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Treatment advances in neonatal neuroprotection and neurointensive care

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Abstract

Knowledge of the nature, prognosis, and ways to treat brain lesions in neonatal infants has increased remarkably. Neonatal hypoxic-ischaemic encephalopathy (HIE) in term infants, mirrors a progressive cascade of excito-oxidative events that unfold in the brain after an asphyxial insult. In the laboratory, this cascade can be blocked to protect brain tissue through the process of neuroprotection. However, proof of a clinical effect was lacking until the publication of three positive randomised controlled trials of moderate hypothermia for term infants with HIE. These results have greatly improved treatment prospects for babies with asphyxia and altered understanding of the theory of neuroprotection. The studies show that moderate hypothermia within 6 h of asphyxia improves survival without cerebral palsy or other disability by about 40% and reduces death or neurological disability by nearly 30%. The search is on to discover adjuvant treatments that can further enhance the effects of hypothermia.

Introduction

Disorders that damage the developing brain are a substantial cause of death or permanent disability such as cerebral palsy. Hypoxic-ischaemic encephalopathy (HIE) in term infants occurs at a rate of about three per thousand live-born infants in developed countries, but the rate is estimated to be higher in the developing world.^{1,2} As many as a million deaths worldwide might be caused by perinatal asphyxia.³ Less common but no less important causes of brain injury in neonates are ischaemic strokes, including venous sinus thrombosis, encephalopathy associated with severe congenital heart disease, intraventricular haemorrhages, and peri ventricular leukomalacia (PVL) in premature infants.^{4–6} Infants with encephalopathy secondary to rare metabolic disorders such as urea-cycle disorders should also be included in this group.⁷ Physicians who care for these children in the period after injury have traditionally provided supportive care with little expectation that their interventions would salvage brain tissue or have an effect on the final outcome. However,

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Contributors

MVJ did the search of published works, prepared figures, wrote, and revised the Review. AF wrote, reviewed, edited, and revised the Review. MAW did research, contributed data, prepared figures, and reviewed the manuscript. FN contributed to the search of published works, writing, revisions, and preparation of the figures.

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this situation has changed over the past 5 years with the publication of positive results from several controlled trials of therapeutic moderate hypothermia for term infants with neonatal HIE.⁸ Publication of these results has led to wider clinical application of therapeutic hypothermia for neonatal encephalopathy. This new therapy is increasingly being given to babies in special neurointensive care nursery units where they can be monitored with amplitude integrated electroencephalography (aEEG) and continuous conventional electroencephalography (EEG) by specially trained nurses.⁹ Time to recovery of a normal background pattern on aEEG seems to be a better predictor of outcome in infants treated with therapeutic hypothermia than in normothermic infants with HIE.¹⁰

These advances in treatment are based on knowledge gained from clinical observation of babies with asphyxia and extensive laboratory research with experimental models.^{11,12} Data from these experiments led to a heuristic model in which hypoxia-ischaemia triggers a delayed series of events that lead to cell death in the brain.¹³ This period of delay, or latent interval, suggested that post-insult interventions could be protective if started in time. Discoveries of the role of glutamate-mediated excitotoxicity, oxidative stress, and cell-death signalling pathways in perinatal brain injuries have provided a foundation for these trials in humans, and continuing research on basic mechanisms of cell death is important to improve treatment and identify new treatment targets. These basic studies suggest that combination of hypothermia with adjuvant treatments, including the anaesthetic gas xenon, erythropoietin, or anticonvulsants, might improve outcome.¹⁴ In this Review we discuss three advances: progress in understanding the cascade of neurochemical events that mediate brain damage after a hypoxic-ischaemic insult in term infants; data from clinical trials that show benefit of therapeutic hypothermia for term infants with HIE; and early clinical results of trials of erythropoietin and other adjuvant drugs that might enhance neuroprotection.

Neonatal encephalopathy in term infants

Signs of encephalopathy in newborn babies older than 34 weeks' gestation can include hyperalertness, lethargy, somnolence or coma, seizures, poor feeding, hypotonia, and respiratory problems that might need mechanical ventilation.¹¹ Sarnat and Sarnat¹⁵ were the first to define this syndrome as neonatal encephalopathy following foetal distress in 21 infants older than 36 weeks' gestation.¹⁵ They distinguished three stages of encephalopathy: stage 1, or mild encephalopathy associated with hyperalertness, sympathetic overdrive, and a normal EEG; stage 2, or moderate encephalopathy marked by obtundation, hypotonia, multifocal seizures, and an EEG showing periodic or continuous delta activity; and stage 3, or severe encephalopathy in which infants were stuporous and flaccid with an isoelectric or periodic EEG. Infants who did not enter stage 3 and who had signs of stage 2 for fewer than 5 days were normal on follow-up, but persistence of stage 2 for a week or failure of the EEG to normalise predicted later neurological impairment or death. Levene and colleagues¹⁶ used a similar system to classify 122 term infants with asphyxia into mild, moderate, or severe groups. On follow-up at 2.5 years of age, all children with mild encephalopathy were normal, whereas 25% with moderate encephalopathy and 76% with severe encephalopathy died or had a disability. The severity of encephalopathy was more predictive of neurological handicap than was the Apgar score, and none of the children with neurological disability had a low Apgar score without also having moderate or severe encephalopathy.

Definition of neonatal encephalopathy as a clinical syndrome was supported by the publication of two papers from the Western Australian case-control study by Badawi and colleagues.^{17,18} This was the first population-based study of neonatal encephalopathy and included all term infants in Western Australia who met the study criteria; however, it included some infants who would not have met criteria for the study by Sarnat and Sarnat¹⁵ or more recent trials of brain cooling. The causes of neonatal encephalopathy in this study

were heterogeneous, and more than 70% of infants had no evidence of intrapartum hypoxia. Antepartum factors that were strongly associated with neonatal encephalopathy included maternal thyroid disease, severe pre-eclampsia, intrauterine growth retardation, and intrapartum factors such as maternal fever and acute obstetrical events. At follow-up, 28% of children with encephalopathy were reported to have genetic disorders, dysmorphic features, or malformations compared with 4% of controls.¹⁹ This study showed that differential diagnosis of encephalopathy in neonates is broad and includes infection, endocrine, genetic, and metabolic disorders.¹¹

Pathogenesis of perinatal brain injury

The excitotoxic cascade

Implicit in the early descriptions of the syndrome of neonatal HIE was the notion that its clinical signs progress after a latent period of hours to days and then resolve within several weeks.¹⁵ Recognition of this concept led to clinical and basic research aimed at understanding the cascade of neurochemical processes responsible for brain injury to improve intervention and salvage brain tissue.^{20–22} An important early discovery made by Reynolds and colleagues^{23,24} was termed “secondary energy failure”. They reported that high-energy-containing phosphate compounds in the brains of term infants fell during the initial period of asphyxia, defined by low Apgar score and severe metabolic acidosis, and rebounded quickly during resuscitation, only to fall again permanently after about 24 h.^{23,25,26} Reynolds’ group also reported this effect in piglets²⁷ and neonatal rat pups²⁸—a noteworthy example of clinical observations promoting laboratory research, the results of which were then translated back to the clinic.^{24,26} The interval between the initial hypoxic-ischaemic insult and delayed energy failure includes the latent period after resuscitation when the infant appears more active for 8–24 h, but then develops seizures and other signs of encephalopathy.^{29,30} The mechanism of brain injury is thought to involve a series of events that we refer to as the “excitotoxic cascade” (figure 1). We prefer this term because experimental evidence suggests that activation of excitatory glutamate receptors, especially NMDA receptors, occurs very early during the initial hypoxic-ischaemic insult. Then oxidative stress associated with worsening mitochondrial dysfunction and mitochondrial failure becomes an important factor that determines whether neurons and glia survive or die by apoptosis or necrosis.^{31–33} This cascade probably accounts for the process of secondary energy failure in mitochondria, in which the brain’s energy supplies fall over a period of about 24 h in babies and sheep models.³⁴

Glutamate-receptor-mediated injury—The first events to occur during a severe hypoxic-ischaemic episode associated with metabolic acidosis are depolarisation of neuronal membranes and power failure of glutamate transporters on perisynaptic glia that ordinarily remove the neurotransmitter from the synaptic cleft (figure 1).^{35,36} Hypoxia-ischaemia reduces ionic gradients across neuronal membranes leading to membrane depolarisation and neurotransmitter release. Glutamate reuptake transporters can operate anaerobically in hypoxaemic conditions, but become impaired³⁷ when ischaemia secondary to falling cardiac output restricts the delivery of glucose. Transporter failure then causes glutamate to accumulate within synapses and spill over into the brain’s extracellular space.³⁶ This accumulation is consistent with notable increases in concentrations of the excitatory aminoacids glutamate and aspartate in the CSF of babies with moderate or severe encephalopathy.^{38,39} Studies in fetal lambs have also shown that hypoxia-ischaemia causes extracellular overflow of glutamate and other aminoacids in the cortex and basal ganglia.⁴⁰ Loss of membrane potential, combined with high concentrations of glutamate, opens calcium-permeable NMDA glutamate channels and voltage-gated calcium channels allowing calcium to move into neurons (figure 2).⁴¹ Strong evidence suggests that many

types of calcium conductance channels are open during sustained hypoxic-ischaemic-induced depolarisation; substantial non-specific transmembrane leakage also occurs.⁴¹ Calcium flooding into cells activates a cascade of events that can cause cell death. Endogenous activation of adenosine A1 receptors during severe asphyxia mediates the initial suppression of neural activity and is an important protective mechanism.⁴² Pretreatment of 7-day-old rats with the NMDA channel blocker MK-801 before hypoxia-ischaemia is strongly protective, but the temporal window for protection by this drug after hypoxic-ischaemia is less than 3 h,²⁰ suggesting that the cascade downstream of NMDA receptors quickly becomes irreversible, self-perpetuating, and unresponsive to channel blockade. Magnesium is neuroprotective against damage caused by HIE in neonatal rodent models, probably due to its ability to block NMDA-receptor channels.⁴³ AMPA-type glutamate receptors are also activated by excess glutamate and probably contribute to seizures during hypoxic-ischaemic encephalopathy.⁴⁴

The prominence of NMDA-mediated injury in the immature brain is related to the fact that NMDA receptors are functionally upregulated in the perinatal period because of their role in activity-dependent neuronal plasticity.⁴⁵ Immature NMDA channels open more easily and stay open longer than do adult channels, and the voltage-dependent magnesium block that is normally present in adult channels at resting membrane potentials is more easily relieved in the perinatal period.⁴⁶ Increased expression of NR2B subunits on NMDA receptors is thought to be responsible for their greater excitability and longer open time during the neonatal period. Normally this increased opening time serves the developing brain well by enhancing physiological forms of activity-dependent plasticity such as long-term potentiation, which is associated with coding of memories.⁴⁵ However, when energy shortages occur, this arrangement becomes a liability, and the increased excitability can lead to neuronal damage. In addition to the enhanced excitability of immature glutamate synapses, the neonatal brain is more excitable than the adult brain because the inhibitory neurotransmitter GABA produces excitation rather than inhibition in the neonatal period.⁴⁷ This excitation occurs because neuronal chloride transporters expressed during the neonatal period raise the intracellular concentration higher than the extracellular concentration.⁴⁷ When GABA binds to its receptor, chloride channels open, which allows chloride to move outward causing depolarisation, rather than moving inward causing hyperpolarisation and inhibition, as it does in the mature brain.⁴⁸

Mitochondrial impairment and oxidative stress—Open NMDA channels allow calcium to enter the intracellular compartment and activate neuronal nitric oxide synthase, leading to production of the oxygen free radical nitric oxide (figure 2). Nitric oxide can form a complex with superoxide to form toxic peroxynitrite molecules that can add nitrate to tyrosine groups on proteins and contribute to the production of hydroxyl radicals, causing lipid peroxidation of proteins and DNA (figure 2). Nitric oxide can also disrupt mitochondrial respiration by impairing the function of cytochrome oxidase (complex 4) and complex 1, which increases production of superoxide and peroxynitrite ions in mitochondria, especially during hypoxia.^{49,50} Accumulation of lactic acid in part reflects progressive loss of mitochondrial membrane potential and mitochondrial failure, and can be measured with proton magnetic resonance spectroscopy during the progression of HIE.¹¹ These ions can enhance the movement of the proapoptotic proteins cytochrome C and apoptosis-inducing factor (AIF) across the outer mitochondrial membrane into the cytoplasm where they trigger apoptosis through the so-called intrinsic pathway. Hagberg and colleagues³⁴ reported that outer mitochondrial membrane permeabilisation in neonates was initiated by the proapoptotic protein Bax and regulated by the anti-apoptotic Bcl proteins (figure 2). They also noted that a Bax-inhibiting peptide reduces neonatal brain injury and functional impairment in a mouse model of HIE,⁵¹ an effect that was confirmed by Han and colleagues.⁵² On the one hand, in the cytoplasm, cytochrome C binds with

caspases in the apoptosome to trigger activation of caspase 3 by proteolysis, and activated caspase 3 in turn can trigger apoptotic DNA fragmentation (figure 2).^{53,54} AIF, on the other hand, moves into the nucleus and triggers DNA fragmentation via a non-caspase-mediated mechanism that is stimulated by heightened activity of the DNA repair enzyme PARP1 (poly-ADP-ribose polymerase; figure 2).⁵⁵ Calcium entering through NMDA receptors can also activate caspase 3 directly without activating neuronal nitric oxide synthase through activation of calpain.⁵⁶ Caspase 3 is expressed to a much greater degree in the immature brain than in the adult brain. Similar to the developmental differences in NMDA and GABA receptors, enhanced activity of caspase 3 is another feature of the neonatal brain that makes it more vulnerable to injury. Apoptosis can also be triggered by the Fas cell-death protein cell-surface receptor through activation of caspase 8 and then caspase 3 in the so-called extrinsic cell-death pathway (figure 2). Transgenic mice that lack Fas receptors are resistant to hypoxic-ischaemic brain injury.⁵⁷

An imbalance between increased reactive oxygen and nitrogen species (superoxide anions, singlet oxygen ions, hydroxyl radicals, and hydrogen peroxide) and marginal or low supplies of antioxidant defenses such as superoxide dismutase, glutathione peroxidase, catalase, glutathione, and vitamin C in the neonatal period make an important contribution to brain injury (figure 2).⁵⁸ X-linked inhibitor of apoptosis protein has strong antioxidant capacity through its roles in upregulating superoxide dismutase 2 and thioredoxin 2 and reducing release of cytochrome C from mitochondria.^{59,60} Studies of genetic manipulation of antioxidant compounds in the brains of mice have shown the importance of this system in modulation of perinatal brain injury.⁶¹ Several drugs can impede oxidative stress and reduce brain injury, including NMDA receptor antagonists and neuronal nitric oxide synthase inhibitors.^{14,34,62} West and colleagues⁶³ reported that antioxidant pomegranate polyphenols and resveratrol protected rat pups from hypoxia-ischaemia on postnatal day 7 when given to the dam before birth. Necrostatin 1, which inhibits receptor interacting protein (RIP-1) kinase and programmed necrosis through a caspase-independent mechanism, also inhibits non-specific oxidative damage to proteins of many molecular weights in the immature brain and provides neuroprotection in the immature mouse model of hypoxia-ischaemia.⁶⁴ Melatonin has also been shown to have antioxidant and protective effects in excitotoxic models of neonatal brain injury.⁶⁵

Inflammation—Inflammation plays an important part in the excito-oxidative cascade of injury in the perinatal period.³⁴ Lipopolysaccharide has been shown to sensitise the perinatal brain to hypoxia-ischaemia and worsen injury;⁶⁶ injury can be reduced by administration of the antioxidant N-acetylcysteine.⁶⁷ This effect seems to depend on activation of microglia and upregulation of inflammatory mediators that are under the control of nuclear factor kappa B (NF- κ B). Treatment with an inhibitor of NF- κ B, after the onset of neonatal hypoxia-ischaemia, has been shown to provide substantial protection against neonatal hypoxia-ischaemia by inhibiting apoptosis.⁶⁸ The mast-cell inhibitor sodium cromoglicate has also been shown to provide neuroprotection against hypoxia-ischaemia in a rodent model,⁶⁹ an effect that was first discovered in an excitotoxic model of injury.⁷⁰ Russell and colleagues⁷¹ showed that pentraxin 1, a member of the large pentraxin family of molecules that have complement activating and opsonic activity, mediates damage in a model of hypoxic-ischaemic perinatal brain injury; this is an example of the molecular links between damage caused by hypoxia-ischaemia and inflammation.

Delayed cell death after hypoxia-ischaemia—Histological studies and MRI show that cell death in the brain exposed to hypoxia-ischaemia is delayed over several days and continues over days to weeks after an injury because groups of cells seem to commit to die.^{72–74} In experimental mouse and rat models, neuropathological studies show a mixture of apoptosis and necrosis and hybrid or continuum phenotypes, depending on the region and

severity of injury.^{75,76} Advances in cell biology and biochemistry suggest that apoptosis or autophagy mediated by programmed cell death mechanisms predominate.⁷⁵ Various forms of programmed cell death make a prominent contribution to degeneration from HIE in animal models and in newborns.⁷⁶ Programmed cell necrosis also contributes to the neurodegeneration seen in animal models of HIE and perhaps in newborns.⁷⁶ Since the process of apoptosis requires energy, a determinant of when cells die is likely to be the ability of mitochondria to provide adequate energy. Another determinant of classic apoptosis is the loss of neuronal connections, which can continue over days to weeks after an injury because groups of cells seem to commit to die.^{75,76} (figure 3). Increased knowledge about the factors that determine when or how cells die after hypoxia-ischaemia is important since it might be possible to salvage tissue through use of drugs, growth factors, or treatment interventions that influence brain activity.^{76,77}

Sex differences in pathways to brain injury

Evidence suggests that sex is an important determinant of which cell-death pathways are activated during HIE, and it can also influence effects of neuroprotective drugs. Hagberg and colleagues⁷⁸ reported that knock-out of *PARP1* preferentially protected male but not female transgenic mice from hypoxic-ischaemic damage. McCullough and colleagues⁷⁹ reported that in adult mice *PARP1* knockout or pharmacological inhibition of *PARP1* protected males from stroke damage, but worsened injury in females. Du and colleagues⁸⁰ also showed that male and female rodent neurons, grown in separate cultures, differ in their activation of cell-death pathways. They reported that male neurons in culture are more sensitive to death from exposure to NMDA and nitric oxide, whereas female neurons were preferentially sensitive to caspase 3 inhibition. When exposed to stress caused by oxygen-glucose deprivation, male neurons preferentially release AIF from mitochondria into the nucleus, whereas female neurons preferentially release cytochrome C from mitochondria to activate caspase 3. In agreement with these results, experiments with AIF-deficient Harlequin mice showed that males but not females are protected from ischaemic brain injury.⁸¹ Male neurons are also more vulnerable to autophagy or autophagocytosis triggered by starvation than are female neurons.⁸² The nitric oxide synthesis inhibitor 7-nitroindazole has been reported to be more protective for male than for female neurons,⁸³ whereas the selective pan-caspase inhibitor QVD-OPh (MP Biochemicals, Illkirch, France) protects female but not male rat pups from stroke damage.⁸⁴ The neuroprotective drug 2-iminobiotin, which inhibits release of cytochrome C and activation of caspase 3, has been reported to protect female but not male 7-day-old rat pups from HIE.⁸⁵ We reported that the competitive glutamate antagonist dextromethorphan is more protective against ischaemia in male than in female immature mice,⁸⁶ whereas erythropoietin has been reported to be more protective in female rat pups.^{87,88} These differences need to be accounted for when designing trials of neuroprotective treatments. Long-term reduction in caspase 3 activity during development leads to upregulation of AIF-dependent pathways, suggesting that there is interplay between these two pathways.⁸⁹ These sexually dimorphic differences in cell-death pathways might contribute to the higher incidence of cerebral palsy and stroke in boys than in girls.^{90,91}

Selective network degeneration after HIE

MRI of the brains of babies with HIE often shows damage to selected neuronal systems rather than global injury. For example, Cowan and colleagues⁹² reported that babies with multiple signs of encephalopathy usually had evidence of bilateral deep basal ganglia injury, cortical injury, or both, whereas babies with seizures alone usually had focal ischaemia or haemorrhage. Term infants exposed to brief (10–30 min) but very intense hypoxia-ischaemia due to umbilical-cord compression in association with cord pH less than 7 and severe lactic acidosis often develop focal brain lesions on MRI in the peri-Rolandic cortex, ventrolateral thalamus, and posterior putamen^{93–95} (figure 4). Infants with these lesions

exhibit abnormalities in generalised movements at 1 month and 3 months of age.⁹⁶ Children with this pattern of injury after asphyxia have also been shown to develop the clinical syndrome of extrapyramidal cerebral palsy including athetosis and dystonia with impaired motor speech and impaired use of their hands compared with their legs.⁹⁷ This selective pattern of injury suggests that some specific neuronal circuits are more vulnerable to injury^{98,99} and has been suggested to mirror damage in areas that are relatively rich in excitatory synapses in term infants;^{32,97} this is supported by evidence that striatal injury in a newborn piglet model of near total asphyxia is mediated by NMDA-receptor activation.¹⁰⁰ A similar systems-selective pattern of network degeneration in the hippocampus has been seen with diffusion tensor MRI in mice with hypoxic-ischaemic injury.¹⁰¹

Neuroprotection mediated by hypothermia

Hypothermia has been used successfully since the 1950s as a protective measure to allow babies with complex congenital heart disease to undergo circulatory arrest for corrective surgery,¹⁰² but its use in neonatological settings was impeded by data showing that it increased the death rate in premature infants.¹⁰³ However, interest in this technique was renewed with the publication of data on the pathogenesis of HIE reviewed above, especially that which showed the importance of delayed secondary energy failure and the excitotoxic cascade in neurological damage. Experiments that showed efficacy and safety in clinically relevant large animal models were especially important in bringing this technique to the clinic.

Mechanisms for hypothermic neuroprotection

Although our understanding of the mechanisms for the neuroprotective effect of hypothermia remains incomplete, experimental studies have shown that it inhibits many steps in the excitotoxic cascade including secondary energy failure,¹⁰⁴ increases in brain lactic acid, glutamate, and nitric oxide concentrations,^{105,106} and epileptic activity.¹⁰⁷ Protective effects have also been associated with inhibition of protease and calpain activation, loss of mitochondrial membrane potential and mitochondrial failure, free radical damage, lipid peroxidation, and inflammation.¹⁰⁸ Experiments in which the glutamate agonist NMDA was injected directly into the brains of 7-day-old rat pups to produce excitotoxic lesions showed that injury was directly proportional to temperature over a range of 25–40°C.¹⁰⁹ This suggests that hypothermia can reduce injury triggered by NMDA-receptor activation alone without hypoxia-ischaemia. Ikonomidou and colleagues¹¹⁰ also showed that hypothermia enhances the protective effect of the non-competitive NMDA channel antagonist MK-801 in a model of hypoxic-ischaemic brain injury in rat pups. Brief periods of moderate hypothermia to 32°C after injury have been shown to delay cell death in 7-day-old rat pups by as much as a week, after which brain atrophy occurs.¹¹¹ This result suggests that moderate hypothermia can delay programmed cell death, independent of its role in other aspects of metabolism, and might be able to extend the therapeutic window for other interventions.

Clinical trials

The publication of three randomised controlled trials of moderate hypothermia or conventional treatment for HIE in term infants, published from 2005 to 2009, is generally thought to have been a milestone in this area of neonatology.^{112–114} The first study, the CoolCap trial, by Gluckman and colleagues,¹¹² included 234 term babies less than 6 h old with evidence of asphyxia at birth (Apgar <5 at 10 min, or severe acidosis) and background abnormality on aEEG. The group was randomised to treatment with a selective head cooling device to maintain rectal temperature at 34.5°C for 72 h or to conventional care. The primary outcome was death or severe disability at 18 months when a neurological and

developmental assessment was done on a total of 218 children who received cooling or conventional treatment. There were no significant differences between groups for the primary outcome for all children but outcome was significantly better for hypothermia when the prestratified group of children with the most severe encephalopathy on aEEG were excluded. This suggested that babies with severe encephalopathy were unresponsive to cooling.

The second study was the National Institutes of Child Health and Human Development (NICHD) Whole-Body Hypothermia for Neonates with HIE by Shankaran and colleagues.¹¹³ They enrolled 208 term infants less than 6 h old with evidence of HIE associated with asphyxia who were randomised to total body cooling to 33.5°C for 72 h or conventional care. Follow-up at 18 months of age in 205 of 208 infants showed that cooling significantly reduced the primary endpoint of death or moderate or severe disability from 62% in controls to 44% in cooled infants.

The third trial was the Total Body Hypothermia for Neonatal Encephalopathy Trial (TOBY) by Azzopardi and colleagues.¹¹⁴ This study enrolled 325 infants with entry criteria that were identical to those of the CoolCap trial, and infants were randomised to cooling at 33.5°C for 72 h or conventional care. Infants in the cooled group were significantly more likely to survive without neurological abnormality (44% cooled *vs* 28% non-cooled), and had lower rates of cerebral palsy (33% cooled *vs* 48% non-cooled) and higher developmental scores than non-cooled infants. aEEG was used to select babies for the CoolCap and TOBY studies but not for the NICHD study. aEEG seems to be useful for staging encephalopathy and has good specificity for detection of seizures,¹¹⁵ and its sensitivity for seizure detection by trained personnel is 78%.^{116,117}

Two meta-analyses of clinical trials of moderate hypothermia for HIE have been published since publication of the TOBY trial, one by Edwards and colleagues⁸ and another by Shah.¹¹⁸ Edwards and colleagues⁸ analysed the TOBY, CoolCap, and the NICHD trials together with seven other trials, including those by Eicher and colleagues,^{119,120} Jacobs and colleagues,¹²¹ and Simbruner and colleagues,¹²² which had follow-ups of at least 18 months. Edwards and colleagues⁸ concluded that cooling reduced the risk of death or disability at 18 months with a number needed to treat of nine with little risk of adverse effects, and Shah's analysis was generally consistent with this assessment. Follow-up studies will be important to assess long-term safety of moderate hypothermia for HIE.

Therapies to augment neuroprotection

In view of the definite but incomplete success of hypothermia after asphyxia for reducing disability from HIE, the hunt is on for treatments that might augment neuroprotection (panel). Phenobarbital and the AMPA-receptor antagonist topiramate are used commonly for seizures in neonates and have good safety profiles.^{145,146} Barks and colleagues¹²³ and Liu and colleagues¹⁴⁷ reported that phenobarbital enhanced the protective effect of hypothermia in a rat pup model of HIE and topiramate extended the temporal window of protection. Phenobarbital treatment has been reported to reduce concentrations of peroxides and antioxidant enzymes in the CSF of babies with asphyxia.¹⁴⁸ A safety study of topiramate in a small number of infants with HIE treated with hypothermia showed no adverse effects.¹²⁴ Levetiracetam might also have potential for use in infants.¹⁴

Chakkarapani and colleagues¹²⁶ reported that inhalation of 50% xenon, a rare, clinically approved anaesthetic gas that acts as an NMDA-receptor antagonist, provided additive neuroprotection in asphyxiated newborn piglets, doubling protection provided by hypothermia alone. Experimental evidence suggests that xenon might exert its protective effect by blocking the glycine site on NMDA receptors.¹⁴⁹ Memantine is another use-

dependent NMDA antagonist that could be used with hypothermia. Erythropoietin might also improve outcome in infants with HIE.^{14,136} Erythropoietin has been shown to have a dose-related protective effect against HIE brain injury in neonatal rodent models,^{87,88} acting through anti-inflammatory, antioxidative, antiapoptotic, and neurotrophic mechanisms, and stimulation of neurogenesis. This is consistent with the probable role of erythropoietin in protection by hypoxic preconditioning, which upregulates genes in the PI3K/AKT pathway in 7-day-old rat pups;¹⁵⁰ two studies of term infants with HIE have shown promising results.^{138,139} These drugs and growth factors could enhance neuroprotection when combined with hypothermia, and warrant further investigation (panel).

In addition to small molecule drugs and growth factors, cell-based treatment has been discussed as a potential therapy for HIE and could also be used with hypothermia.^{151,152} Animal studies support the idea that cord blood and mesenchymal stem cells, cell types that are available in a clinical setting, have a neuroprotective effect in neonatal HIE.¹⁵³ These effects have been attributed to immunomodulation, activation of endogenous stem cells, release of growth factors, and antiapoptosis mechanisms.¹⁵⁴ Only one registered clinical study in the USA is investigating the role of autologous cord-blood transplantation in HIE.¹⁵⁵ This trial administers moderate cooling plus cord-blood cells to term infants with HIE at less than 6 h of age, and cord blood alone to infants with HIE between 6 h and 24 h of age.

Conclusions

Over the past decade, substantial progress has been made in neuroprotection and neurointensive care for neonates. Decades of clinical and basic science research into the pathogenesis of HIE in term infants have been translated into the use of moderate hypothermia for infants with mild or moderate HIE, and much evidence shows that this therapy substantially, although incompletely, reduces neurological disability. This therapy is increasingly being given to babies in special neurointensive care nursery units where they can be monitored with continuous electrophysiological monitoring such as aEEG. More research will be needed to follow-up children treated in this way to determine whether benefits identified at 18 months are maintained into childhood, and whether any harmful effects emerge. Follow-up electrophysiological data also need to be collected to improve understanding of associations between seizures, treatment, and outcome.

Other issues that deserve attention are the optimum timing of initiation and duration of hypothermia and rewarming and whether this technique is useful for other disorders such as perinatal stroke or status epilepticus. Effects of hypothermia on the metabolism and action of other drugs being given in combination with hypothermia also need further investigation. Because the neuroprotective effects of hypothermia for HIE are not fully understood, further research is needed to find adjuvant treatments that are compatible with hypothermia; this will require more basic science research focusing on the mechanisms of injury, especially those associated with mitochondrial failure, inflammation, and genetic mechanisms. Mitochondrial failure seems to be the key to survival for many cells in the brain, and treatments that target the mitochondria might be especially useful. Inflammation enhances the effects of HIE, and anti-inflammatory drugs might turn out to be neuroprotective in human infants. Cell-based treatments are also being explored in preclinical models, and they might act in part by modulating inflammation. Genetic differences are likely to play a part in the variable effects of HIE on infants, and clear differences in cell-death pathways have been established between male and female rodents. Sex should be accounted for when designing and testing new treatments, because preclinical testing shows that treatments might be protective in one sex but ineffective in the other.

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References

1. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev.* 2010; 86:329–338. [PubMed: 20554402]
2. Ellis M, Manandhar DS, Manandhar N, Wyatt J, Bolam AJ, Costello AM. Stillbirths and neonatal encephalopathy in Kathmandu, Nepal: an estimate of the contribution of birth asphyxia to perinatal mortality in a low-income urban population. *Paediatr Perinat Epidemiol.* 2000; 14:39–52. [PubMed: 10703033]
3. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet.* 2005; 365:891–900. [PubMed: 15752534]
4. Nelson KB, Lynch JK. Stroke in newborn infants. *Lancet Neurol.* 2006; 3:150–158. [PubMed: 14980530]
5. Sherlock RL, McQuillen PS, Miller SP. Preventing brain injury in newborns with congenital heart disease: brain imaging and innovative trial designs. *Stroke.* 2009; 40:327–332. [PubMed: 18988911]
6. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol.* 2009; 8:110–124. [PubMed: 19081519]
7. Enns GM. Neurologic damage and neurocognitive dysfunction in urea cycle disorders. *Semin Pediatr Neurol.* 2008; 15:132–139. [PubMed: 18708004]
8. Edwards AD, Brocklehurst P, Gunn AJ, et al. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ.* 2010; 340:c363. [PubMed: 20144981]
9. Hellstrom-Westas L, Rosen I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Arch Dis Child Fetal Neonatal Ed.* 1995; 72:F34–F38. [PubMed: 7743282]
10. Thoresen M, Hellstrom-Westas L, Liu X, De Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics.* 2010; 126:e131–e139. [PubMed: 20566612]
11. Fatemi A, Wilson MA, Johnston MV. Hypoxic-ischemic encephalopathy in the term infant. *Clin Perinatol.* 2009; 36:835–858. [PubMed: 19944838]
12. du Plessis AJ, Volpe JJ. Perinatal brain injury in the preterm and term newborn. *Curr Opin Neurol.* 2002; 15:151–157. [PubMed: 11923628]
13. Johnston MV, Nakajima W, Hagberg H. Mechanisms of hypoxic neurodegeneration in the developing brain. *Neuroscientist.* 2002; 8:212–220. [PubMed: 12061501]
14. Cilio MR, Ferriero DM. Synergistic neuroprotective therapies with hypothermia. *Semin Fetal Neonatal Med.* 2010; 15:293–298. [PubMed: 20207600]
15. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol.* 1976; 33:696–705. [PubMed: 987769]
16. Levene MI, Sands C, Grindulis H, Moore JR. Comparison of two methods of predicting outcome in perinatal asphyxia. *Lancet.* 1986; 1:67–69. [PubMed: 2867316]
17. Badawi N, Kurinczuk JJ, Keogh JM, et al. Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ.* 1998; 317:1537–1538. [PubMed: 9836647]
18. Badawi N, Kurinczuk JJ, Keogh JM, et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ.* 1998; 317:1549–1553. [PubMed: 9836652]
19. Felix JF, Badawi N, Kurinczuk JJ, Bower C, Keogh JM, Pemberton PJ. Birth defects in children with newborn encephalopathy. *Dev Med Child Neurol.* 2000; 42:803–808. [PubMed: 11132253]
20. McDonald JW, Silverstein FS, Johnston MV. MK-801 protects the neonatal brain from hypoxic-ischemic damage. *Eur J Pharmacol.* 1987; 140:359–361. [PubMed: 2820765]

21. Vannucci RC, Towfighi J, Vannucci SJ. Secondary energy failure after cerebral hypoxia-ischemia in the immature rat. *J Cereb Blood Flow Metab.* 2004; 24:1090–1097. [PubMed: 15529009]
22. Hagberg H. Mitochondrial impairment in the developing brain after hypoxia-ischemia. *J Bioenerg Biomembr.* 2004; 36:369–373. [PubMed: 15377874]
23. Hope PL, Costello AM, Cady EB, et al. Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants. *Lancet.* 1984; 2:366–370. [PubMed: 6147452]
24. Hope PL, Reynolds EO. Investigation of cerebral energy metabolism in newborn infants by phosphorus nuclear magnetic resonance spectroscopy. *Clin Perinatol.* 1985; 12:261–275. [PubMed: 3978989]
25. Azzopardi D, Wyatt JS, Cady EB, et al. Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. *Pediatr Res.* 1989; 25:445–451. [PubMed: 2717259]
26. Wyatt JS, Edwards AD, Azzopardi D, Reynolds EO. Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. *Arch Dis Child.* 1989; 64:953–963. [PubMed: 2673061]
27. Lorek A, Takei Y, Cady EB, et al. Delayed (“secondary”) cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res.* 1994; 36:699–706. [PubMed: 7898977]
28. Blumberg RM, Cady EB, Wigglesworth JS, McKenzie JE, Edwards AD. Relation between delayed impairment of cerebral energy metabolism and infarction following transient focal hypoxia-ischaemia in the developing brain. *Exp Brain Res.* 1997; 113:130–137. [PubMed: 9028781]
29. Tan WK, Williams CE, During MJ, et al. Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischemic injury in late gestation fetal sheep. *Pediatr Res.* 1996; 39:791–797. [PubMed: 8726230]
30. Thoresen M. Cooling the newborn after asphyxia—physiological and experimental background and its clinical use. *Semin Neonatol.* 2000; 5:61–73. [PubMed: 10802751]
31. Ferriero DM. Neonatal brain injury. *N Engl J Med.* 2004; 351:1985–1995. [PubMed: 15525724]
32. Johnston MV. Excitotoxicity in perinatal brain injury. *Brain Pathol.* 2005; 15:234–240. [PubMed: 16196390]
33. Johnston MV, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic-ischemic injury in the developing brain. *Pediatric Res.* 2001; 49:735–741.
34. Hagberg H, Mallard C, Rousset CI, Xiaoyang W. Apoptotic mechanisms in the immature brain: involvement of mitochondria. *J Child Neurol.* 2009; 24:1141–1146. [PubMed: 19574577]
35. Taylor DL, Edwards AD, Mehmet H. Oxidative metabolism, apoptosis and perinatal brain injury. *Brain Pathol.* 1999; 9:93–117. [PubMed: 9989454]
36. Silverstein FS, Buchanan K, Johnston MV. Perinatal hypoxia-ischemia disrupts striatal high-affinity [3H]glutamate uptake into synaptosomes. *J Neurochem.* 1986; 47:1614–1619. [PubMed: 2876058]
37. Bak LK, Schousboe A, Sonnewald U, Waagepetersen HS. Glucose is necessary to maintain neurotransmitter homeostasis during synaptic activity in cultured glutamatergic neurons. *J Cereb Blood Flow Metab.* 2006; 26:1285–1297. [PubMed: 16467783]
38. Hagberg H, Thornberg E, Blennow M, et al. Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: relationship to hypoxic-ischemic encephalopathy. *Acta Paediatr.* 1993; 82:925–929. [PubMed: 7906573]
39. Riikonen RS, Kero PO, Simell OG. Excitatory amino acids in cerebrospinal fluid in neonatal asphyxia. *Pediatr Neurol.* 1992; 8:37–40. [PubMed: 1348414]
40. Hagberg H, Andersson P, Kjellmer I, Thiringer K, Thordstein M. Extracellular overflow of glutamate, aspartate, GABA and taurine in the cortex and basal ganglia of fetal lambs during hypoxia-ischemia. *Neurosci Lett.* 1987; 78:311–317. [PubMed: 2888062]
41. Cross JL, Meloni BP, Bakker AJ, Lee S, Knuckey NW. Modes of calcium entry and homeostasis following cerebral ischemia. *Stroke Res Treat.* 2010 published online Nov 1.
42. Takahashi T, Otsuguro K, Ohta T, Ito S. Adenosine and inosine release during hypoxia in the isolated spinal cord of neonatal rats. *Br J Pharmacol.* 2010; 161:1806–1816. [PubMed: 20735412]

43. Cetinkaya M, Alkan T, Ozyener F, Kafa IM, Kurt MA, Koksall N. Possible neuroprotective effects of magnesium sulfate and melatonin as both pre- and post-treatment in a neonatal hypoxic-ischemic rat model. *Neonatology*. 2010; 99:302–310. [PubMed: 21135566]
44. Jensen FE. The role of glutamate receptor maturation in perinatal seizures and brain injury. *Int J Dev Neurosci*. 2002; 20:339–347. [PubMed: 12175872]
45. McDonald JW, Johnston MV. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res Rev*. 1990; 15:41–70. [PubMed: 2163714]
46. Monyer H, Brunashev N, Laurie DJ. Development and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron*. 1993; 12:529–540. [PubMed: 7512349]
47. Dzhalal VI, Kuchibhotla KV, Glykys JC, et al. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci*. 2010; 30:11745–11761. [PubMed: 20810895]
48. Kahle KT, Staley K. Altered neuronal chloride homeostasis and excitatory GABAergic signaling in human temporal lobe epilepsy. *Epilepsy Curr*. 2008; 8:51–53. [PubMed: 18330470]
49. Blomgren K, Hagberg H. Free radicals, mitochondria, and hypoxia-ischemia in the developing brain. *Free Radic Biol Med*. 2006; 40:388–397. [PubMed: 16443153]
50. Robertson CL, Scafidi S, McKenna MC, Fiskum G. Mitochondrial mechanisms of cell death and neuroprotection in pediatric ischemic and traumatic brain injury. *Exp Neurol*. 2009; 218:371–380. [PubMed: 19427308]
51. Wang X, Han W, Du X, et al. Neuroprotective effect of Bax-inhibiting peptide on neonatal brain injury. *Stroke*. 2010; 41:2050–2055. [PubMed: 20671246]
52. Han B, Wang Q, Cui G, Shen X, Zhu Z. Post-treatment of Bax-inhibiting peptide reduces neuronal death and behavioral deficits following global cerebral ischemia. *Neurochem Int*. 2011; 58:224–233. [PubMed: 21167243]
53. Blomgren K, Zhu C, Hallin U, Hagberg H. Mitochondria and ischemic reperfusion damage in the adult and in the developing brain. *Biochem Biophys Res Commun*. 2003; 304:551–559. [PubMed: 12729590]
54. Hagberg H. Mitochondrial impairment in the developing brain after hypoxia-ischemia. *J Bioenerg Biomembr*. 2004; 36:369–373. [PubMed: 15377874]
55. Cregan SP, Dawson VL, Slack RS. Role of AIF in caspase-dependent and caspase-independent cell death. *Oncogene*. 2004; 23:2785–2796. [PubMed: 15077142]
56. Blomgren K, Zhu C, Wang X, Karlsson JO, et al. Synergistic activation of caspase-3 by m-calpain after neonatal hypoxia-ischemia: a mechanism of “pathological apoptosis”? *J Biol Chem*. 2001; 276:10191–10198. [PubMed: 11124942]
57. Graham EM, Sheldon RA, Flock DL, et al. Neonatal mice lacking functional Fas death receptors are resistant to hypoxic-ischemic brain injury. *Neurobiol Dis*. 2004; 17:89–98. [PubMed: 15350969]
58. Gonzalez FF, Ferriero DM. Neuroprotection in the newborn infant. *Clin Perinatol*. 2009; 36:859–880. [PubMed: 19944839]
59. West T, Stump M, Lodygensky G, Neil JJ, Deshmukh M, Holtzman DM. Lack of X-linked inhibitor of apoptosis protein leads to increased apoptosis and tissue loss following neonatal brain injury. *ASN Neuro*. 2009; 1:e00004. [PubMed: 19570023]
60. Zhu C, Xu F, Fukuda A, et al. X chromosome-linked inhibitor of apoptosis protein reduces oxidative stress after cerebral irradiation or hypoxia-ischemia through up-regulation of mitochondrial antioxidants. *Eur J Neurosci*. 2006; 26:3402–3410. [PubMed: 18052985]
61. Tsuru-Aoyagi K, Potts MB, Trivedi A, et al. Glutathione peroxidase activity modulates recovery in the injured immature brain. *Ann Neurol*. 2009; 65:540–549. [PubMed: 19475669]
62. Ishida A, Trescher WH, Lange MS, Johnston MV. Prolonged suppression of brain nitric oxide synthase activity by 7-nitroindazole protects against cerebral hypoxic-ischemic injury in neonatal rat. *Brain Dev*. 2001; 23:349–354. [PubMed: 11504607]
63. West T, Atzeva M, Holtzman DM. Pomegranate polyphenols and resveratrol protect the neonatal brain against hypoxic-ischemic injury. *Dev Neurosci*. 2007; 29:363–372. [PubMed: 17762204]

64. Northington FJ, Chavez-Valdez R, Graham EM, Razdan S, Gauda EB, Martin LJ. Necrostatin decreases oxidative damage, inflammation, and injury after neonatal HI. *J Cereb Blood Flow Metab.* 2010; 31:178–189. [PubMed: 20571523]
65. Gressens P, Schwendimann L, Husson I, et al. Agomelatine, a melatonin receptor agonist with 5-HT(2C) receptor antagonist properties, protects the developing murine white matter against excitotoxicity. *Eur J Pharmacol.* 2008; 588:58–63. [PubMed: 18466899]
66. Wang X, Stridh L, Li W, et al. Lipopolysaccharide sensitizes neonatal hypoxic-ischemic brain injury in a MyD88-dependent manner. *J Immunol.* 2009; 183:7471–7477. [PubMed: 19917690]
67. Wang X, Svedin P, Nie C, et al. N-acetylcysteine reduces lipopolysaccharide-sensitized hypoxic-ischemic brain injury. *Ann Neurol.* 2007; 61:263–271. [PubMed: 17253623]
68. Nijboer CH, Heijnen CJ, Groenendaal F, May MJ, van Bel F, Kavelaars A. Strong neuroprotection by inhibition of NF-kappaB after neonatal hypoxia-ischemia involves apoptotic mechanisms but is independent of cytokines. *Stroke.* 2008; 39:2129–2137. [PubMed: 18420952]
69. Jin Y, Silverman AJ, Vannucci SJ. Mast cells are early responders after hypoxia-ischemia in immature rat brain. *Stroke.* 2009; 40:3107–3112. [PubMed: 19520991]
70. Patkai J, Mesples B, Dommergues MA, et al. Deleterious effects of IL-9-activated mast cells and neuroprotection by antihistamine drugs in the developing mouse brain. *Pediatr Res.* 2001; 50:222–230. [PubMed: 11477207]
71. Russell JC, Kishimoto K, O'Driscoll C, Hossain MA. Neuronal pentraxin 1 induction in hypoxic-ischemic neuronal death is regulated via a glycogen synthase kinase-3alpha/beta dependent mechanism. *Cell Signal.* 2010; 23:673–682. [PubMed: 21130869]
72. Nakajima W, Ishida A, Lange MS, et al. Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat. *J Neurosci.* 2000; 20:7994–8004. [PubMed: 11050120]
73. Edwards AD, Mehmet H. Apoptosis in perinatal hypoxic-ischaemic cerebral damage. *Neuropathol Appl Neurobiol.* 1996; 22:494–498. [PubMed: 9004235]
74. Northington FJ, Ferriero DM, Flock DL, Martin LJ. Delayed neurodegeneration in neonatal rat thalamus after hypoxia-ischemia is apoptosis. *J Neurosci.* 2001; 21:1931–1938. [PubMed: 11245678]
75. Northington FJ, Graham EM, Martin LJ. Apoptosis in perinatal hypoxic-ischemic brain injury: how important is it and should it be inhibited? *Brain Res Brain Res Rev.* 2005; 50:244–257. [PubMed: 16216332]
76. Northington FJ, Zelaya ME, O'Riordan DP, et al. Failure to complete apoptosis following neonatal hypoxia-ischemia manifests as “continuum” phenotype of cell death and occurs with multiple manifestations of mitochondrial dysfunction in rodent forebrain. *Neuroscience.* 2007; 149:822–833. [PubMed: 17961929]
77. Lay CC, Davis MF, Chen-Bee CH, Frostig RD. Mild sensory stimulation completely protects the adult rodent cortex from ischemic stroke. *PLoS One.* 2010; 5:e11270. [PubMed: 20585659]
78. Hagberg H, Wilson MA, Matsushita H, et al. PARP-1 gene disruption in mice preferentially protects males from perinatal brain injury. *J Neurochem.* 2004; 90:1068–1075. [PubMed: 15312162]
79. McCullough LD, Zeng Z, Blizzard KK, Debchoudhury I, Hurn PD. Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: male toxicity, female protection. *J Cereb Blood Flow Metab.* 2005; 25:502–512. [PubMed: 15689952]
80. Du L, Bayir H, Lai Y, et al. Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathway. *J Biol Chem.* 2004; 279:38563–38570. [PubMed: 15234982]
81. Yuan M, Siegel C, Zeng Z, Li J, Liu F, McCullough LD. Sex differences in the response to activation of the poly (ADP-ribose) polymerase pathway after experimental stroke. *Exp Neurol.* 2009; 217:210–218. [PubMed: 19268668]
82. Du L, Hickey RW, Bayir H, et al. Starving neurons show sex difference in autophagy. *J Biol Chem.* 2009; 284:2383–2396. [PubMed: 19036730]
83. Li H, Pin S, Zeng Z, Wang MM, Andreasson KA, McCullough LD. Sex differences in cell death. *Ann Neurol.* 2005; 58:317–321. [PubMed: 15988750]
84. Renolleau S, Fau S, Goyenvalle C, et al. Specific caspase inhibitor Q-VD-OPh prevents neonatal stroke in P7 rat: a role for gender. *J Neurochem.* 2007; 100:1062–1071. [PubMed: 17166174]

85. Nijboer CH, Groenendaal F, Kavelaars A, Hagberg HH, van Bel F, Heijnen CJ. Gender-specific neuroprotection by 2-iminobiotin after hypoxia-ischemia in the neonatal rat via a nitric oxide independent pathway. *J Cereb Blood Flow Metab.* 2007; 27:282–292. [PubMed: 16736041]
86. Comi AM, Highet BH, Mehta P, Hana CT, Johnston MV, Wilson MA. Dextromethorphan protects male but not female mice with brain ischemia. *Neuroreport.* 2006; 17:1319–1322. [PubMed: 16951577]
87. Wen TC, Rogido M, Peng H, Genetta T, Moore J, Sola A. Gender differences in long-term beneficial effects of erythropoietin given after neonatal stroke in postnatal day-7 rats. *Neuroscience.* 2006; 139:803–811. [PubMed: 16581190]
88. Fan X, Heijnen CJ, van der Kooij MA, Groenendaal F, van Bel F. Beneficial effect of erythropoietin on sensorimotor function and white matter after hypoxia-ischemia in neonatal mice. *Pediatr Res.* 2011; 69:56–61. [PubMed: 20856165]
89. West T, Atzeva M, Holtzman DM. Caspase-3 deficiency during development increases vulnerability to hypoxic-ischemic injury through caspase-3-independent pathways. *Neurobiol Dis.* 2006; 22:523–537. [PubMed: 16480886]
90. Golomb MR, Fullerton HJ, Nowak-Gottl U, deVeber G. Male predominance in childhood ischemic stroke: findings from the international pediatric stroke study. *Stroke.* 2009; 40:52–57. [PubMed: 18787197]
91. Johnston MV, Hagberg H. Sex and the pathogenesis of cerebral palsy. *Dev Med Child Neurol.* 2007; 49:74–78. [PubMed: 17209983]
92. Cowan F, Rutherford M, Groenendaal F, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet.* 2003; 361:736–742. [PubMed: 12620738]
93. Menkes JH, Curran J. Clinical and MR correlates in children with extrapyramidal cerebral palsy. *AJNR Am J Neuroradiol.* 1994; 15:451–457. [PubMed: 8197940]
94. Barkovich AJ, Westmark K, Partridge C, Sola A, Ferriero DM. Perinatal asphyxia: MR findings in the first 10 days. *AJNR Am J Neuroradiol.* 1995; 16:427–438. [PubMed: 7793360]
95. Maller AI, Hankins LL, Yeakley JW, Butler IJ. Rolandic type cerebral palsy in children as a pattern of hypoxic-ischemic injury in the full term neonate. *J Child Neurol.* 1998; 13:313–321. [PubMed: 9701479]
96. Ferrari F, Todeschini A, Guidotti I, et al. General movements in full-term infants with perinatal asphyxia are related to basal ganglia and thalamic lesions. *J Pediatr.* 2011 published online Jan 14.
97. Johnston MV, Hoon AH Jr. Possible mechanisms in infants for selective basal ganglia damage from asphyxia, kernicterus, or mitochondrial encephalopathies. *J Child Neurol.* 2000; 15:588–591. [PubMed: 11019789]
98. Barkovich AJ. MR imaging of the neonatal brain. *Neuroimaging Clin N Am.* 2006; 16:117–135. [PubMed: 16543088]
99. Hoon AH Jr, Reinhardt EM, Kelley RI, et al. Brain magnetic resonance imaging in suspected extrapyramidal cerebral palsy: observations in distinguishing genetic-metabolic from acquired causes. *J Pediatr.* 1997; 131:240–245. [PubMed: 9290610]
100. Mueller-Burke D, Koehler RC, Martin LJ. Rapid NMDA receptor phosphorylation and oxidative stress precede striatal neurodegeneration after hypoxic ischemia in newborn piglets and are attenuated with hypothermia. *Int J Dev Neurosci.* 2008; 26:67–76. [PubMed: 17950559]
101. Stone BS, Zhang J, Mack DW, Mori S, Martin LJ, Northington FJ. Delayed neural network degeneration after neonatal hypoxia-ischemia. *Ann Neurol.* 2008; 64:535–546. [PubMed: 19067347]
102. Fuller S, Rajagopalan R, Jarvik GP, et al. J. Maxwell Chamberlain Memorial Paper for congenital heart surgery. Deep hypothermic circulatory arrest does not impair neurodevelopmental outcome in school-age children after infant cardiac surgery. *Ann Thorac Surg.* 2010; 90:1985–1994. [PubMed: 21095350]
103. Gunn AJ, Bennet L. Brain cooling for preterm infants. *Clin Perinatol.* 2008; 35:735–748. [PubMed: 19026337]
104. Thoresen M, Penrice J, Lorek A, et al. Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatr Res.* 1995; 37:667–670. [PubMed: 7603788]

105. Thoresen M, Satas S, Puka-Sundvall M, et al. Post-hypoxic hypothermia reduces cerebrocortical release of NO and excitotoxins. *Neuroreport*. 1997; 8:3359–3362. [PubMed: 9351672]
106. Amess PN, Penrice J, Cady EB, et al. Mild hypothermia after severe transient hypoxia-ischemia reduces the delayed rise in cerebral lactate in the newborn piglet. *Pediatr Res*. 1997; 41:803–808. [PubMed: 9167192]
107. Bennet L, Dean JM, Wassink G, Gunn AJ. Differential effects of hypothermia on early and late epileptiform events after severe hypoxia in preterm fetal sheep. *J Neurophysiol*. 2007; 97:572–578. [PubMed: 17093117]
108. Drury PP, Bennet L, Gunn AJ. Mechanisms of hypothermic neuroprotection. *Semin Fetal Neonatal Med*. 2010; 15:287–292. [PubMed: 20646974]
109. McDonald JW, Chen CK, Trescher WH, Johnston MV. The severity of excitotoxic brain injury is dependent on brain temperature in immature rat. *Neurosci Lett*. 1991; 126:83–86. [PubMed: 1866088]
110. Ikonomidou C, Mosinger JL, Olney JW. Hypothermia enhances protective effect of MK-801 against hypoxic/ischemic brain damage in infant rats. *Brain Res*. 1989; 487:184–187. [PubMed: 2546648]
111. Trescher WH, Ishiwa S, Johnston MV. Brief post-hypoxic-ischemic hypothermia markedly delays neonatal brain injury. *Brain Dev*. 1997; 19:326–338. [PubMed: 9253485]
112. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet*. 2005; 365:663–670. [PubMed: 15721471]
113. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. 2005; 353:1574–1584. [PubMed: 16221780]
114. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med*. 2009; 361:1349–1358. [PubMed: 19797281]
115. Shellhaas RA, Gallagher PR, Clancy RR. Assessment of neonatal electroencephalography (EEG) background by conventional and two amplitude-integrated EEG classification systems. *J Pediatr*. 2008; 153:369–374. [PubMed: 18534239]
116. Shah DK, Mackay MT, Lavery S, et al. Accuracy of bedside electroencephalographic monitoring in comparison with simultaneous continuous conventional electroencephalography for seizure detection in term infants. *Pediatrics*. 2008; 121:1146–1154. [PubMed: 18519484]
117. Frenkel N, Friger M, Meledin I, et al. Neonatal seizure recognition—Comparative study of continuous-amplitude integrated EEG versus short conventional EEG recordings. *Clin Neurophysiol*. 2011 published online Jan 7.
118. Shah PS. Hypothermia: a systematic review and meta-analysis of clinical trials. *Semin Fetal Neonatal Med*. 2010; 15:238–246. [PubMed: 20211588]
119. Eicher DJ, Wagner CL, Katikaneni LP, et al. Moderate hypothermia in neonatal encephalopathy: safety outcomes. *Pediatr Neurol*. 2005; 32:18–24. [PubMed: 15607599]
120. Eicher DJ, Wagner CL, Katikaneni LP, et al. Moderate hypothermia in neonatal encephalopathy: efficacy outcomes. *Pediatr Neurol*. 2005; 32:11–17. [PubMed: 15607598]
121. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev*. 2003; 4:CD003311. [PubMed: 14583966]
122. Simbruner G, Mittal RA, Rohlmann F, Muche R. Systemic hypothermia after neonatal encephalopathy: outcomes of neo. nEURO.network RCT. *Pediatrics*. 2010; 126:e771–e778. [PubMed: 20855387]
123. Barks JD, Liu YQ, Shangguan Y, Silverstein FS. Phenobarbital augments hypothermic neuroprotection. *Pediatr Res*. 2010; 67:532–537. [PubMed: 20098339]
124. Filippi L, Poggi C, la Marca G, et al. Oral topiramate in neonates with hypoxic ischemic encephalopathy treated with hypothermia: a safety study. *J Pediatr*. 2010; 157:361–366. [PubMed: 20553846]
125. Liu C, Lin N, Wu B, Qiu Y. Neuroprotective effect of memantine combined with topiramate in hypoxic-ischemic brain injury. *Brain Res*. 2009; 1282:173–182. [PubMed: 19501064]

126. Chakkarapani E, Dingley J, Liu X, et al. Xenon enhances hypothermic neuroprotection in asphyxiated newborn pigs. *Ann Neurol*. 2010; 68:330–341. [PubMed: 20658563]
127. Dzhalal VI, Kuchibhotla KV, Glykys JC, et al. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci*. 2010; 30:11745–11761. [PubMed: 20810895]
128. Fox C, Dingman A, Derugin N, et al. Minocycline confers early but transient protection in the immature brain following focal cerebral ischemia-reperfusion. *J Cereb Blood Flow Metab*. 2005; 25:1138–1149. [PubMed: 15874975]
129. Tsuji M, Wilson MA, Lange MS, Johnston MV. Minocycline worsens hypoxic-ischemic brain injury in a neonatal mouse model. *Exp Neurol*. 2004; 189:58–65. [PubMed: 15296836]
130. Fan X, van BF. Pharmacological neuroprotection after perinatal asphyxia. *J Matern Fetal Neonatal Med*. 2010; 23(suppl 3):17–19. [PubMed: 20695757]
131. Kelen D, Robertson NJ. Experimental treatments for hypoxic ischaemic encephalopathy. *Early Hum Dev*. 2010; 86:369–377. [PubMed: 20570449]
132. Kaandorp JJ, Benders MJ, Rademaker CM, et al. Antenatal allopurinol for reduction of birth asphyxia induced brain damage (ALLO-Trial); a randomized double blind placebo controlled multicenter study. *BMC Pregnancy Childbirth*. 2010; 10:8. [PubMed: 20167117]
133. Chaudhari T, McGuire W. Allopurinol for preventing mortality and morbidity in newborn infants with suspected hypoxic-ischaemic encephalopathy. *Cochrane Database Syst Rev*. 2008; 2:CD006817. [PubMed: 18425971]
134. Clancy RR. Neuroprotection in infant heart surgery. *Clin Perinatol*. 2008; 35:809–821. [PubMed: 19026342]
135. Ishida A, Trescher WH, Lange MS, Johnston MV. Prolonged suppression of brain nitric oxide synthase activity by 7-nitroindazole protects against cerebral hypoxic-ischemic injury in neonatal rat. *Brain Dev*. 2001; 23:349–354. [PubMed: 11504607]
136. Matsushita H, Johnston MV, Lange MS, Wilson MA. Protective effect of erythropoietin in neonatal hypoxic ischemia in mice. *Neuroreport*. 2003; 14:1757–1761. [PubMed: 14512852]
137. Elmahdy H, El-Mashad AR, El-Bahrawy H, El-Gohary T, El-Barbary A, Aly H. Human recombinant erythropoietin in asphyxia neonatorum: pilot trial. *Pediatrics*. 2010; 125:e1135–e1142. [PubMed: 20385632]
138. McPherson RJ, Juul SE. Erythropoietin for infants with hypoxic-ischemic encephalopathy. *Curr Opin Pediatr*. 2010; 22:139–145. [PubMed: 20090525]
139. Zhu C, Kang W, Xu F, et al. Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy. *Pediatrics*. 2009; 124:e218–e226. [PubMed: 19651565]
140. Holtzman DM, Sheldon RA, Jaffe W, Cheng Y, Ferriero DM. Nerve growth factor protects the neonatal brain against hypoxic-ischemic injury. *Ann Neurol*. 1996; 39:114–122. [PubMed: 8572656]
141. Zhong J, Zhao L, Du Y, et al. Delayed IGF-1 treatment reduced long-term hypoxia-ischemia-induced brain damage and improved behavior recovery of immature rats. *Neurol Res*. 2009; 31:483–489. [PubMed: 19500451]
142. Beresewicz M, Majewska M, Makarewicz D, Vayro S, Zablocka B, Gorecki DC. Changes in the expression of insulin-like growth factor 1 variants in the postnatal brain development and in neonatal hypoxia-ischaemia. *Int J Dev Neurosci*. 2010; 28:91–97. [PubMed: 19766709]
143. Gluckman P, Klempt N, Guan J, et al. A role for IGF-1 in the rescue of CNS neurons following hypoxic-ischemic injury. *Biochem Biophys Res Commun*. 1992; 182:593–599. [PubMed: 1370886]
144. Han BH, Holtzman DM. BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. *J Neurosci*. 2000; 20:5775–5781. [PubMed: 10908618]
145. Meyn DF Jr, Ness J, Ambalavanan N, Carlo WA. Prophylactic phenobarbital and whole-body cooling for neonatal hypoxic-ischemic encephalopathy. *J Pediatr*. 2010; 157:334–336. [PubMed: 20553847]
146. Silverstein FS, Ferriero DM. Off -label use of antiepileptic drugs for the treatment of neonatal seizures. *Pediatr Neurol*. 2008; 39:77–79. [PubMed: 18639748]

147. Liu Y, Barks JD, Xu G, Silverstein FS. Topiramate extends the therapeutic window for hypothermia-mediated neuroprotection after stroke in neonatal rats. *Stroke*. 2004; 35:1460–1465. [PubMed: 15105511]
148. Gathwala G, Marwah A, Gahlaut V, Marwah P. Effect of high-dose phenobarbital on oxidative stress in perinatal asphyxia: an open label randomized controlled trial. *Indian Pediatr*. 2010 published online Nov 30. PII:S097475590900675-1.
149. Dickinson R, Peterson BK, Banks P, et al. Competitive inhibition at the glycine site of the N-methyl-D-aspartate receptor by the anesthetics xenon and isoflurane: evidence from molecular modeling and electrophysiology. *Anesthesiology*. 2007; 107:756–767. [PubMed: 18073551]
150. Gustavsson M, Wilson MA, Mallard C, Rousset C, Johnston MV, Hagberg H. Global gene expression in the developing rat brain after hypoxic preconditioning: involvement of apoptotic mechanisms? *Pediatr Res*. 2007; 61:444–450. [PubMed: 17515869]
151. Comi AM, Cho E, Mulholland JD. Neural stem cells reduce brain injury after unilateral carotid ligation. *Pediatr Neurol*. 2008; 38:86–92. [PubMed: 18206788]
152. de Paula S, Greggio S, Dacosta JC. Use of stem cells in perinatal asphyxia: from bench to bedside. *J Pediatr (Rio J)*. 2010; 86:451–464. [PubMed: 21140037]
153. Pimentel-Coelho PM, Magalhaes ES, Lopes LM, de Azevedo LC, Santiago MF, Mendez-Otero R. Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection in the striatum. *Stem Cells Dev*. 2010; 19:351–358. [PubMed: 19296724]
154. Pimentel-Coelho PM, Mendez-Otero R. Cell therapy for neonatal hypoxic-ischemic encephalopathy. *Stem Cells Dev*. 2010; 19:299–310. [PubMed: 19916801]
155. Cotten, CM.; Kurtzberg, J.; Song, H.; Goldstein, R.; Provenzale, JM. Cord blood for hypoxic-ischemic encephalopathy; NCT00593242. <http://clinicaltrials.gov/ct2/show/NCT00593242>

Panel: Drugs that might augment neuroprotection by mild hypothermia**Anticonvulsant or antiexcitatory**

Phenobarbital,¹²³ topiramate,¹²⁴ levetiracetam,¹⁴ memantine,¹²⁵ xenon,¹²⁶ magnesium sulphate,⁴³ bumetanide¹²⁷

Anti-inflammatory or antioxidant

Sodium cromoglicate,^{69,70} minocycline,^{128,129} indometacin,¹³⁰ melatonin,^{65,131} N-acetylcysteine,⁶⁷ allopurinol,^{132–134} pomegranate polyphenols,⁶³ 7-nitroindazole,¹³⁵ 2-iminobiotin,⁸⁵ necrostatin 1⁶⁴

Multiple mechanisms

Erythropoietin^{82,88,136–139}

Growth factors

Nerve growth factor,¹⁴⁰ insulin-like growth factor 1,^{141–143} brain derived neurotrophic factor¹⁴⁴

Search strategy and selection criteria

We searched all articles listed in PubMed published from 1906, to January, 2011, in English, Japanese, and Chinese using the phrases “neonatal asphyxia”, “neonatal hypoxic-ischemic encephalopathy”, “neonatal encephalopathy”, “neonatal therapeutic hypothermia”, “neonatal excitotoxicity”, “neonatal neuroprotection”, “neonatal neuroinflammation”, “neonatal brain oxidative stress”, “amplitude integrated EEG”, and “neonatal neurointensive care”. Articles reporting controlled clinical trials and meta-analyses of clinical trials of hypothermia for hypoxic-ischaemic encephalopathy and preclinical reports that provided the foundation for these trials or were relevant to potential advances in neonatal neurocritical care were selected.

Figure 1. Excito-oxidative cascade of events that mediate hypoxic-ischaemic brain injury
Severe hypoxia impairs oxidative metabolism leading to neuronal depolarisation and ischaemia. Ischaemia reduces delivery of glucose necessary for anaerobic metabolism, which powers neurotransmitter reuptake pumps on perisynaptic astrocytes. This leads to flooding of the synaptic cleft with glutamate and neuronal depolarisation, which in turn trigger opening of NMDA receptor channels and other calcium channels including acid-sensing ion channels, leading to excess calcium influx into neurons. Calcium flooding through NMDA channels activates the enzyme nitric oxide synthetase leading to high levels of the toxic free radical neurotransmitter nitric oxide. This toxic free radical, along with additional oxygen free radicals generated by reoxygenation of mitochondria following a

period of hypoxia, attack enzymes associated with oxidative phosphorylation and electron transport. Calcium toxicity is also mediated by activation of other enzymes including caspases, calpains, other proteases, and lipases that attack mitochondria and other cellular machinery. Signals released from damaged mitochondria lead to apoptosis or programmed cell death as long as energy supplies persist, but exhaustion of energy supplies leads to necrosis in which cellular membranes are destroyed. Lactic acid accumulates when oxidative phosphorylation within mitochondria is impaired, but its toxicity seems to be less important in the neonatal brain than in adults. Similarly, cerebral oedema occurs when pumps required for water homeostasis are impaired by reduced energy supplies owing to damaged mitochondria. Oedema seems to be a sign of energy failure rather than causing damage on its own. This excito-oxidative cascade occurs over a period of days to weeks. EAAT=excitatory aminoacid transporter. Gln=glutamine. Glu=glutamate. nNOS=neuronal nitric oxide synthase. NO=nitric oxide. VDCC=voltage-dependent calcium channels.

Figure 2. Downstream signalling pathways that mediate the apoptosis–necrosis continuum

Delayed cell death signalling pathways mediate the effects of hypoxia-ischaemia in the brain. The extrinsic pathway mirrors the cells' external environment and begins when inflammatory cytokines (to the right of the figure at the cell surface) bind to and activate Fas-cell death receptors, whereas the intrinsic pathway is activated when signals released from within stressed mitochondria activate caspase and non-caspase-mediated-cell-death pathways within the nucleus. These two cell-death pathways activate common signalling networks within the mitochondria and the nucleus. Mitochondria exposed to caspase-induced stress can release cytochrome C through channels formed by the Bax and Bak proteins in the outer mitochondrial membrane (caspase-mediated cell death) or can release

apoptosis inducing factor (AIF), which activates DNA fragmentation directly (non-caspase pathway). Cytochrome C can combine with Apaf1 and caspase 9 to form the apoptosome, which triggers activation of caspase 3. DNA breaks mediated by free radicals such as nitric oxide (NO·) and peroxynitrite activate poly-ADP-ribose polymerase 1 (PARP1), which consumes NAD⁺ and worsens the energy shortage for mitochondria (shown within the nucleus). Convergence of signalling for the extrinsic (Fas) and intrinsic cell-death pathways are responsible for the interaction between infection (endotoxin) and hypoxia-ischaemia that increases cell death. V_m=membrane potential. VSSC=voltage sensitive calcium channel. Fas=death receptor in tumour necrosis factor family. FADD=fas adaptor death domain protein. BclXL=antiapoptotic proteins in the Bcl2 family of proteins. Bax and Bak=proapoptotic proteins that form channels in outer mitochondrial membrane releasing cytochrome C to trigger apoptosis. tBid=truncated BH3-only proapoptotic protein. Apaf1=apoptotic protein activating factor 1. SMAC=antagonist of inhibitor of apoptosis. IAP=inhibitor of apoptosis. CAD=caspase activating DNase. Cyt C=cytochrome C. nNOS=neuronal nitric oxide synthase. NO=nitric oxide. ROS=reactive oxygen species. PARP1=poly-ADP-ribose polymerase 1. PAR=poly-ADP ribose formed by ribosylation of DNA and proteins. GSH=glutathione, an antioxidant. AIF=apoptosis-inducing factor. Bid=BH3 interacting domain proapoptotic protein. NAD=nicotinamide adenine dinucleotide. FASL=Fas death receptor ligand.

Figure 3. Delayed apoptosis in a Vannucci model of unilateral hypoxia-ischaemia in 7-day-old rat pups

This neonatal model of brain injury shows that neurons continue to commit to programmed cell death over several days after injury.⁷² (A) The top diagram shows the distribution of neurons in coronal slices of brain tissue that express caspase 3, a marker for apoptosis, at intervals up to 168 h after injury. (B) This graph shows the delayed appearance of necrotic or apoptotic cells in the same coronal sections of brain tissue shown in A, using a standard histopathological scoring system.⁷² The Y-axis shows the abundance of these dying cells in the cerebral cortex, corpus striatum, or the CA1 region of the hippocampus. (C) Electron microscopy images (magnification $\times 2500$) of apoptotic neurons at different stages of

programmed cell death from the thalamus (two on the left) and hippocampus (on the right) showing characteristic dark, condensed chromatin. Early apoptotic cells show some nuclear condensation, whereas cells in more advanced stages of cell death show extensive nuclear and cytoplasmic condensation.

Figure 4. T1-weighted MRI of a baby at 2 weeks of age who had undergone severe, near-total asphyxia around the time of birth

Asphyxia was associated with a cord blood pH of 7.6 and base deficit of 25 mEq/L (calculated base deficit in standard arterial blood gases). Injured T1-enhancing areas are very focal and localised to regions of the thalamus, putamen, and peri-Rolandic cerebral cortex that contain synapses that connect the developing motor circuits.