# Molecular, Cellular, and Behavioural Effects Produced by Perinatal Asphyxia: Protection by Poly (ADP-Ribose) Polymerase 1 (PARP-1) Inhibition

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## Contents



**Abstract**

Perinatal asphyxia implies oxygen interruption at birth, leading to death whenever reoxygenation is not promptly reestablished. Reoxygenation triggers a cascade of biochemical events for restoring function at the cost of improper

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homeostasis. The effects observed long after perinatal asphyxia have been explained by overexpression of sentinel proteins, such as poly(ADP-ribose) polymerase 1 (PARP-1), competing for $NAD^+$ during reoxygenation, leading to the idea that sentinel protein inhibition constitutes a suitable therapeutic strategy. Asphyxia also induces transcriptional activation of proinflammatory factors, including NFκB, and its subunit p65, whose translocation to the nucleus was found here, is significantly increased in brain tissue from asphyxia-exposed animals, in tandem with PARP-1 overactivation, suggesting that PARP-1 inhibition downregulates the expression of proinflammatory cytokines. Indeed, TNF-α and IL-1β were found to be increased 8 and 24 h after perinatal asphyxia in mesencephalon and hippocampus of rat neonates.

The possible neuroprotection effect of nicotinamide has been studied in an experimental model of global perinatal asphyxia in rats, inducing the insult by immersing rat fetuses into a water bath for various periods of time. Following asphyxia, the pups are delivered, immediately treated, or given to surrogate dams for nursing, pending further experiments. Systemic administration of nicotinamide was found to rapidly distribute into the brain reaching a steady-state concentration sufficient to inhibit PARP-1 activity for several hours. Nicotinamide prevented several of the long-term consequences elicited by perinatal asphyxia, supporting the idea that it constitutes a lead for exploring compounds with similar or better pharmacological profiles.

**Keywords**

Behavior · Development · Hypoxia · Neonatal · Obstetric complications · Plasticity · Poly(ADP-ribose) polymerase · Rats · Sentinel proteins

**Abbreviations**

| | |
|---|---|
| AIF | Apoptosis-inducing factor |
| AN | Nicotinamide-treated, asphyxia-exposed rats |
| AS | Asphyxia exposed, saline treated |
| ATP | Adenosine triphosphate |
| BAD | Bcl-2-associated death factor |
| BAX | Bcl-2-associated X factor |
| BBB | Blood–brain barrier |
| bFGF | Basic fibroblast growth factor |
| CNS | Central nervous system |
| COX-2 | Cyclooxygenase-2 |
| CREB | cAMP-response element-binding protein |
| CS | Caesarean delivered, saline treated |
| DG | Dentate gyros |
| DNMT1 | DNA(cytosine-5-)-methyl transferase 1 |
| DPQ | 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone |
| DR2313 | 2-methyl-3,5,7,8-tetrahydrothiopyranol[4,3-d]pyrimidine-4-one |

| | |
|---|---|
| E2F | Family of DNA-binding transcription factors |
| EPO | Erythropoietin |
| ERCC2 | Excision repair cross-complementing rodent repair group 2 |
| ERK | Extracellular signal-regulated kinases |
| FOXO | Subclass of forkhead O family of transcription factors |
| FR247304 | 5-Chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl) propyl]-4(3H)-quinazoline |
| GAPDH | Glyceraldehyde 3-phosphate dehydrogenase |
| HDAC | Histone deacetylases |
| HIF | Hypoxia-inducible factor |
| HRE | Hypoxia-responsive elements |
| ICAM-1 | Intercellular adhesion molecule-1 |
| IGF-1 | Insulin-like growth factor-1 |
| IL-1β | Interleukin-1β |
| iNOS | Inducible nitric oxide synthase |
| IQ | Intelligence quotient |
| IκB | Inhibitor of kappa B protein |
| Ku70 | Protein encoded by the XRCC1 gene, required for the non-homologous end joining pathway of DNA repair |
| LIG3 | DNA ligase 3 |
| LPS | Lipopolysaccharides |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NADH | Reduced nicotinamide adenine dinucleotide |
| NFκB | Nuclear factor-κB |
| ONO-1924H | *N*-3-(4-oxo-3,4-dihydrophthalazin-1-yl)phenyl-4-(morpholin-4-yl) butanamide methane sulfonate monohydrate |
| P | Postnatal day |
| p300 | Histone acetyltransferase p300 |
| p65 | Transcription factor p65 |
| PAR | Poly(ADP-ribose) polymers |
| PARG | Poly(ADP-ribose) glycohydrolase |
| PARPs | Poly(ADP-ribose) polymerases |
| PARylated PARP | Poly-ADP-ribosylated PARP |
| PBS | Phosphate-buffered saline |
| PCAF | P300/CBP-associated factor |
| PCr | Phosphocreatine |
| PHD | Prolyl hydroxylase domain |
| PJ34 | [*N*-(6-oxo-5,6-dihydrophenanthridin-2-yL)-*N*,*N*-dimethylacetamide.HCl] |
| POLB, DNA | Polymerase-β |
| ROS | Reactive oxygen species |
| SIRT | Sirtuin |
| SRY | Sex-determining region Y |
| Strep-HRP | Streptavidin-horseradish peroxidase |
| SVZ | Subventricular zone |

| | |
|---|---|
| TH | Tyrosine hydroxylase |
| TNF | Tumor necrosis factor |
| TUNEL | Terminal deoxynucleotidyl transferase dUTP nick end labelling |
| VEGF | Vascular endothelial factor |
| XRCC1 | X-ray cross-complementing factor 1 |

## 1 Introduction

Obstetric complications are important risk factors for several psychiatric and neurological disorders characterized by a delayed clinical onset. Hypoxia is a common factor of obstetric complications, priming brain development by mechanisms not yet established (Basovich, 2010).

Delay in starting pulmonary ventilation at birth implies decreased oxygen saturation in blood and decreased oxygen supply to the brain, which depends upon aerobic metabolism for generating energy and for maintaining the respiratory chain and mitochondrial ATPase activity. Hypoxia implies a switch to glycolysis, which for neurons is a poor metabolic alternative, because of low stores of glucose in brain tissue and deficient ATP output by the glycolysis pathway. Furthermore, glycolysis implies production of lactate, which is accumulated in extracellular compartments, causing acidosis, although there is evidence indicating that lactate can provide a significant source of energy to neurons (Wyss et al., 2011). Prolonged hypoxia further involves suppression of gene expression and translation, favoring the activation of genes for sustained survival, such as hypoxia-inducible factor (HIF) and its target genes (Iyer et al., 1998).

The incidence of perinatal asphyxia is still high, despite improvements of perinatal care (2–6/1,000 term births; De Hann et al., 2006), occurring with higher prevalence in developing countries (Lawn et al., 2010). After asphyxia, infants can develop long-term neurological sequelae, their severity depending upon the extent of the insult. Severe asphyxia has been linked to cerebral palsy, mental retardation, and epilepsy, while mild to severe asphyxia has been associated with attention deficits, hyperactivity, and schizophrenia. No strict correlation has yet been demonstrated between the clinical outcome and the severity and/or the extent of the insult, but the outcome and how the infants recover from the insult are related. In a cohort study monitoring approximately 6,000 children for 8 years, it was demonstrated that resuscitated infants, even if asymptomatic for encephalopathy, showed an increased risk for low IQ score (Odd et al., 2009). At present, there is no therapeutic strategy to significantly prevent the long-term effects produced by perinatal asphyxia, apart from hypothermia, which still is a controversial issue (Robertson et al., 2012).

## 2 Reoxygenation

While the average $pO_2$ in tissue is approximately 65 mmHg, it can decline below 5 mmHg following severe hypoxia. Reoxygenation is mandatory for restoration of function and survival. However, functional constraints of the cells affected by

reduced oxygen may be exacerbated during reoxygenation, implying oxidative stress and production of proinflammatory cytokines, delaying the onset of proper homeostasis and complete recovery (Hagberg et al., 1996; Shalak et al., 2002; Girard et al., 2009). Reoxygenation can even enhance protein synthesis over normal levels (see Fähling, 2009), including ribosomal biogenesis, leading to defects in protein synthesis fidelity, a mechanism associated to several neurodegenerative diseases (Lee et al., 2006). A number of proteins has to be increased upon reoxygenation, triggering (i) cell death, (ii) cell repairing (e.g., basic fibroblast growth factor, bFGF; VEGF, CREB-binding protein; ribosomal protein $S_{19}$; casein kinase 1), and (iii) *neuritogenesis* and *synaptogenesis*, required for maturation of neurocircuitries, also implying neurogenesis. Increased postnatal neurogenesis has been observed in subventricular zones (SVZ) and dentate gyrus (DG) of hippocampus in response to hypoxia/ischemia (Scheepens et al., 2003; Bartley et al., 2005).

The leading role in these early events following hypoxia is taken over by HIF-1α that is both an oxygen sensor and a transcription factor. After heterodimerization with HIF-1β, it binds to hypoxia-responsive elements (HRE) on promoters of genes like VEGF, iNOS, hemoxigenase-1, and EPO or to the oxygen sensors prolyl hydroxylase domain (PHD) proteins 2 and 3. HIF-1 also binds to other transcription factors, such as X-ray cross-complementing factor 1 (XRCC1) (Green et al., 1992), excision repair cross-complementing rodent repair group 2 (ERCC2) (Sung et al., 1993; Chiappe-Gutierrez et al., 1998; Lubec et al., 2002), and poly(ADP-ribose) polymerases (PARPs), mainly PARP-1 (Amé et al., 2004). The coordinated action of the products of these genes is believed to minimize and compensate for the damage induced by hypoxic conditions, acting on genes involved in glycolysis, oxygen transport, cell survival, and apoptosis (see Correia & Moreira, 2010).

## 3 Sentinel Proteins

Most of the adaptive changes elicited by hypoxia begin at the level of the DNA. Indeed, within minutes of cerebral hypoxia/ischemia, oxidative damage of DNA is observed in brain parenchyma, including oxidation of guanosine to 8-oxo-guanine and deamination of cytosine to uracil. A major repair mechanism to reconstitute damaged DNA is base excision repair, shown to be elevated following hypoxia/ischemia, implying activation of PARP-1, XRCC1, ERCC2, DNA polymerase-β (POLB), and DNA ligase (LIG3) (see Ellenberger & Tomkinson, 2008; Herrera-Marschitz et al., 2011).

PARPs, XRCC1, ERCC2, POLB, and LIG3 are rapidly activated whenever there is a risk of genome damage. These proteins play a pivotal role not only in repairing mechanisms but also in other environmentally induced DNA modifications. They can act as both cell survival- and cell death-inducing factors by regulation of DNA repair, chromatin remodelling, and regulation of transcription. PARP-1 overactivation may be elicited by P300/CBP-associated factor (PCAF), resulting in caspase-independent translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus and subsequent cell death by *chromatinolysis*. Indeed, PARP-1 activation is essential for AIF truncation and release from mitochondria

(Kolthur-Seetharam et al., 2006). PARP-1 can be acetylated for enhancing nuclear factor-κB (NFκB)-, FOXO- and Ku70-mediated transcription and for that of tumor suppressor protein p53 (Zampieri et al., 2009). Otherwise, PARP-1 can be deacetylated by proteins of the sirtuin (SIRT) family, histone deacetylases (HDAC) type III, resulting in its inactivation. Activated SIRT also blocks release of truncated AIF from mitochondria (Kolthur-Seetharam et al., 2006; Rajamohan et al., 2009).

PARP-1 activity is strictly controlled to maintain the balance between cell survival and cell death. The equilibrium between the antagonistic actions of SIRT and PARP-1 is maintained through their strict dependency from and competition for $NAD^+$ (Berger, 1985). Increased demand for $NAD^+$ by PARP-1 inhibits SIRT and vice versa. If both are overactive simultaneously, $NAD^+$ depletion generates large quantities of nicotinamide that has been shown to inhibit both SIRT and PARP activities (Rajamohan et al., 2009), probably by an end-product inhibitory mechanism. Thus, PARP and SIRT are at the core of epigenetic actions encompassing DNA and histone modifications.

## 4 Apoptosis

Apoptosis is the prevalent type of delayed cell death in the perinatal brain, mediated by caspase-dependent and caspase-independent mechanisms (Northington et al., 2001; see Morales et al., 2008). Indeed, pro-apoptotic proteins have been observed to be increased following perinatal asphyxia, including Bcl-2-associated X (BAX), and Bcl-2-associated death (BAD) factors, and also anti-apoptotic proteins, including Bcl-2, ERK2, and bFGF, suggesting the activation of neuroprotective and repair pathways (Morales et al., 2008). Extensive and regionally selective nuclear fragmentation has been observed in control and asphyctic rat pups, depending upon the stage of development and the analyzed brain region (Dell'Anna et al., 1997). Signs of apoptosis were observed in para- and presubiculum of both control and asphyxia-exposed animals, independently upon the severity of the insult, with the highest number of cells showing nuclear fragmentation observed at P1-2, decreasing thereafter, with no cells showing nuclear fragmentation at P8 (Fig. 1a). In contrast, no significant nuclear fragmentation was observed in neostriatum of control pups, but a progressive increase in the number of cells showing chromatin fragmentation was observed in asphyxia-exposed animals, with a maximum observed at P8 (Fig. 1b). Similar results were also observed in frontal cortex and cerebellum, increasing with the length of perinatal asphyxia (Dell'Anna et al., 1997).

The effect of perinatal asphyxia on apoptosis is heterogeneous even on a particular region. Indeed, when assayed with an *Apop Tag*$^R$ TUNEL kit (Millipore, Temecula, CA), the number of apoptotic cells was particularly increased in CA1 and CA3 regions of the hippocampus of asphyxia-exposed pups, compared to that from controls (Fig. 2a). A large increase was also observed in the retrosplenial granular cortex (Fig. 2b) (Neira-Peña et al. unpublished), a region connecting the hippocampus with the entorhinal cortex (Wyss & Van Groen, 1992). This is an exciting observation, because entorhinal cortex is a brain area consistently observed to be abnormal in

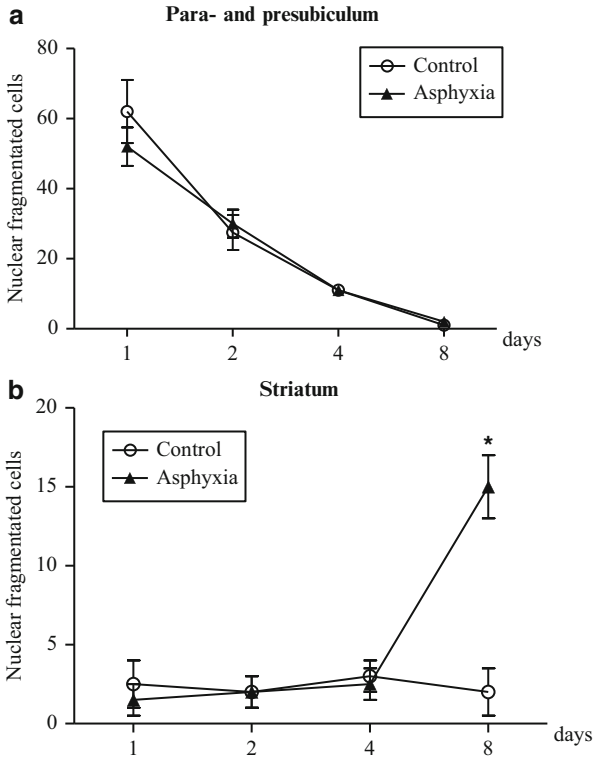


**Fig. 1** *Number of neurons showing nuclear fragmentation in para- and presubiculum* (**a**) *and neostriatum* (**b**) *of asphyxia-exposed and control rat pups 1–8 days after birth*. Brain sections (sagittal 20 μm serial formalin-fixed sections) were obtained from asphyxia-exposed (20–21 min of asphyxia) ($n = 5$–7 animals per group) (*filled triangles*) and the corresponding control ($n = 9$–16) (*open circles*) animals, 1–8 days after delivery. The sections were stained for hematoxylin-eosin and in situ DNA double-strand breaks and analyzed under light microscopy at 40× magnification to detect cellular alterations, using Foster atlas (Foster, 1998) for proper brain region identification (see Dell'Anna et al., 1997). Values are expressed as means ± SEM (*$p < 0.05$; Student's *t*-test)

psychiatric disorders associated to metabolic insults occurring at birth (Bachus et al., 1997). Indeed, entorhinal cortex (Akil et al., 2000) receives dopamine nerve terminals from mesencephalon (Hökfelt et al., 1974). In agreement, as shown in Fig. 2c, apoptosis was also increased in mesencephalon following perinatal asphyxia, both in substantia nigra and VTA (Neira-Peña et al. unpublished).

## 5 Neuroinflammation

Asphyxia can induce transcriptional activation of proinflammatory factors, including NFκB that is normally located in the cytoplasm as a heterodimer, composed of p65 and p50 subunits, coupled to IκB. IκB dissociates from the complex when

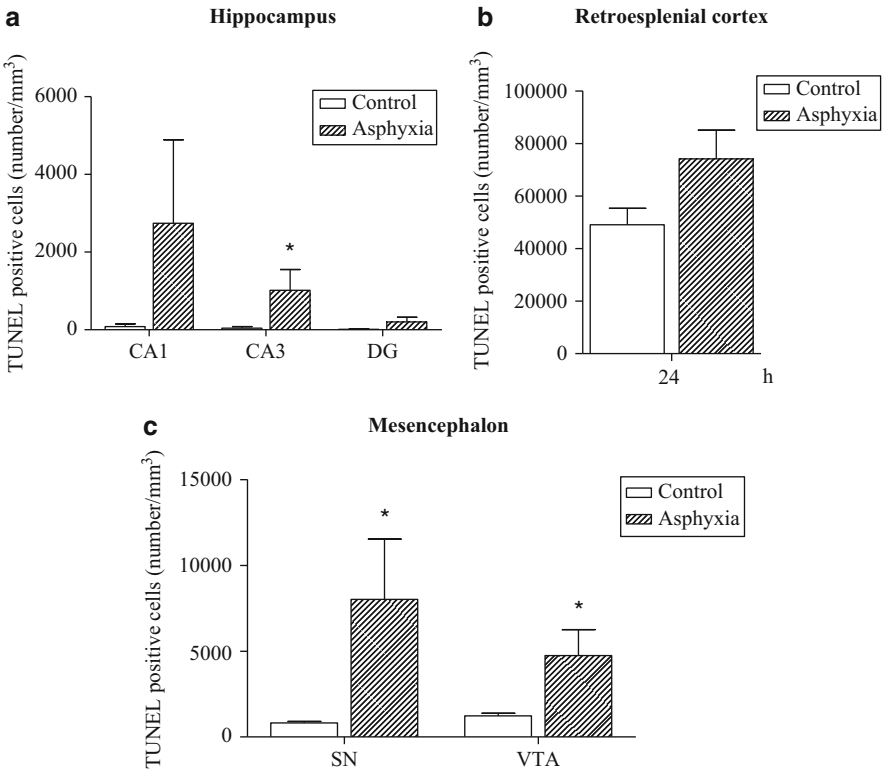


**Fig. 2** *Delayed cell death following perinatal asphyxia*. Twenty-four hours after delivery, asphyxia-exposed and control animals were sacrificed, the brains rapidly removed and fixed in 0.1 M pH 7.4 phosphate-buffered saline (PBS) containing 4% paraformaldehyde, kept in 20% sucrose, embedded in cryomatrix (Thermo Electron Corp, Pittsburgh, PA, USA), and stored at −80 °C. DNA fragmentation was evaluated in coronal brain sections (20 μm thick) with the TUNEL assay (*ApopTag*®; Millipore, Temecula, CA). TUNEL-positive cells (brown) were manually counted in a Nikon TS100 microscope (magnification 100×). The number of TUNEL-positive cells per $mm^3$ was determined in sections showing hippocampus (**a**), retrosplenial granular zone (**b**), and mesencephalon (**c**) (Foster atlas Foster, 1998) from the asphyxia exposed (*dashed bars*) and the corresponding controls (*open bars*). Comparisons were tested with a Student's *t*-test (*$p < 0.05$)

phosphorylated, liberating p65/p50, which are translocated to the nucleus for inducing the transcription of TNF-α, IL-1β intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), iNOS, and IL-6, all genes involved in inflammation (see Girard et al., 2009).

The immature CNS is capable of mounting innate and adaptive immune responses, through microglial and astrocytes, although it has been argued that the immune responses of the developing CNS differ from that of the adult, in part due to immaturity of blood–brain barrier (BBB). Tight junctions are, however, present early during embryonic development (Kniesel et al., 1996), controlling the interchange of proteins, including leukocytes (Engelhardt, 2003; Vexler & Yenari, 2009).

The developing brain is, however, vulnerable to increases of inflammatory cytokines, leading to a substantial cross talk between peripheral and local brain immune components (see Ransohoff et al., 2003; Vexler & Yenari, 2009). Indeed, Qiao et al. (2001) have demonstrated that in neonatal brain, disruption of the BBB to proteins occurs earlier after hypoxic-ischemic insult than in the mature brain.

Microglia provide immunosurveillance to the brain by stimulus-dependent activation (see Vexler & Yenari, 2009). Microglia populate the developing brain at birth, and activated microglia can release a number of cytokines, including IL-1β, IGF-1, and TNF-α. It is not clear yet whether microglia activation contributes or protects against the long-term deficits induced by perinatal asphyxia, but it has been reported that minocycline, a tetracycline derivative with anti-inflammatory properties, protects the neonatal brain against ischemia, partly by inhibiting microglia activation and monocyte infiltration (Arvin et al., 2002; Dommergues et al., 2003; Denker et al., 2007). Nevertheless, to investigate the involvement of different cytokines is complex, depending upon the type of the insult, upon the timing along the development axis, and also upon specific brain regions.

The translocation of NFκB was investigated in tissue samples from mesencephalon, hippocampus, and telencephalon from asphyxia-exposed and control rat pups, 1–24 h after birth, with an antibody specific to p65. It was found (Neira-Peña et al. unpublished) that translocation of p65 to the nucleus was significantly increased (>10×) in mesencephalon of both control and asphyxia-exposed pups 24 h after birth, but that increase was already remarkable 8 h after birth in asphyxia-exposed animals (Fig. 3b). In hippocampus, translocation of p65 was enhanced 1 h after birth in control animals, decreasing thereafter at 8–24 h (Fig. 3a). In contrast, in asphyxia-exposed animals, translocation of p65 was remarkably enhanced in hippocampus 8 h after birth, decreasing thereafter to almost negligible levels at 24 h (Fig. 3a). In telencephalon, translocation of p65 increased (>10×) from 1 to 8–24 h after birth in the controls, but in asphyxia-exposed animals, translocation of p65 was increased compared to the controls at 1 h, decreasing thereafter (Fig. 3c). Furthermore, TNF-α mRNA levels were increased in hippocampus 8 h after perinatal asphyxia (Fig. 4a), while IL-1β mRNA levels were only increased in mesencephalon, 24 h after the insult (Fig. 4b).

DNA damage and cell death elicited by perinatal asphyxia has the potential of activating PARP-1, promoting NFκB activation (see Skaper, 2003; Gagne et al., 2008). In agreement, Ullrich et al. (2001) showed that the microglial migration towards the site of neuronal injury is controlled by PARP-1 overactivation, correlating with NFκB translocation. To evaluate the role of PARP-1 in inflammatory processes, PARP-1 −/− mice were challenged with lipopolysaccharides (LPS), finding that the knockout mice were resistant to the endotoxin shock compared with wild-type animals. The knockout mice also showed low levels of TNF-α, due to reduced transcriptional activity of NFκB (Petrilli et al., 2004).

Inhibition of PARP-1 downregulates the expression of proinflammatory cytokines and suppresses NFκB-dependent gene transcription in microglia. Kauppinen et al. (2006, 2009) demonstrated in a model of bilateral carotid occlusion-reperfusion in rats that treatment with the PARP-1 inhibitor PJ34 ([*N*-(6-oxo-5, 6-dihydrophenanthridin-2-yl)-*N*, *N*-dimethylacetamide.HCl]), 48 h later, rapidly suppressed the ischemia-

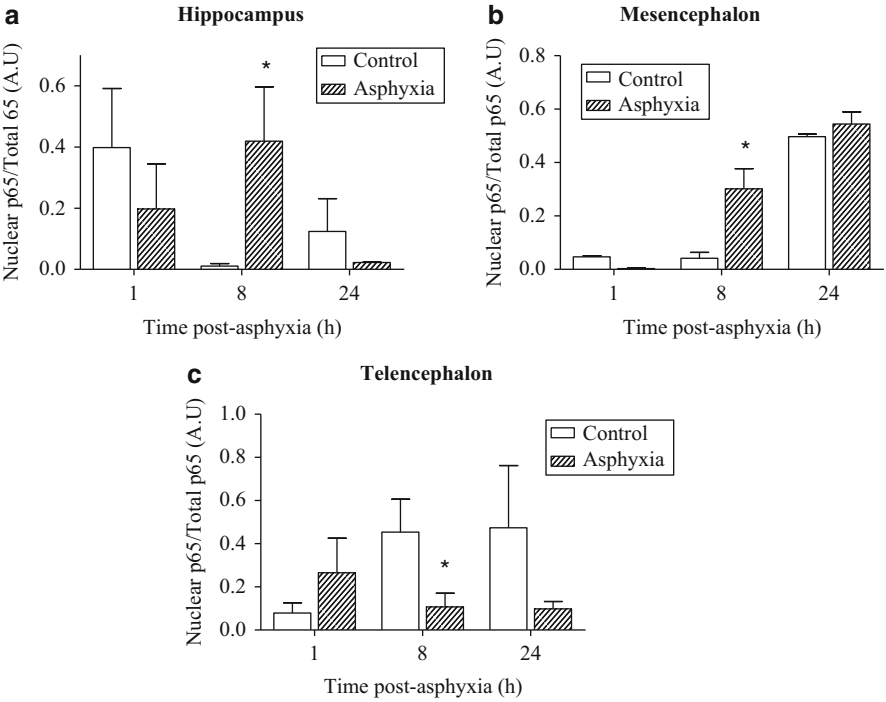


**Fig. 3** *Effect of reoxygenation on p65 activation*. Asphyxia and control rats were sacrificed 1, 8, and 24 h post-birth; the brain was rapidly removed, taking up tissue samples from hippocampus (**a**), mesencephalon (**b**), and telencephalon (**c**), placed in Eppendorf tubes, frozen in liquid nitrogen, and stored at −80 °C, pending further analysis. Cytosolic and nuclear protein was extracted with a ProteoJet™ kit (Fermentas). P65 levels were determined in cytosolic and nuclear protein fractions of tissue samples from asphyxia-exposed (*dashed bars*) and controls (*open bars*) rat pups. Western blot was performed using an anti-NFκB p65 antibody (65 KDa) (Santa Cruz Biotechnology, Inc, Santa Cruz, CA). Tubulin and histone H4 were used as controls for the respective fractions. A density-graphic analysis of the bands was performed. Data are represented as nuclear p65/total p65 ratio of Arbitrary Units (AU) (comparisons were analyzed with a Student's *t*-test; $*p < 0.05$)

induced microglial activation, enhancing long-term neuronal survival, neurogenesis, and spatial memory. Also, in a model of forebrain ischemia, PJ34 produced a near-complete inhibition of microglia activation and a significant reduction of neuronal death in the hippocampus (Hamby et al., 2007). Furthermore, it was reported that minocycline protects cortical neuron cultures against genotoxic agents causing DNA damage. Interestingly, minocycline also inhibits PARP-1 activity (Alano et al., 2006).

## 6 Epigenetics

PARP-1 activity is under control of and controls in a reciprocal way the activity of H1-histone, resulting in exclusion of H1 from a subset of PARP-regulated promoters and subsequent regulation of chromatin structure.

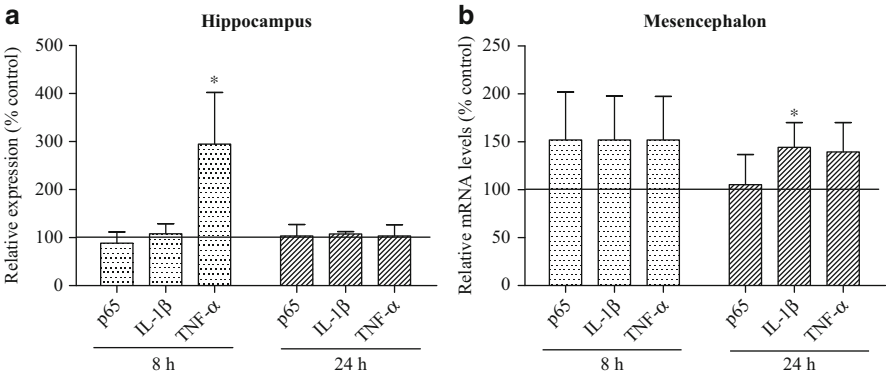


**Fig. 4** *mRNA expression of inflammatory-related genes*. Total RNA was extracted with DNAzol, treated with DNAse, and subsequently verified for optical density 260/280 absorption ratios. The integrity of RNA was analyzed by denaturing gel electrophoresis. Reverse transcription was performed with oligo-dT primers from total RNA to obtain cDNA. Relative mRNA levels to GAPDH of p65, IL-1β, and TNF-α were measured by RT-qPCR in tissue samples from hippocampus (**a**) and mesencephalon (**b**). Figure shows the ratio asphyxia-exposed/control rats, at 8 (*stippled bars*) and 24 h (*dashed bars*) after birth. Results, in triplicates, were analyzed with a MxPro software (comparisons tested by a Student's *t*-test; $*p < 0.05$)

PARP itself can be poly-ADP-ribosylated (PARylated PARP), which is reversed by poly-ADP-ribose glycohydrolase (PARG). There is a link between poly-ADP-ribosylation and DNA methylation implying DNA silencing (Cohen-Armon et al., 2007). Specifically, it has been shown that PARG overexpression, or reduction of PARylated PARP, results in methylation of CpG islands in the DNA (cytosine-5-)-methyltransferase 1 (DNMT1) promoter (Zampieri et al., 2009). Gene silencing leads to widespread DNA hypomethylation, resulting in increased gene expression. Conversely, when PARylated PARP occupies the DNMT1 promoter, it protects its unmethylated state and DNMT transcription. Additionally, other transcription factors, such as p300, SRY, or E2F, are associated with PARP at the DNMT1 promoter (Rajamohan et al., 2009).

Thus, epigenetics is a factor to be considered when studying plastic changes elicited by metabolic insults occurring at birth. There is evidence that the molecular machinery that regulates histone acetylation and DNA methylation might be intimately involved in synaptic plasticity, including learning and memory. Importantly, dysfunction of epigenetic gene expression in the brain may result in neurodegenerative and psychiatric diseases (Sananbenesi & Fischer, 2009). Conversely, drugs that increase histone acetylation (such as HDAC inhibitors) exhibit neuroprotective and neuro-regenerative properties in animal models of neurodegenerative diseases, enhancing cognitive functions (MacDonald & Roskams, 2009).

## 7 A Therapeutic Target

The adverse consequences of long-term effects of overactive PARP-1 in response to asphyxia (Martin et al., 2005) have led to the notion that PARP-1 is a suitable target for therapeutic interventions preventing the long-term effects of perinatal asphyxia

(see Herrera-Marschitz et al., 2011). Nicotinamide has been proposed as a prototype for counteracting PARP-1 overactivation (see Virag & Szabo, 2002), replacing NADH/$NAD^+$ (Zhang et al., 1995), and protecting against oxidative stress (Yan et al., 1999; Wan et al., 1999; Sakakibara et al., 2000) and inflammation (Ducrocq et al., 2000).

It has been reported (Bustamante et al., 2003, 2007) that nicotinamide prevents several of the changes induced by perinatal asphyxia on monoamine contents and dopamine release monitored with in vivo microdialysis 3 months after birth, even if the treatment was delayed for 24 h, suggesting a clinically relevant therapeutic window, supporting the idea that nicotinamide can constitute a therapeutic strategy against the long-term deleterious consequences of perinatal asphyxia, as already proposed for several other pathophysiological conditions (see Virag & Szabo, 2002). Nicotinamide has also been proposed as a treatment of Alzheimer's disease (Green et al., 2008).

The therapeutic doses of nicotinamide (0.8 mmol/kg, i.p.) used in the above-reported studies produce a long-lasting inhibition of PARP-1 activity measured in brain and heart from asphyxia-exposed and control animals (Allende-Castro et al., 2012) and also a decrease of the number of apoptotic nuclei in hippocampus, increased by perinatal asphyxia (Morales et al., 2010).

The use of nicotinamide has, however, been challenged because of its low potency, limited cell uptake, and short cell viability, stimulating the search for more specific compounds, such as 3-aminobenzamide (Ducrocq et al., 2000; Hortobagyi et al., 2003; Koh et al., 2004); 3,4-dihydro-5-[4-(1-piperidinyl) butoxy]-1(2H)-isoquinolinone (DPQ) (Takahashi et al., 1999); PJ34 (Abdelkarim et al., 2001); *N*-3-(4-oxo-3,4-dihydrophthalazin-1-yl)phenyl-4-(morpholin-4-yl) butanamide methane sulfonate monohydrate (ONO-1924H) (Kamanaka et al., 2004); 5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl) propyl]-4(3H)-quinazoline (FR247304) (Iwashita et al., 2004); and 2-methyl-3,5,7,8-tetra-hydrothiopyranol[4,3-d]pyrimidine-4-one (DR2313) (Nakajima et al., 2005). Ultra-potent novel PARP inhibitors are in clinical trials for reducing parenchymal cell necrosis following stroke and/or myocardial infarction, downregulating multiple pathways of inflammation and tissue injury following circulatory shock, colitis, or diabetic complications (Jagtap & Szabo, 2005).

There is, however, concern about applying ultrapotent PARP inhibitors during development, since it has been shown that PARP is required for efficient repair of damaged DNA (Trucco et al., 1998; Schultz et al., 2003), suggesting that moderate PARP-1 inhibitors should be chosen for neuronal protection, whenever used for pediatric patients (Moonen et al., 2005; Geraets et al., 2006).

Nicotinamide is therefore an interesting molecule because of its low potency, which can be an advantage when used for developing animals, antagonizing the effects elicited by PARP-1 overactivation without impairing DNA repair or cell proliferation. Nicotinamide has already been tested in human clinical trials without showing any significant toxicity, although its therapeutic efficacy is still controversial (Macleod et al., 2004). Nicotinamide can constitute a lead for exploring compounds with similar or better pharmacological profiles.

## 8 An Experimental Model for Perinatal Asphyxia

A model for investigating the short- and long-term outcome of perinatal asphyxia was proposed at the Karolinska Institutet, Stockholm, Sweden, in the nineties (Bjelke et al., 1991; Andersson et al., 1992; Herrera-Marschitz et al., 1993). Asphyxia is induced at the time when the rats are delivered providing some features with clinical relevance: (i) it occurs at term, (ii) it is largely noninvasive, (iii) it allows studying peripheral and brain tissue in the same animal, (iv) it allows studying short- and long-term consequences of the insult in the same preparation, and (v) it is highly reproducible among laboratories. Lubec and coworkers in Vienna (Austria) (Lubec et al., 1997a, b; Seidl et al., 2000) have stressed the issue that the model allows to study the early phase of perinatal asphyxia, as observed in the clinical setup.

The model starts by evaluating the estral cycle of young female Wistar rats (~2 months of age), in order to plan for a programmed mating. A vaginal frottis is taken for evaluating the cycle, identifying proestrus, estrus, metaestrus, or diestrus. The female is then exposed to a male at the time of the proestrus for one night, evaluating thereafter for the presence of a vaginal clot. Thus, the time of delivery is calculated, supported by ethological and clinical observations, to predict the exact time of delivery (22 days). At the time of delivery, a first spontaneous birth can be observed before the dams are neck dislocated, and subjected to a caesarean section and hysterectomy. The uterine horns containing the fetuses are immediately immersed into a water bath at 37 °C for various periods of time (0–22 min). Following asphyxia, the pups are removed from the uterine horns and resuscitated by cleaning from fluid and amniotic tissue, freeing the mouth and the nose. Pups exposed to caesarean delivery only (CS, 0 asphyxia) or to mild asphyxia (2–10 min) are rapidly resuscitated, without requiring anything else but removing fluid and amniotic tissue from the mouth. For pups exposed to longer periods of asphyxia (19–21 min), resuscitation implies expert and skilful handling, and it takes a long time (4–6 min) for a first gasping and even longer time for establishing a more or less regular breathing, always supported by gasping. After 60 min of caretaking, the pups are given to surrogate dams for nursing, pending further experiments. An Apgar scale is applied during the recovery period, similar to that observed in neonatal units, including parameters such as weight, sex, color of the skin, respiratory frequency, gasping, vocalization, muscular rigidity, and spontaneous movements (Dell'Anna et al., 1997; Morales et al., 2010; see Herrera-Marschitz et al., 2011). The Apgar evaluation is a critical parameter, because it assesses whether the pups are subjected to mild or to severe asphyxia, which is directly determined by the percentage of survival and recovery (Herrera-Marschitz et al., 1993, 1994). The Apgar evaluation also provides information about the condition shown by the caesarean-delivered control pups, which has to be similar to that shown by vaginally delivered pups. Thus, the Apgar evaluation is a requirement when using the present model of perinatal asphyxia, because it permits to compare results obtained by different laboratories and/or different treatments. The quality of the handling of the pups and the experience of the surrogate dam are

important factors for the acceptance and nursing of both asphyxia-exposed and control pups.

The model has been useful for describing early molecular, metabolic, and physiological effects. Tissue sampling can be collected immediately after delivery, the time when the pups are removed from the uterine horns (0 min, with or without previous immersion into a water bath), or soon after reoxygenation (Lubec et al., 1997a, b; Seidl et al., 2000; Engidawork et al., 2001). Thus, energy-rich phosphates have been measured in brain and peripheral tissue immediately after delivery following short or long periods of perinatal asphyxia, demonstrating that adenosine triphosphate (ATP)/phosphocreatine (PCr) levels were first significantly decreased in kidneys (after 2 min of asphyxia), then in brain tissue (after 10 min), but in heart ATP dropped down after 20 min of asphyxia (Lubec et al., 1997a; Seidl et al., 2000; see Herrera-Marschitz et al., 2011).

## 9 Effect of Nicotinamide on the Long-Term Functional Consequences Elicited by Perinatal Asphyxia

Motor and cognitive alterations of variable severity, including cerebral palsy, seizures, spasticity, attention deficit, hyperactivity, mental retardation, and/or neuropsychiatric syndromes with delayed clinical onset have been associated to perinatal asphyxia (du Plessis & Volpe, 2002; Van Erp et al., 2002; Kaufman et al., 2003; Vannuci & Hagberg, 2004; Odd et al., 2009). With the proposed experimental model, several labs have investigated the behavioral effects associated to perinatal asphyxia, addressing motor function (Bjelke et al., 1991; Chen et al., 1995), emotion (Dell'Anna et al., 1991; Hoeger et al., 2000; Venerosi et al., 2004, 2006; Simola et al., 2008; Morales et al., 2010), and spatial memory (Boksa et al., 1995; Iuvone et al., 1996; Hoeger et al., 2000, 2006; Loidl et al., 2000; Van de Berg et al., 2003; Venerosi et al., 2004).

The issue whether perinatal asphyxia produces long-term effects on cognition has also been investigated. Novel object recognition was studied with a test applied under ethological-like conditions, devoid of any stressful cues or primary reinforcers, such as swimming, food deprivation, or electric shocks (Ennaceur & Delacour, 1988). The animal is tested to discriminate between objects differing in shape and color, without any genuine significance to the rat or being previously associated to any rewarding or aversive stimuli. During a first session, two copies of the same object are presented to the rat for 4 min and then again, during a second session, when one of the previously presented objects is replaced by a novel one, similar in size, but different in shape and/or color. The rat has to recognize the novel object, spending longer time exploring the novel than that previously presented. The first and the second sessions are separated by different time intervals, for evaluating learning consolidation. A good memory would be able to recognize a previously presented object after a long time elapsing between a first and a second session, meaning that the animal would concentrate on exploring the novel object. Novel

object recognition was studied using a 15 or 60 min interval, when the animals were 3 months old (Simola et al., 2008). No differences were observed between asphyxia-exposed (20 min asphyxia) and control animals when a 15 min interval elapsed between the first and the second session. Both asphyxia-exposed and control rats recognized the novel stimulus similarly well, spending longer time exploring the novel object. However, when 60 min elapsed between the first and second session, asphyxia-exposed animals spent less time exploring the novel object, indicating that asphyxia-exposed rats could not recognize its novelty. Conversely, asphyxia-exposed rats could not remember that one of the objects was already presented during the first session (Simola et al., 2008). This was a straightforward experiment showing a subtle consequence of hypoxia occurring at birth, impairing a cognitive function that shows up only after a proper challenge. It is very much reminiscent to the clinical experience revealing effects only when the child starts the primary school (see Odd et al., 2009; Strackx et al., 2010).

In a parallel study, the same condition was repeated, but a cohort of asphyxia-exposed and of control rats received 0.8 mmol/kg, i.p. of nicotinamide 1, 24, and 48 h after birth and evaluated 2–3 month later for the novel object recognition task. Nicotinamide did not affect the performance of the control animals, but prevented the effect of perinatal asphyxia, since the animals receiving nicotinamide performed identically as the controls on novel object recognition (Morales et al., 2010).

Asphyxia-exposed, treated, and control animals were tested in the first 2–3 months after birth for exploratory behavior, monitored with a video camera when animals were placed in a circular arena in a noise-free room. Each animal was left to explore the new environment for 5 min, monitoring the time expended in the center of the arena, exploiting the natural aversion of rodents for open spaces devoid of thigmotactic cues. A decreased time in the center of the arena was considered as a measure of anxiety (Perez de la Mora et al., 2012). Figure 5 shows the tracking of a caesarean-delivered saline-treated (CS), a saline-treated asphyxia-exposed (AS), and a nicotine-treated asphyxia-exposed (AN) rat. As previously reported (Allende-Castro et al., 2012), the AS, but not CS or AN, rats avoided areas devoid of thigmotactic cues, indicating unconditioned fear and anxiety, also observed when analyzing the behavioral profile on an elevated plus maze of parallel experimental cohorts (Morales et al., 2010). Nicotinamide prevented the anxiety-related behavior in both experimental paradigms.

## 10 Pharmacodynamics and Pharmacokinetics of Nicotinamide

It was further investigated whether the effect of nicotinamide was related to PARP-1 inhibition using an enzymatic assay of PARP-1 activity, based on the accumulation of PAR polymers. It was found that a single dose of 0.8 mmol/kg, i.p. of nicotinamide decreased PARP-1 activity by ~70% in brain (mesencephalon, telencephalon) and peripheral (heart) tissue of asphyxia-exposed and caesarean-delivered control

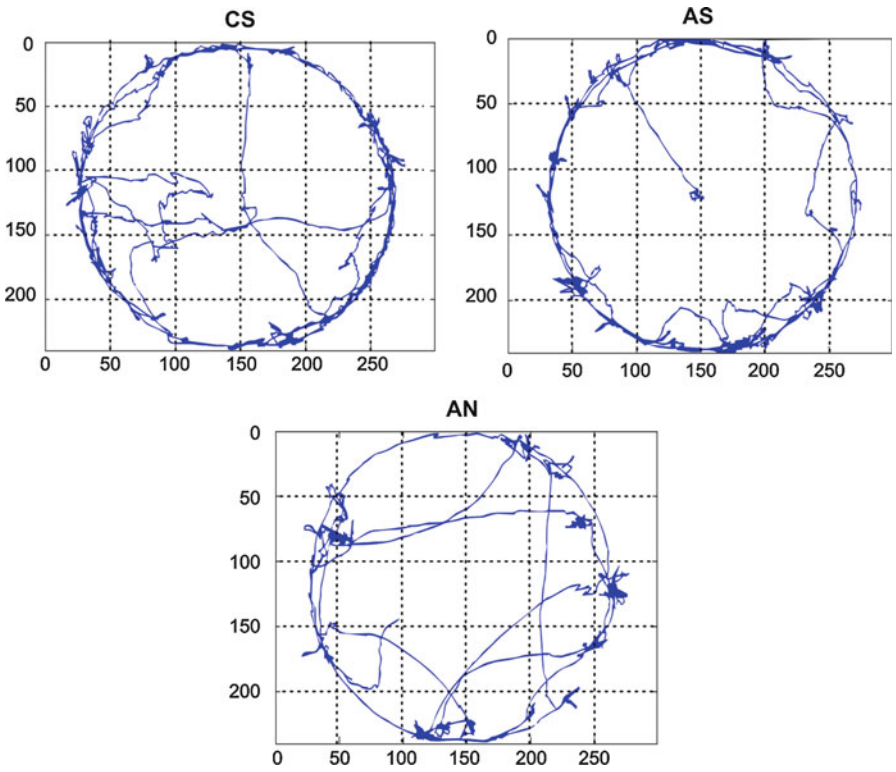


**Fig. 5** *Reversal of the effect of perinatal asphyxia on exploratory behavior by neonatal nicotinamide treatment*. Exploratory behavior was examined in a circular arena placed in a noise-free room. Arena was 140 cm in diameter painted in black, with a continuous wall of 15 cm high and placed 50 cm above the floor. A camera placed 250 cm over the arena was used for recording the rat performance. The camera was connected to a computerized processing unit (*CPU*) placed in the next room where the examiner stayed during the experiment. At 2–3 months of age, control (CS) and asphyxia-exposed (AS) rats treated with saline (3 × 0.1 ml/kg, i.p) or with nicotinamide (AN) (0.8 mmol/kg, i.p.; 1, 24 and 48 h after birth) were placed in the center of the arena free to explore for 5 min (Individual experiments taken from cohorts reported by Allende-Castro et al., 2012)

pups, compared with the corresponding saline controls, 1–24 h after birth. A remarkable PARP-1 inhibition was still observed 24 h after the treatment. In vivo microdialysis experiments in ~8 h-old pups allowed to monitor the distribution of a single dose of nicotinamide (0.8 mmol/kg, i.p.), in peripheral (subcutaneous) and brain (neostriatum) tissue (Allende-Castro et al., 2012), finding a rapid distribution of nicotinamide into the brain, detectable for longer than 6 h at a steady-state concentration of 20 μM. In the same study, theophylline was also investigated, only finding PARP-1 inhibition in peripheral tissue, in agreement with a poor distribution of a single dose of theophylline (0.14 nmol/kg, i.p.) into the brain compartment.

## 11 Conclusion

Perinatal asphyxia is still a health concern worldwide, a risk factor for several mental and neurological disorders with a delayed clinical onset. Hypoxia implies a severe energetic crisis, leading to death if reoxygenation is not promptly restored. The functional constraints produced by the lack of oxygen can be exacerbated by and during the reoxygenation period, implying oxidative stress, synthesis, and release of metabolic by-products delaying the onset of proper homeostasis and recovery. A leading role is played by HIF-1α, promoting the synthesis of proteins thought to minimize or compensate the damage induced by the hypoxic condition, but also to promote proinflammatory cytokines leading to cell death. A number of sentinel proteins are rapidly activated whenever there is a risk of genome damage, stimulating base excision repair. PARP-1 has been shown to play a pivotal role for repairing damaged DNA, but also for eliciting caspase-independent cell death when repairing is not viable, modulating pro- and anti-inflammatory signalling. Furthermore, there is equilibrium between PARP-1 and SIRT proteins, regulating histone acetylation and DNA methylation, the core of epigenetic modification.

PARP-1 overactivation can lead to $NAD^+$ exhaustion, worsening the energy crisis, leading to the hypothesis that PARP-1 is a suitable target for therapeutic interventions preventing the long-term effects of perinatal asphyxia, nicotinamide being a prototype for counteracting PARP-1 overactivation.

The neuroprotection effect of nicotinamide has been studied in an experimental model of global perinatal asphyxia in rats. In this model, asphyxia is induced by immersing rat fetuses into a water bath for various periods of time. Following asphyxia, the pups are delivered, immediately treated, or given to surrogate dams for nursing, pending further experiments.

Following systemic administration, nicotinamide rapidly distributes into the brain and peripheral compartments reaching a steady-state concentration sufficient to inhibit PARP-1 activity for several hours. Nicotinamide prevents several of the long-term consequences elicited by perinatal asphyxia, supporting the idea that it can constitute a lead for exploring compounds with similar or better pharmacological profiles.

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## References

Abdelkarim, G. E., Gertz, K., Harms, C., Katchanov, J., Dimagl, U., Szabo, C., & Endres, M. (2001). Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in in vitro and in vivo models of stroke. *International Journal of Molecular Medicine, 7*, 255–260.

Akil, M., Edgar, C. L., Pierri, J. N., Casali, S., & Lewis, D. A. (2000). Decreased density of tyrosine hydroxylase-immunoreactive axons in the entorhinal cortex of schizophrenic subjects. *Biological Psychiatry, 47*, 361–370.

Alano, C. C., Kauppinen, T. M., Valls, A. V., & Swanson, R. A. (2006). Minocycline inhibits poly (ADP-ribose) polymerase-1 at nanomolar concentrations. *Proceedings of the National Academy Sciences USA, 103*, 9685–9690.

Allende-Castro, C., Espina-Marchant, P., Bustamante, D., Rojas-Mancilla, E., Neira, T., Gutierrez-Hernandez, M. A., Esmar, D., Valdes, J. L., Morales, P., Gebicke-Haerter, P. J., & Herrera-Marschitz, M. (2012). Further studies on the hypothesis of PARP-1 inhibition as a strategy for lessening the long-term effects produced by perinatal asphyxia: Effects of nicotinamide and theophylline on PARP-1 activity in brain and peripheral tissue. *Neurotoxicity Research, 22*, 79–90.

Amé, J.-C., Spenlehauer, C., & de Murcia, G. (2004). The PARP superfamily. *BioEssays, 26*, 882–893.

Andersson, K., Bjelke, B., Bolme, P., & Ögren, S. O. (1992). Asphyxia-induced lesion of the rat hippocampus (CA1, CA3) and the nigro-striatal dopamine system. In J. Gross Hypoxia and Ischemia. CNS. Wissenschafliche Publikationen der Humboldt-Universität zu Berlin, B. *Medizin, 41*, 71–76.

Arvin, K. L., Han, B. H., Du, Y., Lin, S. Z., Paul, S. M., & Holtzman, D. M. (2002). Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. *Annals of Neurology, 52*, 54–61.

Bachus, S. E., Hyde, T. M., Herman, M. M., Egan, M. F., & Kleinman, J. E. (1997). Abnormal cholecystokinin mRNA levels in entorhinal cortex of schizophrenics. *Journal of Psychiatric Research, 31*, 233–256.

Bartley, J., Soltau, T., Wimborne, H., Kim, S., Martin-Sttudard, A., Hess, D., Hill, W., Waller, J., & Carrol, J. (2005). BrdU-positive cells in the neonatal mouse hippocampus following hypoxic-ischemic brain injury. *BMC Neuroscience, 6*, 15.

Basovich, S. N. (2010). The role of hypoxia in mental development and in the treatment of mental disorders. *Bioscience Trends, 4*, 288–296.

Berger, N. A. (1985). Poly (ADP-ribose) in the cellular response to DNA damage. *Radiation Research, 1001*, 4–15.

Bjelke, B., Andersson, K., Ögren, S. O., & Bolme, P. (1991). Asphyctic lesion: Proliferation of tyrosine hydroxylase immunoreactive nerve cell bodies in the rat substantia nigra and functional changes in dopamine transmission. *Brain Research, 543*, 1–9.

Boksa, P., Krishnamurthy, A., & Brooks, W. (1995). Effects of a period of asphyxia during birth on spatial learning in the rat. *Pediatric Research, 37*, 489–496.

Bustamante, D., Goiny, M., Åström, G., Gross, J., Andersson, K., & Herrera-Marschitz, M. (2003). Nicotinamide prevents the long-term effects of perinatal asphyxia on basal ganglia monoamine systems in the rat. *Experimental Brain Research, 148*, 227–232.

Bustamante, D., Morales, P., Torres-Pereyra, J., Goiny, M., & Herrera-Marschitz, M. (2007). Nicotinamide prevents the effect of perinatal asphyxia on dopamine release evaluated with in vivo microdialysis three months after birth. *Experimental Brain Research, 177*, 358–369.

Chen, Y., Ögren, S. O., Bjelke, B., Bolme, P., Eneroth, P., Gross, J., Loidl, F., Herrera-Marschitz, M., & Andersson, K. (1995). Nicotine treatment counteracts perinatal asphyxia-induced changes in the mesostriatal/limbic dopamine systems and in motor behaviour in the four-week old male rat. *Neuroscience, 68*, 531–538.

Chiappe-Gutierrez, M., Kitzmueller, E., Labudova, O., Fuerst, G., Hoeger, H., Hardmeier, R., Nohl, H., Gille, L., & Lubec, B. (1998). mRNA levels of the hypoxia inducible factor (HIF-1) and DNA repair genes in perinatal asphyxia of the rat. *Life Sciences, 63*, 1157–1167.

Cohen-Armon, M., Visochek, L., Rozendal, D., Kalal, A., Geistrikh, I., Klein, R., Bendetz-Nezer, S., Yao, Z., & Seger, R. (2007). DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: A link to histone acetylation. *Molecular Cell, 25*, 297–308.

Correia, S. C., & Moreira, P. I. (2010). Hypoxia-inducible factor 1: A new hope to counteract neurodegeneration? *Journal of Neurochemistry, 112*, 1–12.

De Hann, M., Wyatt, J. S., Vargha-Khadem, F., Gadian, D., & Mishki, M. (2006). Brain and cognitive behavioural development after asphyxia at term birth. *Developmental Science, 9*, 350–358.

Dell'Anna, E., Calzolari, S., Milinari, M., Luvone, L., & Calimici, R. (1991). Neonatal anoxia induces transitory hyperactivity, permanent spatial memory deficits and CA1 cell density reduction in the developing rats. *Behavioural Brain Research, 45*, 125–134.

Dell'Anna, E., Chen, Y., Engidawork, E., Andersson, K., Lubec, G., Luthman, J., & Herrera-Marschitz, M. (1997). Delayed neuronal death following perinatal asphyxia in rat. *Experimental Brain Research, 115*, 105–115.

Denker, S., Ji, S., Lee, S. Y., Dingman, A., Derugin, N., Wendland, M., & Vexler, Z. S. (2007). Macrophages are comprised of resident brain microglia not infiltrating peripheral monocytes acutely after neonatal stroke. *Journal of Neurochemistry, 100*, 893–904.

Dommergues, M. A., Plaisant, F., Verney, C., & Gressens, P. (2003). Early microglia activation following neonatal excitotoxic brain damage in mice: A potential target for neuroprotection. *Neuroscience, 121*, 619–628.

du Plessis, A. J., & Volpe, J. J. (2002). Perinatal brain injury in the preterm and term newborn. *Current Opinion in Neurology, 15*, 151–157.

Ducrocq, S., Benjelloun, N., Plotkine, M., Ben-Ari, Y., & Charriaut-Marlangue, C. (2000). Poly (ADP-ribose) synthase inhibition reduces ischemic injury and inflammation in neonatal rat brain. *Journal of Neurochemistry, 74*, 2504–2511.

Ellenberger, T., & Tomkinson, A. E. (2008). Eukaryotic DNA ligases: Structural and functional insights. *Annual Review of Biochemistry, 77*, 313–338.

Engelhardt, B. (2003). Development of the blood–brain barrier. *Cell and Tissue Research, 314*, 119–129.

Engidawork, E., Loidl, F., Chen, Y., Kohlhauser, C., Stoeckler, S., Dell'Anna, E., Lubec, B., Lubec, G., Goiny, M., Gross, J., Andersson, K., & Herrera-Marschitz, M. (2001). Comparison between hypothermia and glutamate antagonism treatments on the immediate outcome of perinatal asphyxia. *Experimental Brain Research, 138*, 375–383.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. I: Behavioral data. *Behavioural Brain Research, 31*, 47–59.

Fähling, M. (2009). Surviving hypoxia by modulation of mRNA translation rate. *Journal of Cellular and Molecular Medicine, 13*, 2770–2779.

Foster, G. A. (1998). *Chemical neuroanatomy of the prenatal rat brain*. Oxford University Press.

Gagne, J.-P., Isabelle, M., Lo, K. S., Bourassa, S., Hendzel, M. J., Dawson, V. L., Daeson, T. M., & Poirier, G. G. (2008). Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes. *Nucleic Acids Research, 36*, 6959–6976.

Geraets, L., Moonen, H. J. J., Wouters, E. F. M., Bast, A., & Hageman, G. J. (2006). Caffeine metabolites are inhibitors of the nuclear enzyme poly(ADP-ribose)polymerase-1 at physiological concentrations. *Biochemical Pharmacology, 72*, 902–910.

Girard, S., Kadhim, H., Roy, M., Lavoie, K., Brochu, M. E., Larouche, A., & Sebire, G. (2009). Role of perinatal inflammation in cerebral palsy. *Pediatric Neurology, 40*, 168–174.

Green, A., Prager, A., & Stoudt, M. D. (1992). Relationships between DNA damage and the survival of radiosensitive mutant Chinese hamster cell lines exposed to gamma-radiation. Part 1: Intrinsic radiosensitivity. *International Journal of Radiation Biology, 61*, 465–472.

Green, K. N., Steffan, J. S., Martinez-Coria, H., Sun, X., Schreiber, S. S., Thompson, L. M., & LaFerla, F. M. (2008). Nicotinamide restores cognition in Alzheimer's disease transgenic mice via a mechanism involving sirtuin inhibition and selective reduction of Thr231-phosphotau. *The Journal of Neuroscience, 28*, 11500–11510.

Hagberg, H., Gilland, E., Bona, E., Hanson, L. A., Hahlin-Zoric, M., Biennow, M., Holst, M., McRae, A., & Söder, O. (1996). Enhanced expression of interleukin (IL)-1 and IL-6 messenger

RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatric Research, 40*, 603–609.

Hamby, A. M., Suh, S. W., Kauoinen, T. M., & Swanson, R. A. (2007). Use of a poly(ADP-ribose) polymerase inhibitor to suppress inflammation and neuronal death after cerebral ischemia-reperfusion. *Stroke, 38*, 632–636.

Herrera-Marschitz, M., Loidl, C. F., Andersson, K., & Ungerstedt, U. (1993). Prevention of mortality induced by perinatal asphyxia: Hypothermia or glutamate antagonism? *Amino Acids, 5*, 413–419.

Herrera-Marschitz, M., Loidl, C. F., You, Z.-B., Andersson, K., Silveira, R., O'Connor, W. T., & Goiny, M. (1994). Neurocircuitry of the basal ganglia studied by monitoring neurotransmitter release. Effects of intracerebral and perinatal asphyctic lesions. *Molecular Neurobiology, 9*, 171–182.

Herrera-Marschitz, M., Morales, P., Leyton, L., Bustamante, D., Klawitter, V., Espina-Marchant, P., Allende, C., Lisboa, F., Cunich, G., Jara-Caviedes, A., Neira, T., Gutierrez-Hernandez, M. A., Gonzalez-Lira, V., Simola, N., Schmitt, A., Morelli, M., Tasker, R. A., & Gebicke-Haerter, P. J. (2011). Perinatal asphyxia: Current status and approaches towards neuroprotective strategies, focus on sentinel proteins. *Neurotoxicity Research, 19*, 603–627.

Hoeger, H., Engelmann, M., Bernet, G., Seidl, R., Bubna-Littitz, H., Mosgoeller, W., Lubec, B., & Lubec, G. (2000). Long term neurological and behavioral effects of graded perinatal asphyxia in the rat. *Life Sciences, 66*, 947–962.

Hoeger, H., Engidawork, E., Stolzlechner, D., Bubna-Littitz, H., & Lubec, B. (2006). Long-term effect of moderate and profound hyperthermia on morphology, neurological, cognitive and behavioural functions in a rat model of perinatal asphyxia. *Amino Acids, 31*, 385–396.

Hökfelt, T., Ljungdahl, Å., Fuxe, K., & Johansson, O. (1974). Dopamine nerve terminals in the rat limbic cortex: Aspects of the dopamine hypothesis of schizophrenia. *Nature, 184*, 177–179.

Hortobagyi, T., Gorlach, C., Benyo, Z. L., Lacza, Z., Hortobagyi, S., Wahl, M., & Harkany, T. (2003). Inhibition of neuronal nitric oxide synthase-mediated activation of poly(ADP-ribose) polymerase in traumatic brain injury: Neuroprotection by 3-aminobenzamide. *Neuroscience, 121*, 983–990.

Iuvone, L., Geloso, M. C., & Dell'Anna, E. (1996). Changes in open field behavior, spatial memory, and hippocampal parvalbumin immunoreactivity following enrichment in rats exposed to neonatal anoxia. *Experimental Neurology, 139*, 25–33.

Iwashita, A., Tojo, N., Matsuura, S., Yamazaki, S., Kamijo, K., Ishida, J., Yamamoto, H., Hattori, K., Matsuoka, N., & Mutoh, S. (2004). A novel and potent Poly(ADP-Ribose) Polymerase-1 inhibitor, FR247304 (5-chloro-2-[3-4-phenyl-3,6-dihydro-1(2H0-pyridinyl)propyl]-4(3H)-quinazolinone) attenuates neuronal damage in in vitro and in vivo models of cerebral ischemia. *The Journal of Pharmacology and Experimental Therapeutics, 310*, 425–436.

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laugner, E., Wenger, R. H., Gassmann, M., Gearhart, J. D., Lawler, A. M., Yu, A. Y., & Semenza, G. L. (1998). Cellular and developmental control of $O_2$ homeostasis by hypoxia-inducible factor 1 alpha. *Genes & Development, 12*, 149–162.

Jagtap, P., & Szabo, C. (2005). Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nature Review, 4*, 421–440.

Kamanaka, Y., Kondo, K., Ikeda, Y., Kamoshima, W., Kitajima, T., Suzuki, Y., Nakamura, Y., & Umemura, K. (2004). Neuroprotective effects of ONO-1924H, an inhibitor of poly ADP-ribose polymerase (PARP), on cytotoxicity of PC12 cells and ischemic cerebral damage. *Life Sciences, 76*, 151–162.

Kaufman, S. A., Miller, S. P., Ferreiro, D. M., Glidden, D. H., Barkovich, A. J., & Partirdge, J. C. (2003). Encephalopathy as a predictor of magnetic resonance imaging abnormalities in asphyxiated newborns. *Pediatric Neurology, 28*, 342–346.

Kauppinen, T. M., Chan, W. Y., Suh, S. W., Wiggins, A. K., Huang, E. J., & Swanson, R. A. (2006). Direct phosphorylation and regulation of poly(ADP-ribose) polymerase-1 by extracellular

signal-regulated kinases1/2. *Proceedings of the National Academy of Sciences USA, 103*, 7136–7141.

Kauppinen, T. M., Suh, S. W., Berman, A. E., Hamby, A. M., & Swanson, R. A. (2009). Inhibition of poly(ADP-ribose) polymerase suppresses inflammation and promotes recovery after ischemic injury. *Journal of Cerebral Blood Flow and Metabolism, 29*, 820–829.

Kniesel, U., Risau, W., & Wolburgh, H. (1996). Development of blood–brain barrier tight junctions in the rat cortex. *Brain Research and Development Brain Research, 96*, 229–240.

Koh, S.-H., Park, Y., Song, C. W., Kim, J. G., Kim, K., Kim, J., Kim, M.-H., Lee, S. R., Kim, D. W., Yu, H.-J., Chang, D., Hwang, S. J., & Kim, S. H. (2004). The effect of PARP inhibitor on ischemic cell death, its related inflammation and survival signals. *The European Journal of Neuroscience, 20*, 1461–1472.

Kolthur-Seetharam, U., Dantzer, F., McBurney, M. W., de Murcia, G., & Sassone-Corsi, P. (2006). Control of AIF-mediated cell death by the functional interplay of SIRT1 and PARP-1 in response to DNA damage. *Cell Cycle, 5*, 873–877.

Lawn, J. E., Kerber, K., Enweronu-Laryea, C., & Cousens, S. (2010). 3.6 million neonatal deaths-what is progressing and what is not? *Seminars in Perinatology, 34*, 371–386.

Lee, J. W., Beene, K., Nangie, L. A., Longo-Guess, C. M., Cook, S. A., Davisson, M. T., Sundberg, J. P., Schimmel, P., & Ackerman, S. L. (2006). Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature, 443*, 50–55.

Loidl, F., Gavilanes, A. W., Van Dijk, E. H., Vreuls, W., Blokland, A., Vies, J. S., Steinbusch, H. W., & Blanco, C. E. (2000). Effects of hypothermia and gender on survival and behaviour after perinatal asphyxia in rats. *Physiology and Behavior, 68*, 263–269.

Lubec, B., Mark, M., Herrera-Marschitz, M., Labudova, O., Hoeger, H., Gille, L., Nohl, H., Mosgoeller, W., & Lubec, G. (1997a). Decrease of heart protein kinase C and cyclin-dependent kinase precedes death in perinatal asphyxia of the rat. *The FASEB Journal, 11*, 482–492.

Lubec, B., Dell'Anna, E., Fang-Kircher, S., Mark, M., Herrera-Marschitz, M., & Lubec, G. (1997b). Decrease of brain protein kinase C, protein kinase A, and cyclin-dependent kinase correlating with pH precedes neuronal death in neonatal asphyxia of the rat. *Journal of Investigative Medicine, 45*, 284–294.

Lubec, B., Labudova, O., Hoeger, H., Kirchner, L., & Lubec, G. (2002). Expression of transcription factors in the brain of rats with perinatal asphyxia. *Biology of the Neonate, 81*, 266–278.

MacDonald, J. L., & Roskams, A. J. (2009). Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. *Progress in Neurobiology, 88*, 170–183.

Macleod, M. R., O'Collins, T., Howells, D. W., & Donnan, G. A. (2004). Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke, 35*, 1203–1208.

Martin, S. S., Perez-Polo, J. R., Noppens, K. M., & Grafe, M. R. (2005). Biphasic changes in the levels of poly(ADPribose) polymerase-1 and caspase 3 in the immature brain following hypoxia-ischemia. *International Journal of Developmental Neuroscience, 23*, 673–686.

Moonen, H. J. J., Geraets, L., Vaarhorst, A., Wouters, E. F. M., Bast, A., & Hageman, G. J. (2005). Theophylline prevents NAD+ depletion via PARP-1 inhibition in human pulmonary epithelial cells. *Biochemical and Biophysical Research Communications, 338*, 1805–1810.

Morales, P., Fiedler, J. L., Andres, S., Berrios, C., Huaiquin, P., Bustamante, D., Cardenas, S., Parra, E., & Herrera-Marschitz, M. (2008). Plasticity of hippocampus following perinatal asphyxia: Effects on postnatal apoptosis and neurogenesis. *Journal of Neuroscience Research, 86*, 2650–2662.

Morales, P., Simola, N., Bustamante, D., Lisboa, F., Fiedler, J., Gebicke-Haerter, P., Morelli, M., Tasker, R. A., & Herrera-Marschitz, M. (2010). Nicotinamide prevents the effect of perinatal asphyxia on apoptosis, non-spatial working memory and anxiety in rats. *Experimental Brain Research, 202*, 1–14.

Nakajima, H., Kakui, N., Ohkuma, K., Ishikawa, M., & Hasegawa, T. (2005). A newly synthesized Poly(ADPRibose)Polymerase inhibitor, DR2313[2-methyl-3,5,7,8-tetrahydrothiopyranol [4,3-d]-pyrimidine-4-one]: Pharmacological profiles, neuroprotective effects and therapeutic

time window in cerebral ischemia in rats. *The Journal of Pharmacology and Experimental Therapeutics, 312*, 472–481.

Northington, F. J., Ferreiro, D. M., Graham, E. M., Traystman, R. J., & Martin, L. J. (2001). Early neurodegeneration after hypoxia-ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis. *Neurobiology of Disease, 8*, 207–219.

Odd, D. E., Lewis, G., Whitelaw, A., & Gunnell, D. (2009). Resuscitation at birth and cognition at 8 years of age: A cohort study. *Lancet, 373*, 1615–1622.

Perez de la Mora, M., Gallegos-Cari, A., Crespo-Ramirez, M., Marcellino, D., Hansson, A. C., & Fuxe, K. (2012). Distribution of dopamine D2-like receptors in the rat amygdala and their role in the modulation of unconditioned fear and anxiety. *Neuroscience, 201*, 252–266.

Petrilli, V., Herced, Z., Hassa, P. O., Patel, N. S., Di Paola, R., Cortes, U., Dugo, L., Filipe, H. M., Thiemermann, C., Hottiger, M. O., Cuzzocrea, S., & Wang, Z. Q. (2004). Noncleavable poly (ADP-ribose) polymerase-1 regulates inflammation response in mice. *The Journal of Clinical Investigation, 114*, 1072–1081.

Qiao, M., Malisza, K. L., Del Biio, M. R., & Tuor, U. L. (2001). Correlation of cerebral hypoxic-ischemic T2 changes with tissue alterations in water content and protein extravasation. *Stroke, 32*, 958–963.

Rajamohan, S. B., Pillai, V. B., Gupta, M., Sundaresan, N. R., Birukov, K. G., Samant, S., Hottiger, M. A., & Gupta, M. P. (2009). SIRT promote cell survival under stress by deacetylation-dependent deactivation of poly(ADP) polymerase 1. *Molecular and Cellular Biology, 29*, 4116–4129.

Ransohoff, R. M., Kivisakk, P., & Kidd, G. (2003). Three or more routes for leukocyte migration into the central nervous system. *Nature Reviews Immunology, 3*, 569–581.

Robertson, N. J., Tan, S., Groenedaal, F., van Bel, F., Juul, S. E., Bennet, L., Derrick, M., Back, S. A., Valdez, R. C., Northington, F., Gunn, A. J., Mallard, À., & C. (2012). Which neuroprotective agents are ready for bench to bedside translation in the newborn infant? *Pediatrics, 160*, 544–552.

Sakakibara, Y., Mitha, A. P., Ogilvy, C. S., & Maynard, K. I. (2000). Post-treatment with nicotinamide (vitamin B(3)) reduces the infarct volume following permanent focal cerebral ischemia in female Sprague–Dawley and Wistar rats. *Neuroscience Letters, 281*, 111–114.

Sananbenesi, F., & Fischer, A. (2009). The epigenetic bottleneck of neurodegenerative and psychiatric diseases. *Biological Chemistry, 390*, 1145–1153.

Scheepens, A., Wassink, G., Piersma, M. J., Van de Berg, W. D. J., & Blanco, C. E. (2003). A delayed increase in hippocampus following global asphyxia in the neonatal rat. *Brain Research. Developmental Brain Research, 142*, 67–76.

Schultz, N., Lopez, E., Saleh-Gohari, N., & Helleday, T. (2003). Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. *Nucleic Acids Research, 31*, 4959–4964.

Seidl, R., Stoeckler-Ipsiroglu, S., Rolinski, B., Kohlhauser, C., Herkner, K. R., Lubec, B., & Lubec, G. (2000). Energy metabolism in graded perinatal asphyxia of the rat. *Life Sciences, 67*, 421–435.

Shalak, L. F., Laptook, A. R., Jafri, H. S., Ramilo, O., & Perlman, J. M. (2002). Clinical chorioamnionitis elevated cytokines and brain injury in term infants. *Pediatrics, 110*, 673–680.

Simola, N., Bustamante, D., Pinna, A., Pontis, S., Morales, P., Morelli, M., & Herrera-Marschitz, M. (2008). Acute perinatal asphyxia impairs non-spatial memory and alters motor coordination in adult male rats. *Experimental Brain Research, 185*, 595–601.

Skaper, S. D. (2003). Poly(ADP-ribosyl)ation enzyme-1 as a target for neuroprotection in acute central nervous system injury. *Current Drug Targets. CNS and Neurological Disorders, 2*, 279–291.

Strackx, E., Van den Hove, D. L., Prickaerts, J., Zimmermann, L., Steinbusch, H. W., Blanco, C. E., Gavilanes, A. W., & Vles, J. S. (2010). Fetal asphyctic preconditioning protects against perinatal asphyxia-induced behavioral consequences in adulthood. *Behavioural Brain Research, 208*, 343–351.

Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L., & Prakash, S. (1993). Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature, 365*, 852–855.

Takahashi, K., Pieper, A. A., Croul, S. E., Zhang, J., Snyder, S. H., & Greenberg, J. H. (1999). Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia. *Brain Research, 829*, 46–54.

Trucco, C., Oliver, F. J., de Murcia, G., & Menissier de Murcia, J. (1998). DNA repair defect in poly (ADP-ribose) polymerase-deficient cell lines. *Nucleic Acids Research, 26*, 2644–2649.

Ullrich, O., Diestel, A., Evüpoglu, I. Y., & Nitsch, R. (2001). Regulation of microglial expression of integrins by poly(ADP-ribose) polymerase-1. *Nature Cell Biology, 3*, 1035–1042.

Van de Berg, W. D., Kwaijtaal, M., de Louw, A. J., Lissone, N. P., Schmitz, C., Faull, R. L., Blokland, A., Blanco, C. E., & Steinbusch, H. W. (2003). Impact of perinatal asphyxia on the GABAergic and locomotor system. *Neuroscience, 117*, 83–96.

Van Erp, T. G. M., Saleh, P. A., Rosso, P. A., Huttunen, M., Lönnqvist, J., Pirkola, T., Salonen, O., Valanne, L., Poutanen, V.-P., Standersköld-Nordenstam, C.-G., & Cannon, T. D. (2002). Contributions of genetic risk and fetal hypoxia to hippocampal volume in patients with schizophrenia or schizoaffective disorder, their unaffected siblings and healthy unrelated volunteers. *The American Journal of Psychiatry, 159*, 1514–1520.

Vannuci, S., & Hagberg, H. (2004). Hypoxia-ischemia in the immature brain. *The Journal of Experimental Biology, 207*, 3149–3154.

Venerosi, A., Valanzano, A., Cirulli, F., Alleva, E., & Calamandrei, G. (2004). Acute global anoxia during C-section birth affects dopamine-mediated behavioural responses and reactivity to stress. *Behavioural Brain Research, 154*, 155–164.

Venerosi, A., Cutuli, D., Chiarotti, F., & Calamandrei, G. (2006). C-section birth per se or followed by global asphyxia altered emotional behaviour in neonate and adult rats. *Behavioural Brain Research, 168*, 56–63.

Vexler, Z. S., & Yenari, M. A. (2009). Does inflammation after stroke affect the developing brain differently than adult brain? *Developmental Neuroscience, 31*, 378–393.

Virag, L., & Szabo, C. (2002). The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacological Reviews, 54*, 375–429.

Wan, F. J., Lin, H. C., Karg, B. H., Tseng, C. J., & Tung, C. S. (1999). D-amphetamine- induced depletion of energy and dopamine in the rat striatum is attenuated by nicotinamide pretreatment. *Brain Research Bulletin, 50*, 167–171.

Wyss, J. M., & Van Groen, T. (1992). Connections between the retrosplenial cortex and the hippocampal formation in the rat: A review. *Hippocampus, 2*, 1–11.

Wyss, M. T., Jolivet, R., Buck, A., Magistretti, P. J., & Weber, B. (2011). In vivo evidence for lactate as a neuronal energy source. *The Journal of Neuroscience, 31*, 7477–7485.

Yan, Q., Briehl, M., Crowley, C. L., Payne, C. M., Bernstein, H., & Bernstein, C. (1999). The NAD + precursors, nicotinic acid and nicotinamide upregulate glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase mRNA in Jurkat cells. *Biochemical and Biophysical Research Communications, 255*, 133–136.

Zampieri, M., Passananti, C., Calabrese, R., Perilli, M., Corbi, N., De Cave, F., Guastafierro, T., Bacalini, M. G., Reale, A., Amicosante, G., Calabrese, L., Zlatanova, J., & Caiafa, P. (2009). Parp 1 localizes within the Dnmt1 promoter and protects its unmethylated state by its enzymatic activity. *PLoS One, 4*, 4717.

Zhang, J., Pieper, A., & Snyder, S. H. (1995). Poly(ADP-ribose) synthase activation: An early indicator of neurotoxic DNA damage. *Journal of Neurochemistry, 65*, 1411–1414.