

# UCSF

## UC San Francisco Previously Published Works

### Title

Positive and negative conditioning in the neonatal brain.

### Permalink

<https://escholarship.org/uc/item/1247663b>

### Journal

Conditioning Medicine, 1(6)

### Authors

Mallard, Carina  
Hagberg, Henrik  
Vexler, Zinaida

### Publication Date

2018-10-01

Peer reviewed



Published in final edited form as:

*Cond Med.* 2018 October ; 1(6): 279–293.

## Positive and negative conditioning in the neonatal brain

Zinaida S. Vexler<sup>1</sup>, Carina Mallard<sup>2</sup>, and Henrik Hagberg<sup>3</sup>

<sup>1</sup>Department of Neurology, University California San Francisco, San Francisco, California, USA.

<sup>2</sup>Center of Perinatal Medicine and Health, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

<sup>3</sup>Center of Perinatal Medicine and Health, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Sweden.

### Abstract

Brain injury in the perinatal period occurs in many clinical settings, e.g. hypoxic-ischemic encephalopathy (HIE) in term infants, neonatal stroke, encephalopathy of prematurity, and infections. These insults often result in life-long disabilities including cerebral palsy, cognitive deficits, visual dysfunction, hearing impairments, and epilepsy. However, the success of clinical implementation of a broad array of potential neuroprotective strategies tested experimentally has been limited with the exception of therapeutic hypothermia (TH) used within hours of birth in term human babies with mild to moderate HIE. There is an extensive search for adjuvant therapeutic approaches to enhance the outcomes. One strategy is to modify susceptibility in the developing CNS by means of preconditioning or postconditioning using sublethal stress. The pre-clinical and clinical literature has shown that CNS immaturity at the time of ischemic insult plays a central role in the response to injury. Thus, better understanding of the molecular regulation of the endogenous vulnerability of the immature brain is needed. Further, the use of sublethal stressors of different origin may help shed light on mechanistic similarities and distinctions between conditioning strategies. In this review we discuss the mechanisms of protection that are achieved by an interplay of changes on the systemic level and brain level, and via changes of intracellular and mitochondrial signaling. We also discuss the barriers to improving our understanding of how brain immaturity and the type of insult—hypoxic, ischemic or inflammatory—affect the efficacy of conditioning efforts in the immature brain.

### Keywords

preconditioning; postconditioning; hypoxia-ischemia; stroke; immune system; mitochondria; blood-brain barrier

---

Correspondence should be addressed to Zinaida S. Vexler (zena.vexler@ucsf.edu) and Henrik Hagberg (henrik.hagberg@obgyn.gu.se).

Conflicts of interest statement

The authors have no conflicts of interest.

## Introduction

Brain injury in the perinatal period occurs in many clinical settings, e.g. hypoxic-ischemic encephalopathy (HIE) in term infants, neonatal stroke, encephalopathy of prematurity, and infections (Hagberg et al., 2015). These insults often result in life-long disabilities including cerebral palsy, cognitive deficits, visual dysfunction, hearing impairments and epilepsy (Wood et al., 2000; Delobel-Ayoub et al., 2009; Fernandez-Lopez et al., 2014; Hagberg et al., 2016). A broad array of potential neuroprotective strategies has been tested pre-clinically, targeting various signaling pathways to attenuate neuronal cell death and/or limit toxic aspects of oxidative stress and inflammation caused by injury. However, the success of clinical implementation has been limited. Therapeutic hypothermia (TH) used within hours of birth in term human babies with HIE has been the only successful treatment thus far (Edwards et al., 2010). TH has been approved for treating HIE since 2010 in atterm human neonates and it has become the standard of care in many countries. However, beneficial effects of TH are limited to only mild-to-moderate brain injury, and many infants still develop significant adverse outcomes. There is an extensive search for adjuvant therapeutic approaches to improve the outcomes. One strategy is to better understand the molecular regulation of the endogenous vulnerability of the immature brain by using sublethal stressors of different origin. The present review will focus on various ways to modify susceptibility in the developing CNS by means of preconditioning (pre-C) or postconditioning (post-C) stressors.

## Conditioning

A sublethal stress (e.g., hypoxia, ischemia) or drug administration can induce resistance to a subsequent potentially lethal insult, a phenomenon known as pre-C (Janoff, 1964). Pre-C was first described in the heart (Murry et al., 1986) but can be induced in most organs, including the brain (Chen and Simon, 1997; Dirnagl et al., 2003; Gidday, 2006; Thompson et al., 2015). In the adult, the application of pre-C and post-C stressors have been extensively studied as protective strategies in models of cerebral ischemia. Multiple lines of evidence have suggested that protection is achieved by an array of mechanisms at the systemic level, at the neurovascular unit interface, and by modifying cell-cell interactions and intracellular signalling and, in particular, mitochondrial function. Altered temporo-spatial patterns of cerebral blood flow (CBF) upon reperfusion, immune-neurovascular interactions, suppression of neuroinflammation, and changes in mitochondrial function in injured adult brain are among the most important changes caused by pre-C and post-C stressors.

Pre-C seems to be particularly robust in the immature brain (Gidday et al., 1994). In most studies, conditioning is induced by exposure to the stressor or drug some time prior to the severe insult (usually > 6–12 h), but tolerance can also be triggered by exposure to the stressor after the main insult, referred to as post-C (Zhao et al., 2006; Teo et al., 2015). Conditioning stimuli can both reduce immature brain vulnerability (positive conditioning; Gidday et al., 1994) or increase vulnerability (negative conditioning; Eklind et al., 2001). Furthermore, subthreshold stress in a peripheral organ (e.g. repeated limb ischemia) can induce conditioning in the developing brain, a phenomenon called remote pre-C or post-C (R-pre-C or R-post-C) (Tropak et al., 2011; Khan et al., 2015; Ezzati et al., 2016).

Conditioning can be induced by sublethal hypoxia, ischemia, epileptic seizures, hyperbaric oxygenation, hyperthermia, toll-like receptor (TLR) agonists, or drugs such as magnesium sulphate, glucocorticoids or anesthetics (e.g. xenon) (Barks et al., 1991; Gidday et al., 1994; Chen and Simon, 1997; Eklind et al., 2001; Dirnagl et al., 2003; Gidday, 2006; Koning et al., 2017). As in the adult brain, several pre-C and post-C mechanisms have been proposed to alter vulnerability in the developing brain. Some of the different regimens of conditioning applied in the immature brain are summarized in Table 1.

## Pre-C and post-C as modulators of the neurovascular unit in the injured immature brain

In the adult, CBF is not affected by pre-C itself, but the decrease in CBF during a subsequent episode of ischemia is attenuated (Nakamura et al., 2006). Similarly, we have found that hypoxic pre-C in the immature rat attenuates the drop in CBF during a severe HI insult induced 24 h after pre-C (Gustavsson et al., 2007). This effect was associated with upregulation of vascular genes (e.g. Angpt2, VEGF, Flt1, Kdr, Pdgfra) and an increase in vascular density. Recently, it was shown that the pre-C-induced preservation of vascular density after HI was accompanied by an increase in VEGFR2, and the pre-C effect was reversed by the angiotensin II receptor subtype 2 (AT2-R) inhibitor (PD123319), offering support for a vascular component in pre-C (Lopez-Aguilera et al., 2012). The pre-C effect of erythropoietin (EPO) could also at least partly be explained by vascular effects. Lipopolysaccharide (LPS)-induced pre-C diminished the extent of secondary perfusion deficits (rather than primary perfusion deficits) in adult stroke by induction of endothelial nitric oxide synthase (eNOS) (Dawson et al., 1999), and NOS inhibition by L-NAME abolished the pre-C effects, indicating a role for NOS in LPS-mediated positive pre-C. Similarly, in neonatal rats, Akt-mediated eNOS upregulation in neurons and vascular endothelial cells was required for LPS-induced positive pre-C against HI (Lin et al., 2010). However, in hypoxic pre-C, the CBF was not affected by L-nitroarginine (Gustavsson et al., 2007), implying NO has other targets besides the vasculature. There is no data yet in adult or immature brains with ischemia models demonstrating that pre-C neuroprotection predominantly depends on vascular adaptations (c.f. Gidday, 2006). However, in an excitotoxicity model, the protective effect of hypoxic pre-C was reversed by VEGFR2 antibodies, and VEGF reduced brain injury to a similar extent as hypoxic pre-C (Laudenbach et al., 2007). However, we can not rule out non-vascular actions mediated by VEGF-VEGFR2, considering VEGF also has neurotropic effects. Furthermore, the pre-C effect of MgSO<sub>4</sub> was not associated with improvement in CBF either before or during a subsequent period of HI (Koning et al., 2017), suggesting that vascular modifications are not always required for induction of pre-C in the immature brain.

Disruption of blood-brain barrier (BBB) integrity is a major contributor to the pathophysiology of stroke. In adult stroke models, brief repetitive hypoxic episodes (Stowe et al., 2011) and exercise pre-C (Davis et al., 2007) were shown to limit BBB dysfunction, in part by downregulating endothelial adhesion molecules, limiting leukocyte infiltration, as well as by preserving basal lamina. The use of low thrombin concentrations (thrombin preconditioning; TPC) before middle cerebral artery occlusion (MCAO) attenuated brain

edema, BBB disruption and brain hemorrhage (Stetler et al., 2009). Effects were mediated by mechanisms that include induction of heat shock proteins, particularly HSP27/HSP25, modulation of downstream cellular protective p44/42 MAPK/p90RSK/HSP25 pathways and protease-activated receptors (PAR) (Stetler et al., 2009). Pre-C also enhanced recognition and chaperoning of damaged or misfolded proteins, stabilizing actin filaments and limiting the stress-response cascade upstream of the mitochondrial cell death machinery (Stetler et al., 2009; Shi et al., 2017).

Literature has shown that BBB integrity greatly depends on the maturational stage of the brain at the time of stroke (Fernandez-Lopez et al., 2012; Kratzer et al., 2014), as well as the injury model used (Fernandez-Lopez et al., 2012; Ek et al., 2015; Mallard and Vexler, 2015). Individual components of the neurovascular unit, including endothelial cells, the extracellular matrix (ECM) and glial cells, undergo unsynchronized developmental changes and increases astrocytic and pericyte vessel coverage in postnatal brain, affecting both acute structural-functional BBB responses and delayed angiogenic processes.

Data on the role of the neurovascular unit in mediating pre-C in neonates, while scarce, suggest that pre-C protects the ECM and BBB integrity. One study showed that administration of plasminogen activator inhibitor-1 (PAI-1) before HI reduced brain injury in a dose-dependent manner (Yang et al., 2009). Protection was preceded by a marked reduction of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator, affecting MMP-9 activation, brain edema and BBB permeability, and was associated with reduced axonal degeneration (Yang et al., 2009). Another study demonstrated that intra-cerebroventricular injection of a stable-mutant form of PAI-1 attenuates pro-inflammatory response and BBB disruption in, an LPS injury model followed by HI in P7 rats (Yang et al., 2013). Hyperthermic pre-C also prevented BBB disruption produced by HI in newborn rat (Ikeda et al., 1999).

Several studies have suggested that inflammation during gestation can prime the vasculature, producing long-lasting changes in BBB function but not necessarily short-term changes in BBB integrity. While LPS pre-C followed by HI induced significant changes in BBB integrity in P12 rats, there was little evidence of acute neutrophil infiltration into the brain or increased albumin leakage after a single injection of LPS (Brochu et al., 2011). Newborn rats given multiple intraperitoneal LPS injections at P0-P8 did not have significantly increased sucrose leakage through the BBB at either P9 or P20 (Stolp et al., 2011), but strikingly, LPS led to significantly higher brain sucrose concentration during adulthood (Stolp et al., 2005). Deficiency in omega-3 lipids during gestation negatively conditioned pups by making them more prone to HI postnatally by enhancing neuroinflammation, adversely affecting the vasculature and BBB integrity (Zhang et al., 2010; Zhang et al., 2015). Together, these results suggest that the brain ECM plays a modulatory role in BBB integrity and injury after neonatal HI.

## Innate immune system modulation as positive and negative pre-C in the immature brain

Data have been accumulating on the role of innate immunity in injury to the immature brain and the possibility to attenuate injury by modifying immune responses. *In utero* infections, neonatal sepsis and chorioamnionitis, are linked to both preterm birth and neurological deficits later in life (Shatrov et al., 2010; Salas et al., 2013; Soraisham et al., 2013; Shankaran et al., 2014). Children born with bacteremia very preterm perform worse on neurocognitive tests in the first weeks of life (Bright et al., 2017) and demonstrate neurological dysfunction at school age (Kavas et al., 2017).

Induction of cerebral innate immunity via TLR has been thoroughly established in the perinatal brain injury field (Mallard et al., 2009). TLRs are innate immune receptors that sense “pathogen-associated molecular pattern molecules” (PAMP) expressed by viral and bacterial micro-organisms or “damage-associated molecular pattern molecules” (DAMP) that are released from injured tissue. Individual TLRs differ in their ligand specificity, signal transduction pathways they activate, and types of infection to which they respond. TLR4 mediates gram-negative bacterial infections (induced by LPS/endotoxin), TLR2 is mostly associated with gram-positive bacteria (mimicked by i.p. injection of the synthetic lipopeptide Pam(3) CSK(4); PAM), and TLR3 mediates viral infections (mimicked by i.p. injection of poly I:C). Several studies have shown that excessive activation of TLRs, either by bacterial stimuli or by injury, can have direct negative effects on the developing brain. For example, chronic exposure to LPS or PAM impairs both grey and white matter development in neonatal mice (Du et al., 2011), and TLR2 gene-deficient mice are protected from neonatal HI (Stridh et al., 2011). There is also growing evidence demonstrating that TLR-mediated mechanisms can modulate the vulnerability of the immature brain to other insults, resulting in either positive or negative pre-C. Negative pre-C effects on HI neonatal brain injury have been observed following activation of several TLR subtypes, including TLR2 (Falck et al., 2017; Mottahedin et al., 2017), TLR3 (Stridh et al., 2013) and TLR4 (Eklind et al., 2001; Eklind et al., 2005; Wang et al., 2007) (Table 1). Immune challenges during gestation have also demonstrated a broad range of negative conditioning effects in maturing rodents. Indeed, LPS administration during gestation increased brain cytokine levels and made pups more vulnerable to HI at P1 (Girard et al., 2008). The LPS-induced increased vulnerability to neonatal HI (negative pre-C) is dependent on the TLR adaptors MyD88 (Wang et al., 2009) and TRIF (Stridh et al., 2013), while in adult stroke, positive pre-C after TLR4 signaling depends on TRIF but not on MyD88 signaling (Vartanian et al., 2011), emphasizing age-dependent differences in immune pre-C mechanisms.

The time interval between TLR activation and the main insult is another important factor that determines whether there are positive or negative immune pre-C effects on brain injury. For example, when administered 2–4 days before MCAO in adult rats, LPS enhanced protection, with maximal effect at 3 days, whereas no protection was observed when LPS was administered 1 day or 1 week before MCAO (Tasaki et al., 1997). The magnitude of TLR activation is also important in determining positive or negative outcome, with higher doses of LPS abolishing positive pre-C in MCAO-induced injury (Bordet et al., 2000).

Similar to the adult, we have shown in neonates that, depending on the time interval between insults, the same dose of LPS can both increase and decrease vulnerability to HI in P7 rat pups. Administration of a low LPS dose 4 h, 6 h, or 3 days before HI exacerbated injury (Eklind et al., 2001) and produced short-term memory impairment (Ikeda et al., 2004), while a 24-h interval was protective (Eklind et al., 2005). Literature is also emerging on the lasting effects of brain reprogramming following gestational immune challenges affecting susceptibility to injury during adulthood. Remarkably, while intrauterine LPS administration sensitized (negative pre-C) to early postnatal HI, it served as a protective pre-C stimulus in adult HI (Wang et al., 2007).

The need for new protein synthesis for induction of pre-C has been shown in many settings including LPS-induced effects (Bordet et al., 2000). Protective pre-C LPS effects on MCAO injury were blocked by dexamethasone or indomethacin administration, supporting a role for inflammatory mechanisms (Bordet et al., 2000), whereas we reported that up-regulation of endogenous corticosterone protects the immature brain in LPS-induced pre-C 24 h prior to HI (Ikeda et al., 2006). Using microarray techniques, we compared the cerebral gene response following positive and negative LPS-induced pre-C. We showed that the increased vulnerability to HI (negative pre-C) seen at 6 h after LPS is associated with significant gene regulation, including genes associated with protein metabolism, immune and inflammatory response, chemotaxis, and cell death (Eklind et al., 2006). These changes in inflammatory genes were very prominent and distinct from the positive pre-C scenario (i.e., 24 h after LPS administration) (Eklind et al., 2006). Further, expression of transcript changes differed from those induced by HI only (Hedtjarn et al., 2004; Hedtjarn et al., 2004), indicating specificity in the pre-C response. Further evidence of involvement of immune responses in sensitizing effects on the immature brain was obtained in a study that utilized immunomodulator IDR-1018 in P9 mice subjected to LPS combined with HI (Bolouri et al., 2014). Importantly, IDR-1018 was protective when administered as pre-treatment or post-treatment (Bolouri et al., 2014); however, whether pre-C-specific responses were affected remains to be determined.

The source of the inflammatory response in the immature brain associated with TLR-induced pre-C has not been fully elucidated. While it is clear that microglia, the endogenous brain immune cells, respond to antenatal and postnatal systemic TLR activation, as well as non-infectious inflammation, the mechanisms that link peripheral signals to activated microglia remain obscure. We showed that microglia are the predominant source of TLR2 expression during normal postnatal brain development but that LPS stimulation and intracerebral IL-1 $\beta$  injection in P9-P10 mice produce stimuli-dependent induction of inflammatory mediators (Lalancette-Hebert et al., 2017). Administration of LPS at P5 leads to a transient increase in absolute number and cell density of Iba1-positive microglia, as well as a persistent alteration in hippocampal inflammatory status in microglia (Smith et al., 2014). Further, repeated injections of PAM at P3-P11 decreased the volume of cerebral grey and white matter in association with elevated levels of several pro-inflammatory cytokines and chemokines in the brain as well as increased microglial density (Du et al., 2011). In fetal sheep, LPS administered either to the fetus (Duncan et al., 2002; Mallard et al., 2003; Dean et al., 2011) or into the amniotic fluid (Nitsos et al., 2006) at a gestational age comparable to that of the preterm human infant led to CNS inflammation and increased numbers of

microglia with activated morphologic phenotype. Comparative analysis of TLR2 and TLR4 stimulation has shown stimulus-dependent patterns of cytokine induction and leukocyte infiltration into immature brain (Mottahedin et al., 2017). The magnitude and particulars of induced infiltration of CD11<sup>+</sup>/CD45<sup>hi</sup> leukocytes were particularly prominent following PAM administration (Mottahedin et al., 2017). The role of increased microglia proliferation and leukocyte trafficking in preconditioning remains unclear, but, interestingly, it was recently shown that positive LPS pre-C induces a Ly6Chi monocyte response that protects the brain after MCAO (Garcia-Bonilla et al., 2018).

## Intracellular mechanisms of hypoxic pre-C in the immature brain

There is a vast amount of information on intracellular mechanisms of pre-C in response to various stress stimuli, and there seems to be a high degree of similarity across organs and species. It appears that some general endogenously protective cellular strategies have evolved in order to cope with dangerous exposures such as hypoxia/ischemia, substrate deficiency and infections. There isn't a single neuroprotective factor, but pre-C is the result of the combined action of multiple, partly overlapping cellular and molecular events that counteracts cell death, reduces the injurious effects of inflammation, adapts mitochondrial metabolism and enhances regenerative processes (Dirnagl et al., 2003; Gidday, 2006). In some reviews, the different components involved in pre-C have been divided into sensors, transducers, and effectors (Dirnagl and Meisel, 2008). However, in the immature brain, the sequence of events is still uncertain, but an approximate staging of the mechanisms is indicated in Figure 1.

## Gene activation and protein translation

It has been shown that pre-C induces a number of potentially neuroprotective genes in the brain (Jones and Bergeron, 2001; Bernaudin et al., 2002; Gustavsson et al., 2007), indicating that gene programming is important for development of pre-C also in the immature brain in addition to posttranslational and other adaptations (Gidday, 2006). Studies in the adult brain demonstrate that ischemic pre-C is inhibited by the protein translational inhibitor cycloheximide (Barone et al., 1998). Xenon-induced pre-C in the neonatal brain is also inhibited by cycloheximide (Ma et al., 2006), indicating that pre-C depends on gene transcription and *de novo* protein synthesis. However, no studies using transcription or translation inhibitors have been performed for hypoxic or other types of pre-C in the developing brain.

Hypoxic or ischemic pre-C induces a number of transcription factors including activating protein 1 (AP1), cyclic AMP response-element binding-protein (CREB), nuclear factor kappa B (NF- $\kappa$ B), early growth response 1 (EGR1), and the redox-regulated transcriptional activator specificity protein 1 (SP1) (Gidday, 2006). However, the best characterized transcription factor induced after hypoxic pre-C is probably hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), which is strongly associated with preconditioning in the immature brain (Bergeron et al., 2000; Sharp and Bernaudin, 2004; Sheldon et al., 2009). Selective neuronal HIF-1 $\alpha$  gene deletion blocked hypoxic pre-C in mice (Sheldon et al., 2009), indicating that pre-C depends on HIF-1 $\alpha$ . However, the interpretation is complicated by the fact that



HIF-1 $\alpha$  gene deficiency confers heightened vulnerability also in response to the HI insult itself (Sheldon et al., 2009), and it is difficult to tell if HIF-1 $\alpha$  is reducing vulnerability during the pre-C phase or during the HI insult or both. Ischemic pre-C in the neonatal brain was associated with a marked and sustained activation of CREB after 24 h (Lee et al., 2004). Intracerebroventricular infusions of antisense CREB oligodeoxynucleotides significantly reduced the pre-C neuroprotection and induction of CREB signaling with rolipram 24 h before HI protected newborn rats, supporting a role of CREB in neonatal pre-C (Lee et al., 2004).

## Survival kinases

Several so-called survival kinases (e.g. protein kinase C $\epsilon$  (PKC $\epsilon$ ), phosphoinositide 3 kinase (PI3K)–AKT, and glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ )) are upregulated in the immature brain after hypoxic pre-C (Gustavsson et al., 2007) (Figure 1). PKC $\epsilon$  phosphorylates and activates mitoK<sub>ATP</sub> (see below) in the inner mitochondrial membrane (Raval et al., 2007; Bednarczyk, 2009), and inhibition of PKC $\epsilon$  has been shown to reverse pre-C protection (Raval et al., 2007). PKC $\epsilon$  is also critical for maintenance of mitochondrial nicotinamide adenine dinucleotide (NAD) or nicotinamide phosphoribosyltransferase (Nampt) pools (Morris-Blanco et al., 2014) known to be critical for immature brain integrity (Robertson et al., 2004; Fiskum et al., 2008; Galindo et al., 2017).

The PI3K/PKB/Akt/GSK3 $\beta$  pathway has been shown to reduce brain vulnerability in ischemia (Zhao et al., 2006) and in the neonatal brain (Yin et al., 2007; Zhao et al., 2013), and has been implicated in pre-C of neuronal cells (Hillion et al., 2006) and the neonatal brain (Feng et al., 2010). Activation of this pathway leads to phosphorylation of Bcl-2 family proteins, decreases the pro- vs. anti-apoptotic balance, and prevents Bax-dependent mitochondrial permeabilization (Baines et al., 2003; Brywe et al., 2005; Maurer et al., 2006; Dirnagl and Meisel, 2008; Feng et al., 2010), which is implicated as an important cell death mechanism in the neonatal brain (Wang et al., 2009) (Figure 1). Neuroprotection induced by hypoxic pre-C of the neonatal brain also induces changes in mRNA for Bcl-2 family proteins and other apoptosis-related proteins enforcing anti-cell death actions (Gustavsson et al., 2007). Recently, hypoxic pre-C was shown to also counteract caspase-Bax-dependent apoptosis induced by propofol (Lv et al., 2018). The cellular inhibitor of apoptosis-1 (cIAP1) is also important in pre-C of the immature brain, offering further support of the apoptotic pathway (Lin et al., 2013). Inhibition of PI3K/Akt using LY294002 attenuated pre-C neuroprotection and promoted the expression of NF-kappa-B, COX-2, and CD68. Proteomic microarray analysis revealed that pre-C inhibited expression of proinflammatory cytokines induced by HI (Yin et al., 2007).

The PI3K/AKT pathway is also stimulated by EPO which, in spite of being a HIF-1 target gene, was only moderately upregulated at the gene level (mainly in astrocytes) early after hypoxic pre-C in the immature brain compared to other organs (Bernaudin et al., 2002). The level of the EPO protein was not increased after hypoxic pre-C, at least not 0.5 h, 6 h, 12 h or 24 h after pre-C (Jones and Bergeron, 2001). However, EPO was induced by pharmacologic pre-C with deferoxamine (Mu et al., 2005) or MgSO<sub>4</sub> (Koning et al., 2017), suggesting that EPO certainly could contribute to the development of tolerance, especially as

it is well known to exert protective effects in the immature brain (Rangarajan and Juul, 2014). Furthermore, in the adult brain, EPO itself induces pre-C, and the protective effect of hypoxic pre-C is reversed by soluble EPO receptors (Prass et al., 2003) and EPO antisense (Liu et al., 2005), supporting its importance at least in some forms of pre-C.

## **Involvement of mitochondria: energy metabolism, mitoK<sub>ATP</sub> and biogenesis/mitophagy**

In the adult brain and heart, ischemic or hypoxic pre-C prevents mitochondrial swelling, protects membrane integrity, reduces ATP consumption and improves mitochondrial energy metabolism during a subsequent episode of cerebral ischemia (Zhang et al., 2003; Dirnagl and Meisel, 2008). Sirt1 and HSP90 have been proposed to be involved in mitochondrial protection via activation of PKC $\epsilon$  (Thompson et al., 2015). In the neonatal brain, hypoxic pre-C also preserves mitochondrial function and attenuates the primary energy depletion during a subsequent period of HI (Brucklacher et al., 2002), and there is some evidence that AMP-activated protein kinase (AMPK) is involved (Rousset et al., 2015).

We found recently that a pre-C bolus of magnesium sulphate (MgSO<sub>4</sub>) applied 12 h to 6 days prior to neonatal HI reduces brain injury dramatically (Table 1). The protection was accompanied by downregulation of genes for metabolic enzymes, reduction of succinate during the subsequent HI, as well as preservation of high energy phosphates, mitochondrial respiration and attenuation of mitochondrial reactive oxygen species (ROS) production (Koning et al., 2017; Koning et al., 2018). It is important to emphasize that the serum levels of magnesium were normalized at the time of the HI insult and the treatment was ineffective if applied immediately prior, during or after the insult, suggesting that a pre-C mechanism is at play rather than a pre-treatment effect during HI (c.f. Riepe et al., 1997).

Opening of mitoK<sub>ATP</sub> channels leads to influx of K<sup>+</sup> into the mitochondrial matrix, increased oxygen consumption, and depolarization of the inner membrane (moderate uncoupling), reducing production of ROS mitochondrial Ca<sup>2+</sup> accumulation, and Bax-dependent mitochondrial permeabilization (Bajgar et al., 2001; Liu et al., 2002; Bednarczyk, 2009). Brain mitochondria have higher concentrations of mitoK<sub>ATP</sub> than do liver or heart mitochondria (Bajgar et al., 2001). Hypoxic pre-C in the neonatal brain induced upregulation of the protein level of the Kir6.2 isoform and enhanced current activities of mitoK<sub>ATP</sub> channels (Sun et al., 2015). The mitoK<sub>ATP</sub> opener diazoxide also reduced injury in the immature brain whereas inhibition of mitoK<sub>ATP</sub> (using tolbutamide) blocked the protective effects of pre-C (Rajapakse et al., 2002; Wang et al., 2011; Sun et al., 2015).

Mitophagy of injured mitochondria and their replacement through biogenesis is an important process to maintain viability of postmitotic cells. A compelling notion is that cells with renewed mitochondria might be more resistant to severe stress. Ischemic pre-C of the heart depends on mitophagy through a parkin-SQSTM1-dependent mechanism (Huang et al., 2011), and mitophagy was recently also suggested to be involved in pre-C of neurons (Lizama et al., 2018). Under hypoxic conditions, HIF-1 $\alpha$  (see above) induces BNIP3L-dependent and BNIP3-dependent mitophagy, probably through interaction with the outer mitochondrial membrane protein FUNDC1 (Ashrafi and Schwarz, 2013). Exposure of mice

to hypoxic pre-C is also reported to increase mitochondrial DNA content, PGC-1 $\alpha$  activity, and subsequent mitochondrial biogenesis (Gutsaeva et al., 2008). Hypoxic pre-C of mice induced expression of mRNA for Bnip3l and Bnip3, accompanied by an increase in the concentrations of the biogenesis markers PGC-1 $\alpha$  and nuclear respiratory factors 1 and 2, suggesting that the mitophagy–biogenesis cycle might also be associated with pre-C of the immature brain (Gustavsson et al., 2008).

In summary, there is strong evidence that signaling cascades induced by pre-C converge on mitochondria in the brain of both adults (Liu et al., 2002) and neonates (Busija et al., 2005; Sun et al., 2015), as well as in other organs (Boengler et al., 2018). Interestingly, recent results in adult brain suggest that cross talk between mitochondria and endoplasmic reticulum with regard to Ca<sup>2+</sup> regulation may be an additional critical mechanistic component in pre-C (Sisalli et al., 2015) that has to be addressed also in the immature brain in the future.

### **Nitric oxide (NO)-mediated conditioning**

Nitric oxide (NO) synthase is upregulated after pre-C in the immature brain and generates NO and nitrite (Gidday et al., 1999; Gustavsson et al., 2007), and this inhibits production of ROS at complex 1 of the electron transport chain, improves mitochondrial respiration at complex II-IV and in turn reduces the threshold of mitochondrial permeabilization in heart and liver (Shiva et al., 2007). Indeed, non-specific inhibitors of NO synthase block hypoxic pre-C in the immature brain (Gidday et al., 1999). However, specific blockade of nNOS or iNOS had no effect whereas eNOS inhibition prevented hypoxic pre-C (Gidday et al., 1999), indicating that vascular factors are involved.

### **R-pre-C and R-post-C as conditioning paradigms in the immature brain**

R-pre-C and R-post-C refer to a sublethal shear stress therapy by repetitive inflation-deflation of a blood pressure cuff on limbs before or after a major insult. R-pre-C and R-post-C have been demonstrated to be safe and are being actively studied in adult animal stroke models (Hess et al., 2013; Hess et al., 2015) and in small stroke clinical trials. A growing number of studies show that eNOS/NO/nitrite play an important role in improved perfusion in mice after stroke, and in protection conveyed by R-post-C (Hess et al., 2016) and effectiveness after embolic stroke in male and ovariectomized female mice (Hoda et al., 2014). Chronic R-post-C was recently demonstrated to induce vascular remodeling and protection in the adult (Khan et al., 2018). R-post-C was found effective alone and in combination with intravenous tPA in embolic stroke in adult mice (Hoda et al., 2012).

In neonates, while potential for protection by R-post-C has been demonstrated in a few studies, efficacy and the mechanisms of R-pre-C and R-post-C are not sufficiently understood. Available data have been discussed extensively in a recent review in this journal (Adstamongkonkul and Hess, 2017) and will not be discussed in detail in this review. As examples, in P10 rats subjected to HI, R-post-C induced immediately after the end of hypoxia by four 10-min ischemia/reperfusion cycles on both hind limbs reduced infarct volume at 48 h and improved functional outcomes at 4 weeks after HI, in part via

involvement of the opioid receptor and PI3K/Akt signaling pathways (Zhou et al., 2011). In a piglet asphyxia model, immediate R-post-C reduced brain nitrotyrosine formation (Rocha-Ferreira et al., 2016) and protected oligodendrocytes in the corpus callosum and in the periventricular white matter but did not attenuate injury in grey matter (Ezzati et al., 2016).

In humans, effects of early and late R-pre-C were determined in infants who underwent surgery for congenital heart disease. In that pilot randomized controlled trial, R-pre-C proved to be feasible but did not produce significant differences in acute outcomes (Guerra et al., 2017). In another study conducted in neonates to 17-year-old children who underwent cardiac surgery, R-pre-C was not associated with improvements in clinical outcomes or physiological markers (McCrindle et al., 2014). Given the complexity of the studied effects and dependence of the effects on the type and severity of insult, age, the timing and exact protocol(s) of conditioning, additional animal studies and larger clinical trials are necessary to uncover the extent of the therapeutic potential for R-pre-C and R-post-C in the immature brain.

### **Noble gases and anesthetics as a pre-C strategy in injured immature brain**

Anesthetic agents and noble gases have been tested for their efficacy in pre-C and post-C. The pros for such an approach are that anesthetic agents are commonly used in injured neonates and may provide additional benefits. The cons are that newborn brain is susceptible not only to widespread neuronal apoptosis resultant from ischemic, oxidative and excitotoxic injury (Ikonomidou et al., 1989; Ikonomidou et al., 1999; Hu et al., 2000) but also neuronal apoptosis induced by anesthetic agents (Olney et al., 2004; Schifilliti et al., 2010). In fact, models of neonatal toxicity were developed using prolonged exposure to the anesthetic agents isoflurane and sevoflurane (Shu et al., 2010).

Xenon (Xe), a noble gas that has been recognized as an anesthetic for more than 50 years, was found less neurotoxic to the developing brain compared to other anesthetics, effective as an anesthetic adjuvant in animals, and safe as a conditioning strategy in clinical trials in neonates with neurologic injury (Azzopardi et al., 2016; Alam et al., 2017). Xe has a small blood-gas partition coefficient, readily crosses the BBB and does not cause hemodynamic depression. Over the years, Xe was shown to exert neuroprotection (Preckel et al., 2000; Banks et al., 2010) and cardioprotection (Pagel, 2010; Schwiebert et al., 2010) in different adult animal models. Yet, Xe is not widely applied in clinical practice mainly because of its high cost and requirement for highly trained personnel.

Several mechanisms of Xe action have been identified. In neuronal-glia cell co-cultures subjected to oxygen-glucose deprivation, Xe pre-C produced a concentration-dependent reduction of lactate dehydrogenase release, an effect abolished by a protein synthesis inhibitor, and decreased cell death (Ma et al., 2006). Xe-induced pre-C reduced infarct size during subchronic injury and provided long-term neurological functional improvement in P7 rats subjected to HI (Ma et al., 2006). Xe pre-C and post-C was suggested to produce protection via induction of pro-survival proteins in models of neonatal HI (Ma et al., 2006), asphyxia (Luo et al., 2008) and prolonged (toxic) isoflurane exposure (Shu et al., 2010), such as Bcl-2 and BDNF. Xe enhanced CREB phosphorylation (Luo et al., 2008) and

activity-dependent neuroprotective protein (ADNP) (Cattano et al., 2008) and decreased cytochrome-C release from mitochondria. Xe potently non-competitively inhibited NMDA receptors, with only minor effects on GABA receptors and non-NMDA receptors (Franks et al., 1998). At high dose, Xe inhibited the  $\text{Ca}^{2+}$ -ATPase in rat brain synaptic plasma membranes (Franks et al., 1995) by inducing conformational changes in the lipid membrane (Lopez and Kosk-Kosicka, 1995). Xe administration alone showed neuroprotection after HI in neonatal rats (Dingley et al., 2006; Zhuang et al., 2012) and newborn pigs (Chakkarapani et al., 2010) as a post-C approach. Side-by-side use of Xe and hypoxic pre-C demonstrated positive pre-C by Xe but negative effects of hypoxic pre-C (i.e., injury exacerbation and lack of protective protein induction) (Shu et al., 2010). Additional protective mechanisms of Xe pre-C from ischemia/reperfusion injury, via mitochondrial  $\text{K}^{+}$ -ATPase, were reported in immature rabbit heart (Li et al., 2013). Given the approval of TH for HIE in atterm human infants and inclusion of TH as a standard of care in many countries, efficacy of Xe post-treatment combined with immediate or delayed TH was examined. The presence or lack of beneficial effects of Xe administered after injury was shown to strongly depend on the severity of HI, Xe dosing, the timing of administration, and coordination between TH and Xe administration after injury. We limit the discussion of Xe effects to pre-C and do not focus on post-treatment in this review.

In cooled human newborns with neonatal encephalopathy, a small, single-arm, dose-escalation study showed the feasibility of using Xe in combination with TH (Dingley et al., 2014). No acute adverse respiratory, inflammatory or cardiovascular effects or adverse effects at 18 months' follow-up were observed (Dingley et al., 2014). Safety and feasibility of Xe was also confirmed in a recent TOBY-Xe trial. This open-label, randomized controlled trial compared effects of moderate whole-body cooling alone in moderately to severely injured 36–43-week-old infants to effects of moderate hypothermia combined with 30% Xe for 24 h (Azzopardi et al., 2016). At the same time, monitoring of multiple MRI/MRS markers showed lack of Xe-induced effects, suggesting that Xe is unlikely to enhance the neuroprotective effect of cooling after birth asphyxia (Azzopardi et al., 2016). While recent data in small and large newborn animal models and clinical trials have indicated safety and some neuroprotective potential of Xe, additional randomized clinical trials are necessary to corroborate these findings and confirm the feasibility of its routine use and its optimal timing, concentration, and duration for human neonatal HIE (Amer and Oorschot 2018).

Other noble gases, argon and helium, were also shown to be beneficial as pre-C approaches in animal studies. When administered at the same rate as Xe, argon and helium improved cell survival, brain structural integrity, and neurologic function at P40 in a model of neonatal asphyxia in rats (Zhuang et al., 2012). Argon reduced infarct volume following more severe HI injury (Zhuang et al., 2012). Protection was associated with increased expression of Bcl-2 and increased Bcl-xL and Bax expression as well (Zhuang et al., 2012). The safety of argon was established in newborn pigs under physiological conditions and following hypoxia (Alderliesten et al., 2014).

Testing of the commonly used anesthetic isoflurane as a post-C approach in HI revealed that brief exposure carries beneficial potential and that neuroprotection was concentration- and time-dependent (Xu et al., 2016). Short-term and long-term protection were partly mediated

via the GluR2 AMPA receptor (Xu et al., 2016) and inhibition of the mitochondrial permeability transition pore following neonatal HI (Zhao et al., 2014). Sevoflurane post-C also improved long-term learning and memory following HI. As with hypoxic pre-C, protection was mediated via the PI3K/Akt-mPTP pathway (Lai et al., 2016). Dexmedetomidine post-C reduced brain injury after HI in neonatal rats as well (Ren et al., 2016). Taken together, the results show the central role of mitochondria in anesthetics-induced pre-C protection.

## Effects of pre-C and post-C on injury after neonatal focal arterial stroke

In adult stroke models, while restoration of CBF is protective after a short ischemic episode, it can be damaging following sustained perfusion deficits because of hyperemia in previously occluded regions and subsequent secondary hypoperfusion known as “no-reflow phenomenon” (Aronowski et al., 1997). The latter phenomenon has been attributed to leukocytes, and to neutrophils in particular. Ischemic post-C induced by serial short interruptions of CBF during early reperfusion either interrupted hyperemia (Zhao et al., 2006) or shortened its length (Wang et al., 2008) and reduced infarct volume, pointing to ischemic post-C as a neuroprotective strategy. Involvement of multiple mechanisms has been described, such as functional normalization of receptor and ion exchanger systems (Pignataro et al., 2011; Pignataro et al., 2011) and effects on inflammation (Joo et al., 2013). Effects of ischemic post-C on cell-cell interactions were also identified, such as neuronal VEGF regulation and microglial polarization (Esposito et al., 2018).

There is now ample evidence that the developmental stage of the brain at stroke onset plays a key role in injury (Ferriero, 2004; Yager and Ashwal, 2009; Semple et al., 2013; Fernandez-Lopez et al., 2014; Hagberg et al., 2015; Mallard and Vexler, 2015), including distinctions in the neuroinflammatory mechanisms, and the role of microglial cells and BBB responses between perinatal and adult stroke (Denker et al., 2007; Faustino et al., 2011; Fernandez-Lopez et al., 2012; Fernandez-Lopez et al., 2016). Compared to adult stroke models in which pre-C and post-C paradigms have been extensively studied, information on effectiveness and magnitude of conditioning-induced protection following neonatal arterial stroke is scant, largely due to too few laboratories performing neonatal arterial focal stroke models.

In contrast to the presence of hyperemia in adult stroke models, induction of focal arterial stroke in P7 rats by electrocoagulation of left MCAO combined with concomitant transient occlusion of both common carotid arteries (CCA) did not lead to hyperemia early reperfusion, as was evident from CBF measurement by 2D-color-coded ultrasound imaging and laser Doppler flowmetry (Leger et al., 2012). Post-C induced by a series of re-occluding single or both CCA for various short time periods (up to 5 min) did not reduce infarct size 72 h after MCAO (Leger et al., 2012). In the same model in P7 rats, pre-C with NO inhalation was beneficial, as it induced NO in the brain, improved CBF and protected neonatal brain against stroke (Charriaut-Marlangue et al., 2012). In contrast, based on increased infarct size, post-C with NO inhalation exacerbated injury (Charriaut-Marlangue et al., 2012). Taken together, these data are consistent with the notion that the pattern of CBF changes upon reperfusion is a critically important mediating mechanism of post-C

protection. The constraint of these data is the contribution of both local and systemic perfusion deficits. In a transient 3-h suture MCAO occlusion in P7 rats, using perfusion-sensitive contrast-enhanced MRI, we observed partial to complete reperfusion of previously occluded regions at a single acute time point, 30 min after suture retraction, as well as perfusion of injured regions 24 h after reperfusion (Fernandez-Lopez et al., 2012). There was essentially no neutrophil infiltration 1–24 h after reperfusion and only a limited number of marginated neutrophils within previously occluded regions (Fernandez-Lopez et al., 2012). While post-C was not used in the latter study, the lack of neutrophil infiltration and only modest BBB leakage in neonatal tMCAO (Fernandez-Lopez et al., 2012; Fernandez-Lopez et al., 2016), as opposed to the presence of marked BBB disruption and apparent neutrophil margination that clog the vessels following tMCAO in adult brain, indirectly point to the importance of brain immaturity in the cerebrovascular responses that can affect efficacy of conditioning efforts in neonatal stroke.

### **Summary: progress and limitations in the understanding of beneficial effects of pre-C and post-C in the immature brain**

In this review we focused on experimental models of pre-C and post-C that are most commonly applied, models that utilize brief hypoxic or ischemic exposures, immune conditioning or conditioning with pharmacological agents such as EPO, magnesium or anesthetic agents. We also discussed mechanistic insights at the intracellular levels, with emphasis on common and distinct model-specific mechanisms. Yet, the magnitude of conditioning-induced benefits and specifics in the immature brain are far from being well understood. One important lesson learned from these studies is that because of the immaturity of the brain, information can't be easily transferred from the adult field. While the general conceptual framework is similar regardless of age—the same intracellular mechanisms can be involved in pre-C and post-C and the time interval between insults is important in determining the pathological outcome—the dynamic changes that occur *in utero*, in the early postnatal brain, and in immune system development create a delicate balance on the timing of sublethal exposure on the systemic, cell-to-cell and intracellular responses that can render the brain either more or less vulnerable to the severe insult. The field is evolving and other models of conditioning for the immature brain are being tested, namely hypobaric and hyperbaric hypoxia (Gamdzyk et al., 2016; Hentia et al., 2018), corticosteroids, resveratrol, and stem cells. Models where cells are conditioned prior to injection are also being developed (Sandvig et al., 2017). Overcoming some of the caveats should enhance our understanding. For example, the majority of rodent pre-C studies were performed in P7 rats, a rodent age that is no longer thought to correspond to brain development of at-term human infants, but rather to correspond to human brain at 32–36 gestation weeks (Semple et al., 2013). The HI injury manifests itself not in exactly the same ways in P7 and P10 rodents (Patel et al., 2015). Thus, there is a gap in knowledge of conditioning effects at-term. Another caveat is insufficient understanding of whether conditioning mechanisms can be expected to be similarly efficacious after arterial stroke as they are after HI. Literature on sex differences in pre-C and post-C effects in immature brain is essentially non-existent. Moving forward, while understanding of pre-C is important, post-C might be a more valuable strategy given the unknowns of the timing of insult in newborn

humans and, as we discussed, that interventions that are effective as pre-C might not be effective or may even be damaging in the immature brain. Finally, identification of biomarkers of conditioning efficacy could make a difference in translating the findings from animal studies to humans.

## Acknowledgements

We gratefully acknowledge the support from ERA-net (EU;VR 529-2014-7551), Wellcome Trust (WT094823), Swedish Medical Research Council (VR 2015-02493, HH; VR-2017-01409, CM), Brain Foundation (HH, CM), Åhlen Foundation (HH, CM), ALF-GBG (426401, HH; 722491, CM), and the Leducq Foundation (DSRRP34404), Torsten Söderberg (M98/15, CM), RO1 NS44025 (ZSV), RO1 NS76726 (ZSV), R21NS098514 (ZSV), R01 HL139685 (ZSV, CM), R01 NS103483 (ZSV, CM), AHA17IRG33430004 (ZSV), CP Alliance PG0816 (ZSV).

## References

- Adstamongkonkul D and Hess DC (2017) Ischemic conditioning and neonatal hypoxic ischemic encephalopathy: a literature review. *Conditioning Medicine* 1:9–16. [PubMed: 30215057]
- Alam A, Suen KC, Hana Z, Sanders RD, Maze M and Ma D (2017) Neuroprotection and neurotoxicity in the developing brain: an update on the effects of dexmedetomidine and xenon. *Neurotoxicol Teratol* 60:102–116. [PubMed: 28065636]
- Alderliesten T, Favie LM, Neijzen RW, Auwarter V, Nijboer CH, Marges RE, Rademaker CM, Kempf J, van Bel F and Groenendaal F (2014) Neuroprotection by argon ventilation after perinatal asphyxia: a safety study in newborn piglets. *PLoS One* 9:e113575. [PubMed: 25460166]
- Amer AR and Oorschot DE (2018) Xenon Combined With Hypothermia in Perinatal Hypoxic-Ischemic Encephalopathy: A Noble Gas, a Noble Mission. *Pediatr Neurol* 84:5–10. [PubMed: 29887039]
- Aronowski J, Strong R and Grotta JC (1997) Reperfusion injury: demonstration of brain damage produced by reperfusion after transient focal ischemia in rats. *J Cereb Blood Flow Metab* 17:1048–1056. [PubMed: 9346429]
- Ashrafi G and Schwarz TL (2013) The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ* 20:31–42. [PubMed: 22743996]
- Azzopardi D, Robertson NJ, Bainbridge A, Cady E, Charles-Edwards G, Deierl A, Fagiolo G, Franks NP, Griffiths J, Hajnal J, Juszczak E, Kapetanakis B, Linsell L, Maze M, Omar O, Strohm B, Tusor N and Edwards AD (2016) Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): a proof-of-concept, open-label, randomised controlled trial. *Lancet Neurol* 15:145–153. [PubMed: 26708675]
- Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL, Guo Y, Bolli R, Cardwell EM and Ping P (2003) Protein kinase Cepsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. *Circ Res* 92:873–880. [PubMed: 12663490]
- Bajgar R, Seetharaman S, Kowaltowski AJ, Garlid KD and Paucek P (2001) Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. *J Biol Chem* 276:33369–33374. [PubMed: 11441006]
- Banks P, Franks NP and Dickinson R (2010) Competitive inhibition at the glycine site of the N-methyl-D-aspartate receptor mediates xenon neuroprotection against hypoxia-ischemia. *Anesthesiology* 112:614–622. [PubMed: 20124979]
- Barks JD, Post M and Tuor UI (1991) Dexamethasone prevents hypoxic-ischemic brain damage in the neonatal rat. *Pediatr Res* 29:558–563. [PubMed: 1866211]
- Barone FC, White RF, Spera PA, Ellison J, Currie RW, Wang X and Feuerstein GZ (1998) Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. *Stroke* 29:1937–1950; discussion 1950–1931. [PubMed: 9731622]
- Bednarczyk P (2009) Potassium channels in brain mitochondria. *Acta Biochim Pol* 56:385–392. [PubMed: 19759922]



- Bergeron M, Gidday JM, Yu AY, Semenza GL, Ferriero DM and Sharp FR (2000) Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann Neurol* 48:285–296. [PubMed: 10976634]
- Bernaudin M, Tang Y, Reilly M, Petit E and Sharp FR (2002) Brain genomic response following hypoxia and re-oxygenation in the neonatal rat. Identification of genes that might contribute to hypoxia-induced ischemic tolerance. *J Biol Chem* 277:39728–39738. [PubMed: 12145288]
- Boengler K, Lochnit G and Schulz R (2018) Mitochondria “THE” target of myocardial conditioning. *Am J Physiol Heart Circ Physiol*.
- Bolouri H, Savman K, Wang W, Thomas A, Maurer N, Dullaghan E, Fjell CD, Ek CJ, Hagberg H, Hancock RE, Brown KL and Mallard C (2014) Innate defense regulator peptide 1018 protects against perinatal brain injury. *Ann Neurol* 75:395–410. [PubMed: 24339166]
- Bordet R, Deplanque D, Maboudou P, Puisieux F, Pu Q, Robin E, Martin A, Bastide M, Leys D, Lhermitte M and Dupuis B (2000). Increase in endogenous brain superoxide dismutase as a potential mechanism of lipopolysaccharide-induced brain ischemic tolerance. *J Cereb Blood Flow Metab* 20:1190–1196. [PubMed: 10950379]
- Bright HR, Babata K, Allred EN, Erdei C, Kuban KCK, Joseph RM, O’Shea TM, Leviton A, Dammann O and Investigators ES (2017) Neurocognitive Outcomes at 10 Years of Age in Extremely Preterm Newborns with Late-Onset Bacteremia. *J Pediatr* 187:43–49 e41. [PubMed: 28526224]
- Brochu ME, Girard S, Lavoie K and Sebire G (2011) Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: An experimental study. *J Neuroinflammation* 8:55. [PubMed: 21599903]
- Brucklacher RM, Vannucci RC and Vannucci SJ (2002) Hypoxic preconditioning increases brain glycogen and delays energy depletion from hypoxia-ischemia in the immature rat. *Dev Neurosci* 24:411–417. [PubMed: 12640180]
- Brywe KG, Mallard C, Gustavsson M, Hedtjarn M, Leverin AL, Wang X, Blomgren K, Isgaard J and Hagberg H (2005) IGF-I neuroprotection in the immature brain after hypoxia-ischemia, involvement of Akt and GSK3beta? *Eur J Neurosci* 21:1489–1502. [PubMed: 15845077]
- Busija DW, Katakam P, Rajapakse NC, Kis B, Grover G, Domoki F and Bari F (2005) Effects of ATP-sensitive potassium channel activators diazoxide and BMS-191095 on membrane potential and reactive oxygen species production in isolated piglet mitochondria. *Brain Res Bull* 66:85–90. [PubMed: 15982523]
- Cattano D, Valleggi S, Ma D, Kastsuchenka O, Abramo A, Sun P, Cavazzana AO, Natale G, Maze M and Giunta F (2008) Xenon induces transcription of ADNP in neonatal rat brain. *Neurosci Lett* 440:217–221. [PubMed: 18571851]
- Chakkarapani E, Dingley J, Liu X, Hoque N, Aquilina K, Porter H and Thoresen M (2010) Xenon enhances hypothermic neuroprotection in asphyxiated newborn pigs. *Ann Neurol* 68:330–341. [PubMed: 20658563]
- Charriaut-Marlangue C, Bonnin P, Gharib A, Leger PL, Villapol S, Pocard M, Gressens P, Renolleau S and Baud O (2012) Inhaled nitric oxide reduces brain damage by collateral recruitment in a neonatal stroke model. *Stroke* 43:3078–3084. [PubMed: 22949477]
- Chen J and Simon R (1997) Ischemic tolerance in the brain. *Neurology* 48:306–311. [PubMed: 9040711]
- Davis W, Mahale S, Carranza A, Cox B, Hayes K, Jimenez D and Ding Y (2007) Exercise preconditioning ameliorates blood-brain barrier dysfunction in stroke by enhancing basal lamina. *Neurol Res* 29:382–387. [PubMed: 17626734]
- Dawson DA, Furuya K, Gotoh J, Nakao Y and Hallenbeck JM (1999) Cerebrovascular hemodynamics and ischemic tolerance: lipopolysaccharide-induced resistance to focal cerebral ischemia is not due to changes in severity of the initial ischemic insult, but is associated with preservation of microvascular perfusion. *J Cereb Blood Flow Metab* 19:616–623. [PubMed: 10366191]
- Dean JM, van de Looij Y, Sizonenko SV, Lodygensky GA, Lazeyras F, Bolouri H, Kjellmer I, Huppi PS, Hagberg H and Mallard C (2011) Delayed cortical impairment following lipopolysaccharide exposure in preterm fetal sheep. *Ann Neurol* 70:846–856. [PubMed: 22002627]

- Delobel-Ayoub M, Arnaud C, White-Koning M, Casper C, Pierrat V, Garel M, Burguet A, Roze JC, Matis J, Picaud JC, Kaminski M, Larroque B and Group ES (2009) Behavioral problems and cognitive performance at 5 years of age after very preterm birth: the EPIPAGE Study. *Pediatrics* 123:1485–1492. [PubMed: 19482758]
- Denker S, Ji S, Lee SY, Dingman A, Derugin N, Wendland M and Vexler ZS (2007) Macrophages are Comprised of Resident Brain Microglia not Infiltrating Peripheral Monocytes Acutely after Neonatal Stroke. *J Neurochem* 100:893–904. [PubMed: 17212701]
- Dingley J, Tooley J, Liu X, Scull-Brown E, Elstad M, Chakkarapani E, Sabir H and Thoresen M (2014) Xenon ventilation during therapeutic hypothermia in neonatal encephalopathy: a feasibility study. *Pediatrics* 133:809–818. [PubMed: 24777219]
- Dingley J, Tooley J, Porter H and Thoresen M (2006) Xenon provides short-term neuroprotection in neonatal rats when administered after hypoxia-ischemia. *Stroke* 37:501–506. [PubMed: 16373643]
- Dirnagl U and Meisel A (2008) Endogenous neuroprotection: mitochondria as gateways to cerebral preconditioning? *Neuropharmacology* 55:334–344. [PubMed: 18402985]
- Dirnagl U, Simon RP and Hallenbeck JM (2003) Ischemic tolerance and endogenous neuroprotection. *Trends Neurosci* 26:248–254. [PubMed: 12744841]
- Du X, Fleiss B, Li H, D'Angelo B, Sun Y, Zhu C, Hagberg H, Levy O, Mallard C and Wang X (2011) Systemic Stimulation of TLR2 Impairs Neonatal Mouse Brain Development. *PLoS One* 6:e19583. [PubMed: 21573120]
- Duncan JR, Cock ML, Scheerlinck JP, Westcott KT, McLean C, Harding R and Rees SM (2002) White matter injury after repeated endotoxin exposure in the preterm ovine fetus. *Pediatr Res* 52:941–949. [PubMed: 12438674]
- Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, Strohm B, Thoresen M, Whitelaw A and Azzopardi D (2010) Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 340:c363. [PubMed: 20144981]
- Ek CJ, D'Angelo B, Baburamani AA, Lehner C, Leverin AL, Smith PL, Nilsson H, Svedin P, Hagberg H and Mallard C (2015) Brain barrier properties and cerebral blood flow in neonatal mice exposed to cerebral hypoxia-ischemia. *J Cereb Blood Flow Metab* 35:818827.
- Eklind S, Hagberg H, Wang X, Savman K, Leverin AL, Hedtjarn M and Mallard C (2006) Effect of lipopolysaccharide on global gene expression in the immature rat brain. *Pediatr Res* 60:161–168. [PubMed: 16864697]
- Eklind S, Mallard C, Arvidsson P and Hagberg H (2005) Lipopolysaccharide Induces Both a Primary and a Secondary Phase of Sensitization in the Developing Rat Brain. *Pediatr Res* 58:112–116. [PubMed: 15879289]
- Eklind S, Mallard C, Leverin AL, Gilland E, Blomgren K, Mattsby-Baltzer I and Hagberg H (2001) Bacterial endotoxin sensitizes the immature brain to hypoxic--ischaemic injury. *Eur J Neurosci* 13:1101–1106. [PubMed: 11285007]
- Espósito E, Hayakawa K, Ahn BJ, Chan SJ, Xing C, Liang AC, Kim KW, Arai K and Lo EH (2018) Effects of ischemic post-conditioning on neuronal VEGF regulation and microglial polarization in a rat model of focal cerebral ischemia. *J Neurochem* 146:160–172. [PubMed: 29570780]
- Ezzati M, Bainbridge A, Broad KD, Kawano G, Oliver-Taylor A, Rocha-Ferreira E, Alonso-Alconada D, Fierens I, Rostami J, Jane Hassell K, Tachtsidis I, Gressens P, Hristova M, Bennett K, Lebon S, Fleiss B, Yellon D, Hausenloy DJ, Golay X and Robertson NJ (2016) Immediate remote ischemic postconditioning after hypoxia ischemia in piglets protects cerebral white matter but not grey matter. *J Cereb Blood Flow Metab* 36:1396–1411. [PubMed: 26661194]
- Falck M, Osredkar D, Maes E, Flatebo T, Wood TR, Sabir H and Thoresen M (2017) Hypothermic neuronal rescue from infection-sensitized hypoxic-ischaemic brain injury Is pathogen dependent. *Dev Neurosci* 39:238–247. [PubMed: 28407632]
- Faustino J, Wang X, Jonhson C, Klibanov A, Derugin N, Wendland M and Vexler ZS (2011) Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. *J Neurosci* 31:12992–13001. [PubMed: 21900578]
- Feng Y, Rhodes PG and Bhatt AJ (2010) Hypoxic preconditioning provides neuroprotection and increases vascular endothelial growth factor A, preserves the phosphorylation of Akt-Ser-473 and

- diminishes the increase in caspase-3 activity in neonatal rat hypoxic-ischemic model. *Brain Res* 1325:1–9. [PubMed: 20153736]
- Fernandez-Lopez D, Faustino J, Daneman R, Zhou L, Lee SY, Derugin N, Wendland MF and Vexler ZS (2012) Blood-brain barrier permeability is increased after acute adult stroke but not neonatal stroke in the rat. *J Neurosci* 32:9588–9600. [PubMed: 22787045]
- Fernandez-Lopez D, Faustino J, Klibanov AL, Derugin N, Blanchard E, Simon F, Leib SL and Vexler ZS (2016) Microglial Cells Prevent Hemorrhage in Neonatal Focal Arterial Stroke. *J Neurosci* 36:2881–2893. [PubMed: 26961944]
- Fernandez-Lopez D, Natarajan N, Ashwal S and Vexler ZS (2014) Mechanisms of perinatal arterial ischemic stroke. *J Cereb Blood Flow Metab* 34 921–932. [PubMed: 24667913]
- Ferriero DM (2004) Neonatal brain injury. *N Engl J Med* 351:1985–1995. [PubMed: 15525724]
- Fiskum G, Danilov CA, Mehrabian Z, Bambrick LL, Kristian T, McKenna MC, Hopkins I, Richards EM and Rosenthal RE (2008) Postischemic oxidative stress promotes mitochondrial metabolic failure in neurons and astrocytes. *Ann N Y Acad Sci* 1147:129–138. [PubMed: 19076438]
- Franks JJ, Horn JL, Janicki PK and Singh G (1995) Halothane, isoflurane, xenon, and nitrous oxide inhibit calcium ATPase pump activity in rat brain synaptic plasma membranes. *Anesthesiology* 82:108–117. [PubMed: 7832292]
- Franks NP, Dickinson R, de Sousa SL, Hall AC and Lieb WR (1998) How does xenon produce anaesthesia? *Nature* 396:324. [PubMed: 9845069]
- Galindo R, Banks Greenberg M, Araki T, Sasaki Y, Mehta N, Milbrandt J and Holtzman DM (2017) NMNAT3 is protective against the effects of neonatal cerebral hypoxia-ischemia. *Ann Clin Transl Neurol* 4:722–738. [PubMed: 29046881]
- Gamzyk M, Malek M, Bratek E, Koks A, Kaminski K, Ziembowicz A and Salinska E (2016) Hyperbaric oxygen and hyperbaric air preconditioning induces ischemic tolerance to transient forebrain ischemia in the gerbil. *Brain Res* 1648:257–265. [PubMed: 27431936]
- Garcia-Bonilla L, Brea D, Benakis C, Lane DA, Murphy M, Moore J, Racchumi G, Jiang X, Iadecola C and Anrather J (2018) Endogenous Protection from Ischemic Brain Injury by Preconditioned Monocytes. *J Neurosci* 38:6722–6736. [PubMed: 29946039]
- Gidday JM (2006) Cerebral preconditioning and ischaemic tolerance. *Nat Rev Neurosci* 7:437–448. [PubMed: 16715053]
- Gidday JM, Fitzgibbons JC, Shah AR and Park TS (1994) Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat. *Neurosci Lett* 168:221–224. [PubMed: 8028780]
- Gidday JM, Shah AR, Maceren RG, Wang Q, Pelligrino DA, Holtzman DM and Park TS (1999) Nitric oxide mediates cerebral ischemic tolerance in a neonatal rat model of hypoxic preconditioning. *J Cereb Blood Flow Metab* 19:331–340. [PubMed: 10078885]
- Girard S, Kadhim H, Larouche A, Roy M, Gobeil F and Sebire G (2008) Pro-inflammatory disequilibrium of the IL-1 beta/IL-1ra ratio in an experimental model of perinatal brain damages induced by lipopolysaccharide and hypoxia-ischemia. *Cytokine* 43:54–62. [PubMed: 18511291]
- Guerra GG, Joffe AR, Seal R, Phillipos E, Wong M, Moez EK, Dinu IA, Duff JP, Ross D, Rebeyka I and Robertson CM (2017) Pilot randomized controlled trial on early and late remote ischemic preconditioning prior to complex cardiac surgery in young infants. *Paediatr Anaesth* 27:433–441. [PubMed: 28300357]
- Gustavsson M, Barmark G, Larsson J, Muren E and Ronne H (2008) Functional genomics of monensin sensitivity in yeast: implications for post-Golgi traffic and vacuolar H<sup>+</sup>-ATPase function. *Mol Genet Genomics* 280:233–248. [PubMed: 18612650]
- Gustavsson M, Mallard C, Vannucci SJ, Wilson MA, Johnston MV and Hagberg H (2007) Vascular response to hypoxic preconditioning in the immature brain. *J Cereb Blood Flow Metab* 27:928–938. [PubMed: 17033689]
- Gutsaeva DR, Carraway MS, Suliman HB, Demchenko IT, Shitara H, Yonekawa H and Piantadosi CA (2008) Transient hypoxia stimulates mitochondrial biogenesis in brain subcortex by a neuronal nitric oxide synthase-dependent mechanism. *J Neurosci* 28:2015–2024. [PubMed: 18305236]
- Hagberg H, David Edwards A and Groenendaal F (2016) Perinatal brain damage: The term infant. *Neurobiol Dis* 92:102–112. [PubMed: 26409031]

- Hagberg H, Mallard C, Ferriero D, Vannucci S, Levison S, Vexler Z and Gressens P (2015) The role of inflammation in perinatal brain injury. *Nat Rev Neurol* 11:192–208. [PubMed: 25686754]
- Hedtjarn M, Mallard C, Eklind S, Gustafson-Brywe K and Hagberg H (2004) Global gene expression in the immature brain after hypoxia-ischemia. *J Cereb Blood Flow Metab* 24:1317–1332. [PubMed: 15625407]
- Hedtjarn M, Mallard C and Hagberg H (2004) Inflammatory gene profiling in the developing mouse brain after hypoxia-ischemia. *J Cereb Blood Flow Metab* 24:1333–1351. [PubMed: 15625408]
- Hentia C, Rizzato A, Camporesi E, Yang Z, Muntean DM, Sandesc D and Bosco G (2018) An overview of protective strategies against ischemia/reperfusion injury: The role of hyperbaric oxygen preconditioning. *Brain Behav* 8:e00959. [PubMed: 29761012]
- Hess DC, Blauenfeldt RA, Andersen G, Hougaard KD, Hoda MN, Ding Y and Ji X (2015) Remote ischaemic conditioning—a new paradigm of self-protection in the brain. *Nat Rev Neurol* 11:698–710. [PubMed: 26585977]
- Hess DC, Hoda MN and Bhatia K (2013) Remote limb preconditioning [corrected] and postconditioning: will it translate into a promising treatment for acute stroke? *Stroke* 44:1191–1197. [PubMed: 23339961]
- Hess DC, Hoda MN and Khan MB (2016) Humoral Mediators of Remote Ischemic Conditioning: Important Role of eNOS/NO/Nitrite. *Acta Neurochir Suppl* 121:45–48. [PubMed: 26463921]
- Hillion JA, Li Y, Maric D, Takanohashi A, Klimanis D, Barker JL and Hallenbeck JM (2006) Involvement of Akt in preconditioning-induced tolerance to ischemia in PC12 cells. *J Cereb Blood Flow Metab* 26:1323–1331. [PubMed: 16511503]
- Hoda MN, Bhatia K, Hafez SS, Johnson MH, Siddiqui S, Ergul A, Zaidi SK, Fagan SC and Hess DC (2014) Remote ischemic preconditioning is effective after embolic stroke in ovariectomized female mice. *Transl Stroke Res* 5:484–490. [PubMed: 24385308]
- Hoda MN, Siddiqui S, Herberg S, Periyasamy-Thandavan S, Bhatia K, Hafez SS, Johnson MH, Hill WD, Ergul A, Fagan SC and Hess DC (2012) Remote ischemic preconditioning is effective alone and in combination with intravenous tissue-type plasminogen activator in murine model of embolic stroke. *Stroke* 43:2794–2799. [PubMed: 22910893]
- Hu BR, Liu CL, Ouyang Y, Blomgren K and Siesjo BK (2000) Involvement of caspase-3 in cell death after hypoxia-ischemia declines during brain maturation. *J Cereb Blood Flow Metab* 20:1294–1300. [PubMed: 10994850]
- Huang C, Andres AM, Ratliff EP, Hernandez G, Lee P and Gottlieb RA (2011) Preconditioning involves selective mitophagy mediated by Parkin and p62/SQSTM1. *PLoS One* 6:e20975. [PubMed: 21687634]
- Ikedo T, Mishima K, Aoo N, Egashira N, Iwasaki K, Fujiwara M and Ikenoue T (2004) Combination treatment of neonatal rats with hypoxia-ischemia and endotoxin induces long-lasting memory and learning impairment that is associated with extended cerebral damage. *Am J Obstet Gynecol* 191:2132–2141. [PubMed: 15592303]
- Ikedo T, Xia XY, Xia YX and Ikenoue T (1999) Hyperthermic preconditioning prevents blood-brain barrier disruption produced by hypoxia-ischemia in newborn rat. *Brain Res Dev Brain Res* 117:53–58. [PubMed: 10536232]
- Ikedo T, Yang L, Ikenoue T, Mallard C and Hagberg H (2006) Endotoxin-induced hypoxic-ischemic tolerance is mediated by up-regulation of corticosterone in neonatal rat. *Pediatr Res* 59:56–60. [PubMed: 16327010]
- Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovskva V, Turski L and Olney JW (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* 283:70–74. [PubMed: 9872743]
- Ikonomidou C, Mosinger JL, Salles KS, Labruyere J and Olney JW (1989) Sensitivity of the developing rat brain to hypobaric/ischemic damage parallels sensitivity to N-methyl-aspartate neurotoxicity. *J Neurosci* 9:2809–2818. [PubMed: 2671294]
- Janoff A (1964) Alterations in Lysosomes (Intracellular Enzymes) during Shock; Effects of Preconditioning (Tolerance) and Protective Drugs. *Int Anesthesiol Clin* 2:251–269. [PubMed: 14125012]

- Jones NM and Bergeron M (2001) Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. *J Cereb Blood Flow Metab* 21:1105–1114. [PubMed: 11524615]
- Joo SP, Xie W, Xiong X, Xu B and Zhao H (2013) Ischemic postconditioning protects against focal cerebral ischemia by inhibiting brain inflammation while attenuating peripheral lymphopenia in mice. *Neuroscience* 243:149–157. [PubMed: 23590905]
- Kavas N, Arisoy AE, Bayhan A, Kara B, Gunlemez A, Turker G, Oruc M and Gokalp AS (2017). Neonatal sepsis and simple minor neurological dysfunction. *Pediatr Int* 59:564–569. [PubMed: 27935218]
- Khan MB, Hafez S, Hoda MN, Baban B, Wagner J, Awad ME, Sangabathula H, Haigh S, Elsalanty M, Waller JL and Hess DC (2018) Chronic Remote Ischemic Conditioning Is Cerebroprotective and Induces Vascular Remodeling in a VCID Model. *Transl Stroke Res* 9:51–63. [PubMed: 28755277]
- Khan MB, Hoda MN, Vaibhav K, Giri S, Wang P, Waller JL, Ergul A, Dhandapani KM, Fagan SC and Hess DC (2015) Remote ischemic postconditioning: harnessing endogenous protection in a murine model of vascular cognitive impairment. *Transl Stroke Res* 6:69–77. [PubMed: 25351177]
- Koning G, Leverin AL, Nair S, Schwendimann L, Ek J, Carlsson Y, Gressens P, Thornton C, Wang X, Mallard C and Hagberg H (2017) Magnesium induces preconditioning of the neonatal brain via profound mitochondrial protection. *J Cereb Blood Flow Metab*: 271678X17746132.
- Koning G, Lyngfelt E, Svedin P, Leverin AL, Jinnai M, Gressens P, Thornton C, Wang X, Mallard C and Hagberg H (2018) Magnesium sulphate induces preconditioning in preterm rodent models of cerebral hypoxia-ischemia. *Int J Dev Neurosci*. S0736–5748(17)30324–6.
- Kratzer I, Chip S and Vexler ZS (2014) Barrier mechanisms in neonatal stroke. *Front Neurosci* 8:359. [PubMed: 25426016]
- Lai Z, Zhang L, Su J, Cai D and Xu Q (2016) Sevoflurane postconditioning improves long-term learning and memory of neonatal hypoxia-ischemia brain damage rats via the PI3K/Akt-mPTP pathway. *Brain Res* 1630:25–37. [PubMed: 26541582]
- Lalancette-Hebert M, Faustino J, Thammisetty SS, Chip S, Vexler ZS and Kriz J (2017) Live imaging of innate immune response in neonates reveals differential TLR-2 dependent activation patterns in sterile inflammation and infection. *Brain Behav Immun* 65:312–327. [PubMed: 28579520]
- Laudenbach V, Fontaine RH, Medja F, Carmeliet P, Hicklin DJ, Gallego J, Leroux P, Marret S and Gressens P (2007) Neonatal hypoxic preconditioning involves vascular endothelial growth factor. *Neurobiol Dis* 26:243–252. [PubMed: 17306552]
- Lee HT, Chang YC, Wang LY, Wang ST, Huang CC and Ho CJ (2004) cAMP response element-binding protein activation in ligation preconditioning in neonatal brain. *Ann Neurol* 56:611–623. [PubMed: 15470752]
- Leger PL, Bonnin P, Nguyen T, Renolleau S, Baud O and Charriaut-Marlangue C (2012) Ischemic postconditioning fails to protect against neonatal cerebral stroke. *PLoS One* 7:e49695. [PubMed: 23251348]
- Li Q, Lian C, Zhou R, Li T, Xiang X and Liu B (2013) Pretreatment with xenon protected immature rabbit heart from ischaemia/reperfusion injury by opening of the mitoKATP channel. *Heart Lung Circ* 22:276–283. [PubMed: 23261327]
- Lin HY, Wu CL and Huang CC (2010) The Aktendothelial nitric oxide synthase pathway in lipopolysaccharide preconditioning-induced hypoxicischemic tolerance in the neonatal rat brain. *Stroke* 41:1543–1551. [PubMed: 20508195]
- Lin WY, Chang YC, Ho CJ and Huang CC (2013) Ischemic preconditioning reduces neurovascular damage after hypoxia-ischemia via the cellular inhibitor of apoptosis 1 in neonatal brain. *Stroke* 44:162–169. [PubMed: 23192759]
- Liu D, Lu C, Wan R, Auyeung WW and Mattson MP (2002) Activation of mitochondrial ATP-dependent potassium channels protects neurons against ischemia-induced death by a mechanism involving suppression of Bax translocation and cytochrome c release. *J Cereb Blood Flow Metab* 22:431–443. [PubMed: 11919514]
- Liu J, Narasimhan P, Yu F and Chan PH (2005) Neuroprotection by hypoxic preconditioning involves oxidative stress-mediated expression of hypoxia-inducible factor and erythropoietin. *Stroke* 36:1264–1269. [PubMed: 15890996]

- Lizama BN, Palubinsky AM, Raveendran VA, Moore AM, Federspiel JD, Codreanu SG, Liebler DC and McLaughlin B (2018) Neuronal Preconditioning Requires the Mitophagic Activity of C-terminus of HSC70-Interacting Protein. *J Neurosci*. pii: 0699–18. doi: 10.1523/JNEUROSCI.0699-18.2018.
- Lopez MM and Kosk-Kosicka D (1995) How do volatile anesthetics inhibit Ca<sup>2+</sup>-ATPases? *J Biol Chem* 270:28239–28245. [PubMed: 7499320]
- Lopez-Aguilera F, Plateo-Pignatari MG, Biaggio V, Ayala C and Seltzer AM (2012) Hypoxic preconditioning induces an AT2-R/VEGFR-2(Flk-1) interaction in the neonatal brain microvasculature for neuroprotection. *Neuroscience* 216:1–9. [PubMed: 22569153]
- Luo Y, Ma D, Ieong E, Sanders RD, Yu B, Hossain M and Maze M (2008) Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. *Anesthesiology* 109:782–789. [PubMed: 18946288]
- Lv J, Liang Y, Tu Y, Chen J and Xie Y (2018) Hypoxic preconditioning reduces propofol-induced neuroapoptosis via regulation of Bcl-2 and Bax and downregulation of activated caspase-3 in the hippocampus of neonatal rats. *Neurol Res*:1–7.
- Ma D, Hossain M, Pettet GK, Luo Y, Lim T, Akimov S, Sanders RD, Franks NP and Maze M (2006) Xenon preconditioning reduces brain damage from neonatal asphyxia in rats. *J Cereb Blood Flow Metab* 26:199–208. [PubMed: 16034370]
- Mallard C and Vexler ZS (2015) Modeling Ischemia in the Immature Brain: How Translational Are Animal Models? *Stroke* 46:3006–3011. [PubMed: 26272384]
- Mallard C, Wang X and Hagberg H (2009) The role of Tolllike receptors in perinatal brain injury. *Clin Perinatol* 36:763–772. [PubMed: 19944834]
- Mallard C, Welin AK, Peebles D, Hagberg H and Kjellmer I (2003) White matter injury following systemic endotoxemia or asphyxia in the fetal sheep. *Neurochem Res* 28:215–223. [PubMed: 12608695]
- Maurer U, Charvet C, Wagman AS, Dejardin E and Green DR (2006) Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. *Mol Cell* 21:749–760. [PubMed: 16543145]
- McCordle BW, Clarizia NA, Khaikin S, Holtby HM, Manlhiot C, Schwartz SM, Caldarone CA, Coles JG, Van Arsdell GS, Scherer SW and Redington AN (2014) Remote ischemic preconditioning in children undergoing cardiac surgery with cardiopulmonary bypass: a single-center double-blinded randomized trial. *J Am Heart Assoc* 3(4).
- Morris-Blanco KC, Cohan CH, Neumann JT, Sick TJ and Perez-Pinzon MA (2014) Protein kinase C epsilon regulates mitochondrial pools of Nampt and NAD following resveratrol and ischemic preconditioning in the rat cortex. *J Cereb Blood Flow Metab* 34:1024–1032. [PubMed: 24667915]
- Mottahedin A, Smith PL, Hagberg H, Ek CJ and Mallard C (2017) TLR2-mediated leukocyte trafficking to the developing brain. *J Leukoc Biol* 101:297–305. [PubMed: 27493242]
- Mu D, Chang YS, Vexler ZS and Ferriero DM (2005) Hypoxia-inducible factor 1alpha and erythropoietin upregulation with deferoxamine salvage after neonatal stroke. *Exp Neurol* 195:407–415. [PubMed: 16023639]
- Murry CE, Jennings RB and Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74:1124–1136. [PubMed: 3769170]
- Nakamura H, Katsumata T, Nishiyama Y, Otori T, Katsura K and Katayama Y (2006) Effect of ischemic preconditioning on cerebral blood flow after subsequent lethal ischemia in gerbils. *Life Sci* 78:1713–1719. [PubMed: 16253278]
- Nitsos I, Rees SM, Duncan J, Kramer BW, Harding R, Newnham JP and Moss TJ (2006) Chronic exposure to intra-amniotic lipopolysaccharide affects the ovine fetal brain. *J Soc Gynecol Investig* 13:239–247.
- Olney JW, Young C, Wozniak DF, Ikonomidou C and Jevtovic-Todorovic V (2004) Anesthesia-induced developmental neuroapoptosis. Does it happen in humans? *Anesthesiology* 101:273–275. [PubMed: 15277906]
- Pagel PS (2010) Cardioprotection by noble gases. *J Cardiothorac Vasc Anesth* 24:143–163. [PubMed: 19467886]

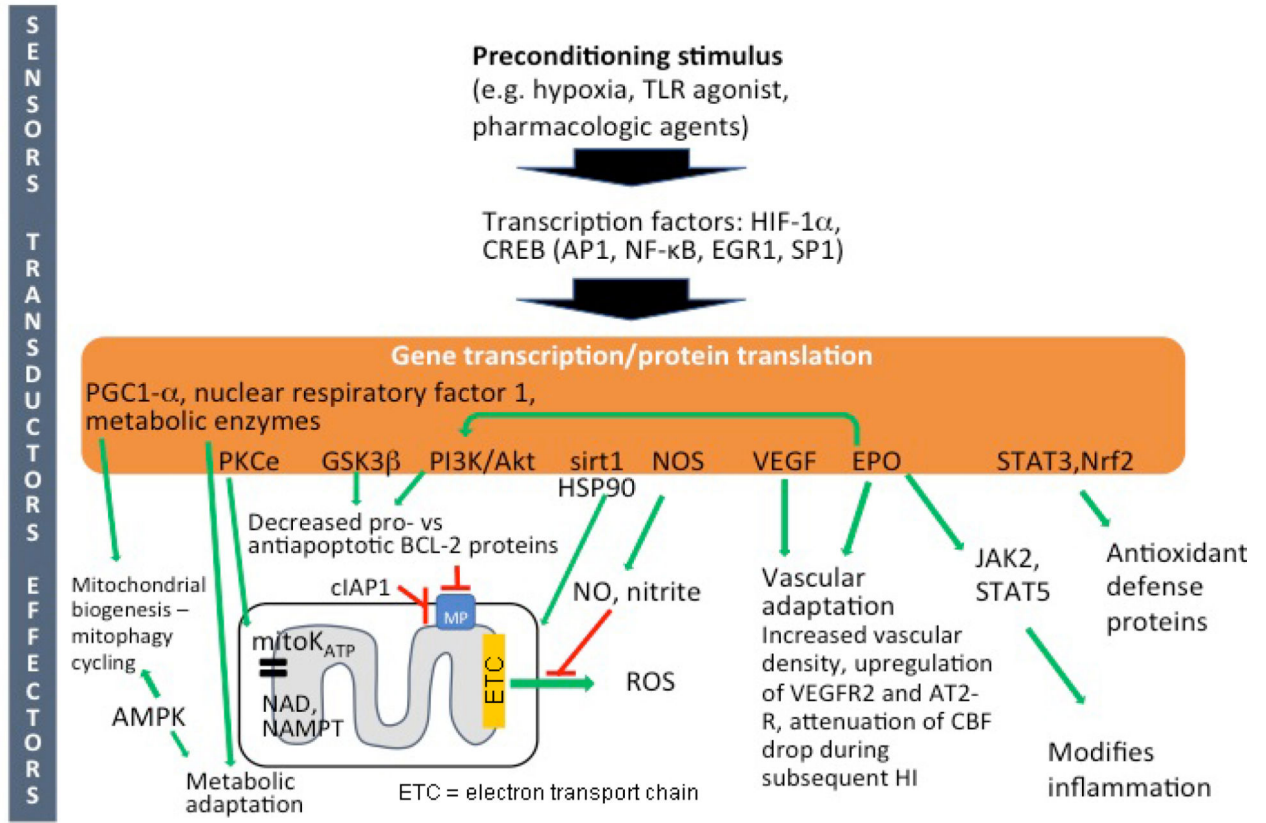
- Patel SD, Pierce L, Ciardiello A, Hutton A, Paskewitz S, Aronowitz E, Voss HU, Moore H and Vannucci SJ (2015) Therapeutic hypothermia and hypoxia-ischemia in the term-equivalent neonatal rat: characterization of a translational preclinical model. *Pediatr Res* 78:264–271. [PubMed: 25996893]
- Pignataro G, Cuomo O, Esposito E, Sirabella R, Di Renzo G and Annunziato L (2011) ASIC1a contributes to neuroprotection elicited by ischemic preconditioning and postconditioning. *Int J Physiol Pathophysiol Pharmacol* 3:1–8. [PubMed: 21479097]
- Pignataro G, Esposito E, Cuomo O, Sirabella R, Boscia F, Guida N, Di Renzo G and Annunziato L (2011) The NCX3 isoform of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger contributes to neuroprotection elicited by ischemic postconditioning. *J Cereb Blood Flow Metab* 31:362–370. [PubMed: 20628398]
- Prass K, Scharff A, Ruscher K, Lowl D, Muselmann C, Victorov I, Kapinya K, Dirnagl U and Meisel A (2003) Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. *Stroke* 34:1981–1986. [PubMed: 12829864]
- Preckel B, Mullenheim J, Moloschavij A, Thamer V and Schlack W (2000) Xenon administration during early reperfusion reduces infarct size after regional ischemia in the rabbit heart *in vivo*. *Anesth Analg* 91:1327–1332. [PubMed: 11093973]
- Rajapakse N, Shimizu K, Kis B, Snipes J, Lacza Z and Busija D (2002) Activation of mitochondrial ATP-sensitive potassium channels prevents neuronal cell death after ischemia in neonatal rats. *Neurosci Lett* 327:208–212. [PubMed: 12113913]
- Rangarajan V and Juul SE (2014) Erythropoietin: emerging role of erythropoietin in neonatal neuroprotection. *Pediatr Neurol* 51:481–488. [PubMed: 25266611]
- Raval AP, Dave KR, DeFazio RA and Perez-Pinzon MA (2007) epsilonPKC phosphorylates the mitochondrial  $\text{K}^+$  (ATP) channel during induction of ischemic preconditioning in the rat hippocampus. *Brain Res* 1184:345–353. [PubMed: 17988655]
- Ren X, Ma H and Zuo Z (2016) Dexmedetomidine Postconditioning Reduces Brain Injury after Brain Hypoxia-Ischemia in Neonatal Rats. *J Neuroimmune Pharmacol* 11:238–247. [PubMed: 26932203]
- Riepe MW, Esclaire F, Kasischke K, Schreiber S, Nakase H, Kempfski O, Ludolph AC, Dirnagl U and Hugon J (1997) Increased hypoxic tolerance by chemical inhibition of oxidative phosphorylation: “chemical preconditioning”. *J Cereb Blood Flow Metab* 17:257–264. [PubMed: 9119898]
- Robertson CL, Bucci CJ and Fiskum G (2004) Mitochondrial response to calcium in the developing brain. *Brain Res Dev Brain Res* 151:141–148. [PubMed: 15246700]
- Rocha-Ferreira E, Rudge B, Hughes MP, Rahim AA, Hristova M and Robertson NJ (2016) Immediate Remote Ischemic Postconditioning Reduces Brain Nitrotyrosine Formation in a Piglet Asphyxia Model. *Oxid Med Cell Longev* 2016:5763743. [PubMed: 27379176]
- Rousset CI, Leiper FC, Kichev A, Gressens P, Carling D, Hagberg H and Thornton C (2015) A dual role for AMP-activated protein kinase (AMPK) during neonatal hypoxic-ischaemic brain injury in mice. *J Neurochem* 133:242–252. [PubMed: 25598140]
- Salas AA, Faye-Petersen OM, Sims B, Peralta-Carcelen M, Reilly SD, McGwin G Jr., Carlo WA and Ambalavanan N (2013) Histological characteristics of the fetal inflammatory response associated with neurodevelopmental impairment and death in extremely preterm infants. *J Pediatr* 163:652–657 e651–652. [PubMed: 23664630]
- Sandvig I, Gadjanski I, Vlaski-Lafarge M, Buzanska L, Loncaric D, Sarnowska A, Rodriguez L, Sandvig A and Ivanovic Z (2017) Strategies to Enhance Implantation and Survival of Stem Cells After Their Injection in Ischemic Neural Tissue. *Stem Cells Dev* 26:554–565. [PubMed: 28103744]
- Schifilliti D, Grasso G, Conti A and Fodale V (2010) Anaesthetic-related neuroprotection: intravenous or inhalational agents? *CNS Drugs* 24:893–907. [PubMed: 20932063]
- Schwiebert C, Huhn R, Heinen A, Weber NC, Hollmann MW, Schlack W and Preckel B (2010) Postconditioning by xenon and hypothermia in the rat heart *in vivo*. *Eur J Anaesthesiol* 27:734–739. [PubMed: 20051868]
- Semple BD, Blomgren K, Gimlin K, Ferriero DM and Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol* 106:107:1–16.

- Shankaran S, Laptook AR, Pappas A, McDonald SA, Das A, Tyson JE, Poindexter BB, Schibler K, Bell EF, Heyne RJ, Pedroza C, Bara R, Van Meurs KP, Grisby C, Huitema CM, Garg M, Ehrenkranz RA, Shepherd EG, Chalak LF, Hamrick SE, Khan AM, Reynolds AM, Laughon MM, Truog WE, Dysart KC, Carlo WA, Walsh MC, Watterberg KL, Higgins RD, Eunice H Kennedy Shriver National Institute of Child and N. Human Development Neonatal Research (2014) Effect of depth and duration of cooling on deaths in the NICU among neonates with hypoxic ischemic encephalopathy: a randomized clinical trial. *JAMA* 312:2629–2639. [PubMed: 25536254]
- Sharp FR and Bernaudin M (2004) HIF1 and oxygen sensing in the brain. *Nat Rev Neurosci* 5:437–448. [PubMed: 15152194]
- Shatrov JG, Birch SC, Lam LT, Quinlivan JA, McIntyre S and Mendz GL (2010) Chorioamnionitis and cerebral palsy: a meta-analysis. *Obstet Gynecol* 116:387–392. [PubMed: 20664400]
- Sheldon RA, Osredkar D, Lee CL, Jiang X, Mu D and Ferriero DM (2009) HIF-1 alpha-deficient mice have increased brain injury after neonatal hypoxia-ischemia. *Dev Neurosci* 31:452–458. [PubMed: 19672073]
- Shi Y, Jiang X, Zhang L, Pu H, Hu X, Zhang W, Cai W, Gao Y, Leak RK, Keep RF, Bennett MV and Chen J (2017) Endothelium-targeted overexpression of heat shock protein 27 ameliorates blood-brain barrier disruption after ischemic brain injury. *Proc Natl Acad Sci U S A* 114:E1243–E1252. [PubMed: 28137866]
- Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ and Gladwin MT (2007) Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J Exp Med* 204:2089–2102. [PubMed: 17682069]
- Shu Y, Patel SM, Pac-Soo C, Fidalgo AR, Wan Y, Maze M and Ma D (2010) Xenon pretreatment attenuates anesthetic-induced apoptosis in the developing brain in comparison with nitrous oxide and hypoxia. *Anesthesiology* 113:360–368. [PubMed: 20613483]
- Sisalli MJ, Annunziato L and Scorziello A (2015) Novel Cellular Mechanisms for Neuroprotection in Ischemic Preconditioning: A View from Inside Organelles. *Front Neurol* 6:115. [PubMed: 26074868]
- Smith PL, Hagberg H, Naylor AS and Mallard C (2014) Neonatal peripheral immune challenge activates microglia and inhibits neurogenesis in the developing murine hippocampus. *Dev Neurosci* 36:119–131. [PubMed: 24642725]
- Soraisham AS, Trevenen C, Wood S, Singhal N and Sauve R (2013) Histological chorioamnionitis and neurodevelopmental outcome in preterm infants. *J Perinatol* 33:70–75. [PubMed: 22555781]
- Stetler RA, Gao Y, Signore AP, Cao G and Chen J (2009) HSP27: mechanisms of cellular protection against neuronal injury. *Curr Mol Med* 9:863–872. [PubMed: 19860665]
- Stolp HB, Dziegielewska KM, Ek CJ, Potter AM and Saunders NR (2005) Long-term changes in blood-brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat. *Eur J Neurosci* 22:2805–2816. [PubMed: 16324115]
- Stolp HB, Johansson PA, Habgood MD, Dziegielewska KM, Saunders NR and Ek CJ (2011) Effects of neonatal systemic inflammation on blood-brain barrier permeability and behaviour in juvenile and adult rats. *Cardiovasc Psychiatry Neurol* 2011:469046. [PubMed: 21547250]
- Stowe AM, Altay T, Freie AB and Gidday JM (2011) Repetitive hypoxia extends endogenous neurovascular protection for stroke. *Ann Neurol* 69:975–985. [PubMed: 21437933]
- Stridh L, Mottahedin A, Johansson ME, Valdez RC, Northington F, Wang X and Mallard C (2013) Toll-like receptor-3 activation increases the vulnerability of the neonatal brain to hypoxia-ischemia. *J Neurosci* 33:12041–12051. [PubMed: 23864690]
- Stridh L, Smith PL, Naylor AS, Wang X and Mallard C (2011) Regulation of Toll-like receptor 1 and -2 in neonatal mice brains after hypoxia-ischemia. *J Neuroinflammation* 8:45. [PubMed: 21569241]
- Sun HS, Xu B, Chen W, Xiao A, Turlova E, Alibraham A, Barszczyk A, Bae CY, Quan Y, Liu B, Pei L, Sun CL, Deurloo M and Feng ZP (2015) Neuronal K(ATP) channels mediate hypoxic preconditioning and reduce subsequent neonatal hypoxic-ischemic brain injury. *Exp Neurol* 263:161–171. [PubMed: 25448006]



- Tasaki K, Ruetzler CA, Ohtsuki T, Martin D, Nawashiro H and Hallenbeck JM (1997) Lipopolysaccharide pretreatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. *Brain Res* 748:267–270. [PubMed: 9067475]
- Teo JD, Morris MJ and Jones NM (2015) Hypoxic postconditioning reduces microglial activation, astrocyte and caspase activity, and inflammatory markers after hypoxia-ischemia in the neonatal rat brain. *Pediatr Res* 77:757–764. [PubMed: 25751571]
- Thompson JW, Narayanan SV, Koronowski KB, Morris-Blanco K, Dave KR and Perez-Pinzon MA (2015) Signaling pathways leading to ischemic mitochondrial neuroprotection. *J Bioenerg Biomembr* 47:101–110. [PubMed: 25262285]
- Tropak MB, Shi H, Li J, Dai X, Redington AN and Askalan R (2011) Potent neuroprotection induced by remote preconditioning in a rat model of neonatal cerebral hypoxic-ischemic injury. *J Thorac Cardiovasc Surg* 142:233–235. [PubMed: 21555139]
- Vartanian KB, Stevens SL, Marsh BJ, Williams-Karnesky R, Lessov NS and Stenzel-Poore MP (2011) LPS preconditioning redirects TLR signaling following stroke: TRIF-IRF3 plays a seminal role in mediating tolerance to ischemic injury. *J Neuroinflammation* 8:140. [PubMed: 21999375]
- Wang JY, Shen J, Gao Q, Ye ZG, Yang SY, Liang HW, Bruce IC, Luo BY and Xia Q (2008) Ischemic postconditioning protects against global cerebral ischemia/ reperfusion-induced injury in rats. *Stroke* 39:983–990. [PubMed: 18239163]
- Wang L, Zhu QL, Wang GZ, Deng TZ, Chen R, Liu MH and Wang SW (2011) The protective roles of mitochondrial ATP-sensitive potassium channels during hypoxia-ischemia-reperfusion in brain. *Neurosci Lett* 491:63–67. [PubMed: 21215294]
- Wang X, Carlsson Y, Basso E, Zhu C, Rousset CI, Rasola A, Johansson BR, Blomgren K, Mallard C, Bernardi P, Forte MA and Hagberg H (2009) Developmental shift of cyclophilin D contribution to hypoxic-ischemic brain injury. *J Neurosci* 29:2588–2596. [PubMed: 19244535]
- Wang X, Hagberg H, Nie C, Zhu C, Ikeda T and Mallard C (2007) Dual role of intrauterine immune challenge on neonatal and adult brain vulnerability to hypoxia-ischemia. *J Neuropathol Exp Neurol* 66:552–561. [PubMed: 17549015]
- Wang X, Stridh L, Li W, Dean J, Elmgren A, Gan L, Eriksson K, Hagberg H and Mallard C (2009) Lipopolysaccharide sensitizes neonatal hypoxic-ischemic brain injury in a MyD88-dependent manner. *J Immunol* 183:7471–7477. [PubMed: 19917690]
- Wood NS, Marlow N, Costeloe K, Gibson AT and Wilkinson AR (2000) Neurologic and developmental disability after extremely preterm birth. EPICure Study Group. *N Engl J Med* 343:378–384. [PubMed: 10933736]
- Xu Y, Xue H, Zhao P, Yang Y, Ji G, Yu W, Han G, Ding M and Wang F (2016) Isoflurane postconditioning induces concentration- and timing-dependent neuroprotection partly mediated by the GluR2 AMPA receptor in neonatal rats after brain hypoxia-ischemia. *J Anesth* 30:427–436. [PubMed: 26810756]
- Yager JY and Ashwal S (2009) Animal models of perinatal hypoxic-ischemic brain damage. *Pediatr Neurol* 40:156–167. [PubMed: 19218028]
- Yang D, Nemkul N, Shereen A, Jone A, Dunn RS, Lawrence DA, Lindquist D and Kuan CY (2009) Therapeutic administration of plasminogen activator inhibitor-1 prevents hypoxic-ischemic brain injury in newborns. *J Neurosci* 29:8669–8674. [PubMed: 19587273]
- Yang D, Sun YY, Nemkul N, Baumann JM, Shereen A, Dunn RS, Wills-Karp M, Lawrence DA, Lindquist DM and Kuan CY (2013) Plasminogen activator inhibitor-1 mitigates brain injury in a rat model of infection-sensitized neonatal hypoxia-ischemia. *Cereb Cortex* 23:1218–1229. [PubMed: 22556277]
- Yin W, Signore AP, Iwai M, Cao G, Gao Y, Johnnides MJ, Hickey RW and Chen J (2007) Preconditioning suppresses inflammation in neonatal hypoxic ischemia via Akt activation. *Stroke* 38:1017–1024. [PubMed: 17272774]
- Zhang HX, Du GH and Zhang JT (2003) Ischemic pre-conditioning preserves brain mitochondrial functions during the middle cerebral artery occlusion in rat. *Neurol Res* 25:471–476. [PubMed: 12866194]

- Zhang W, Hu X, Yang W, Gao Y and Chen J (2010) Omega-3 polyunsaturated fatty acid supplementation confers long-term neuroprotection against neonatal hypoxic-ischemic brain injury through anti-inflammatory actions. *Stroke* 41:2341–2347. [PubMed: 20705927]
- Zhang W, Liu J, Hu X, Li P, Leak RK, Gao Y and Chen J (2015) n-3 Polyunsaturated Fatty Acids Reduce Neonatal Hypoxic/Ischemic Brain Injury by Promoting Phosphatidylserine Formation and Akt Signaling. *Stroke* 46:2943–2950. [PubMed: 26374481]
- Zhao H, Sapolsky RM and Steinberg GK (2006) Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats. *J Cereb Blood Flow Metab* 26:1114–1121. [PubMed: 16736038]
- Zhao H, Sapolsky RM and Steinberg GK (2006) Phosphoinositide-3-kinase/akt survival signal pathways are implicated in neuronal survival after stroke. *Mol Neurobiol* 34:249–270. [PubMed: 17308356]
- Zhao J, Qu Y, Wu J, Cao M, Ferriero DM, Zhang L and Mu D (2013) PTEN inhibition prevents rat cortical neuron injury after hypoxia-ischemia. *Neuroscience* 238:242–251. [PubMed: 23458710]
- Zhao P, Ji G, Xue H, Yu W, Zhao X, Ding M, Yang Y and Zuo Z (2014) Isoflurane postconditioning improved long-term neurological outcome possibly via inhibiting the mitochondrial permeability transition pore in neonatal rats after brain hypoxia-ischemia. *Neuroscience* 280:193–203. [PubMed: 25241064]
- Zhou Y, Fathali N, Lekic T, Ostrowski RP, Chen C, Martin RD, Tang J and Zhang JH (2011) Remote limb ischemic postconditioning protects against neonatal hypoxic-ischemic brain injury in rat pups by the opioid receptor/Akt pathway. *Stroke* 42:439–444. [PubMed: 21183744]
- Zhuang L, Yang T, Zhao H, Fidalgo AR, Vizcaychipi MP, Sanders RD, Yu B, Takata M, Johnson MR and Ma D (2012) The protective profile of argon, helium, and xenon in a model of neonatal asphyxia in rats. *Crit Care Med* 40:1724–1730. [PubMed: 22610177]



**Figure 1.** Intracellular mechanisms of preconditioning in neonatal brain. Abbreviations: Activator protein-1 (AP-1); AMP-activated protein kinase (AMPK); cellular inhibitor of apoptosis-1 (cIAP1); cyclic AMP response-element binding-protein (CREB); early growth response 1 (EGR1); erythropoietin (EPO); hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ); Janus kinase 2 (JAK2); mitochondrial permeabilization (MP); nicotinamide adenine dinucleotide (NAD); nicotinamide phosphoribosyltransferase (Namp); nitric oxide synthase (NOS); nitric oxide (NO); nuclear factor kappa B (NF $\kappa$ B); peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 $\alpha$ ); phosphokinase B (PKB)/AKT; phosphoinositide 3 kinase (PI3K); phosphokinase C (PKC); reactive oxygen species (ROS); signal transducer and activator of transcription (STATs) specificity protein 1 (SP1); vascular endothelial growth factor (VEGF).

Table 1.

Examples of conditioning paradigms in the developing brain.

conditioning stimulus	pre-/ post-remote, +/- conditioning	severe insult	model	outcome/remarks	reference
3 h of 8% hypoxia 24 h prior to insult	Pre +	3 h of HI	7-day-old rats	After 1 week no brain injury in pre-C group compared with 34% hemispheric loss in sham group.	Gidday et al., 1994
3 h of 8% hypoxia 24 h prior to insult	Pre +	75 min of HI	7-day-old rats	Performance in cylinder rearing test improved at 4, 6 & 8 weeks and in Morris water maze at 7 weeks in pre-C vs. sham; brain injury reduced by 72% in pre-C group.	Gustavsson et al., 2005
LPS (TLR4 ligand) 0.3 mg/kg 2 h, 4 h, 6 h or 72 h prior to HI	Pre -	10–50 min of HI	7–10-day-old rats	LPS given 2 h, 4 h, 6 h or 72 h prior to 20–50 min of HI in 7-, 8- or 10-day-old rats significantly increased brain injury (vs. saline) at 3 days or 2 weeks after HI.	Eklind et al., 2001; 2015
LPS 0.3 mg/kg 24 h prior to HI	Pre +	20 or 50 min of HI	8-day-old rats	LPS administered 24 h prior to 50 min (but not 20 min) of HI significantly reduced brain injury overall and in hippocampus, striatum and cortex 2 weeks after HI.	Eklind et al., 2015
PAM3CSK4 (TLR2 ligand) 5 mg/kg 14 h prior to HI	Pre -	50 min of HI	9-day-old mice	PAM3CSK4 increased grey (by 14%) and white (by 13.7%) matter injury 5 days after insult.	Motahedin et al., 2017
Poly I:C(TLR3 agonist) 10 mg/kg 14 h prior to HI	Pre -	50 min HI	9-day-old mice	Poly I:C increased (vs. saline) infarction 5 fold, total tissue loss by 90% and white matter loss 5-fold 5 days after HI.	Stridh et al., 2013
Limb ischemia (5 min, 3 times) immediately prior to HI	Remote, Pre +	65 min HI	14-day-old rats	Remote limb ischemia prior to HI reduced brain infarction by 71 % 7 days after HI.	Tropak et al., 2011
Bilateral Limb ischemia (10 min, 4 times) immediately after HI	Remote, Post +	Cerebral HI adjusted depending on ATP peak measured by <sup>31</sup> P MRS	1-day-old (<30 h) piglets (model of HIE)	Limb ischemia reduced the HI-Induced increase in white matter proton MRS lactate/N-acetyl aspartate and increased whole brain <sup>31</sup> P MRS ATP over 48 h after HI. Cell death and inflammation were reduced in several brain areas at 48 h.	Ezzati et al., 2016
Hypoxia (8% O <sub>2</sub> for 1 h/day for 5 d) 24 h after HI	Post +	3 h HI	7-day-old rats	Delayed hypoxic post-C reduced tissue loss, IL-10, astroglia and microglia activation 7 days after HI.	Teo et al., 2015
Dexamethasone (0.1 mg/kg) 24 h prior to HI	Pre +	3 h HI	7-day-old rats	A single dose of dexamethasone 24 h (but not 3 h or 0 h) prior to HI prevented the development of brain injury 7 days after HI.	Barks et al., 1991
Xenon (2 h of 70% xenon/30% O <sub>2</sub> ) 424p h prior to HI	Pre +	2 h HI	7-day-old rats	Xenon applied 4 h, 8 h or 24 h (but not 2 h) prior to HI substantially reduced brain injury 4 days after HI and motor and neurological coordination at 30 days.	Ma et al., 2006
MgSO <sub>4</sub> (1.1 mg/g) 12 h-6 days prior to HI	Pre +	1 h HI	7-day-old rats	MgSO <sub>4</sub> reduced brain injury in white and grey matter when given 12 h-6 days prior to HI but not 0 n, 3 h before or after HI. Brain injury was reduced by >80% when MgSO <sub>4</sub> was given 24 h prior to Insult.	Koning et al., 2017

Abbreviations: Hypoxia-ischemia (HI); hypoxic-ischemic encephalopathy (HIE); lipopolysaccharide (LPS); magnetic resonance spectroscopy (MRS); magnesium sulphate (MgSO<sub>4</sub>); negative conditioning (-); polyminosinic; polycyidylic acid (poly I:C); positive conditioning (+).