation of murine microglia by mild trypsinization. Glia 44(3):183-189.

Saver JL. 2006. Time is brain--quantified. Stroke 37(1):263-6.

Sawada M, Kondo N, Suzumura A, Marunouchi T. 1989. Production of tumor necrosis factor-alpha by microglia and astrocytes in culture. Brain Research 491(2):394-397.

Schilling M, Besselmann M, Leonhard C, Mueller M, Ringelstein EB, Kiefer R. 2003. Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone marrow chimeric mice. Exp Neurol 183(1):25-33.

Schmued LC, Albertson C, Slikker W. 1997. Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res 751(1):37-46.

Schwartz M, Butovsky O, BrÅck W, Hanisch UK. 2006. Microglial phenotype: is the commitment reversible? Trends in Neuroscience 29 (2):68-74.

Seino S, Miki T. 2003. Physiological and pathophysiological roles of ATP-sensitive K+ channels. Progress in Biophysics and Molecular Biology 81(2):133-76.

Shaked I, Tchoresh D, Gersner R, Meiri G, Mordechai S, Xiao X, Hart RP, Schwartz M. 2005. Protective autoimmunity: interferon-gamma enables microglia to remove glutamate without evoking inflammatory mediators. J Neurochem 92(5):997-1009.

Shimada IS, Peterson BM, Spees JL. 2010. Isolation of Locally Derived Stem/Progenitor Cells From the Peri-Infarct Area That Do Not Migrate From the Lateral Ventricle After Cortical Stroke. Stroke 41(9):e552-e560.

Sica A, Schioppa T, Mantovani A, Allavena P. 2006. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur J Cancer 42(6):717-27.

Sierra A, Encinas JM, Deudero JJP, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M. 2010. Microglia Shape Adult Hippocampal Neurogenesis through Apoptosis-Coupled Phagocytosis. Cell Stem Cell 7(4):483-495.

Simard JM, Chen M, Tarasov KV, Bhatta S, Ivanova S, Melnitchenko L, Tsymbalyuk N, West GA, Gerzanich V. 2006. Newly expressed SUR1-regulated $NC_{Ca\text{-}ATP}$ channel mediates cerebral edema after ischemic stroke. Nat Med 12(4):433-440.

Simard JM, Kent TA, Kunte H. 2011. Letter by Simard et al regarding article, "Sulfonylurea use before stroke does not influence outcome". Stroke 42(7):e409; author reply e410.

Simard JM, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Yurovsky V, Gerzanich V. 2010. Glibenclamide Is Superior to Decompressive Craniectomy in a Rat Model of Malignant Stroke. Stroke 41(3):531-7.

Simard JM, Woo SK, Bhatta S, Gerzanich V. 2008. Drugs acting on SUR1 to treat CNS ischemia and trauma. Curr Opin Pharmacol 8(1):42-49.

Simard JM, Yurovsky V, Tsymbalyuk N, Melnichenko L, Ivanova S, Gerzanich V. 2009. Protective effect of delayed treatment with low-dose glibenclamide in three models of ischemic stroke. Stroke 40(2):604-9.

Skaper SD. 2007. The brain as a target for inflammatory processes and neuroprotective strategies. Ann N Y Acad Sci 1122:23-34.

Slevin M, Kumar P, Gaffney J, Kumar S, Krupinski J. 2006. Can angiogenesis be exploited to improve stroke outcome? Mechanisms and therapeutic potential. Clin Sci 111(3):171-83.

Soulet D, Rivest S. 2008. Bone-marrow-derived microglia: myth or reality? Curr Opin Pharmacol 8(4):508-18.

Spranger M, Kiprianova I, Krempien S, Schwab S. 1998. Reoxygenation increases the release of reactive oxygen intermediates in murine microglia. J Cereb Blood Flow Metab 18(6):670-4.

Sriram K, O'Callaghan JP. 2007. Divergent roles for tumor necrosis factor-alpha in the brain. J Neuroimmune Pharmacol. p 140-53.

Stellwagen D, Malenka RC. 2006. Synaptic scaling mediated by glial TNF-α. Nature 440(7087):1054-9.

Stoll G, Jander S, Schroeter M. 1998. Inflammation and glial responses in ischemic brain lesions. Prog Neurobiol 56(2):149-71.

Streit WJ. 2002. Microglia as neuroprotective, immunocompetent cells of the CNS. Glia 40(2):133-9.

Streit WJ. 2005. Microglia and neuroprotection: implications for Alzheimer's disease. Brain Res Brain Res Rev 48(2):234-9.

Sun X-L, Hu G. 2009. ATP-sensitive potassium channels: A promising target for protecting neurovascular unit function in stroke. Clin Exp Pharmacol Physiol 37(2):243-52.

# T

The National Institute of Neurological Disorders G. 1995. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. N Engl J Med 333(24):1581-7.

Thored P, Arvidsson A, Cacci E, Ahlenius H, Kallur T, Darsalia V, Ekdahl CT, Kokaia Z, Lindvall O. 2006. Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem Cells 24(3):739-747.

Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J, Nygren JM, Jacobsen S-EW, Ekdahl CT, Kokaia Z and others. 2009. Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. Glia 57(8):835-849.

Thored P, Wood J, Arvidsson A, Cammenga J, Kokaia Z, Lindvall O. 2007. Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke 38(11):3032-9.

Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, van Praag H, Martone ME, Ellisman MH, Gage FH. 2007. Synapse formation on neurons born in the adult hippocampus. Nat Neurosci 10(6):727-34.

Toulorge D, Guerreiro S, Hirsch EC, Michel PP. 2010. KATP channel blockade protects midbrain dopamine neurons by repressing a glia-to-neuron signaling cascade that ultimately disrupts mitochondrial calcium homeostasis. J Neurochem(114):553-564.

Towfighi A, Saver JL. 2011. Stroke declines from third to fourth leading cause of death in the United States: historical perspective and challenges ahead. Stroke 42(8):2351-5.

Tran PB, Banisadr G, Ren D, Chenn A, Miller RJ. 2006. Chemokine receptor expression by neural progenitor cells in neurogenic regions of mouse brain. J Comp Neurol 500(6):1007-1034.

Tremblay M-È, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. 2011. The role of microglia in the healthy brain. J Neurosci 31(45):16064-9.

Turbic A, Leong SY, Turnley AM. 2011. Chemokines and inflammatory mediators interact to regulate adult murine neural precursor cell proliferation, survival and differentiation. PLoS ONE 6(9):e25406.

# V

Valente T, Hidalgo J, Bolea I, Ramirez B, Anglés N, Reguant J, Morelló JR, Gutiérrez C, Boada M, Unzeta M. 2009. A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain. J Alzheimers Dis 18(4):849-65.

Van Groen T, Puurunen K, Mäki H-M, Sivenius J, Jolkkonen J. 2005. Transformation of diffuse β-amyloid precursor protein and β-amyloid deposits to plaques in the thalamus after transient occlusion of the middle cerebral artery in rats. Stroke 36(7):1551-6.

van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. 2002. Functional neurogenesis in the adult hippocampus. Nature 415(6875):1030-1034.

Veroni C, Gabriele L, Canini I, Castiello L, Coccia E, Remoli ME, Columba-Cabezas S, Aricò E, Aloisi F, Agresti C. 2010. Activation of TNF receptor 2 in microglia promotes induction of anti-inflammatory pathways. Mol Cell Neurosci 45(3):234-44.

Virgili N, Espinosa-Parrilla JF, Mancera P, Pastén-Zamorano A, Gimeno-Bayon J, Rodríguez MJ, Mahy N, Pugliese M. 2011. Oral administration of the KATP channel opener diazoxide ameliorates disease progression in a murine model of multiple sclerosis. J Neuroinflammation 2;8:149.

Vukovic J, Blackmore DG, Jhaveri D, Bartlett PF. 2011. Activation of neural precursors in the adult neurogenic niches. Neurochem Int 59(3):341-346.

# W

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. 2009. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29(13):3974-80.

Walberer M, Nedelmann M, Ritschel N, Mueller C, Tschernatsch M, Stolz E, Bachmann G, Blaes F, Gerriets T. 2010. Intravenous immunoglobulin reduces infarct volume but not edema formation in acute stroke. Neuroimmunomodulation 17(2):97-102.

Walker TL, White A, Black DM, Wallace RH, Sah P, Bartlett PF. 2008. Latent stem and progenitor cells in the hippocampus are activated by neural excitation. J Neurosci 28(20):5240-7.

Walton NM, Sutter BM, Laywell ED, Levkoff LH, Kearns SM, Marshall GP, Scheffler B, Steindler DA. 2006. Microglia instruct subventricular zone neurogenesis. Glia 54(8):815-825.

Wang H, Zhang Y-l, Tang X-C, Feng H-S, Hu G. 2004. Targeting ischemic stroke with a novel opener of ATP-sensitive potassium channels in the brain. Mol Pharmacol 66(5):1160-8.

Wang L, Zhu Q-L, Wang G-Z, Deng T-Z, Chen R, Liu M-H, Wang S-W. 2011. The protective roles of mitochondrial ATP-sensitive potassium channels during hypoxia-ischemia-reperfusion in brain. Neurosci Lett 491(1):63-7.

Weston RM, Jones NM, Jarrott B, Callaway JK. 2007. Inflammatory cell infiltration after endothelin-1-induced cerebral ischemia: histochemical and myeloperoxidase correlation with temporal changes in brain injury. J Cereb Blood Flow Metab 27(1):100-14.

Wheeler A, Wang C, Yang K, Fang K, Davis K, Styer AM, Mirshahi U, Moreau C, Revilloud J, Vivaudou M and others. 2008. Coassembly of different sulfonylurea receptor subtypes extends the phenotypic diversity of ATP-sensitive potassium (KATP) channels. Mol Pharmacol 74(5):1333-44.

Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC. 2009. Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. J Neurochem 108(6):1343-1359.

Widera D, Holtkamp W, Entschladen F, Niggemann B, Zänker K, Kaltschmidt B, Kaltschmidt C. 2004. MCP-1 induces migration of adult neural stem cells. Eur J Cell Biol 83(8):381-7.

Wiltrout C, Lang B, Yan Y, Dempsey RJ, Vemuganti R. 2007. Repairing brain after stroke: a review on post-ischemic neurogenesis. Neurochem Int 50(7-8):1028-1041.

# Y

Yamada K, Inagaki N. 2005. Neuroprotection by KATP channels. J Mol Cell Cardiol 38(6):945-949.

Yamada K, Ji JJ, Yuan H, Miki T, Sato S, Horimoto N, Shimizu T, Seino S, Inagaki N. 2001. Protective Role of ATP-Sensitive Potassium Channels in Hypoxia-Induced Generalized Seizure. Science 292(5521):1543-1546.

Yamaguchi T, Sano K, Takakura K, Saito I, Shinohara Y, Asano T, Yasuhara H. 1998. Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. Stroke 29(1):12-7.

Yamashita T, Ninomiya M, Hernández Acosta P, García-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T and others. 2006. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 26(24):6627-36.

Yamori Y, Horie R, Handa H, Sato M, Fukase M. 1976. Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. Stroke 7(1):46-53.

Yan Y-P, Sailor KA, Vemuganti R, Dempsey RJ. 2006. Insulin-like growth factor-1 is an endogenous mediator of focal ischemia-induced neural progenitor proliferation. Eur J Neurosci 24(1):45-54.

Yang Y, Rosenberg GA. 2011. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke 42(11):3323-8.

Yu C-Z, Li C, Pei D-S, Zong Y-Y, Shi Q, Wen X-R, Guan Q-H, Hang D, Hou X-Y, Zhang G-Y. 2009. Neuroprotection against transient focal cerebral ischemia and oxygen-glucose deprivation by interference with GluR6-PSD95 protein interaction. Neurochem Res. p 2008-21.

# Z

Zausinger S, Hungerhuber E, Baethmann A, Reulen H, Schmid-Elsaesser R. 2000. Neurological impairment in rats after transient middle cerebral artery occlusion: a comparative study under various treatment paradigms. Brain Res 863(1-2):94-105.

Zerangue N, Schwappach B, Jan YN, Jan LY. 1999. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron 22(3):537-48.

Zhang RL, Zhang ZG, Chopp M. 2005. Neurogenesis in the adult ischemic brain: generation, migration, survival, and restorative therapy. The Neuroscientist. p 408-16.

Zhang RL, Zhang ZG, Zhang L, Chopp M. 2001. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 105(1):33-41.

Zhao CS, Puurunen K, Schallert T, Sivenius J, Jolkkonen J. 2005. Effect of cholinergic medication, before and after focal photothrombotic ischemic cortical injury, on histological and functional outcome in aged and young adult rats. Behav Brain Res 156(1):85-94.

Zhu P, Hata R, Cao F, Gu F, Hanakawa Y, Hashimoto K, Sakanaka M. 2008. Ramified microglial cells promote astrogliogenesis and maintenance of neural stem cells through activation of Stat3 function. FASEB J 22(11):3866-77.

# APPENDIX I

| Primary Antibodies | Host | Company | Technique | Dilution | Blocking time | Blocking buffer (IHC or IF) | Immunobuffer (IHC or IF) |
|---|---|---|---|---|---|---|---|
| **Polyclonal anti-SUR1 (C-16 clone)** | Goat | Santa Cruz Biotec. | ICF / IF / **WB** | 1:100 | 3 | 0,3 % Saponin + 10 % NGS + 10 % BSA | 0,5 % triton + 1 % NGS + 1% BSA |
| **Polyclonal anti-SUR2B (C-15 clone)** | Goat | Santa Cruz Biotec. | ICF / IF | 1:100 | 3 | 0,3 % Saponin + 10 % NGS + 10 % BSA | 0,5 % triton + 1 % NGS + 1% BSA |
| **Monoclonal anti-neuronal (NeuN)** | Mouse | Chemicon | IHC / IF | 1:150 | 2 | 0,5 % triton + 5 % NGS + 5% BSA | 0,5 % triton + 1 % NGS + 1% BSA |
| **Monoclonal anti-CD3 (PC3/188A)** | Mouse | Santa Cruz Biotec. | IF | 1:100 | 2 | 0,3 % triton + 5 % NGS + 5% BSA | 0,3 % triton + 1 % NGS + 1% BSA |
| **Monoclonal anti-rat CD11b (OX-42 clone)** | Mouse | Serotec | IF | 1:500 | 2 | 0,5 % triton + 5 % NGS + 1% BSA | 0,5 % triton + 1 % NGS + 1% BSA |
| **Monoclonal anti-Glial fibrillary acidic protein (GFAP)** | Mouse | Sigma-Aldrich | IHC<br>ICF<br>IF | 1:400<br>1:750<br>1:750 | 2 | 0,5 % triton + 5 % NGS + 5% BSA | 0,5 % triton + 1 % NGS + 1% BSA |
| **Monoclonal anti-alpha-tubulin FITC conjugate** | Mouse | Sigma-Aldrich | ICF | 1:500 | 2 | 0,5 % triton + 5 % NGS + 1% BSA | 0,5 % triton + 1 % NGS + 1% BSA |
| **Polyclonal anti-Kir6.1** | Rabbit | Alomone Labs | IF<br>**WB** | 1:400<br>1:500 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |
| **Polyclonal anti-Kir6.2** | Rabbit | Alomone Labs | ICF<br>IF<br>**WB** | 1:400<br>1:400<br>1:500 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |
| **Polyclonal anti-S100β** | Rabbit | Dako | IHC | 1:800 | 2 | 0,5 % triton + 5 % NRS | 0,5 % triton + 1 % NRS |
| **Tyrosine Hydroxilase** | Rabbit | AbCam | IF | 1:5000 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |
| **Caspase-3 (5A1E clone)** | Rabbit | Cell Signaling | IF | 1:500 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |
| **DCX (C-18 clone)** | Goat | Santa Cruz Biotec. | IHC | 1:150 | 2 | 0,5 % triton + 5 % NGS + 1% BSA | 0,3 % triton + 1 % NGS + 1% BSA |
| **RECA-1** | Rabbit | Serotec | IHC | 1:100 | 2 | 0,3 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Isolectine B4 Peroxidase-conjugated (IB4)** | - | Sigma-Aldrich | IHC | 1:25 | 2 | 0,3 % triton + 5 % NGS + 5% BSA | 0,3 % triton + 1 % NGS + 1% BSA |
| **Calbindin** | Rabbit | Swant | IF | 1:500 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |
| **Calretinin** | Rabbit | Swant | IF | 1:2000 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |
| **Parvalbumin** | Rabbit | Swant | IF | 1:2000 | 2 | 0,5 % triton + 5 % NRS + 1% BSA | 0,5 % triton + 1 % NRS + 1% BSA |

| **Note** | **Immunobuffer (WB)** |
|---|---|
| | TBST + 5 % NFDM + 1 % BSA |

| Secondary Antibodies | Company | Technique | Dilution | Immunobuffer |
|---|---|---|---|---|
| **Anti-mouse IgG biotin conjugated** | Sigma-Aldrich | IHC / IF | 1:250 | 0,5 % triton + 1 % NGS + 1% BSA |
| **Anti-rabbit IgG biotin conjugated** | Sigma-Aldrich | IHC | 1:250 | 0,5 % triton + 1 % NRS + 1% BSA |
| **Anti-goat IgG biotin conjugated** | Sigma-Aldrich | IHC | 1:250 | 0,5 % triton + 1 % NGS + 1% BSA |
| **ExtrAvidin-Peroxidase** | Sigma-Aldrich | IHC | 1:250 | PBS 0.01 M |
| **Anti-mouse AlexaFluor-555** | Invitrogen | ICF<br>IF | 1:300<br>1:500 | PBS 0.01 M |
| **Anti-rabbit AlexaFluor-488** | Invitrogen | ICF<br>IF | 1:300<br>1:500 | PBS 0.01 M |
| **Anti-goat AlexaFluor-488** | Invitrogen | ICF<br>IF | 1:300<br>1:500 | PBS 0.01 M |
| **ExtrAvidin-FITC** | Sigma-Aldrich | IF | 1:250 | PBS 0.01 M |
| **Anti-mouse IgG-HRP conjugated** | Sigma-Aldrich | WB | 1:2500 | TBST + 5 % NFDM + 1 % BSA |
| **Anti-rabbit IgG-HRP conjugated** | Sigma-Aldrich | WB | 1:2500 | TBST + 5 % NFDM + 1 % BSA |
| **Anti-goat IgG-HRP conjugated** | Sigma-Aldrich | WB | 1:2500 | TBST + 5 % NFDM + 1 % BSA |

# APPENDIX II

## Protocols for RT-PCR:

### 1.- Kir 6.1:

**Reaction components for RT-PCR-**

| Master Mix | Volume 1 rx (µL) |
|---|---|
| 10X Qiagen RT-Buffer | 2,5 |
| dNTP Mix | 0,5 |
| Taq DNA polimerase | 0,125 |
| 5X Q-Solution | - |
| Mg $Cl_2$ | - |
| Free RNase Water | 16,875 |
| Reverse Primer | 1,5 |
| Forward Primer | 1,5 |
| Template cDNA | 2 |
| Final Volume | **25** |

**Thermal Cycler conditions-**

| Steps | Time | Temperature (ºC) |
|---|---|---|
| Initial denaturing and activation time | 15′ | 95º |
| Denaturation | 30′′ | 95º |
| Annealing | 30′′ | 60º |
| Extension | 45′′ | 72º |
| Number of Cycles | x35 | |
| Final Extension | 5′ | 72º |

### 2.- Kir 6.2:

**Reaction components for RT-PCR-**

| Master Mix | Volume 1 rx (µL) |
|---|---|
| 10X Qiagen RT-Buffer | 2,5 |
| dNTP Mix | 0,5 |
| Taq DNA polimerase | 0,125 |
| 5X Q-Solution | - |
| Mg $Cl_2$ | 0,5 |
| Free RNase Water | 16,375 |
| Reverse Primer | 1,5 |
| Forward Primer | 1,5 |
| Template cDNA | 2 |
| Final Volume | **25** |

**Thermal Cycler conditions-**

| Steps | Time | Temperature (ºC) |
|---|---|---|
| Initial denaturing and activation time | 15′ | 95º |
| Denaturation | 30′′ | 95º |
| Annealing | 30′′ | 60º |
| Extension | 45′′ | 72º |
| Number of Cycles | x35 | |
| Final Extension | 5′ | 72º |

**3.- SUR1:**

**Reaction components for RT-PCR-**

| Master Mix | Volume 1 rx (µL) |
|---|---|
| 10X Qiagen RT-Buffer | 2,5 |
| dNTP Mix | 0,5 |
| Taq DNA polimerase | 0,125 |
| 5X Q-Solution | - |
| Mg $Cl_2$ | - |
| Free RNase Water | 16,875 |
| Reverse Primer | 1,5 |
| Forward Primer | 1,5 |
| Template cDNA | 2 |
| Final Volume | **25** |

**Thermal Cycler conditions-**

| Steps | Time | Temperature (ºC) |
|---|---|---|
| Initial denaturing and activation time | 15′ | 95º |
| Denaturation | 30′′ | 95º |
| Annealing | 30′′ | 60º |
| Extension | 45′′ | 72º |
| Number of Cycles | x35 | |
| Final Extension | 5′ | 72º |

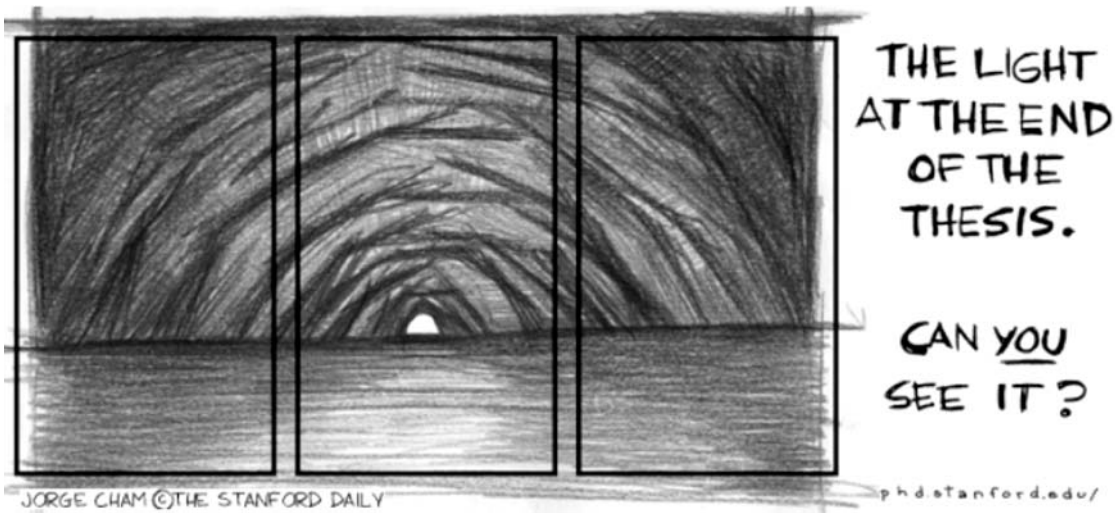
**4.- SUR2A/B:**

**Reaction components for RT-PCR-**

| Master Mix | Volume 1 rx (µL) |
|---|---|
| 10X Qiagen RT-Buffer | 2,5 |
| dNTP Mix | 0,5 |
| Taq DNA polimerase | 0,125 |
| 5X Q-Solution | - |
| Mg $Cl_2$ | - |
| Free RNase Water | 16,875 |
| Reverse Primer | 1,5 |
| Forward Primer | 1,5 |
| Template cDNA | 2 |
| Final Volume | **25** |

**Thermal Cycler conditions-**

| Steps | Time | Temperature (ºC) |
|---|---|---|
| Initial denaturing and activation time | 3′ | 94º |
| Denaturation | 45′′ | 94º |
| Annealing | 45′′ | 57º |
| Extension | 1′ | 72º |
| Number of Cycles | x35 | |
| Final Extension | 10′ | 72º |



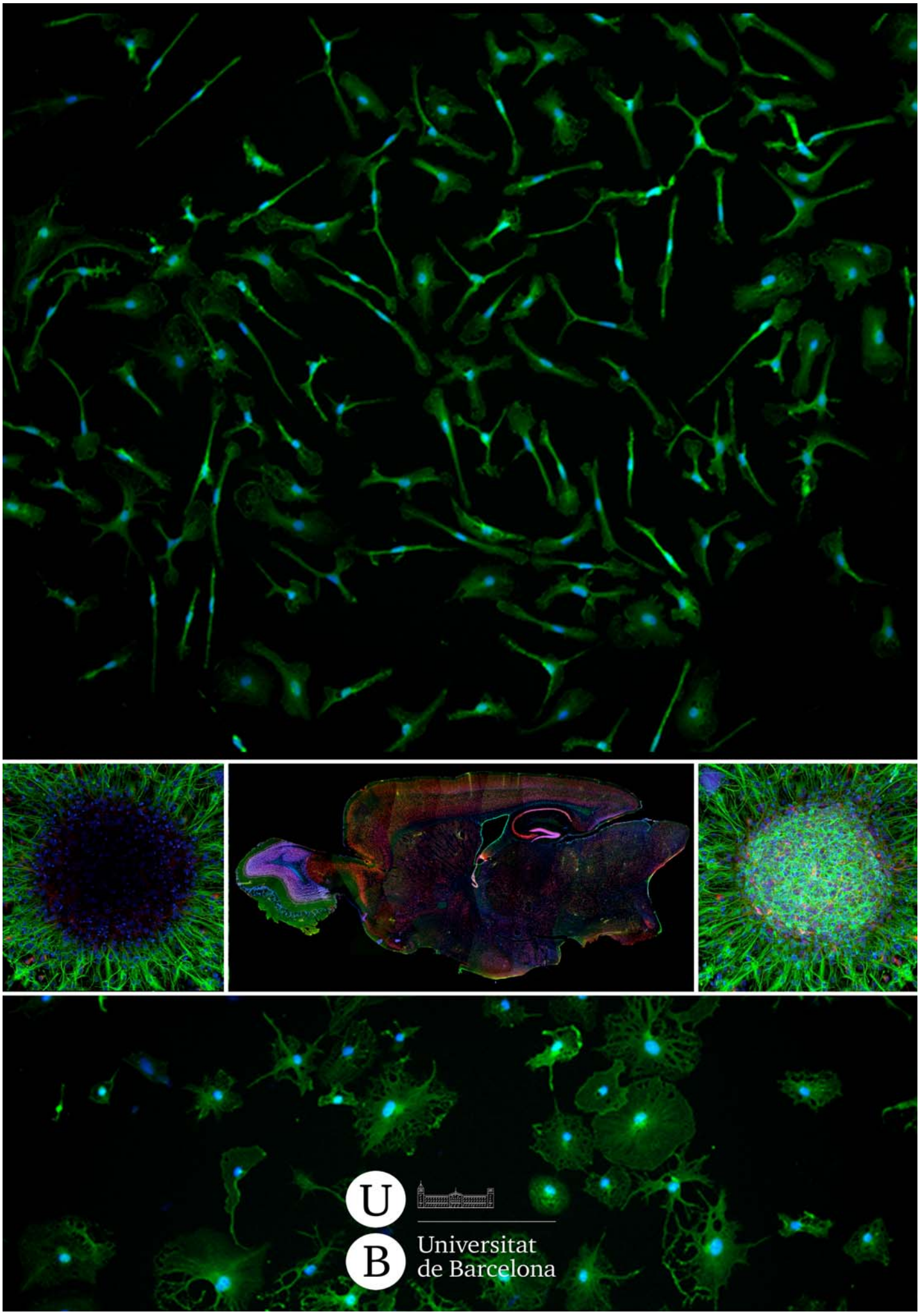

# *Post hoc vs Post-Doc*

***The Post hoc Fallacy***
*To incorrectly assume "A" is the cause of "B" just because "A" preceded "B".*

*e.g. "All Professors have Ph.D.'s, therefore getting a Ph.D. means you'll get a Professor job (right?)"*

***The Post-Doc Fallacy***
*To incorrectly assume you'll have a job just because you have a PhD.*

*e.g. "Now what??"*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