Title: MODELS AND METHODS FOR CONDITIONING THE ISCHEMIC BRAIN

Authors: Antonio Vinciguerra, Ornella Cuomo, Pasquale Cepparulo, Serenella Anzilotti, Paola Brancaccio, Rossana Sirabella, Natascia Guida, Lucio Annunziato, Giuseppe Pignataro

PII: S0165-0270(18)30296-6
DOI: https://doi.org/10.1016/j.jneumeth.2018.09.029
Reference: NSM 8131

To appear in: *Journal of Neuroscience Methods*

Received date: 15-5-2018
Revised date: 13-9-2018
Accepted date: 26-9-2018

Please cite this article as: Vinciguerra A, Cuomo O, Cepparulo P, Anzilotti S, Brancaccio P, Sirabella R, Guida N, Annunziato L, Pignataro G, MODELS AND METHODS FOR CONDITIONING THE ISCHEMIC BRAIN, *Journal of Neuroscience Methods* (2018), https://doi.org/10.1016/j.jneumeth.2018.09.029

MODELS AND METHODS FOR CONDITIONING THE ISCHEMIC BRAIN

Antonio Vinciguerra, PhD*; Ornella Cuomo, PhD*; Pasquale Cepparulo, PhD; Serenella Anzilotti, PhD[1]; Paola Brancaccio, PhD; Natascia Guida, PhD[1]; Lucio Annunziato, MD[1]; Giuseppe Pignataro, MD, PhD

Division of Pharmacology, Department of Neuroscience, School of Medicine, "Federico II" University of Naples, Via Pansini, 5, 80131, Naples, Italy.

[1]IRCCS SDN, Via Gianturco, 113, 80143, Naples, Italy

***Equal Contribution**

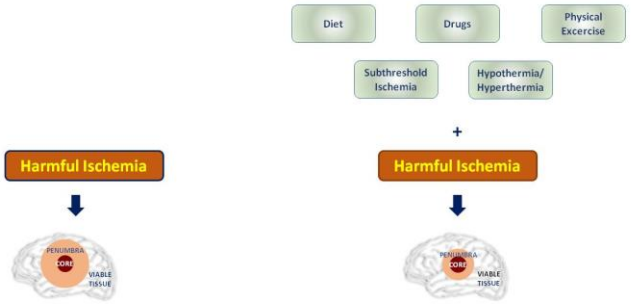
Corresponding Author: Giuseppe Pignataro, M.D., PhD, Division of Pharmacology, Department of Neuroscience, School of Medicine, "Federico II" University of Naples, Via Pansini 5, 80131 Naples, Italy.

Phone: +39-81-7463332;

Fax: +39-81-7463323

E-mail: giuseppe.pignataro@unina.it

Graphical abstract



HIGHLIGHTS

- Ischemic conditioning is an adaptive biological process
- Brain conditioning may be achieved through the exposure to a sub-threshold insult.
- Conditioning stimuli include hypoxia, physical exercise, temperature and drugs.

- Conditioning can be delivered before, PreC, or after, PostC, the harmful event.

ABSTRACT

Background

In last decades the need to find new neuroprotective targets has addressed the researchers to investigate the endogenous molecular mechanisms that brain activates when exposed to a conditioning stimulus. Indeed, conditioning is an adaptive biological process activated by those interventions able to confer resistance to a deleterious brain event through the exposure to a sub-threshold insult. Specifically, preconditioning and postconditioning are realized when the conditioning stimulus is applied before or after, respectively, the harmul ischemia.

Aims and Results

The present review will describe the most common methods to induce brain conditioning, with particular regards to surgical, physical exercise, temperature-induced and pharmacological approaches. It has been well recognized that when the subliminal stimulus is delivered after the ischemic insult, the achieved neuroprotection is comparable to that observed in models of ischemic preconditioning. In addition, subjecting the brain to both preconditioning as well as postconditioning did not cause greater protection than each treatment alone.

Conclusions

The last decades have provided fascinating insights into the mechanisms and potential application of strategies to induce brain conditioning. Since the identification of intrinsic cell-survival pathways should provide more direct opportunities for translational neuroprotection trials an accurate examination of the different models of preconditioning and postconditioning is mandatory before starting any new project.

Keywords: preconditioning, postconditioning, neuroprotection, stroke, brain ischemia

1. INTRODUCTION

Ischemic conditioning is used to group together a number of different stressors or interventions able to confer resistance to a deleterious brain event as an adaptive biological process. Ischemic conditioning through the exposure to a sub-threshold insult as above mentioned, can confer neuroprotection both if conditioning stimuli is applied before, as preconditioning stimulus, or if it is delivered after the harmful ischemia, as occurs in postconditioning. Indeed, ischemic preconditioning has been mechanistically characterized temporarly before postconditioning in such organs like brain, heart, and liver and it is referred to an endogenous protective process induced by a subliminal ischemic event which increases the tissue tolerance or, in other terms, increases the specific organ resistance to a subsequent, normally lethal, episode of ischemia. Since its original description in brain by Kitagawa et al. in 1990, non-ischemic preconditioning stimuli have

been classified as different stressors like toxins, seizure, anoxia, hyperthermia, and spreading depression that, applied at sub-threshold levels, are able to induce tolerance to normally lethal episodes of the same type of insult. Importantly, non-ischemic conditioning stimuli are able to promote additional neuroprotection to an ischemic insult, a phenomenon known as “cross-tolerance” (Gidday, 2006; Plamondon et al., 1999; Tauskela and Blondeau, 2009). In addition, when the subliminal stimulus is delivered after the ischemic insult, the achieved neuroprotection is comparable to that observed in models of ischemic preconditioning. However, subjecting the brain to both preconditioning as well as postconditioning did not cause greater protection than each treatment alone (Pignataro et al., 2007). It is now well established that dangerous signals evoked in the brain by the stressing preconditioning or postconditioning stimuli trigger complex endogenous protective mechanisms resulting to a latent protective phenotype. When the lethal ischemic insult is applied within this latent protective phenotype, a separate set of responses which strikingly differ from the unprimed or unpreconditioned brain’s phenotype are activated, thus establishing the so-called ischemia-tolerant phenotype. Therefore, thanks to conditioning induction, the negative outcome of brain cells induced by focal or global ischemia is shifted from death to survival (Pignataro et al., 2012).

Interestinghly it has been described a rapid preconditioning, in which neuroprotection take place within minutes from the subliminal stimulus, and a delayed preconditioning, that occurs within hours. It is obvious that the mechanisms that mediate these temporal distinct phenomena are different and imply the activation of separate cascade of events. In the next paragraphs the most common methods used to confer conditioning neuroprotection in ischemic brain will be reviewed and summarized.

2. SURGICAL METHODS TO INDUCE BRAIN PRE AND POSTCONDITIONING

Most of the published surgical protocols of pre- and postconditioning require the transient mechanical interruption followed by reperfusion of the same artery occluded during stroke in order to reduce the reperfusion injury. Different protocols may be described for the induction of pre- and post-conditioning in models of focal or global ischemia (Table 1 and 2).

2.1 Ischemic Preconditioning

Evidences from current literature show that the parameters that must be taken into consideration in setting on a surgical strategy to induce ischemic preconditioning (IPC) are: the typology of ischemic subliminal insult (focal versus global), the duration (transient versus permanent), the possible number of conditioning cycles, and the time interval between preconditioning and injurious stimulus. Therefore, it is possible to classify the different preconditioning/harmful ischemia situations as: Focal/Permanent Focal,

Focal/Focal, Focal/Global, Global/Focal or Global/Global, where the first term indicates the type of preconditioning stimulus and the second term indicates the type of harmful ischemia.

2.1.1 Transient Focal Preconditioning/Permanent Focal Ischemia

The first published method to induce rat ischemic preconditioning consisted in 10 minutes of transient middle cerebral artery occlusion (tMCAO), as the preconditioning (IPC) non-injurious stimulus, applied before a permanent MCAO (pMCAO) as the injurious ischemia (Barone et al. 1998). In addition, the best protocol of IPC is able to protect the brain in a time window ranging between 2 and 7 days before pMCAO (Barone et al., 1998).

2.1.2 Transient Focal Preconditioning/ Transient Focal Harmful Ischemia

In the transient focal preconditioning/transient focal harmful ischemia model, both stimuli are induced by transiently occluding the MCA. As previously shown, protocols used as preconditioning stimuli consist in a series of brief interruption of blood flow. Indeed, one (Naylor et al., 2005; Hao et al., 2003; Lee et al., 2007) or three times of 10 minutes of MCAO protects from subsequent 120 minutes of tMCAO in rats (Alkayed et al., 2002; Chen et al., 1996). Shorter durations (2 and 3 minutes) of tMCAO were sufficient to induce delayed ischemic tolerance, but did not provide early tolerance to transient ischemia (Glantz et el., 2005; Puisieux et al., 2004). According to another recent protocol to induce tolerance, rats were subjected to a 30 minutes of tMCAO followed, 3 days later, by 100 minutes of injurious tMCAO. The protection was already evident if the time interval between the preconditioning stimulus and the injurious stimulus was of 1 day, but it reaches the maximum benefit when the harmful stimulus was delivered 3 days after preconditioning, whereas, the duration of the protection was lost in 7 days (Lusardi et al., 2011; Pignataro et al., 2012, Cuomo et al., 2016). Interestingly, transient focal-focal preconditioning neuroprotection works also in mice and in spontaneously hypertensive rats (Hoyte et al., 2006). Recently in a mouse model of preconditioning, delayed preconditioning effects have been observed by inducing 2 cycles of 5-minute tMCAO as the IPC stimulus, followed, 3 days later, by 90-minute tMCAO(Zhang et al., 2008). A similar protocol of delayed preconditioning can be applied to mice, but in that case the duration of the IPC stimulus was 15 minutes whereas the duration of the injurious middle cerebral artery occlusion was 60 minutes. Once again, it is evident also from another laboratory that in order to reach the best protection, the time interval between preconditioning and harmful ischemia must be three days (Lusardi et al., 2011). Conversely, the rapid preconditioning has also been induced in mice by MCA occlusion for either 5 minutes or three cycles of 5 minutes separated by 10 minutes' reperfusion and followed, 30 minutes later, by 60 minutes occlusion of the MCA (Atochin et al., 2003).

2.1.3 Focal Preconditioning/Global Harmful Ischemia

It has been observed that unilateral occlusion of the MCA induced significant protection from global ischemia in both gerbils (Miyashita et al., 1994) and rats (Belayev et al., 1996). Interestingly, transient (20 minutes) occlusion of the distal MCA protected only ipsilateral parietal cortex from global ischemia induced by 10 minutes of both common carotid arteries and vertebral arteries occlusion (2 vessel occlusion, 2-VO) (Glazier et al., 1994).

2.1.4 Global Preconditioning/Focal Harmful Ischemia

Brief global ischemia can protect from both subsequent transient and permanent focal ischemia (Simon et al., 1993). Chiarugi's team reported that 5-minute bilateral common carotid artery occlusion (BCCAO) in the mouse prompted reduction of infarct volumes triggered 24 hours later by 20-minute middle cerebral artery occlusion (Faraco et al., 2009).

2.1.5 Global Preconditioning/Global Harmful Ischemia

In the first description of preconditioning in the brain, Kitagawa and coworkers demonstrated that a single 2-min ischemia performed in gerbils by BCCAO 1 or 2 days before 5 minutes of BCCAO exhibited only partial protective effects against delayed neuronal death (Kitagawa et al., 1990). However, two cycles of two minutes of BCCAO, performed 2 days before 5 minutes of ischemia, drastically reduced neuronal death. What is evident is that a specific combination in duration and time intervals of ischemic treatment was needed, because neither 1-minute ischemia nor 2-minute ischemia exhibited protective effects alone. Besides the gerbil a recent paper described a new model of global preconditioning/global in mice. In this model, adult Swiss albino mice were subjected to global cerebral ischemia induced by occluding both the carotid arteries; after 17 minutes of global cerebral ischemia, reperfusion was allowed for 24 hours, whereas preconditioning stimulus was delivered according to two different experimental protocols. For rapid preconditioning, three brief cycles of carotid arteries occlusion for a period of 1 minute followed by 1 minute of reperfusion time were immediately performed before BCCAO, whereas, for delayed preconditioning, the same three cycles of artery occlusion were applied 24 hours before BCCAO (Rehni et al., 2010). The protection achieved with both rapid and delayed preconditioning methods were able to produce a reduction in the infarct volume of more than 50% compared to ischemia alone.

2.2 Other surgical methods to induce preconditioning

In recent years a growing interest in the field of preconditioning has raised attention to several studies to generate new models of IPC/ischemia. Beside the myriad of new models proposed it is interesting to spend a few words on the method of IPC induction in neonatal animals. To settle up this model P7 rat pups have been used, where unilateral

common carotid artery ligation followed 1 hour later by 8% oxygen hypoxia for 2 hours was performed. This Hypoxic/Ischemic stimulus produces selective damage in the ipsilateral hemisphere to the occluded artery that resembles HI damage to the human neonatal brain (Cerullo et al., 2018). When the carotid artery ligation was performed 24 hours before hypoxia, a remarkable neuroprotection was achieved, thus showing that the preconditioning phenomenon can be induced also in neonatal mice. Notably, the same stimulus represented by the ligation of the carotid artery applied 1 hour before subjecting neonatal rats to hypoxia was not able to induce any protection (Lee et al., 2004). Another model has been proposed in 2005 by Mishima and coworkers (Mishima et al., 2005). They demonstrated that electroconvulsive shock (ECS) can be used as a preconditioning stimulus in what is commonly called cross-protection. In particular, both single and repetitive ECS application confers neuroprotection from subsequent global ischemia induced in rats by BCCAO (Winston et al., 1990). Single groups received ECS only 2 days before 8 minutes of global ischemia, whereas repetitive ECS group received ECS once a day for 9 consecutive days until 2 days before global ischemia induction. No differences were found in the neuroprotection induced by the two experimental procedures (Mishima et al., 2005).

2.3 Ischemic Postconditioning

The neuroprotective strategy of ischemic postconditioning (IPOSTC), defined as a repetitive series of brief reperfusion/occlusions applied after ischemia, is a relatively new concept with respect to ischemic preconditioning (IPC) (Zhao et al., 2006). Initially shown to be able to reduce infarct size after cardiac ischemia in both clinical (Staat et al., 2005) and preclinical conditions (Gidday, 2010), ischemic postconditioning has recently been shown to be effective in attenuating neuronal damage in the rodent spinal cord injury models (Jiang et al., 2006) and both in focal (Pignataro et al., 2008) and in global models of cerebral ischemia (Wang et al., 2008).

.

2.3.1 Focal Harmful Ischemia/Focal Postconditioning

All the surgical methods of IPOSTC utilize the modeling of reperfusion in the same artery as that affected by focal ischemic stroke. Indeed, during the reperfusion phase, after the canonical 100 minutes of MCAO, the cerebral blood flow may be interrupted by reinsertion of the surgical filament. The time window and the duration of re-occlusion may influence the entity of the protection. Indeed, it has been demonstrated that after 100 minutes of MCAO, reperfusion was established for 5 minutes after which the MCA was occluded again for 5 minutes, followed by two additional cycles of 5 minutes of reperfusion and 5 minutes of occlusion (total of three cycles). Protection has been established 24 hours after the last occlusion. In an equally protective protocol, after 100 minutes of MCAO, reperfusion was established for 10 minutes after which the MCA was occluded for 10 minutes. Longer intervals of reperfusion were not protective (Pignataro et al., 2008).

2.3.2 Global Harmful Ischemic/Global Postconditioning

The protection in a model of global ischemia is achieved by different schedules of cycles of reperfusion/reocclusion of the common carotid arteries. In particular, Wang et al. published four different protocols that are equally effective in mediating neuroprotection: (1) 3 cycles of 15 seconds/15 seconds reperfusion/reocclusion after 10 minutes of ischemia, (2) 3 cycles of 30 seconds/30 seconds reperfusion/reocclusion, (3) 3 cycles of 60 seconds/15 seconds reperfusion/reocclusion, (4) 3 cycles of 15 seconds/15 seconds reperfusion/ reocclusion applied after 45 seconds reperfusion (Wang et al., 2007)

3. Whole body conditioning stimuli

It has been widely demonstrated that physical exercise is able to exert neuroprotective effects both in the preclinical and clinical settings. Several studies have shown that exercise can be a promising conditioning method able to induce neuroprotection through the promotion of angiogenesis, mediation of the inflammatory response, inhibition of over-activation of glutamate, protection of the blood-brain barrier (BBB) and inhibition of apoptosis (Zhang et al. 2011) (See Figure 1)

3.1 Physical exercise contrasts brain deterioration

Several meta-analysis established that physical activity positively correlates with the increase in neuroprotection in subjects affected by both hemorrhagic and ischemic infarcts (Lee et al., 2003; Wendel-Vos et al., 2004). Much of the neuroprotective effect is due to positive changes in risk factors related to stroke such as elevated blood pressure, lipid profile, weight, hypertension and diabetes (Evenson et al., 1999, Gillum et al., 1996; Hu et al., 2004). In human studies it has been demonstrated that exercise improves memory, learning and executive functions by contrasting the phenomena of dementia and cognitive decline, at the same time, constant training is able to reduce in the general population the risk of Alzheimer's, Parkinson's and Huntington's (Cotman et al., 2007) through a strengthening of the structural function of BBB and an increase in neuronal viability. Whether exercise can be considered a real preconditioning stimulus remains to be established, as for definition, a preconditioning stimulus represent a toxic stimulus applied at sub-threshold event.

3.1.1 Methods to prevent stroke damage by physical exercise

It has been proven a direct correlation between the duration and intensity of an exercise and the quality of life improvement associated with these activities since several researchers have found that the best stroke outcomes correlate with moderate intensity and duration of exercise (Larson et al. 2006). Nevertheless, despite any evidence, no specific guidelines or methods have been established for exercise preconditioning able to transfer either cardioprotection or neuroprotection (Abete et al. 2001; Kloner 2001). However, in rats, exercise for at least 2 weeks until 12 weeks was able to decrease infarct

and brain neuronal damage and this effect was still present 3 weeks after exercise ceased (Ang et al. 2003; Stummer et al. 1994; Wang et al. 2001; Curry et al. 2010; Davis et al. 2007; Ding et al 2004b; Ding et al. 2004c). A recent study found that 1 or 2 weeks of pre-ischemic exercise did not reduce brain infarction after ischemic stroke compared with a non-exercise group, but that at least 3 weeks of pre-training was necessary for neuroprotection (Liebelt et al., 2010). Different types of exercise, such as treadmill, running wheel, and environmental enrichment, have been shown to induce neuroprotection.

3.1.2 Methods of Post-stroke Exercise

While preconditioning exercise confers neuroprotection when applied before an acute ischemic heart attack occurs, the use of physical exercise as a core component of post-stroke rehabilitation programs is more consolidated, due to its capability to confer improved clinical outcome in infarcted patients during rehabilitation (Rabaldi 2007). Post-ischemic complex exercise, which involves balancing and coordination, if compared to simple exercise with treadmill is able to increase the expression of neurotrophins and neurogenesis, and also induces an increase in synaptogenesis in the brain regions (Jones et al 1999, Stranahan et al., 2007).

3.2 Hypothermia

Conditioning mediated by hypothermia is obtained by controlled lowering of the body temperature, at a precise time interval from the beginning of the ischemia. A series of experiments evaluating the effectiveness of hypothermic conditioning have indicated that a controlled reduction of body temperature by ice packs, cooling blanckets, alcohol applied to the body surface, from values of 31.5°C, 28.5°C, up to 25.5°C was able to evoke a parallel reduction of the cerebral ischemic volume after focal ischemia in the rat. In these experiments the duration of hypothermia was 20 minutes, while the total time in which the body temperature of the animal remained below the physiological temperature was 120 minutes. Several studies have shown that hypothermia is able to induce neuroprotection against hypoxia/ischemia (Nioshio et al. 1999, 2000; Yunoki et al. 2002, 2003; Mitchell et al. 2010; Yuan et al. 2004). In a model of transient ischemia obtained by both common carotid and one vertebral artery occlusion (three-vessel occlusion, 3-VO), it has been shown that the duration and the magnitude of the body temperature reduction are responsible for a tolerance effect subsequent to the ischemic insult that can be either rapid or delayed as it occurs for ischemic preconditioning (Yunoki et al 2003, Stetler et al 2008, Mitchell et al., 2010). Selective cooling by intracarotid saline infusion has been demonstrated to produce robust neuroprotection (Ding et al. 2004; Luan et al. 2004; Li et al. 2004). From that moment on, it is clear that regionally selective vascular cooling is more effective in reducing brain injury and improving functional outcome in rats compared to external cooling (Wang et al. 2010).

3.2.1 Rapid vs. Delayed protection

The maximum degree of neuroprotection induced by hypothermia is obtained when it is induced 20 to 60 minutes before the ischemic insult, for a duration of the conditioning stimulus equal to at least 20 minutes. This form of rapid preconditioning clearly cannot

depend on de novo protein synthesis and it depends almost exclusively on the intensity of the conditioning stimulus (Yunoki et al., 2003); On the contrary, delayed preconditioning exerts its best neuroprotective effects when the hypothermic stimulus is administered from 6 hours to 48 hours before the ischemic insult. This second type of neuroprotective response, due to the de novo synthesis of proteins, reaches its peak of efficacy in proximity of 24 hours, and is completely lost at 7 days from the initial stimulus; duration of the stimulus must be at least 20 minutes (Nishio et al. 2000). In addition, the best temporal and intensity combination able to induce the greatest reduction of brain infarct volume is obtained when a slight hypothermia (33°C) is maintained for 120 minutes, at most 180, compared to only 20-60 minutes used in other preclinical studies (Yuan et al. 2004).

3.2.2 Intra- and Post-ischemic hypothermic stimulus application

Hypothermia applied during the ischemic insult, the intra-ischemic hypothermia, is considered the gold standard due to its ability to reduce ischemic cerebral damage in a large number of in vivo animal studies (Barone et al. 1997; Corbett and Thornhill 2000; Miyazawa et al. 2003). At the same time, post-ischemic hypothermia was shown to be effective in tight relationship to the duration of hypothermia (Miyzawa et al., 2003). Some clinical studies have revealed that the neuroprotective actions of mild or moderate hypothermia in acute ischemic infarction can be achieved either from the early onset of post-stroke, onset of reflation or, alternatively, prolonging hypothermia up to 48-72 hours (Schwab et al. 1998, 2001; Kammersgaard et al. 2000; Steiner et al. 2000). Therefore a prolonged application of post-ischemic hypothermia seems necessary to obtain a significant and persistent neuroprotection (Coulborne et al. 1997; Inamasu et al. 2000; Kollmar et al. 2002). The common mechanism of beneficial action seems to be linked to a slowing of neuronal damage caused by ischemia.

3.2.3 Hypothermia induced by drugs

Drug-induced mild hypothermia (35.1 ± 1.1 to 36.0 ± 0.5°C for <20 hours), capable of reducing ischemic brain lesion, has been investigated in rats by Johansen and collaborators with the dopamine D_2 receptor agonist, talipexole continuously infused for 20 hours after 60 minutes of MCAO (Johansen et al., 2003). The talipexole-induced hypothermic regime has been investigated in moderately sized cortical infarcts in rats before and after cerebral ischemia. In preconditioning experiments, authors investigated the effects of drug-induced hypothermia given before cerebral ischemia in two well-known models of either 60-minute middle cerebral artery occlusion (MCAO) or 10 minutes of global cerebral ischemia (2-vessel occlusion model with hypotension). In postconditioning experiments drug-induced hypothermia has shown to be effective also if the stimulus was delivered as long as 90 days after the insult or if treatment was delayed for 3 hours after the experimental stroke (Johansen et al., 2014).

3.2.4 Safety concerns

Despite the clinical efficacy in stroke, the induction of body temperature below 28°C is able to trigger cardiac arrhythmia (Polderman 2009). This may represent an obstacle to the use of the maximum neuroprotective potential of this conditioning stimulus, which exerts its best effects by bringing the body temperature of the animal below 25.5°C, when a delayed tolerance is sought (Yunoki et al., 2002). Another critical issue represented by the achievement of low body temperature values, for prolonged periods, is the increased risk of systemic infections (Polderman 2009). These considerations impose to be carefully in translating this paradigm to humans.

3.3 Hyperthermia

Hyperthermia is able to induce neuroprotection when administered at precise time intervals prior to cerebral ischemia. (Chopp et al., 1989; Zhang et al., 2000; Xu et al., 2002; Kelty et al., 2002; Du et al., 2010). In a model of transient cerebral ischemia of 120-min duration, rats were heated inside a humidified chamber and kept at a body temperature of 41.5°C for 15 minutes. This treatment showed a significant reduction in neuronal loss subsequent to ischemia with an optimal neuroprotection effect (Chopp et al 1989; Xu et al. 2002). The effect of this kind of conditioning stimulus on the reduction of cerebral ischemic infarction was observed only when hypothermia was induced 18 to 24 hours before stroke, whereas the neuroprotective effect was lost after 48 hours (Chopp et al. 1989; Zhang et al., 2000; Xu et al., 2002).

4. Brain conditioning by diet

In recent years the role of nutrition as a conditioning factor for stroke has been increasingly affirmed. Prevention based on diet it is affirming itself more and more in stroke prevention. Whereas some risk factors including cardiovascular complications, hypertension, diabetes, hypercholesterolemia, cigarette smoking, inflammatory markers, obesity and dyslipidemia have to been mandatory addressed by pharmacology to reduce the possibility to have a stroke, modifiable risk factors, like diet, often coexist and have been estimated to account for 60-80% of stroke incidence in general population (Allen and Bayraktutan 2008; Moskovitz et al. 2010). Improper lifestyle and nutrition causing imbalances in essential vitamin and nutriments can affect modifiable risk factors. Many epidemiological and clinical studies have shown that deficiencies in omega-3, α-lnolenic acid (ALA), eicosapentaenoic acid (EPA) and Docosaexaenoic acid (DHA) represent a risk factors for cardiovascular and cerebral diseases, including stroke (Donald B. et al. 2012, Blondeau et al. 2015; Berressem B. et al., 2016).

4.1 PUFAs as a rapid conditioner

The primary role of polyunsaturated fatty acids (PUFAs) like EPA, ALA and DHA, is to act as structural components at the level of cell membranes. Neuroprotective effects of n-3 PUFAs against ischemic brain injury have been shown in several stroke models (Black et al., 1979; Marcheselli et al., 2003; Akbar et al., 2005; Belayev et al., 2005; Moreira et al., 2010; Zhang et al., 2010). These studies focused on the acute phase of ischemia assessed from 24 hours to 7 days postinjury in focal ischemic stroke models. Recently, it has been expanded the therapeutic window to 21 days postinjury. Our data therefore demonstrate that oral administration of fish oil for 6 weeks before ischemia improved outcomes demonstrating the long term efficacy of omega-3 diet (Zhang et al. 2014). In a seminal paper by Pulsinelli and Brierley, it has been described an acute neuroprotective effect of ALA and DHA (linoleic acid and docohexanoic acid) in an in vivo model of transient global ischemia, in which CA1 hippocampal pyramidal cells were killed by glutamate exitotoxicity (Pulsinelli and Brierley, 1979). When ALA was administered intravenously (i.v. 500 nmol/kg) or intracerebroventricularly (i.c.v. 10 uM/5ul) within 30 minutes prior or after ischemia, it preserved 80% of the CA1 neurons. Conversely, intraperitoneal injection of AA or DHA 1 hour after transient ischemia aggravated injury (Eckert GP et al. 2013). In vitro, the same range of concentrations known to be protective both in vivo with i.c.v. injection and in vitro on granule cells prevented hippocampal neuronal death triggered by the addition of an exitotoxic concentration of glutamate (50 uM) for 24 hours (Blondeau et al., 2009). This finding demonstrated postsynaptic mediated neuroprotection by PUFAs. In 1984 was shown that intravenous administration of EPA in gerbils improved CBF after ischemia and reperfusion, compared to LA-treated animals (Black et al., 1984). The in vivo and in vitro neuroprotective 10 and 100 uM of ALA, respectively, increased the diameter of the basilar but not carotid artery (Blondeau et al., 2007). The omega-3 induced relaxation of cerebral resistance arteries, as those of the cerebral vascular bed, without affecting the systemic blood pressure as carotid arteries with elastic properties. Another evidence supporting supplementation of omega-3 in diet is that ischemic rats treated by daily gavage with EPA for 4 weeks, starting 1 day following ischemia, showed a better glucose utilization and CBF increase in peri-infarcted areas whereas no effect was observed in ischemic core (Katsumata et al. 1999). Thus, ALA is able to counteract glutamate excitotoxicity and induces vasodilatation of brain arteries in penumbra, which potentially contribute to protect brain against ischemic stroke (See Figure 2a)

4.2 Alfa-linoleic acid as a conditioning stimulus

ALA has been demonstrated to be a “natural” conditioner able to induce delayed cross-tolerance to ischemia. When administered 2 hours post ischemia, ALA induced the maximal neuroprotection, and this effect lasted until 6 hours, with the window of interest dissipated 12 hours after reperfusion. Conversely the saturated palmitic fatty acid failed to reduce the infarct volume when injected 2h after reperfusion. At the same time, an ALA injection providing the best cerebral protection measured 24h post stroke did not display any beneficial effect on long-term survival rate. To obtain a significant improvement in the

survival rate at 10 days and 1 month post ischemia, repeated injections were required (Blondeau et al., 2009; Herteaux et al., 2006). This positive effect was mediated by the suppression of glutamate exitotoxicity but it failed to prevent later stages of cell death including apoptosis and associated inflammatory events. Also if administered 3 days before a transient focal ischemia in mice ALA significantly reduced infarct volume (Blondeau et al., 2009). Deficiency in omega-3 intake, as reported in numerous epidemiologic studies, represents an important risk factor in the development and/or deterioration of neurovascular diseases, like stroke. Several papers demonstrate a correlation between ALA supplementation and decreased infarct volumes in stroked mice. In addition, supplementation of 0.4 g/kg/day of EPA/DHA during 14 days before surgery may reduce apoptosis of hippocampal neurons in ischemic rat (Bas et al., 2007; Ozen et al., 2008).

4.3 Omega-3 brain conditioning effect by chronic administration

Daily oral administration of DHA in gerbils for 4-10 weeks suppressed neuronal injury due to subsequent transient ischemia (Cao et al., 2004, 2005, 2006, 2007) and a supplementation of fish-oil for 6 weeks in rats prior to ischemia reduced infarct volumes by ca. 30% (Choi-Kwon et al. 2004). Rats subjected to 2-vessel occlusion at 3 months after being fed a diet with a decreased omega-6/omega-3 ratio immediately after birth, displayed an improved BBB function and behavior measured at 7 months (de Wilde et al., 2002). A decreased apoptotic death was also displayed when rats were fed a diet supplemented with fish n-3 fatty acids for 2 weeks prior to global ischemia (Bas et al., 2007; Ozen et al., 2008). 2 weeks of fish-oil supplemented diets prior to ischemia resulted in an improvement in spatial memory deficits despite no improvement in CA1 hippocampal neurons was observed (Plamondon and Roberge, 2008).

4.4 Security

The use of omega-3 ad preconditioning stimulus can cause deleterious effects or toxicity. The potential toxicity of PUFAs has been evaluated in Eskimo population, which consumes 15-20 g/day of omega-3. A chronic and massive omega-3 ingestion causes nausea. An increased delay in erythrocyte sedimentation when intake EPA and DHA exceeds 9 g/day has been observed; the raccomendation for DHA is to not exceed more than 15 times the DRI (Martin, 2001).

5. Medical gases for brain conditioning

Among different stimuli able to induce the conditioning phenomenon there are medical gases including anesthetics and hyperbaric oxygen (Zheng and Zuo, 2004; Ostriwski et al., 2008). These gases are clinically relatively safe; they usually cross the blood-brain barrier rapidly so they can be applied systemically to induce neuroprotection. Regarding anesthetics, pre- and postconditioning by isoflurane or sevoflurane have all been

demonstrated to significantly reduce cerebral infarct size and improve recovery of neurological function after cerebral ischemia (McBride et al., 2015) (See Figure 2b).

5.1 Isoflurane

Neuroprotective potential of isoflurane has been shown constantly in both rats and mice when the interval between isoflurane exposure and brain ischemia is 24 hours or shorter whereas the neuroprotection disappeared if the time interval was of 48 hours (Kapinya et al., 2002). Thus, the most in vivo studies use 24 hours as interval. One exposure to 1-2% isoflurane for 30 minutes to 4 hours is commonly used to precondition the animals, also multiple exposures to isoflurane for 5 consecutive days have been used successfully (Kitano et al., 2007). Animals exposed to 1.2% or 2% isoflurane for 1 hour for 5 consecutive days (Sun et al., 2015; Tong et al., 2015), 1.5% isoflurane for 30 minutes (Li et al., 2013) or 1% isoflurane for 4 hours (Zhu et al., 2010) before middle cerebral artery occlusion (MCAO) were all shown to exhibit significantly alleviated neurological deficits and reduced infarct volume (Wang et al., 2016).A dose-dependent neuroprotection, paralleled with a long-term neurological outcome improvement was observed in adult rat models of both transient and permanent focal brain ischemia, global brain ischemia, or intracerebral hemorrhage, and neonatal rat HI insult. This evidence was not confirmed in aged female mice after focal brain ischemia, suggesting a gender difference in isoflurane induced preconditioning. (Kitano et al., 2007).. In a rat MCAO model, postconditioning significantly decreased neurobehavioral deficit scores and infarct volume (Li et al., 2014). Additionally, isoflurane postconditioning decreased the numbers of PI-positive cells 24 hours after reperfusion compared with the ischemia/reperfusion group (Wang et al., 2016). In terms of the paradigm of isoflurane postconditioning, postconditioning with 1.5%, 2% and 3.0% isoflurane for 1 hour since reperfusion has all been demonstrated as effective in previous animal studies (Lin et al., 2011).

5.2 Sevoflurane

Preconditioning with the single inhalation of sevoflurane enabled to protect animals from cerebral ischemic insults, while repeated preconditioning of sevoflurane also provided neuroprotection against focal or global brain damage induced by ischemia/reperfusion in short period (3 days) after ischemia (Wang et al., 2011; Wang, 2016). Sevoflurane postconditioning also yields protection against cerebral ischemia consistently. Postconditioning with sevoflurane significantly decreased apoptotic cell counts at 3 days (Kim et al., 2016) and preserved the CA1 neuron histology and reduced necrotic or apoptotic cells at 7 days after global cerebral ischemia in rats (Seo et al., 2013). In this study, the postconditioning paradigms ranged from single treatment of 10 minutes after transient global ischemia to two repeats of 5 minutes treatment of 2.5% sevoflurane and a subsequent washout time of 10 minutes after ischemia (Seo et al., 2013).. An improvement of long-term neurological function by sevoflurane preconditioning has been found in studies in neonatal rats and mice after HI insult (Luo et al. 2008; McAuliffe et al. 2007). Finally a study clearly demonstrated the neuroprotective potential of sevoflurane

preconditioning effects in the spinal cord ischemia by 3.7% concentrations for 30-min at 1 h before rats were subjected to a 12-min spinal cord ischemia. There was a significative improvement in neurological outcome at 48 h after ischemia.

5.3 Desflurane

The neuroprotective effect of desflurane has been demonstrated in focal cerebral ischemia in rats and also in newborn pigs under deep hypothermic circulatory arrest (Haelewyn et al., 2003; Tsai et al., 2004). In neonatal rats with incomplete cerebral ischemia and low-flow cardiopulmonary bypass, neurologic function was improved with desflurane anesthesia (Kurth et al., 2001). Desflurane postconditioning was suggested as protective in an in vitro study showing that LDH release at 1 hour after OGD was reduced by desflurane postconditioning in the human neuroblastoma cell line (Lin et al., 2011). Unfortunately, studies on neuroprotective effect of desflurane preconditioning and postconditioning in cerebral ischemia are still limited (Wang et al., 2016).

5.4 Xenon and Halothane

Halothane was demonstrated to attenuate cerebral ischemic injury both in cats and rodents 16 hours to 7 days after ischemia and hypoxia (Zausinger et al., 2002; Haelewyn et al., 2003). For Xenon, a delayed phase of preconditioning-induced neuroprotection has been shown in neuronal-glial co-cultures, hippocampal slices, and intact brains of rodents (Bantel et al. 2009; Luo et al. 2008; Ma et al. 2006; Limatola et al. 2010).Post-MCAO administration of xenon showed reduced cortical damage in animal models (David et al., 2003; Abraini et al., 2005).

5.5 Hyperbaric Oxygen (HBO)

A 5-day preconditioning protocol of HBO 2.5 atmosphere absolute (ATA) in 100% oxygen for 1 h every day for 5 days is very usual; this protocol was also effective in neonatal rats after HI insult. In all studies in which HBO preconditioning was applied 24 h before a detrimental insult it was protective against HI insult (Freiberger et al., 2006; Li et al., 2008). The degree of neuroprotection by HBO depends on the number of HBO episodes (5 episodes may be better than 3) and on the degree of ATA (3.5 ATA appears better than 2 ATA) used as preconditioning stimuli. The effective time-window between conditioning stimulus and detrimental is less than 72 hours. Finally, this stimulus was not effective in permanent focal ischemia. In spinal cord ischemia the same protocol of 5-days HBO preconditioning as above provided protection against 20-minute spinal cord ischemia in rabbits (Dong et al. 2002). In a model of perinatal hypoxia-ischemia (HI) in rats it has been demonstrated that postconditioning treatment, performed 1 or 6 hours after HI by HBO, was able to ameliorate ischemic brain damage (Gamdzyk et al., 2016).

6.Pharmacological conditioning

Recently, it has been suggested that conditioning may also be stimulated with various pharmacologic approaches. The perspective of the rapid administration of a drug to induce a pre- or postconditioning state is very attractive particularly for situations such as traumatic injury where other forms of preconditioning stimuli like hyperbaric oxygen and transient ischemia could be not easy to handle. A large number of agents can induce ischemic conditioning but only seven: thrombin, erythropoietin (EPO), deferoxamine, erythromicine, opioids, diazoxide and lipopolysaccharide (LPS) all acting by upregulating brain defenses with EPO and thrombin are endogenous compounds modifying stroke, LPS as an exogenous stimulating endogenous defenses (See Figure 3).

6.1 beta-methylamino-Lalanine (L-BMAA)

Cycad neurotoxin (L-BMAA), is an excitatory non-protein amino acid produced by cyanobacteria associated with amyotrophic lateral sclerosis-Parkinson dementia complex (ALS-PDC), in Guam indigenous population. In a recent paper it has been shown that a sub-toxic acute exposure to L-BMAA for 8 weeks works as preconditioning stimulus being able to delay ALS progression in G93A mice (Anzilotti et al., 2018). This effect is mainly due by targeting a protein, NCX3, a membrane transporter able to handle the deregulation of ionic homeostasis occurring during ALS (Anzilotti et al., 2018).This represents a proof of concept on the effect of pharmacological conditioning in different neurological disorders.

6.2 Thrombin

Thrombin is an endogenous serine protease with a preminent role in the coagulation cascade by cleaving fibrinogen to fibrin (Coughlin, 2000). Thrombin inhibition by antitrhombin systemic administration protects brain from ischemic damage induced by both tMCAO and pMCAO (Cuomo et al. 2007).

6.3 Diazoxide

In a postconditioning study, Robin and collaborators have reported that mitoKATP opening by diazoxide right before the start of reperfusion conferred significant neuroprotection. In this study, diazoxide was used in conjunction with ischemic postconditioning comprising three episodes of 30 s of occlusion and reperfusion. The authors found that diazoxide resulted in a 60% decrease in infarction volume, and this effect was abolished by mitoKATP blocker 5-hydroxydecanoate (5-HD). Additionally, no delayed postconditioning effect was observed, as postC applied 5 min after the onset of reperfusion did not yield neuroprotection (Jin et al., 2016).

6.4 Deferoxamine

Deferoxamine is a potent iron chelator used for decades in the treatment of iron overload secondary to poisoning (Selim, 2009). Administered subcutaneously, deferoxamine can penetrate the blood-brain barrier acting by the inhibition of iron-mediated free radicals taking part in long-term deficits following hemorrhagic stroke in a rat model (Hua et al., 2006, Nakamura et al., 2003).

6.5 Erythromycin

Erythromycin is an antibiotic of the macrolide family used to treat a wide variety of gram-positive infections (MC Kendrick, 1979). When administered as a conditioning stimulus prior to ischemi insult, erythromycin reduces hippocampal and parietal cortex neuronal loss and improves scores on tests of neurological function (Brambrink et al., 2006).

6.6 Opioids

A variety of studies have shown significant neuroprotective effects with morphine preconditioning. In vivo studies indicate that opioids may have a delayed preconditioning effect since infarct volume was reduced following MCAO in rats treated with morphine 24 h prior to the ischemic event (Lehmann, 1997). The opioid fentanyl improves blood flow in ischemic regions compared to controls (Chi et al., 2010). Instead, naloxone, an opioid receptor antagonist, abolish acute preconditioning.

6.7 Lipopolysaccharide

LPS is a potent endotoxin that plays a central role in the development of gram-negative sepsis (Davies and Cohen, 2011). A lot of experiments demonstrate that LPS conditioning in fact decreases subsequent brain injury in animal models of ischemic stroke (Ahmed et al. 2000). LPS seems to play a role in improving blood flow following infarction, with one study showing improved blood flow in the preinfarct area minutes and days after occlusion (Furuya et al. 2005).

6.8 Miscellaneous

Other pharmacological treatments have been proved to induce brain conditioning. Indeed, in the rat global ischemia model induced by four-vessel occlusion, pharmacological postconditioning obtained by injection of 3-nitropropionic acid (3-NP), norepinephrine or bradykinin, is able to exert a remarkable neuroprotection (Zheng et al., 2003; Zhao et al., 2012). Another study reports that after global ischemia, intracerebroventricular kainate application is able to prevent hippocampal neuronal damage 2 days after ischemia induction (Birnbaum et al., 2007; Liem et al., 2002).

7. Conclusions

In conclusion, the last decades have provided fascinating insights into the mechanisms and potential application of neuroprotection of the ischemic brain.

However, in order to obtain consistent results and valid indications on the direction to follow for the development of a new effective treatment in the management of diseases such as cerebral ischemia, it is appropriate to choose the right experimental model. Until now, the tolerance field is populated by a myriad of animal models and putative protective compounds.

From what appeared in the scientific literature, there is a majority of data about the neuroprotective effect of preconditioning in comparison with postconditioning. The concept of postconditioning it is relatively newer but at the same time more promising because of its relative simplicity in a clinical setting.

Furthermore, the timing between induction of pre- and postconditioning and harmful stimulus of stroke is a matter of crucial interest and the correct execution of the experimental protocol is of fundamental importance for obtaining the best results and comparing the results of different laboratories. Likewise, in case of other stressors like pharmacological compounds, anesthetics, hypoxic conditions, PUFAs specific therapeutic protocols are required and what comes out is that chronic post-ischemic treatment correlates with a better outcome than the acute treatment. Since the identification of intrinsic cell-survival pathways should provide more direct opportunities for translational neuroprotection trials an accurate examination of the different models of preconditioning and postconditioning is mandatory before starting any new project.

9. Acknowledgment

This work was supported by the following grants: ConSLA from ARISLA, Programma Operativo Nazionale (PON_01602 and PON03PE_00146_1) from MIUR to L.A.; POR Campania FESR 2007-2013 FARMABIONET (B25C1300023007) to G.P.; POR Campania FESR 2007-2013 MOVIE (B25C1300024007) to L.A.

10. Conflict of interest

The authors declare no competing financial interests.

References

Abete, P., Ferrara, N., Cacciatore, F., Sagnelli, E., Manzi, M., Carnovale, V., ... & Napoli, C. (2001). High level of physical activity preserves the cardioprotective effect of preinfarction angina in elderly patients. Journal of the American College of Cardiology, 38(5), 1357-1365.

Abraini, J. H., David, H. N., & Lemaire, M. (2005). Potentially neuroprotective and therapeutic properties of nitrous oxide and xenon. Annals of the New York Academy of Sciences, 1053(1), 289-300.

Ahmed, S. H., He, Y. Y., Nassief, A., Xu, J., Xu, X. M., & Hsu, C. Y. (2000). Effects of lipopolysaccharide priming on acute ischemic brain injury. Stroke, 31(1), 193-199.

Akbar, M., Calderon, F., Wen, Z., & Kim, H. Y. (2005). Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. Proceedings of the National Academy of Sciences of the United States of America, 102(31), 10858-10863.

Alkayed, N. J., Goyagi, T., Joh, H. D., Klaus, J., Harder, D. R., Traystman, R. J., & Hurn, P. D. (2002). Neuroprotection and P450 2C11 upregulation after experimental transient ischemic attack. Stroke, 33(6), 1677-1684.

Allen, C. L., & Bayraktutan, U. (2008). Risk factors for ischaemic stroke. International journal of Stroke, 3(2), 105-116.

Ang, E. T., Wong, P. T. H., Moochhala, S., & Ng, Y. K. (2003). Neuroprotection associated with running: is it a result of increased endogenous neurotrophic factors?. Neuroscience, 118(2), 335-345.

Anzilotti, S., Brancaccio, P., Simeone, G., Valsecchi, V., Vinciguerra, A., Secondo, A., ... & Pignataro, G. (2018). Preconditioning, induced by sub-toxic dose of the neurotoxin L-BMAA, delays ALS progression in mice and prevents Na+/Ca 2+ exchanger 3 downregulation. Cell death & disease, 9(2), 206.

Ara, J., Fekete, S., Frank, M., Golden, J. A., Pleasure, D., & Valencia, I. (2011). Hypoxic-preconditioning induces neuroprotection against hypoxia–ischemia in newborn piglet brain. Neurobiology of disease, 43(2), 473-485.

Atochin, D. N., Clark, J., Demchenko, I. T., Moskowitz, M. A., & Huang, P. L. (2003). Rapid cerebral ischemic preconditioning in mice deficient in endothelial and neuronal nitric oxide synthases. Stroke, 34(5), 1299-1303.

Bantel, C., Maze, M., & Trapp, S. (2009). Neuronal Preconditioning by Inhalational AnestheticsEvidence for the Role of Plasmalemmal Adenosine Triphosphate–sensitive Potassium Channels. Anesthesiology: The Journal of the American Society of Anesthesiologists, 110(5), 986-995.

Barone, F. C., Feuerstein, G. Z., & White, R. F. (1997). Brain cooling during transient focal ischemia provides complete neuroprotection. Neuroscience & Biobehavioral Reviews, 21(1), 31-44.

Barone, F. C., White, R. F., Spera, P. A., Ellison, J., Currie, R. W., Wang, X., & Feuerstein, G. Z. (1998). Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. Stroke, 29(9), 1937-1951.

Barone, F. C., White, R. F., Spera, P. A., Ellison, J., Currie, R. W., Wang, X., & Feuerstein, G. Z. (1998). Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. Stroke, 29(9), 1937-1951.

Bas, O., Songur, A., Sahin, O., Mollaoglu, H., Ozen, O. A., Yaman, M., ... & Yagmurca, M. (2007). The protective effect of fish n-3 fatty acids on cerebral ischemia in rat hippocampus. Neurochemistry international, 50(3), 548-554.

Belayev, L., Ginsberg, M. D., Alonso, O. F., Singer, J. T., Zhao, W., & Busto, R. (1996). Bilateral ischemic tolerance of rat hippocampus induced by prior unilateral transient focal ischemia: relationship to c-fos mRNA expression. Neuroreport, 8(1), 55-59.

Belayev, L., Marcheselli, V. L., Khoutorova, L., De Turco, E. B. R., Busto, R., Ginsberg, M. D., & Bazan, N. G. (2005). Docosahexaenoic acid complexed to albumin elicits high-grade ischemic neuroprotection. Stroke, 36(1), 118-123.

Berressem, D., Koch, K., Franke, N., Klein, J., & Eckert, G. P. (2016). Intravenous treatment with a long-chain Omega-3 lipid emulsion provides neuroprotection in a murine model of ischemic stroke–A pilot study. PloS one, 11(11), e0167329.

Birnbaum Y, Hale SL, Kloner RA (1997). Ischemic preconditioning at a distance: reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit. Circulation;96:1641–6.

Black, K. L., Culp, B., Madison, D., Randall, O. S., & Lands, W. E. (1979). The protective effects of dietary fish oil on focal cerebral infarction. Prostaglandins and medicine, 3(5), 257-268.

Black, K. L., Hsu, S., Radin, N. S., & Hoff, J. T. (1984). Effect of intravenous eicosapentaenoic acid on cerebral blood flow, edema and brain prostaglandins in ischemic gerbils. Prostaglandins, 28(4), 545-556.

.

Blondeau, N., Lipsky, R. H., Bourourou, M., Duncan, M. W., Gorelick, P. B., & Marini, A. M. (2015). Alpha-linolenic acid: an omega-3 fatty acid with neuroprotective properties—Ready for use in the stroke clinic?. BioMed research international, 2015.

Blondeau, N., Nguemeni, C., Debruyne, D. N., Piens, M., Wu, X., Pan, H., ... & Marini, A. M. (2009). Subchronic alpha-linolenic acid treatment enhances brain plasticity and exerts an antidepressant effect: a versatile potential therapy for stroke. Neuropsychopharmacology, 34(12), 2548.

Blondeau, N., Pétrault, O., Manta, S., Giordanengo, V., Gounon, P., Bordet, R., ... & Heurteaux, C. (2007). Polyunsaturated fatty acids are cerebral vasodilators via the TREK-1 potassium channel. Circulation research, 101(2), 176-184.

Brambrink, A. M., Koerner, I. P., Diehl, K., Strobel, G., Noppens, R., & Kempski, O. (2006). The antibiotic erythromycin induces tolerance against transient global cerebral ischemia in rats (pharmacologic preconditioning). Anesthesiology: The Journal of the American Society of Anesthesiologists, 104(6), 1208-1215.

Cao, D. H., Xu, J. F., Xue, R. H., Zheng, W. F., & Liu, Z. L. (2004). Protective effect of chronic ethyl docosahexaenoate administration on brain injury in ischemic gerbils. Pharmacology Biochemistry and Behavior, 79(4), 651-659.

Cao, D., Li, M., Xue, R., Zheng, W., Liu, Z., & Wang, X. (2005). Chronic administration of ethyl docosahexaenoate decreases mortality and cerebral edema in ischemic gerbils. Life sciences, 78(1), 74-81.

Cao, D., Yang, B., Hou, L., Xu, J., Xue, R., Sun, L., ... & Liu, Z. (2007). Chronic daily administration of ethyl docosahexaenoate protects against gerbil brain ischemic damage through reduction of arachidonic acid liberation and accumulation. The Journal of nutritional biochemistry, 18(5), 297-304.

Cao, D., Yang, B., Hou, L., Xu, J., Xue, R., Sun, L., ... & Liu, Z. (2007). Chronic daily administration of ethyl docosahexaenoate protects against gerbil brain ischemic damage through reduction of arachidonic acid liberation and accumulation. The Journal of nutritional biochemistry, 18(5), 297-304.

Cao, D., Zhou, C., Sun, L., Xue, R., Xu, J., & Liu, Z. (2006). Chronic administration of ethyl docosahexaenoate reduces gerbil brain eicosanoid productions following ischemia and reperfusion. The Journal of nutritional biochemistry, 17(4), 234-241.

Cerullo, P., Brancaccio, P., Anzilotti, S., Vinciguerra, A., Cuomo, O., Fiorino, F., ... & Pignataro, G. (2018). Acute and long-term NCX activation reduces brain injury and restores behavioral functions in mice subjected to neonatal brain ischemia. Neuropharmacology, 135, 180-191.

Chen, J., Graham, S. H., Zhu, R. L., & Simon, R. P. (1996). Stress proteins and tolerance to focal cerebral ischemia. Journal of Cerebral Blood Flow & Metabolism, 16(4), 566-577.

Chi, O. Z., Hunter, C., Liu, X., Chokshi, S. K., & Weiss, H. R. (2010). Effects of fentanyl pretreatment on regional cerebral blood flow in focal cerebral ischemia in rats. Pharmacology, 85(3), 153-157.

Choi-Kwon, S., Park, K. A., Lee, H. J., Park, M. S., Lee, J. H., Jeon, S. E., ... & Park, K. C. (2004). Temporal changes in cerebral antioxidant enzyme activities after ischemia and reperfusion in a rat focal brain ischemia model: effect of dietary fish oil. Developmental brain research, 152(1), 11-18.

Choi-Kwon, S., Park, K. A., Lee, H. J., Park, M. S., Lee, J. H., Jeon, S. E., ... & Park, K. C. (2004). Temporal changes in cerebral antioxidant enzyme activities after ischemia and reperfusion in a rat focal brain ischemia model: effect of dietary fish oil. Developmental brain research, 152(1), 11-18.

Chopp, M., Chen, H., Ho, K. L., Dereski, M. O., Brown, E., Hetzel, F. W., & Welch, K. M. A. (1989). Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. Neurology, 39(10), 1396-1396.

Colbourne, F., Sutherland, G., & Corbett, D. (1997). Postischemic hypothermia. Molecular neurobiology, 14(3), 171-201.

Corbett, D., & Thornhill, J. (2000). Temperature modulation (hypothermic and hyperthermic conditions) and its influence on histological and behavioral outcomes following cerebral ischemia. Brain pathology, 10(1), 145-152.

Cotman, C. W., Berchtold, N. C., & Christie, L. A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends in neurosciences, 30(9), 464-472.

Coughlin, S. R. (2000). Thrombin signalling and protease-activated receptors. Nature, 407(6801), 258.

Cuomo, O., Pignataro, G., Gala, R., Scorziello, A., Gravino, E., Piazza, O., ... & Annunziato, L. (2007). Antithrombin reduces ischemic volume, ameliorates neurologic deficits, and prolongs animal survival in both transient and permanent focal ischemia. Stroke, 38(12), 3272-3279.

Cuomo O, Pignataro G, Sirabella R, Molinaro P, Anzilotti S, Scorziello A, Sisalli MJ1, Di Renzo G, Annunziato L. Sumoylation of LYS590 of NCX3 f-Loop by SUMO1 Participates in Brain Neuroprotection Induced by Ischemic Preconditioning. Stroke. 2016;47(4):1085-93.

Curry, A., Guo, M., Patel, R., Liebelt, B., Sprague, S., Lai, Q., ... & Ding, Y. (2010). Exercise pre-conditioning reduces brain inflammation in stroke via tumor necrosis factor-α, extracellular signal-regulated kinase 1/2 and matrix metalloproteinase-9 activity. Neurological research, 32(7), 756-762.

David, H. N., Leveille, F., Chazalviel, L., MacKenzie, E. T., Buisson, A., Lemaire, M., & Abraini, J. H. (2003). Reduction of ischemic brain damage by nitrous oxide and xenon. Journal of Cerebral Blood Flow & Metabolism, 23(10), 1168-1173.

Davies, B., & Cohen, J. (2011). Endotoxin removal devices for the treatment of sepsis and septic shock. The Lancet infectious diseases, 11(1), 65-71.

Davis, W., Mahale, S., Carranza, A., Cox, B., Hayes, K., Jimenez, D., & Ding, Y. (2007). Exercise pre-conditioning ameliorates blood–brain barrier dysfunction in stroke by enhancing basal lamina. Neurological research, 29(4), 382-387.

Dawson, T. M. (2002). Preconditioning-mediated neuroprotection through erythropoietin?. Lancet (London, England), 359(9301), 96.

Ding, Y. H., Luan, X. D., Li, J., Rafols, J. A., Guthinkonda, M., Diaz, F. G., & Ding, Y. (2004). Exercise-induced overexpression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke. Current neurovascular research, 1(5), 411-420.

Ding, Y., Li, J., Clark, J., Diaz, F. G., & Rafols, J. (2003). Synaptic plasticity in thalamic nuclei enhanced by motor skill training in rat with transient middle cerebral artery occlusion. Neurological Research, 25(2), 189-194.

Ding, Y., Li, J., Luan, X., Ding, Y. H., Lai, Q., Rafols, J. A., ... & Diaz, F. G. (2004). Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. Neuroscience, 124(3), 583-591.

Do Lee, C., Folsom, A. R., & Blair, S. N. (2003). Physical activity and stroke risk: a meta-analysis. Stroke, 34(10), 2475-2481.

Dong, H., Xiong, L., Zhu, Z., Chen, S., Hou, L., & Sakabe, T. (2002). Preconditioning with hyperbaric oxygen and hyperoxia induces tolerance against spinal cord ischemia in rabbits. Anesthesiology: The Journal of the American Society of Anesthesiologists, 96(4), 907-912.

Du, F., Zhu, L., Qian, Z. M., Wu, X. M., Yung, W. H., & Ke, Y. (2010). Hyperthermic preconditioning protects astrocytes from ischemia/reperfusion injury by up-regulation of HIF-1 alpha expression and binding activity. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1802(11), 1048-1053.

Eckert, G. P., Lipka, U., & Muller, W. E. (2013). Omega-3 fatty acids in neurodegenerative diseases: focus on mitochondria. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 88(1), 105-114.

Eid, T., & Brines, M. (2002). Recombinant human erythropoietin for neuroprotection: what is the evidence?. Clinical breast cancer, 3, S109-S115.

Evenson, K. R., Rosamond, W. D., Cai, J., Toole, J. F., Hutchinson, R. G., Shahar, E., & Folsom, A. R. (1999). Physical activity and ischemic stroke risk: the Atherosclerosis Risk in Communities Study. Stroke, 30(7), 1333-1339.

Faraco, G., Pittelli, M., Cavone, L., Fossati, S., Porcu, M., Mascagni, P., ... & Chiarugi, A. (2009). Histone deacetylase (HDAC) inhibitors reduce the glial inflammatory response in vitro and in vivo. Neurobiology of disease, 36(2), 269-279.

Freiberger, J. J., Suliman, H. B., Sheng, H., McAdoo, J., Piantadosi, C. A., & Warner, D. S. (2006). A comparison of hyperbaric oxygen versus hypoxic cerebral preconditioning in neonatal rats. Brain research, 1075(1), 213-222.

Furuya, K., Zhu, L., Kawahara, N., Abe, O., & Kirino, T. (2005). Differences in infarct evolution between lipopolysaccharide-induced tolerant and nontolerant conditions to focal cerebral ischemia. Journal of neurosurgery, 103(4), 715-723.

Gamdzyk, M., Małek, M., Bratek, E., Koks, A., Kaminski, K., Ziembowicz, A., & Salinska, E. (2016). Hyperbaric oxygen and hyperbaric air preconditioning induces ischemic tolerance to transient forebrain ischemia in the gerbil. Brain research, 1648, 257-265.

Gidday, J. M. (2006). Cerebral preconditioning and ischaemic tolerance. Nature Reviews Neuroscience, 7(6), 437.

Gidday, J. M. (2010). Pharmacologic preconditioning: translating the promise. Translational stroke research, 1(1), 19-30.

Gillum, R. F., Mussolino, M. E., & Ingram, D. D. (1996). Physical activity and stroke incidence in women and men: the NHANES I Epidemiologic Follow-up Study. American journal of epidemiology, 143(9), 860-869.

Glantz, L., Avramovich, A., Trembovler, V., Gurvitz, V., Kohen, R., Eidelman, L. A., & Shohami, E. (2005). Ischemic preconditioning increases antioxidants in the brain and peripheral organs after cerebral ischemia. Experimental neurology, 192(1), 117-124.

Glazier, S. S., O'Rourke, D. M., Graham, D. I., & Welsh, F. A. (1994). Induction of ischemic tolerance following brief focal ischemia in rat brain. Journal of Cerebral Blood Flow & Metabolism, 14(4), 545-553.

Haelewyn, B., Yvon, A., Hanouz, J. L., MacKenzie, E. T., Ducouret, P., Gerard, J. L., & Roussel, S. (2003). Desflurane affords greater protection than halothane against focal cerebral ischaemia in the rat. British journal of anaesthesia, 91(3), 390-396.

Hao, Y., Luo, Z., Zhou, D., & Gao, L. (2003). Cerebral ischemic tolerance induced by focal ischemic preconditioning in rats. Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical science edition, 34(3), 455-458.

Hasselblatt, M., Ehrenreich, H., & Sirén, A. L. (2006). The brain erythropoietin system and its potential for therapeutic exploitation in brain disease. Journal of neurosurgical anesthesiology, 18(2), 132-138.

Heurteaux, C., Laigle, C., Blondeau, N., Jarretou, G., & Lazdunski, M. (2006). Alpha-linolenic acid and riluzole treatment confer cerebral protection and improve survival after focal brain ischemia. Neuroscience, 137(1), 241-251.

Hoyte, L. C., Papadakis, M., Barber, P. A., & Buchan, A. M. (2006). Improved regional cerebral blood flow is important for the protection seen in a mouse model of late phase ischemic preconditioning. Brain research, 1121(1), 231-237.

Hu, G., Barengo, N. C., Tuomilehto, J., Lakka, T. A., Nissinen, A., & Jousilahti, P. (2004). Relationship of physical activity and body mass index to the risk of hypertension: a prospective study in Finland. Hypertension, 43(1), 25-30.

Hua, Y., Nakamura, T., Keep, R. F., Wu, J., Schallert, T., Hoff, J. T., & Xi, G. (2006). Long-term effects of experimental intracerebral hemorrhage: the role of iron. Journal of neurosurgery, 104(2), 305-312.

Inamasu, J., Suga, S., Sato, S., Horiguchi, T., Akaji, K., Mayanagi, K., & Kawase, T. (2000). Post-ischemic hypothermia delayed neutrophil accumulation and microglial activation following transient focal ischemia in rats. Journal of neuroimmunology, 109(2), 66-74.

Jhansen, F. F., Jørgensen, H. S., & Reith, J. (2003). Prolonged drug-induced hypothermia in experimental stroke. Journal of Stroke and Cerebrovascular Diseases, 12(2), 97-102.

Jiang, X., Shi, E., Nakajima, Y., & Sato, S. (2006). Postconditioning, a series of brief interruptions of early reperfusion, prevents neurologic injury after spinal cord ischemia. Annals of surgery, 244(1), 148.

Jin, Z., Wu, J., & Yan, L. J. (2016). Chemical conditioning as an approach to ischemic stroke tolerance: mitochondria as the target. International journal of molecular sciences, 17(3), 351.

Johansen, F. F., Hasseldam, H., Rasmussen, R. S., Bisgaard, A. S., Bonfils, P. K., Poulsen, S. S., & Hansen-Schwartz, J. (2014). Drug-induced hypothermia as beneficial treatment before and after cerebral ischemia. Pathobiology, 81(1), 42-52.

Johansen, F. F., Hasseldam, H., Rasmussen, R. S., Bisgaard, A. S., Bonfils, P. K., Poulsen, S. S., & Hansen-Schwartz, J. (2014). Drug-induced hypothermia as beneficial treatment before and after cerebral ischemia. Pathobiology, 81(1), 42-52.

Jones, T. A., Chu, C. J., Grande, L. A., & Gregory, A. D. (1999). Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. Journal of Neuroscience, 19(22), 10153-10163.

Jump, D. B., Depner, C. M., & Tripathy, S. (2012). Omega-3 fatty acid supplementation and cardiovascular disease Thematic Review Series: New lipid and lipoprotein targets for the treatment of cardiometabolic diseases. Journal of lipid research, 53(12), 2525-2545.

Kammersgaard, L. P., Rasmussen, B. H., Jørgensen, H. S., Reith, J., Weber, U., & Olsen, T. S. (2000). Feasibility and safety of inducing modest hypothermia in awake patients with acute stroke through surface cooling: a case-control study: the Copenhagen Stroke Study. Stroke, 31(9), 2251-2256.

Katsumata, T., Katayama, Y., Obo, R., Muramatsu, H., Ohtori, T., & Terashi, A. (1999). Delayed administration of ethyl eicosapentate improves local cerebral blood flow and metabolism without affecting infarct volumes in the rat focal ischemic model. European journal of pharmacology, 372(2), 167-174.

Kelty, J. D., Noseworthy, P. A., Feder, M. E., Robertson, R. M., & Ramirez, J. M. (2002). Thermal preconditioning and heat-shock protein 72 preserve synaptic transmission during thermal stress. Journal of Neuroscience, 22(1), RC193-RC193.

Kim, H. C., Kim, E., Bae, J. I., Lee, K. H., Jeon, Y. T., Hwang, J. W., ... & Park, H. P. (2017). Sevoflurane postconditioning reduces apoptosis by activating the JAK-STAT pathway after transient global cerebral ischemia in rats. Journal of neurosurgical anesthesiology, 29(1), 37-45.

Kirino, T. (2002). Ischemic tolerance. Journal of Cerebral Blood Flow & Metabolism, 22(11), 1283-1296.

Kitagawa, K., Matsumoto, M., Tagaya, M., Hata, R., Ueda, H., Niinobe, M., ... & Kamada, T. (1990). 'Ischemic tolerance'phenomenon found in the brain. Brain research, 528(1), 21-24.

Kitagawa, K., Matsumoto, M., Tagaya, M., Hata, R., Ueda, H., Niinobe, M., ... & Kamada, T. (1990). 'Ischemic tolerance'phenomenon found in the brain. Brain research, 528(1), 21-24.

Kitano, H., Kirsch, J. R., Hurn, P. D., & Murphy, S. J. (2007). Inhalational anesthetics as neuroprotectants or chemical preconditioning agents in ischemic brain. Journal of Cerebral Blood Flow & Metabolism, 27(6), 1108-1128.

Kloner, R. A. (2001). Preinfarct angina and exercise: yet another reason to stay physically active.

Kollmar, R., Schäbitz, W. R., Heiland, S., Georgiadis, D., Schellinger, P. D., Bardutzky, J., & Schwab, S. (2002). Neuroprotective effect of delayed moderate hypothermia after focal cerebral ischemia: an MRI study. Stroke, 33(7), 1899-1904.

Kurth, C. D., Priestley, M., Watzman, H. M., McCann, J., & Golden, J. (2001). Desflurane confers neurologic protection for deep hypothermic circulatory arrest in newborn pigs. Anesthesiology: The Journal of the American Society of Anesthesiologists, 95(4), 959-964.

Larson, E. B., Wang, L., Bowen, J. D., McCormick, W. C., Teri, L., Crane, P., & Kukull, W. (2006). Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Annals of internal medicine, 144(2), 73-81.

Lee, H. T., Chang, Y. C., Wang, L. Y., Wang, S. T., Huang, C. C., & Ho, C. J. (2004). cAMP response element-binding protein activation in ligation preconditioning in neonatal brain. Annals of neurology, 56(5), 611-623.

Lee, S. H., Kim, Y. J., Lee, K. M., Ryu, S., & Yoon, B. W. (2007). Ischemic preconditioning enhances neurogenesis in the subventricular zone. Neuroscience, 146(3), 1020-1031.

Lehmann, K. A. (1997). Opioids: overview on action, interaction and toxicity. Supportive care in cancer, 5(6), 439-444.

Li, G., Jia, J., Fu, J., Wang, H., Ji, K., & Zang, B. (2014). The effects of preconditioning and postconditioning with isoflurane on focal cerebral ischemi/reperfusion injury in rats. Zhonghua wei zhong bing ji jiu yi xue, 26(6), 431-435.

Li, J., Luan, X., Clark, J. C., Rafols, J. A., & Ding, Y. (2004). Neuroprotection against transient cerebral ischemia by exercise pre-conditioning in rats. Neurological research, 26(4), 404-408.

Li, L., Deng, J., & Zuo, Z. (2013). Glutamate transporter type 3 mediates isoflurane preconditioning-induced acute phase of neuroprotection in mice. Brain research bulletin, 98, 23-29.

Li, Z., Liu, W., Kang, Z., Lv, S., Han, C., Yun, L., ... & Zhang, J. H. (2008). Mechanism of hyperbaric oxygen preconditioning in neonatal hypoxia–ischemia rat model. Brain research, 1196, 151-156.

Liebelt, B., Papapetrou, P., Ali, A., Guo, M., Ji, X., Peng, C., ... & Ding, Y. (2010). Exercise preconditioning reduces neuronal apoptosis in stroke by up-regulating heat

shock protein-70 (heat shock protein-72) and extracellular-signal-regulated-kinase 1/2. Neuroscience, 166(4), 1091-1100.

Liem DA, Verdouw PD, Ploeg H, Kazim S, Duncker DJ (2002). Sites of action of adenosine in interorgan preconditioning of the heart. Am J Physiol Heart Circ Physiol.;283:H29–37.

Limatola, V., Ward, P., Cattano, D., Gu, J., Giunta, F., Maze, M., & Ma, D. (2010). Xenon preconditioning confers neuroprotection regardless of gender in a mouse model of transient middle cerebral artery occlusion. Neuroscience, 165(3), 874-881.

Lin, D., Li, G., & Zuo, Z. (2011). Volatile anesthetic post-treatment induces protection via inhibition of glycogen synthase kinase 3β in human neuron-like cells. Neuroscience, 179, 73-79.

Luan, X., Li, J., McAllister, J. P., Diaz, F. G., Clark, J. C., Fessler, R. D., & Ding, Y. (2004). Regional brain cooling induced by vascular saline infusion into ischemic territory reduces brain inflammation in stroke. Acta neuropathologica, 107(3), 227-234.

Luo, Y., Ma, D., Ieong, E., Sanders, R. D., Yu, B., Hossain, M., & Maze, M. (2008). Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology: The Journal of the American Society of Anesthesiologists, 109(5), 782-789.

Lusardi, T. A., Farr, C. D., Faulkner, C. L., Pignataro, G., Yang, T., Lan, J., ... & Saugstad, J. A. (2010). Ischemic preconditioning regulates expression of microRNAs and a predicted target, MeCP2, in mouse cortex. Journal of Cerebral Blood Flow & Metabolism, 30(4), 744-756.

Marcheselli, V. L., Hong, S., Lukiw, W. J., Tian, X. H., Gronert, K., Musto, A., ... & Bazan, N. G. (2003). Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. Journal of Biological Chemistry, 278(44), 43807-43817.

Marti, H. H., Wenger, R. H., Rivas, L. A., Straumann, U., Oigicaylioglu, M., Henn, V., ... & Gassmann, M. (1996). Erythropoietin gene expression in human, monkey and murine brain. European Journal of Neuroscience, 8(4), 666-676.

Martin, A. (2001). The" apports nutritionnels conseilles (ANC)" for the French population. Reproduction Nutrition Development, 41(2), 119-128.

McAuliffe, J. J., Joseph, B., & Vorhees, C. V. (2007). Isoflurane-delayed preconditioning reduces immediate mortality and improves striatal function in adult mice after neonatal hypoxia–ischemia. Anesthesia & Analgesia, 104(5), 1066-1077.

McBride, D. W., Klebe, D., Tang, J., & Zhang, J. H. (2015). Correcting for brain swelling's effects on infarct volume calculation after middle cerebral artery occlusion in rats. Translational stroke research, 6(4), 323-338.

McKendrick, M. W. (1979). Erythromycin revisited. Journal of Antimicrobial Chemotherapy, 5(5), 495-497.

MISHIMA, Y., HARADA, H., SUGIYAMA, K., MIYAGAWA, Y., UEHARA, N., & KANO, T. (2005). Induction of neuronal tolerance by electroconvulsive shock in rats subjected to forebrain ischemia. The Kurume medical journal, 52(4), 153-160.

Mitchell, H. M., White, D. M., Domowicz, M. S., & Kraig, R. P. (2011). Cold pre-conditioning neuroprotection depends on TNF-α and is enhanced by blockade of interleukin-11. Journal of neurochemistry, 117(2), 187-196.

Miyashita, K., Abe, H., Nakajima, T., Ishikawa, A., Nishiura, M., Sawada, T., & Naritomi, H. (1994). Induction of ischaemic tolerance in gerbil hippocampus by pretreatment with focal ischaemia. Neuroreport, 6(1), 46-48.

Miyazawa, T., Tamura, A., Fukui, S., & Hossmann, K. A. (2003). Effect of mild hypothermia on focal cerebral ischemia. Review of experimental studies. Neurological research, 25(5), 457-464.

Moreira, J. D., Knorr, L., Thomazi, A. P., Simão, F., Battú, C., Oses, J. P., ... & Perry, M. L. (2010). Dietary omega-3 fatty acids attenuate cellular damage after a hippocampal ischemic insult in adult rats. The Journal of nutritional biochemistry, 21(4), 351-356.

Moskowitz, M. A. (2010). Brain protection: maybe yes, maybe no. Stroke, 41(10 suppl 1), S85-S86.

Nakamura, T., Keep, R. F., Hua, Y., Schallert, T., Hoff, J. T., & Xi, G. (2004). Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. Journal of neurosurgery, 100(4), 672-678.

Naylor, M., Bowen, K. K., Sailor, K. A., Dempsey, R. J., & Vemuganti, R. (2005). Preconditioning-induced ischemic tolerance stimulates growth factor expression and neurogenesis in adult rat hippocampus. Neurochemistry international, 47(8), 565-572.

Nishio, S., CHEN, Z. F., Yunoki, M., Toyoda, T., Anzivino, M., & Lee, K. S. (1999). Hypothermia-Induced Ischemic Tolerance. Annals of the New York Academy of Sciences, 890(1), 26-41.

Nishio, S., Yunoki, M., Chen, Z. F., Anzivino, M. J., & Lee, K. S. (2000). Ischemic tolerance in the rat neocortex following hypothermic preconditioning. Journal of neurosurgery, 93(5), 845-851.

Ostrowski, R. P., Graupner, G., Titova, E., Zhang, J., Chiu, J., Dach, N., ... & Zhang, J. H. (2008). The hyperbaric oxygen preconditioning-induced brain protection is mediated by a reduction of early apoptosis after transient global cerebral ischemia. Neurobiology of disease, 29(1), 1-13.

Ozen, O. A., Cosar, M., Sahin, O., Fidan, H., Eser, O., Mollaoglu, H., ... & Songur, A. (2008). The protective effect of fish n-3 fatty acids on cerebral ischemia in rat prefrontal cortex. Neurological Sciences, 29(3), 147.

Pignataro, G., Meller, R., Inoue, K., Ordonez, A. N., Ashley, M. D., Zhigang, X., & Simon, R. P. (2008). In vivo and in vitro characterization of a novel neuroprotective strategy for stroke: ischemic postconditioning. Journal of Cerebral Blood Flow & Metabolism, 28(2), 232-241.

Pignataro, G., Meller, R., Inoue, K., Ordonez, A. N., Ashley, M. D., Zhigang, X., & Simon, R. P. (2008). In vivo and in vitro characterization of a novel neuroprotective strategy for stroke: ischemic postconditioning. Journal of Cerebral Blood Flow & Metabolism, 28(2), 232-241.

Pignataro, G., Scorziello, A., Di Renzo, G., & Annunziato, L. (2009). Post-ischemic brain damage: effect of ischemic preconditioning and postconditioning and identification of potential candidates for stroke therapy. The FEBS journal, 276(1), 46-57.

Plamondon, H., & Roberge, M. C. (2008). Dietary PUFA supplements reduce memory deficits but not CA1 ischemic injury in rats. Physiology & behavior, 95(3), 492-500.

Plamondon, H., Blondeau, N., Heurteaux, C., & Lazdunski, M. (1999). Mutually protective actions of kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: involvement of adenosine A1 receptors and KATP channels. Journal of Cerebral Blood Flow & Metabolism, 19(12), 1296-1308.

Polderman, K. H. (2009). Mechanisms of action, physiological effects, and complications of hypothermia. Critical care medicine, 37(7), S186-S202.

Puisieux, F., Deplanque, D., Bulckaen, H., Maboudou, P., Gelé, P., Lhermitte, M., ... & Bordet, R. (2004). Brain ischemic preconditioning is abolished by antioxidant drugs but does not up-regulate superoxide dismutase and glutathion peroxidase. Brain research, 1027(1-2), 30-37.

Pulsinelli, W. A., & Brierley, J. B. (1979). A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke, 10(3), 267-272.

Rabadi, M. H. (2007). Randomized clinical stroke rehabilitation trials in 2005. Neurochemical research, 32(4-5), 807-821.

Rehni, A. K., Singh, T. G., Bhateja, P., Singh, N., & Arora, S. (2010). Involvement of cyclic adenosine diphosphoribose receptor activation in ischemic preconditioning induced protection in mouse brain. Brain research, 1309, 75-82.

Schwab, S., Georgiadis, D., Berrouschot, J., Schellinger, P. D., Graffagnino, C., & Mayer, S. A. (2001). Feasibility and safety of moderate hypothermia after massive hemispheric infarction. Stroke, 32(9), 2033-2035.

Schwab, S., Schwarz, S., Aschoff, A., Keller, E., & Hacke, W. (1998). Moderate hypothermia and brain temperature in patients with severe middle cerebral artery infarction. In Intracranial Pressure and Neuromonitoring in Brain Injury (pp. 131-134). Springer, Vienna.

Selim, M. (2009). Deferoxamine mesylate: a new hope for intracerebral hemorrhage: from bench to clinical trials. Stroke, 40(3 suppl 1), S90-S91.

Seo, J. H., Park, H. P., Jeon, Y. T., Lim, Y. J., Nam, K., & Hwang, J. W. (2013). Combined treatment with celecoxib and sevoflurane after global cerebral ischaemia has no additive neuroprotective effects in rats. British journal of anaesthesia, 110(6), 988-995.

Simon, R. P., Niiro, M., & Gwinn, R. (1993). Prior ischemic stress protects against experimental stroke. Neuroscience letters, 163(2), 135-137.

Sirén, A. L., Knerlich, F., Poser, W., Gleiter, C. H., Brück, W., & Ehrenreich, H. (2001). Erythropoietin and erythropoietin receptor in human ischemic/hypoxic brain. Acta neuropathologica, 101(3), 271-276.

Staat, P., Rioufol, G., Piot, C., Cottin, Y., Cung, T. T., L'Huillier, I., ... & Ovize, M. (2005). Postconditioning the human heart. Circulation, 112(14), 2143-2148.

Stetler, R. A., Zhang, F., Liu, C., & Chen, J. (2008). Ischemic tolerance as an active and intrinsic neuroprotective mechanism. Handbook of clinical neurology, 92, 171-195.

Stranahan, A. M., Khalil, D., & Gould, E. (2007). Running induces widespread structural alterations in the hippocampus and entorhinal cortex. Hippocampus, 17(11), 1017-1022.

Strokin, M., Chechneva, O., Reymann, K. G., & Reiser, G. (2006). Neuroprotection of rat hippocampal slices exposed to oxygen–glucose deprivation by enrichment with docosahexaenoic acid and by inhibition of hydrolysis of docosahexaenoic acid-containing phospholipids by calcium independent phospholipase A2. Neuroscience, 140(2), 547-553.

Stummer, W., Weber, K., Tranmer, B., Baethmann, A., & Kempski, O. (1994). Reduced mortality and brain damage after locomotor activity in gerbil forebrain ischemia. Stroke, 25(9), 1862-1869.

Sun, M., Deng, B., Zhao, X., Gao, C., Yang, L., Zhao, H., ... & Sun, X. (2015). Isoflurane preconditioning provides neuroprotection against stroke by regulating the expression of the TLR4 signalling pathway to alleviate microglial activation. Scientific reports, 5, 11445.

Tauskela, J. S., & Blondeau, N. (2009). Prescription for stroke: should preconditioning be investigated as a drug?. Ischemic Tolerance of the Brain, Research Signpost, 85-135.

Tong, L., Wu, Z., Ran, M., Chen, Y., Yang, L., Zhang, H., ... & Xiong, L. (2015). The role of SUMO-conjugating enzyme Ubc9 in the neuroprotection of isoflurane preconditioning against ischemic neuronal injury. Molecular neurobiology, 51(3), 1221-1231.

Tsai, S. K., Lin, S. M., Hung, W. C., Mok, M. S., Chih, C. L., & Huang, S. S. (2004). The effect of desflurane on ameliorating cerebral infarction in rats subjected to focal cerebral ischemia-reperfusion injury. Life sciences, 74(20), 2541-2549.

Wang, F., Luo, Y., Ling, F., Wu, H., Chen, J., Yan, F., ... & Ding, Y. (2010). Comparison of neuroprotective effects in ischemic rats with different hypothermia procedures. Neurological research, 32(4), 378-383.

Wang, H., Lu, S., Yu, Q., Liang, W., Gao, H., Li, P., ... & Gao, Y. (2011). Sevoflurane preconditioning confers neuroprotection via anti-inflammatory effects. Frontiers in bioscience (Elite edition), 3, 604-615.

Wang, H., Shi, H., Yu, Q., Chen, J., Zhang, F., & Gao, Y. (2016). Sevoflurane preconditioning confers neuroprotection via anti-apoptosis effects. In Brain Edema XVI (pp. 55-61). Springer International Publishing.

Wang, J. Y., Shen, J., Gao, Q., Ye, Z. G., Yang, S. Y., Liang, H. W., ... & Xia, Q. (2008). Ischemic postconditioning protects against global cerebral ischemia/reperfusion-induced injury in rats. Stroke, 39(3), 983-990.

Wang, R. Y., Yang, Y. R., & Yu, S. M. (2001). Protective effects of treadmill training on infarction in rats. Brain research, 922(1), 140-143.

Wendel-Vos, G. C. W., Schuit, A. J., Feskens, E. J. M., Boshuizen, H. C., Verschuren, W. M. M., Saris, W. H. M., & Kromhout, D. (2004). Physical activity and stroke. A meta-analysis of observational data. International journal of epidemiology, 33(4), 787-798.

Winston, S. M., Hayward, M. D., Nestler, E. J., & Duman, R. S. (1990). Chronic Electroconvulsive Seizures Down–Regulate Expression of the Immediate-Early Genes c-fos and c-jun in Rat Cerebral Cortex. Journal of neurochemistry, 54(6), 1920-1925.

Xu, H., Aibiki, M., & Nagoya, J. (2002). Neuroprotective effects of hyperthermic preconditioning on infarcted volume after middle cerebral artery occlusion in rats: role of adenosine receptors. Critical care medicine, 30(5), 1126-1130.

Yuan, H. B., Huang, Y., Zheng, S., & Zuo, Z. (2004). Hypothermic preconditioning increases survival of purkinje neurons in rat cerebellar slices after an in vitro simulated ischemia. Anesthesiology: The Journal of the American Society of Anesthesiologists, 100(2), 331-337.

Yunoki, M., Nishio, S., Ukita, N., Anzivino, M. J., & Lee, K. S. (2002). Characteristics of hypothermic preconditioning influencing the induction of delayed ischemic tolerance. Journal of neurosurgery, 97(3), 650-657.

Yunoki, M., Nishio, S., Ukita, N., Anzivino, M. J., & Lee, K. S. (2003). Hypothermic preconditioning induces rapid tolerance to focal ischemic injury in the rat. Experimental neurology, 181(2), 291-300.

Zausinger, S., Baethmann, A., & Schmid-Elsaesser, R. (2002). Anesthetic methods in rats determine outcome after experimental focal cerebral ischemia: mechanical ventilation is required to obtain controlled experimental conditions. Brain research protocols, 9(2), 112-121.

Zhang, J., Yang, Z. J., Klaus, J. A., Koehler, R. C., & Huang, J. (2008). Delayed tolerance with repetitive transient focal ischemic preconditioning in the mouse. Stroke, 39(3), 967-974.

Zhang, M., Wang, S., Mao, L., Leak, R. K., Shi, Y., Zhang, W., ... & Xu, Y. (2014). Omega-3 fatty acids protect the brain against ischemic injury by activating Nrf2 and upregulating heme oxygenase 1. Journal of Neuroscience, 34(5), 1903-1915.

Zhang, N., Yin, Y., Han, S., Jiang, J., Yang, W., Bu, X., & Li, J. (2011). Hypoxic preconditioning induced neuroprotection against cerebral ischemic injuries and its cPKCγ-mediated molecular mechanism. Neurochemistry international, 58(6), 684-692.

Zhang, P., Abraham, V. S., Kraft, K. R., Rabchevsky, A. G., Scheff, S. W., & Swain, J. A. (2000). Hyperthermic preconditioning protects against spinal cord ischemic injury. The Annals of thoracic surgery, 70(5), 1490-1495.

Zhao, H., Sapolsky, R. M., & Steinberg, G. K. (2006). Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats. Journal of Cerebral Blood Flow & Metabolism, 26(9), 1114-1121.

Zhao H, Ren C, Chen X, Shen G (2012). From Rapid to Delayed and Remote Postconditioning: the Evolving Concept of Ischemic Postconditioning in Brain Ischemia. Curr Drug Targets. 2012 Feb; 13(2): 173–187.

Zheng, S., & Zuo, Z. (2004). Isoflurane preconditioning induces neuroprotection against ischemia via activation of P38 mitogen-activated protein kinases. Molecular pharmacology, 65(5), 1172-1180.

Zheng S, Zuo Z. (2003) Isoflurane preconditioning reduces purkinje cell death in an in vitro model of rat cerebellar ischemia. Neuroscience;118:99–106.

Zhu, W., Wang, L., Zhang, L., Palmateer, J. M., Libal, N. L., Hurn, P. D., ... & Murphy, S. J. (2010). Isoflurane preconditioning neuroprotection in experimental focal stroke is androgen-dependent in male mice. Neuroscience, 169(2), 758-769.

Zhu P, Zhan L, Zhu T, Liang D, Hu J, Sun W et al (2014) The roles of p38 MAPK/MSK1 signaling pathway in the neuroprotection of hypoxic postconditioning against transient global cerebral ischemia in adult rats. Mol Neurobiol 49:1338–1349.

Zhu T, Zhan L, Liang D, Hu J, Lu Z, Zhu X et al (2014) Hypoxiainducible factor 1a mediates neuroprotection of hypoxic postconditioning against global cerebral ischemia. J Neuropathol Exp Neurol 73:975–986.

LEGENDS OF FIGURES

Figure 1: Schematic representation of whole body conditioning stimuli.

Figure 2: Schematic representation of conditioning induced by diet (2a) or by gas anaesthetics (2b)

Figure 3: Schematic representation of pharmacological conditioning stimuli

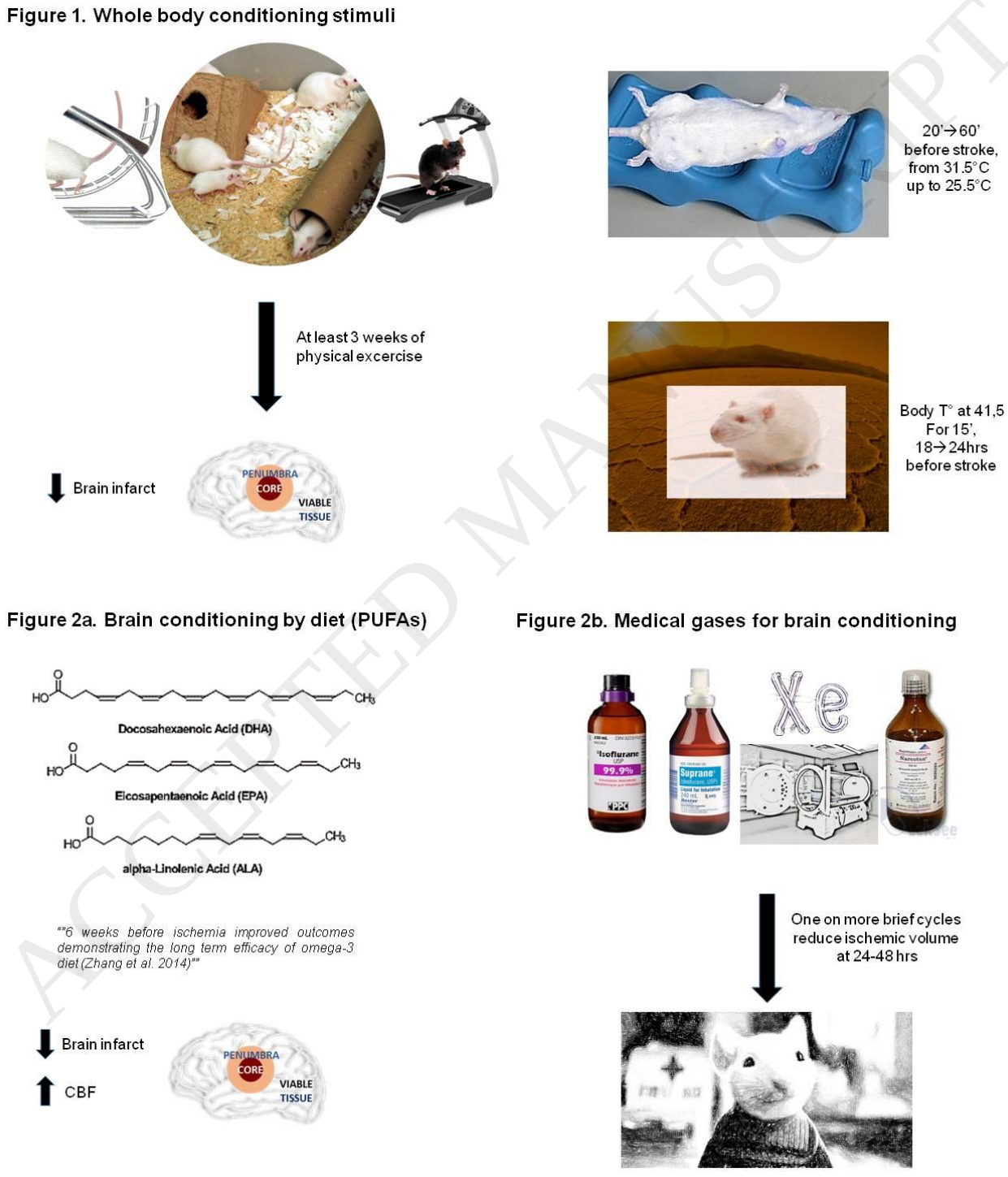


Figure 3. Pharmacological conditioning

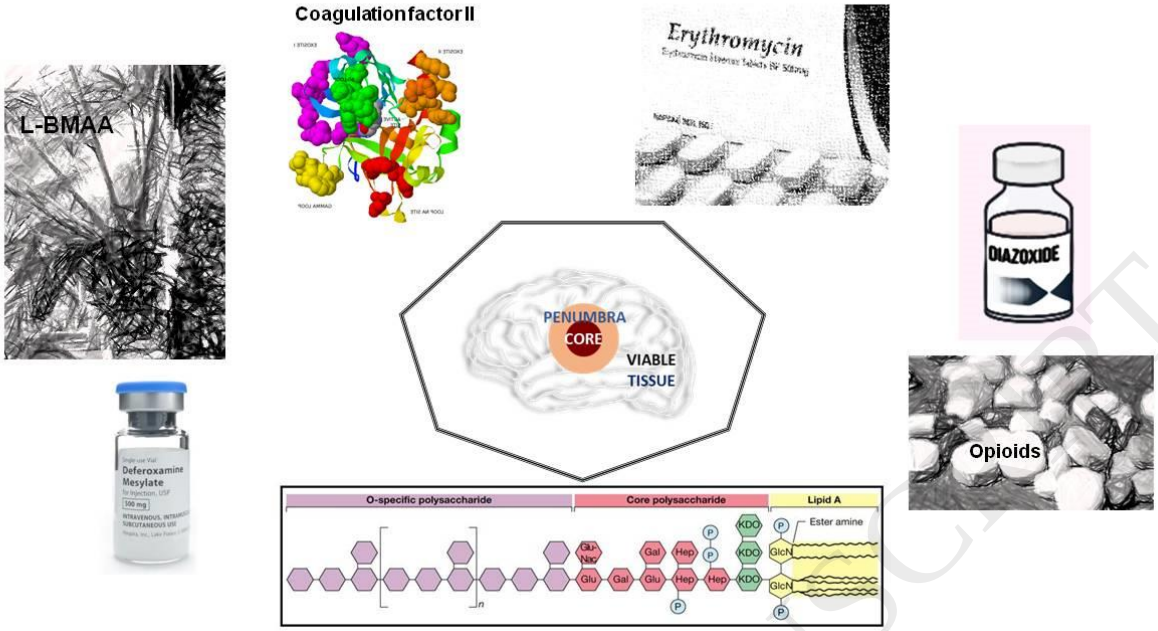


Table 1: Surgical Methods To Induce Ischemic Preconditioning, PreC. MCAO, Middle Cerebral Artery Occlusion; pMCAO, Permanent Middle Cerebral Artery Occlusion; BCCAO, Bilateral Common Carotid Artery Occlusion.

Kind of PreC/Ischemia	*Animal Model*	*PreC stimulus*	*Reperfusion Time from PreC*	*Harmful Ischemia*	*References*
Focal/Focal	Rat	10 minutes MCAO	1 to 7 days	pMCAO	Barone et al., 1998
	Spontaneously hypertensive rat	10 minutes MCAO	72 hours	60 minutes MCAO	Naylor et al., 2005
	Rat	10 minutes MCAO	72 hours	120 minutes MCAO	Hao et al., 2003
	Rat	Three cycles of 10-minute MCAO	72 hours	120 minutes MCAO	Alkayed et al., 2002
	Rat	2 minutes MCAO	24 hours	90 minutes MCAO	Glantz et al., 2005
	Mouse	15 minutes MCAO	72 hours	45 minutes MCAO	Hoyte et al., 2006
	Mouse	Two cycles of 5-minute MCAO	72 hours	90 minutes MCAO	Zhang et al., 2008
	Rat	30 minutes MCAO	72 hours	100 minutes MCAO	Lusardi et al., 2011; Pignataro et al., 2012
	Mouse	15 minutes MCAO	72 hours	60 minutes MCAO	Lusardi et al., 2011
	Mouse	Three cycles of 5-minute MCAO	30 minutes	60 minutes MCAO	Atochin et al., 2003
Focal/Global	Rat	20 minutes MCAO	24 hours	10 minutes 2-VO	Glazier et al., 1994
	Gerbil	Transient unilateral MCAO	72 hours	5 min BCCAO	Miyashita et al., 1994
Global/Focal	Mouse	5 minutes BCCAO	24 hours	20 minutes MCAO	Faraco et al., 2009
Global/Global	Gerbil	Two cycles of 2-minute BCCAO	24 or 48 hours	5 minutes BCCAO	Kitagawa et al., 1990
	Mouse	Three cycles of 1-minute BCCAO	Null or 24h	17 minutes BCCAO	Rehni et al., 2010

Other surgical methods	P7 rat pup	Unilateral common carotid artery ligation	6 or 24 hours	2 hours 8% oxygen hypoxia	Lee et al., 2004
	Rat	Electroconvulsive shock	48 hours	8 minutes BCCAO	Mishima et al., 2005

Table 2: Surgical Methods To Induce Ischemic Postconditioning, PostC. MCAO, Middle Cerebral Artery Occlusion; 4-VO,4 Vessel Occlusion.

Kind of Ischemia/PostC	*Animal Model*	*Harmful Ischemia*	*Reperfusion Time from Harmful Ischemia*	*PostC stimulus*	*References*
Focal/Focal	Rat	100 minutes MCAO	10 minutes reperfusion	10 minutes MCAO	Pignataro et al., 2008
	Rat	100 minutes MCAO	Three cycles of 5-minute reperfusion	Three cycles of 5-minute MCAO	Pignataro et al., 2008
Global/Global	Rat	10 minutes 4-VO	15 seconds reperfusion	Three cycles of 15 second/15 second reperfusion/reocclusion	Wang et al., 2008
	Rat	10 minutes 4-VO	30 seconds reperfusion	Three cycles of 30 second/30 second reperfusion/reocclusion	Wang et al., 2008
	Rat	10 minutes 4-VO	60 seconds reperfusion	Three cycles of 60 second/15 second reperfusion/reocclusion	Wang et al., 2008
	Rat	10 minutes 4-VO	45 seconds reperfusion	Three cycles of 15 second/15 second reperfusion/reocclusion	Wang et al., 2008