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REVIEW ARTICLE/BRIEF REVIEW

Preclinical neuroprotective actions of xenon and possible implications for human therapeutics: a narrative review

Action neuroprotectrice préclinique du xénon et implications possibles pour la thérapeutique humaine: un compte rendu narratif

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Abstract

Purpose *The purpose of this report is to facilitate an understanding of the possible application of xenon for neuroprotection in critical care settings. This narrative review appraises the literature assessing the efficacy and safety of xenon in preclinical models of acute ongoing neurologic injury.*

Source *Databases of the published literature (MEDLINE® and EMBASE™) were appraised for peer-reviewed manuscripts addressing the use of xenon in both preclinical models and disease states of acute ongoing neurologic injury. For randomized clinical trials not yet reported, the investigators' declarations in the National Institutes of Health clinical trials website were considered.*

Principal findings *While not a primary focus of this review, to date, xenon cannot be distinguished as superior for surgical anesthesia over existing alternatives in adults. Nevertheless, studies in a variety of preclinical disease models from multiple laboratories have consistently shown xenon's neuroprotective properties. These properties are enhanced in settings where xenon is combined with hypothermia. Small randomized clinical trials are underway to explore xenon's efficacy and safety in clinical settings of acute neurologic injury where hypothermia is the current standard of care.*

Conclusion *According to the evidence to date, the neuroprotective efficacy of xenon in preclinical models and its safety in clinical anesthesia set the stage for the launch of randomized clinical trials to determine whether these encouraging neuroprotective findings can be translated into clinical utility.*

Résumé

Objectif *L'objectif de ce compte rendu est d'aider le lecteur à mieux saisir les applications possibles du xénon pour favoriser la neuroprotection dans les contextes de soins critiques. Ce compte rendu narratif examine la littérature évaluant l'efficacité et l'innocuité du xénon dans des modèles précliniques de lésion neurologique aiguë et en développement.*

Source *Les bases de données de littérature publiée (MEDLINE® et EMBASE™) ont été passées en revue pour en extraire les manuscrits révisés par les pairs portant sur l'utilisation du xénon dans des modèles précliniques ou des états maladifs de lésion neurologique aiguë et en développement. Dans le cas d'études cliniques randomisées pas encore rapportées, nous nous sommes fondés sur les déclarations des chercheurs sur le site des études cliniques des Instituts nationaux de santé (National Institutes of Health).*

Constatations principales *Bien que ce ne soit pas le sujet principal de ce compte rendu, à ce jour, on ne peut déterminer que le xénon est supérieur aux alternatives existantes pour l'anesthésie chirurgicale pratiquée chez l'adulte. Toutefois, les études traitant de divers modèles précliniques de maladies et provenant de plusieurs laboratoires reconnaissent, de façon constante, les propriétés neuroprotectrices du xénon. Ces propriétés sont encore rehaussées dans les contextes où le xénon est*

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combiné à l'hypothermie. De petites études cliniques randomisées sont en cours afin d'explorer l'efficacité et l'innocuité du xénon dans les contextes cliniques de lésion neurologique aiguë, où l'hypothermie constitue la norme actuelle de soins.

Conclusion *Selon les données probantes à ce jour, l'efficacité neuroprotectrice du xénon dans les modèles précliniques et son innocuité en anesthésie clinique ouvrent la voie pour la réalisation d'études cliniques randomisées afin de déterminer si ces effets neuroprotecteurs encourageants peuvent se traduire en une utilité clinique.*

Interest in the clinical applications of xenon, a monoatomic gas, has waxed and waned over the last 70 years. The advent of the market authorization of xenon for use as an anesthetic is an auspicious time to reflect on concerns and expectations of its future use in critical care settings. In particular, within the last decade, several findings have propelled xenon into the frame as a neuroprotective agent to guard the central nervous system against imminent and acute ongoing damage. This review provides a critical appraisal of reports of xenon in preclinical models retrieved in the MEDLINE® and EMBASE™ databases using the search terms (xenon) AND (neuroprotect*). Pertinent references from manuscripts were also reviewed. Where appropriate, attention is drawn to ongoing clinical trials identified through the National Institutes of Health website (https://clinicaltrials.gov/).

It is worth pointing out the vast number of casualties that litter the clinical trial pathway in the as yet unsuccessful pursuit of a safe and effective neuroprotectant.[1] The focus of attention has been on the reasons why the U.S. Food and Drug Administration has yet to approve a single neuroprotective agent despite encouraging preclinical data. The veracity of preclinical findings is questioned in the absence of randomization, blinded assessment, and power analysis in the preclinical studies.[2]

History of xenon

Xenon derives its name from the Greek word for "stranger" because of its rarity,[3] representing no more than 0.0875 ppm in the atmosphere.[4] Xenon is a colourless, odourless, tasteless, and inert gas that was discovered by Ramsay and Travers in 1898. It is used commercially for lasers,[5] high-intensity lamps,[6] plasma screens,[7] propellant in the aerospace industry,[8] *x-ray* tubes, and medical applications, including imaging[9] and anesthesia.

Use of xenon in anesthesia

Nearly 70 years ago, a group of physiologists at the University of California reported xenon's anesthetic properties in animals.[10] Within five years, this preclinical finding was advanced to patients in what was the first report in the English literature of its clinical use for general anesthesia.[11] It took a further five decades, however, before marketing authorization was granted for the use of xenon as a general anesthetic. The Russian Federation was first to approve its use, possibly because of its notoriety as the purported anesthetic for the Russian President's heart surgery in 1996.[A] Since then, western Europe has approved its use, but xenon remains without marketing authorization for use as an anesthetic in North America. Certain factors have driven up the price of xenon to a point where its value is difficult to justify for general anesthesia, including its scarcity, the high cost of xenon purification from the atmosphere by fractional distillation, as well as competition posed by high-spend industries, such as aerospace, for its anesthetic indication. To date, studies have not shown a compelling advantage for the use of xenon over lower cost alternatives as the general anesthetic of choice in adults. Xenon has yet to be tested for pediatric anesthesia where its potential in preventing anesthetic-induced developmental toxicity may direct its use as an anesthetic for this vulnerable patient population.[12-14]

Mechanisms for the biological properties of xenon

Xenon's physicochemical properties prevent covalent bonding with other molecules under biologic conditions. Despite this chemical inertness, biological interactions can occur through non-covalent van der Waals intermolecular forces. Xenon's outer electron shell is full, which prevents covalent binding under biological conditions, but xenon is highly polarizable, which enables its attraction to surrounding molecules. Franks *et al.*[15] were the first to show that xenon can interact with the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor, a plausible site for its anesthetic properties, by competing for binding at the glycine coactivation site[16] through an interaction with a phenylalanine residue.[17] While other targets may also contribute to xenon's anesthetic properties, including other glutamate receptor subtypes[18] and molecular species inducing potassium leak currents,[19] the importance of the NMDA subtype of the glutamate

[A] *The New York Times.* Yeltsin Has 7-Hour Heart Surgery And Doctors Say It Was a Success. Available from URL: www.nytimes.com/1996/11/06/world/yeltsin-has-7-hour-heart-surgery-and-doctors-say-it-was-a-success.html (accessed August 2015).

receptor in the excitotoxic phase of acute neurologic injury spurred a series of studies exploring xenon's neuroprotective properties.

Cellular and molecular candidate mechanisms for neuroprotective properties of xenon

Excitotoxicity

Overactivation of glutamate receptors is involved in a number of pathological processes. Excessive entry of calcium, mediated by NMDA receptors, triggers biochemical cascades that ultimately lead to neuronal cell death. This neurotoxicity due to overactivation of NMDA receptors, termed *excitotoxicity* by Olney,[20] is considered to underlie the acute neuronal injury observed following insults such as stroke, cardiac arrest, and traumatic brain injury (TBI). *N*-methyl-D-aspartate receptor antagonists are neuroprotective in *in vitro* and *in vivo* brain injury models.[21]

Following the discovery that xenon inhibits NMDA receptors,[15] it was shown that xenon could protect neuronal cell cultures against injury induced by NMDA, glutamate, or oxygen-glucose deprivation.[22] The same study showed xenon to be neuroprotective *in vivo* against neuronal injury caused by subcutaneous injection of *N*-methyl-D,L-aspartate in rats. Subsequently, Petzelt *et al*. corroborated this finding in an *in vitro* model of hypoxia[23] and in an *in vivo* model of stroke.[24] Several NMDA antagonists have been clinically evaluated for their putative neuroprotective properties. Gavestinel (with activity at the glycine co-agonist site) and magnesium sulfate (an open pore blocker) were among the more recent, but neither had sufficient efficacy, possibly on the basis of poor central nervous system (CNS) penetrability.[25,26]

Other NMDA receptor antagonists, such as nitrous oxide, ketamine, and dizocilpine (MK-801) have intrinsic neurotoxicity,[27] but xenon not only appears to be devoid of these neurotoxic effects but also ameliorates the injury produced by other NMDA antagonists.[28] Reasons for xenon's relative lack of neurotoxicity may relate to both the site of its action on the NMDA receptor as well its neutral effect on spontaneous dopamine release that the other NMDA antagonists enhance.[29]

Existing data distinguish the actions of xenon as a competitive inhibitor of NMDA receptors from well-established open-channel blockers of NMDA receptors, such as ketamine and MK-801. Open-channel blockers of NMDA receptors invariably show changed kinetics following the application of the agonist in the presence of the inhibitor. For example, when NMDA is applied to NMDA receptors, the rate of closure of the channel is always much faster when an open-channel blocker, such as ketamine or MK-801, is present.[30] In the presence of xenon, there is no increase in the rate of closure of the NMDA response.[15,16] Also, open-channel blockers, such as ketamine, invariably increase the decay of excitatory postsynaptic currents,[31] but this is not observed with xenon.[32] Thus, with both heterologous expression systems and intact synapses, xenon does not behave as an open-channel blocker, which may be an additional reason why it lacks the neurotoxicity seen with other NMDA antagonists.

Further reasons for the lack of neurotoxicity are provided below in the description of xenon's other mechanisms of neuroprotective action.

Modulation of background ("leak") potassium conductance

Activation of the two-pore potassium channels (K_{2p} channels) tends to hyperpolarize the membrane potential, taking it farther from an activation threshold. Xenon was shown to activate a species of these channels (TREK-1),[B] with the activation being critically dependent on a specific amino acid residue (Glu306). These TREK-1 channels are the mediating mechanism whereby intracellular acidification as well as polyunsaturated fatty acids produce neuroprotection.[33] Genetically modified mice that lack TREK-1 channels fare poorly in models of cerebral ischemia, highlighting the importance of this molecular species in the organism's defence against acute neuronal injury.[34] It is notable that other NMDA antagonists, including nitrous oxide and cyclopropane, also activate TREK-1.[19]

Modulation of neuroapoptosis

Acute neuronal ischemic injury provokes sequential waves of signalling mechanisms, which successively results in neuronal death at different times. While excitotoxicity features early in the processes, resulting in neuronal death, apoptosis (i.e., programmed cell death) follows later through signalling mechanisms that are well defined.[35] Xenon reduces the expression of pro-apoptotic genes, such as *BAX*,[36] and increases anti-apoptotic proteins, such as Bcl-x_L[36] and Bcl-2,[36,37] resulting in a significant decline in neuroapoptosis.[36]

Modulation of neuroinflammation

Several investigators have reported that circulating immune cells traverse the blood-brain barrier following

[B] TREK-1 (also called KCNK2) is part of the subfamily of mechano-gated potassium channels that are present in mammalian neurons.

acute injury;[38] furthermore, the ischemic brain activates the resident microglia cells. These activated macrophages propagate the ongoing neuronal damage through several pathways, including through the elaboration of pro-inflammatory cytokines that further injures the penumbra around an infarcted core.[39] This process selectively prevents neuroinflammation and attenuates brain ischemic injury.[40-42] In several types of organ injury models, xenon has been shown to exert anti-inflammatory effects[43,44] and decrease neuronal dysfunction associated with neuroinflammation.[45,46]

Induction of hypoxia-inducible factor 1alpha (HIF-1α)

Xenon potently increases the translational efficiency and upregulation of the oxygen sensor, HIF-1α, under normoxic conditions.[47] Downstream effectors of HIF-1α, including erythropoietin (EPO), have been shown to exert important neuroprotective properties.[48] Nevertheless, it should be emphasized that, when EPO was administered to patients with anemia due to diabetes-induced renal failure, there was a twofold increase in stroke.[49] In the setting of organ injury, xenon-induced upregulation of HIF-1α has been shown to be cytoprotective in the kidney,[47,50] lung,[43] heart,[51] and brain.[52] The Russian Federation's Biomedical Agency exploited xenon's ability to induce EPO by pretreating its endurance athletes. Some have speculated that this may have contributed to the athletes' superior performance at the Sochi 2014 Winter Olympics. Since September 1, 2014, xenon has been banned for use in competitive athletics.[C]

Modulation of adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP} channels)

Hypoxic-ischemic neuronal injury can be preempted by activation of K_{ATP} channels,[53,54] and its absence exacerbates cerebral ischemic injury.[55] Pretreatment with xenon prevents glucose- and oxygen-deprived neuronal cells from dying in primary cultures. A key mechanism involves xenon's activation of the K_{ATP} channels.[56,57]

Neuroprotective efficacy of xenon in preclinical models

Tables 1, 2, 3, 4 and 5 summarize the preclinical studies assessing the effects of xenon in models of acute neurological injury. The studies are arranged to address each of the putative indications for which xenon may be beneficial, including neonatal hypoxic ischemic encephalopathy (HIE) from intrapartum asphyxia (Table 1), stroke (Table 2), TBI (Table 3), anesthetic-induced developmental toxicity (Table 4), and cardiac arrest (Table 5).

Synergistic interaction between xenon and therapeutic hypothermia

There are convincing scientific data to extend the potential neuroprotective properties of xenon to settings in which hypothermia (i.e., therapeutic hypothermia/targeted temperature management [TH/TTM]) is provided. Data - published in eight peer-reviewed manuscripts from four different laboratories involving four preclinical injury models - show that xenon's neuroprotective action is most effective when body temperature is reduced (Table 1). Unlike other neuroprotective strategies, including a different NMDA antagonist (gavestinel), xenon alone exhibits this enhanced efficacy when temperature is reduced. In order to determine the possible mechanism for the superior neuroprotection that xenon provides in the presence of hypothermia, further studies were performed *in vitro* using an oxygen-glucose deprivation model to induce ischemic damage (lactate dehydrogenase release) in cultured neurons (Figure).[36] Using the slope of a van't Hoff plot to quantify the overall heat transfer (increase in enthalpy) that occurs during ischemic damage, the addition of xenon (12.5%) significantly altered the mean (SD) slope from 34.8 (4.5) to 177 (12) $kJ \cdot mol^{-1}$. This was not noted with another neuroprotectant (gavestinel) that does not have a synergistic interaction with hypothermia.[36] The precise mechanism for the increase in enthalpy has not been determined.

The following two preclinical models mimicking neonatal asphyxia and cardiac arrest are described in greater detail because of their relevance to subsequent clinical trials.

In a Rice-Vannucci rat model of neonatal asphyxia,[36] neither xenon (20%) alone nor temperature reduction (35°C) alone was capable of preventing long-term neurological consequences; however, the combination of xenon (20%) and 35°C was highly effective. Even when initiated at different times, the combination of TH/TTM plus xenon was efficacious, whereas neither treatment alone was effective.[58,59] In a Rice-Vannucci rat model of neonatal asphyxia, xenon (50%) at normothermia was ineffective; however, adding xenon (50%) to hypothermia (32°C) proved to be more effective than either intervention alone.[60]

[C] *The Economist.* An Obscure Gas Improves Athletes' Performance. Available from URL: http://www.economist.com/news/science-and-technology/21595890-obscure-gas-improves-athletes-performance-breathe-it (accessed August 2015).

Table 1 Xenon in hypoxic-ischemic encephalopathy models

Author/Journal/Year of publication	Species	Injury	Results/Conclusions
Wilhelm *et al.*, Anesthesiology 2002[22]	Mouse cell culture Rat	Injury provoked by oxygen-glucose deprivation and assessed by the release of lactate dehydrogenase into the culture medium; N-methyl-D,L-Aspartate	Xenon, when co-administered with the injurious agent, exerts a concentration-dependent neuroprotective effect at concentrations below which anesthesia is produced
Ma *et al.*, J Cereb Blood Flow Metab 2006[107]	Rat	Hypoxia-ischemia	Protective in preconditioning
Dingley *et al.*, Stroke 2006[108]	Rat	Hypoxia-ischemia	Neuroprotective post injury
Rajakumaraswamy *et al.*, Neurosci Lett 2006[109]	Mouse cell culture Rat	Oxygen-glucose deprivation; Hypoxia-ischemia	Synergistic neuroprotection between xenon and dexmedetomidine
Dingley *et al.*, Anesth Analg 2008[110]	Rat	Hypoxia-ischemia	Effect of xenon and hypothermia on respiration
Cattano *et al.*, Neurosci Lett 2008[111]	Rat	No injury	Xenon induces ADNP
Valleggi *et al.*, J Neurosurg Anesthesiol 2008[112]	Rat	No injury	Xenon induces 6 genes involved in preconditioning pathways
Luo *et al.*, Anesthesiology 2008[113]	Cell culture Rat	Oxygen-glucose deprivation; Hypoxia-ischemia	Xenon preconditions through phospho-CREB
Bantel *et al.*, Anesthesiology 2010[56]	Cell culture	Oxygen-glucose deprivation	Preconditioning via K_{ATP} plasmalemmal channels
Chakkarapani *et al.*, Anesth Analg 2009[114]	Pig	Hypoxia-ischemia	Effectiveness of xenon delivery device
Jawad *et al.*, Neurosci Lett 2009[115]	Fetal neuronal culture	Oxygen-glucose deprivation	Xenon most efficacious noble gas for neuroprotection
Faulkner *et al.*, Ann Neurol 2011[62]	Pig	Hypoxia-ischemia	Decrease Lac/NAA by xenon and hypothermia
Yang *et al.*, PLoS One 2012[116]	Rat	Hypoxia-ischemia	Maternal exposure provides neuroprotective preconditioning for fetus
Chakkarapani *et al.*, Intensive Care Med 2012[117]	Pig	Hypoxia-ischemia	Neuroprotection and hemodynamic stability from xenon and hypothermia
Chakkarapani *et al.*, J Cereb Blood Flow Metab 2012[118]	Pig	Hypoxia-ischemia	Cerebrovascular reactivity maintained during xenon and hypothermia

ADNP = activity-dependent neuroprotective protein; CREB = cyclic adenosine monophosphate response element binding protein; K_{ATP} = adenosine triphosphate potassium; Lac/NAA = lactate to N-acetylaspartate ratio

In piglets with global cerebral ischemia for 45 min,[61] the combination of xenon (50%) and hypothermia (33.5°C) produced superior protection than either intervention alone when assessed three days later. In piglets with global cerebral ischemia produced by bilateral vertebral artery occlusion and hypoxia,[62] neither xenon (50%) nor hypothermia to 33.5°C was effective at preventing evidence of cerebral energy failure by nuclear magnetic resonance (NMR) spectroscopy; however, the combination of both xenon and hypothermia for 24 hr prevented post-global ischemia energy failure (Table 6).

In an adult pig model of cardiac arrest followed by cardiopulmonary resuscitation,[63] xenon (70%) did *not* improve either neurocognitive outcomes or histopathological evidence of global ischemia. Nevertheless, one hour of xenon (70%) with 16 hr of

Table 2 Xenon in stroke models

Author/Journal/Year of publication	Species	Injury	Results/Conclusions
Homi *et al*., Anesthesiology 2003[119]	Mouse	Xenon *vs* N_2O in middle cerebral artery occlusion (MCAO)	Xenon neuroprotective in morphological and functional assay at 24 hr
David *et al*., J Cereb Blood Flow Metab 2003[24]	Cortical neuronal-glial culture	NMDA-induced Calcium (Ca^{++}) influx;	Xenon decreases Ca^{++} influx;
	Rat	Xenon *vs* N_2O in MCAO	Xenon but not N_2O decreases ischemic brain damage in resistant region
Limatola *et al*., Neuroscience 2010[52]	Mouse	MCAO	Xenon preconditions through HIF-1α
David *et al*., J Cereb Blood Flow Metab 2010[120]	Rat	MCAO	Xenon protective after MCAO and against hemorrhagic properties of tPA*;
			Xenon blocks tPA* activity
Sheng *et al*., Anesthesiology 2012[105]	Rat	Temporary focal ischemia	Concentration- and time-dependent synergistic neuroprotection with hypothermia

* tPA = tissue plasminogen activator. HIF-1α = hypoxia-inducible factor-1-alpha

Table 3 Xenon in traumatic brain injury models

Author/Journal/Year of publication	Species	Injury	Results/Conclusions
Coburn *et al*., Crit Care Med 2008[103]	Mouse *in vitro*	Trauma to hippocampal slice	Xenon mitigates injury
Harris *et al*., Anesthesiology 2013[121]	Mouse *in vitro*	Trauma to hippocampal slice	Xenon is best noble gas against injury
Campos-Pires *et al*., Crit Care Med 2015[104]	Mouse *in vivo*	Controlled cortical impact trauma	Xenon improves functional and histological outcomes

hypothermia (33°C) was significantly more effective than either alone.[64] Fries *et al.*'s study[64] merits further detailed consideration because of its relative importance for the conduct of the recently concluded Xe-hypotheca trial (https://clinicaltrials.gov/ct2/results?term=Xe-Hypotheca&Search=Search). After a ten-minute ventricular fibrillation-induced cardiac arrest and respiratory arrest (through termination of mechanical ventilation during general anesthesia in the presence of muscle relaxation), the pigs were resuscitated with compression and mechanical ventilation (F_iO_2 = 1.0; tidal volume of 15 $mL{\cdot}kg^{-1}$) at a ratio of 30:2. After six minutes of resuscitation, spontaneous circulation was restored by injecting epinephrine directly into the right atrium through a pulmonary artery catheter. It is notable that the arrest, time to resuscitation, and methods of resuscitation are quite similar to those performed clinically. Thereafter, the 15 resuscitated pigs with return of spontaneous circulation (ROSC) were randomized into one of three groups (n = 5), namely, Control group (in which the pigs received fluid therapy and maintenance of normothermia), mild therapeutic hypothermia group (MTH group, in which pigs received fluid therapy with surface cooling to achieve a core temperature of 33°C), and mild therapeutic hypothermia with xenon group (Xe+MTH group, in which pigs also received one hour of xenon at 70% through a closed-system ventilator). In the animals that received MTH, surface cooling lasted 16 hr and then rewarming occurred at a rate of $1°C{\cdot}hr^{-1}$. The pigs were weaned off the ventilator and returned to their housing pen. Post-arrest, the neurologic status was scored with a validated neurologic deficit score in which four items (level of consciousness, respiration, posture, and feeding behaviour) were evaluated to yield a score that varies from 0 = no neurologic impairment to 100 = brain death. Apart from the specific evaluation of the CNS, an overall performance score was used. Five days after resuscitation, the pigs were sacrificed during general anesthesia and neurohistopathology was performed, including an assessment of neuroinflammation. Both hypothermia groups (MTH and Xe+MTH) showed better overall performance scores. Interestingly, there was a premature death in each of the Control and MTH groups but not in the Xe+MTH group. While both hypothermia groups had less histopathological damage than the normothermic group, the Xe+MTH group was distinctive in exhibiting reduced astrogliosis and microgliosis. Overall, the authors concluded that the

Table 4 Xenon in anesthetic-induced developmental neurotoxicity models

Author/Journal/Year of publication	Species	Injury	Results/Conclusions
Ma *et al*., Anesthesiology 2007[12]	Neonatal rat Hippocampal slice preparation	Effect of xenon *vs* N_2O on isoflurane-induced neurotoxicity	Xenon prevents, while N_2O enhances, isoflurane-induced neurotoxicity; Xenon alone does not produce neurotoxicity
Cattano *et al*., Can J Anesth 2008[13]	Neonatal mouse	Effect of xenon on isoflurane-induced neurotoxicity	Xenon prevents isoflurane-induced neurotoxicity; Xenon alone produces modest neurotoxicity
Cattano *et al*., Neurosci Lett 2008[111]	Neonatal rat	None	Xenon upregulates brain ADNP
Shu *et al*., Anesthesiology 2010[101]	Neonatal rat	Exposure to xenon *vs* N_2O or hypoxia on isoflurane neurotoxicity	Xenon prevents while N_2O and hypoxia exacerbate isoflurane-induced neurotoxicity; anti-apoptotic factors induced by xenon
Cattano *et al*., Minerva Anestesiol 2011[122]	Neonatal rat	None	Xenon upregulates anti-apoptotic genes
Brosnan *et al*., Anesthesiology 2013[102]	Hippocampal slice preparation	Xenon *vs* isoflurane *vs* sevoflurane	At 1 MAC, each produced apoptosis; less with 1 MAC xenon; at 0.75 MAC xenon produced no apoptosis
Sabir *et al*., Anesthesiology 2013[14]	Piglet	Xenon *vs* isoflurane on neurotoxicity	Xenon does not produce neuroapoptosis

ADNP = activity-dependent neuroprotective protein; MAC = minimum alveolar concentration

Table 5 Xenon in cardiac arrest models

Author/Journal/Year of publication	Species	Injury	Results/Conclusions
Schmidt *et al*., Anesthesiology 2005[123]	Pig	Effect of xenon *vs* total intravenous anesthesia exposure before 4 min of cardiac arrest	Xenon preconditions against brain injury
Fries *et al*., Crit Care Med 2008[124]	Pig	Xenon after 8 min cardiac arrest + 4 min resuscitation	Xenon improves functional and histopathological outcome
Fries *et al*., Crit Care Med 2012[64]	Pig	Xenon combined with hypothermia after 10 min cardiac arrest	Xenon improved neurological and cardiac functional outcome

addition of xenon to hypothermia resulted in a significant improvement in functional outcome (no deaths and fewer neurologic deficits over time) and ameliorated myocardial dysfunction.

In the above-mentioned preclinical studies, dose-concentrations are limited to $< 70\%$ by the need to provide sufficient oxygen for adequate saturation. Unless accompanied by therapeutic hypothermia, concentrations of xenon $< 20\%$ appear to be ineffective. Therefore, the clinical effective range is likely to reside in a relatively narrow range of 20-70%.

Clinical studies of neurological injury treated with a combination of Xe+TH

For patients undergoing coronary artery bypass grafting while on cardiopulmonary bypass

There is an increased incidence of postoperative neurocognitive deficit (POCD) following cardiac surgery with cardiopulmonary bypass (CPB).[65] Moreover, the pathogenic mechanisms involved in the development of POCD may be similar to those involved in the propagation

Table 6 Neuroprotection through a combination of xenon and hypothermia

Author/Journal/Year of publication	Species	Injury	Results/Conclusions
Ma *et al.*, Ann Neurol 2005[36]	Rat pup	Rice-Vannucci model of neonatal asphyxia	Synergistic interaction between Xe and TH
Martin *et al.*, Br J Anaesth 2007[58]	Rat pup	Rice-Vannucci model of neonatal asphyxia	Synergy even when Xe + TH delivered asynchronously
Hobbs *et al.*, Stroke 2008[125]	Rat pup	Rice-Vannucci model of neonatal asphyxia	Synergistic interaction between Xe and TH
Thoresen *et al.*, J Cereb Blood Flow Metab 2009[59]	Rat pup	Rice-Vannucci model of neonatal asphyxia	Additivity even when Xe + TH delivered asynchronously
Chakkarapani *et al.*, Ann Neurol 2010[61]	Piglet	Global ischemia for 45 min	Superior protection with Xe +TH
Faulkner *et al.*, Ann Neurol 2011[62]	Piglet	Global ischemia until energy loss	Synergistic interaction between Xe and TH
Fries *et al.*, Crit Care Med 2012[64]	Pig	Xenon combined with hypothermia after 10 min cardiac arrest	Xenon improved neurological and cardiac functional outcome
Sabir *et al.*, PLoS One 2014[60]	Rat Pup	Rice-Vannucci model of neonatal asphyxia	Superior protection with Xe +TH

TH = therapeutic hypothermia; Xe = xenon

of acute neuronal injury from other causes.[66] Hence, xenon has been investigated as a potential prophylactic pharmacological intervention.[67] To test the feasibility and safety of delivering xenon to patients undergoing coronary artery bypass graft surgery while on CPB, xenon was administered to patients in an open-label dose-escalation study (0, 20, 35, 50% xenon in oxygen and air; $n = 4$ per group). Xenon was delivered throughout the surgical portion of the procedure, including prior to, during, and after CPB. Xenon concentration (partial pressures) was measured at five defined points before, during, and after CPB using gas chromatography. Because of a theoretical concern regarding gas bubble emboli, middle cerebral artery Doppler was used to assess embolic load. Blood markers, S100-β and troponin I, were also used to assess adverse effects of emboli on major organ system function.

Xenon was delivered at the chosen partial pressures using a closed-circle breathing system that recirculated the gas after scrubbing out the carbon dioxide and supplementing oxygen to ensure adequate oxygenation.

Neither the duration of tracheal intubation nor length of stay in the intensive care unit (ICU) and hospital was different between the control (no xenon) and any of the xenon concentration groups. Patients administered xenon had no major organ dysfunction, and concerns about possible damage created by theoretical expansion of air bubbles did not materialize. This finding is consistent with a previous report in which diffusion of gas into a closed space, and hence its expansion, was considered in a preclinical model of ileus. While xenon increased gas diffusion, this was considerably less than was seen with nitrous oxide.[68] This small open-label trial showed the tolerability of xenon in patients with cardiac disease and was an important precursor for the subsequent Xe-hypotheca study in which patients were randomized.

Xenon and therapeutic hypothermia for out-of-hospital cardiac arrest (OHCA)

The Xe-hypotheca study was a Phase II drug clinical trial in adult victims of OHCA with an initial cardiac rhythm that was "shockable", i.e., either ventricular fibrillation or pulseless ventricular tachycardia. One hundred ten patients were randomized in a 1:1 ratio to receive either mild therapeutic hypothermia treatment alone for 24 hr (MTH group) or in combination with inhaled xenon. A report of the feasibility and safety of the first 36 patients enrolled in the trial has been published.[69]

After assessment and emergent treatment (i.e., mechanical ventilation and correction of cardiovascular instability) in the emergency department, patients underwent a brain computerized tomography scan and transthoracic echocardiography to exclude a possible cerebral origin of the cardiac arrest and to assess myocardial function. If an ST-elevation myocardial infarction was established, primary percutaneous coronary intervention was performed before ICU admission. Subsequent care was performed according to the Utstein style, and the recommendations of the International Liaison Committee on Resuscitation were followed.[70,71]

Cooling of patients was conducted with the use of an Alsius CoolGard^{TM} 3000 thermal regulation system (Zoll Medical Corporation, Chelmsford, MA, USA), an invasive intravascular temperature management device, to achieve a target core temperature of 33°C and then to maintain the

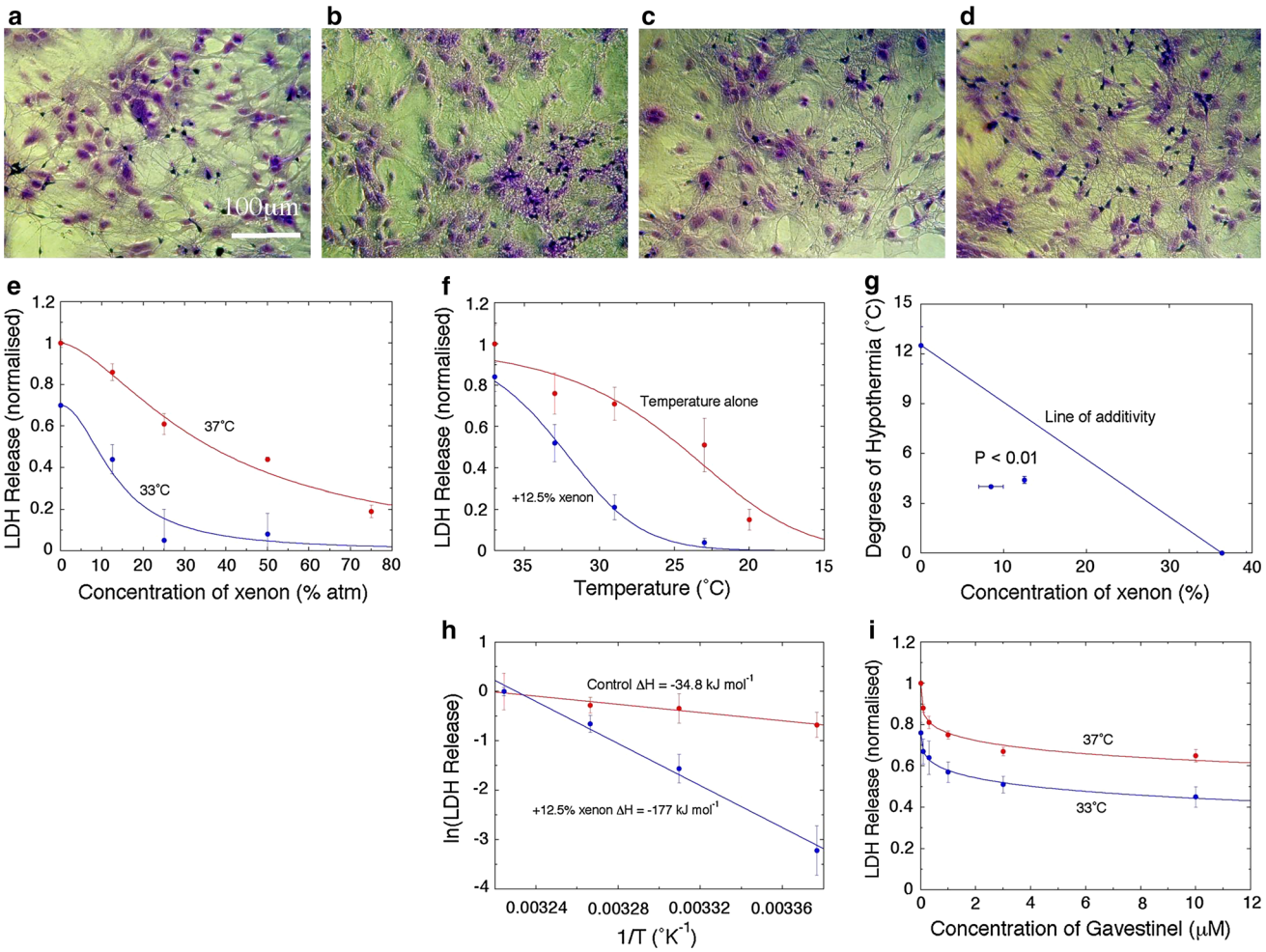

FIGURE Neuroprotective effects of xenon and hypothermia *in vitro*. (A) Photomicrographs of normal Nissl-stained neurons, (B) after oxygen-glucose deprivation (OGD), (C) after OGD in the presence of xenon (75%), and (D) after OGD in the presence of hypothermia at 20°C. (E) Lactate dehydrogenase (LDH) release from OGD-injured cultured neurons exposed to increasing concentrations of xenon at normothermia (37°C) and hypothermia (33°C). (F) LDH release from OGD-injured cultured neurons exposed to decreasing temperature with or without xenon (12.5%). (G) Isobologram illustrating the protective efficacy of combining xenon and temperature on injured cultured neurons. Protection from damage to OGD-injured neurons is synergistically enhanced when provided in combination as reflected by IC50 values for the interventions that were significantly to the left of the "line of additivity" joining the IC50 of the individual interventions. (H) van't Hoff plot showing the temperature dependence of neuroprotection for hypothermia alone and hypothermia in the presence of xenon. The enthalpy change (H) is a measure of the overall heat flow during the process. (I) LDH release from OGD-injured cultured neurons exposed to increasing concentrations of gavestinel at normothermia (37°C) and hypothermia (33°C). (Reprinted with permission from: *Ma D, Hossain M, Chow A, et al.* Xenon and hypothermia combine to provide neuroprotection from neonatal asphyxia. Ann Neurol 2005; 58: 182-93).[36]

core temperature for 24 hr. Subsequent rewarming was performed at a maximum rate of 0.5°C·hr^{-1}. Sedation was accomplished with a continuous infusion of propofol (1-5 mg·kg^{-1}·hr^{-1}) and fentanyl (50-100 μg·hr^{-1}), and shivering was terminated with cisatracurium. Exposure to xenon was accomplished through a PhysioFlex^{TM} closed-system ventilator (Dräger, Lübeck, Germany) to achieve an end-tidal xenon concentration of at least 40%. This was delivered continuously until completion of rewarming.

At the time of the first report, no adverse reactions attributable to xenon were identified.[69] In the 28 patients with coronary artery disease, the median (interquartile range [IQR]) delta troponin T value at 72 hr was 0.62 [0.18-2.39] μg·L^{-1} in the control group and 0.08 [0.0-0.67] μg·L^{-1} in the xenon-treated group.

These favourable cardiovascular features, together with the use of xenon at higher concentrations in surgical settings (albeit for shorter durations), portend safe use of

xenon when combined with mild therapeutic hypothermia in patients with cardiovascular disease.

Hypoxic ischemic encephalopathy

The 2010 International Liaison Committee on Resuscitation guidelines state that infants born at (or near) term with moderate to severe HIE should be offered therapeutic hypothermia. Treatment should be initiated and conducted under clearly defined protocols at neonatal intensive care facilities that provide multidisciplinary care and follow-up.[72] A meta-analysis that included 767 neonates concluded that therapeutic hypothermia significantly reduced the risk of death or disability at 18 months *vs* standard care (relative risk, 0.81; 95% CI, 0.71 to 0.93). The number needed to treat of neonates for one newborn to be free of death or disability was 9 (95% CI, 5 to 25).[73] Thus, the therapeutic benefit of hypothermia is modest and further neuroprotective interventions are urgently needed. This was recognized by the National Institute of Child Health and Human Development who arranged two workshops to discuss the next steps.[74,75] From this initiative, studies have evolved to identify adjunctive agents that have focused around the use of EPO and xenon. While early studies had shown the safety and possible additional benefit of EPO treatment,[76,77] randomized trials need to be undertaken with this adjuvant. The current evidence is insufficient to recommend adjunctive EPO therapy for newborns with HIE who are undergoing hypothermia.

For the use of xenon as an adjunctive to therapeutic hypothermia, two groups have independently pursued clinical studies to ascertain the dose range, duration, feasibility, and safety (i.e., the CoolXenon Study; ISRCTN75602528, www.isrctn.com/ISRCTN75602528) and to compare xenon and therapeutic hypothermia *vs* therapeutic hypothermia alone (i.e., the TOBY Xe Trial; NCT00934700, https://clinicaltrials.gov/ct2/show/NCT00934700).

The TOBY Xe Trial (NCT00934700)

This randomized proof-of-concept clinical trial was approved by the National Research Ethics Board in the UK (June 2010), and according to its website (see www.npeu.ox.ac.uk/toby-xe for a detailed description of the trial protocol), it has recruited 89 patients at four sites: Hammersmith Hospital (Imperial College Hospital Trust), St. Thomas Hospital (King's College Hospital Trust), University College London Hospital, and Liverpool Women's Hospital.

The investigators have reported preliminary findings regarding the potentially beneficial effect of xenon on seizure activity.[78] These data are very encouraging because the severity of seizures in asphyxiated encephalopathic neonates is strongly associated with more extensive brain injury[79] and worse clinical outcome.[80]

The CoolXenon Study (ISRCTN75602528)

In this single-arm dose-escalation study led by Prof. Marianne Thoresen and Dr. John Dingley, xenon was administered to 14 infants who received therapeutic hypothermia for HIE.[81] The primary outcomes (i.e., cardiorespiratory stability, electroencephalogram (EEG) parameters, and feasibility) were compared with 42 case-matched neonates who received therapeutic hypothermia alone. For the secondary outcome of safety, the *Bayley Scales of Infant Development II* was used to assess infants aged 18-20 months. It is noteworthy that most of the xenon-treated patients (13/14) received 50% xenon for 18 hr during the 72 hr of therapeutic hypothermia. As with the TOBY Xe study, an anticonvulsant effect of xenon was noted. Prior to starting xenon, 8/14 patients had seizure activity clinically or by EEG, while only 1/14 had seizure activity during xenon administration. There was cardiorespiratory stability throughout the period that xenon was administered. At 18 months, seven of 14 patients survived with a good clinical outcome; three died while in the hospital, and four survivors incurred disability. Therefore, death and disability occurred in 50% of patients. Historically, this number is 75%. Based on these encouraging data, the investigators have proposed randomized trials comparing xenon and therapeutic hypothermia *vs* therapeutic hypothermia alone (Thoresen M, CoolXenon2, NCT01545271, 2012, https://clinicaltrials.gov/ct2/show/NCT01545271; and Thoresen M, CoolXenon3, NCT02071394, 2014, https://clinicaltrials.gov/ct2/show/NCT02071394).

Future applications of xenon's neuroprotective properties

Anesthetic-induced developmental neurotoxicity

Despite the reversible nature of general anesthesia, when immature organisms are exposed to compounds that produce the anesthetized state, brain injury can occur and result in long-term neurobehavioural deficits.[82] This anesthetic-induced developmental neurotoxicity (AIDN) is unlikely due to physiological derangements that may

accompany the anesthetized state because blood gas tensions were normal,[83] and anesthetic-induced toxicity can be reproduced in *in vitro* neuronal cell cultures[84] as well as in *ex vivo* brain slices (Table 4).[12,85,86] Indeed, this AIDN has been noted with structurally unrelated compounds spanning the entire panoply of general anesthetic agents approved for pediatric use.[82,87-90] It is also evident across species - from nematodes[91] to rodents,[82] to piglets,[92] as well as to non-human primates.[93-97]

In contrast to the preponderance of preclinical studies establishing AIDN, this has yet to be confirmed in well-controlled prospective randomized clinical trials. In fact, observational data obtained from databases collected for other reasons[98,99] represent the most compelling support for an *association* between exposure to general anesthesia and subsequent cognitive and behavioural problems later in human life. This association is strongest for multiple anesthetics administered to children younger than three years.[100]

Based on the known neuroprotective effects of xenon, we and others have investigated whether xenon itself produces AIDN and whether it can prevent AIDN.[12-14,101,102] Apart from the *in vitro* preparation in which concentrations of xenon above 1 MAC produced injury, each of the studies in neonatal rats, neonatal mice, and piglets showed attenuation of anesthetic-induced neurotoxicity and/or lack of AIDN when exposure to xenon was included.[12-14,101] Nevertheless, it is noteworthy that xenon alone did produce significant, albeit modest, neuroapoptosis (though less than isoflurane) when administered alone to mice.[13] Nevertheless, xenon attenuated the injury produced by isoflurane in this species, as well as in other species.[13]

Traumatic brain injury

Approximately 1.7 million patients per year require medical care in the United States for TBI, incurring an annual medical cost burden of over $70 billion.[D] The severity of the condition is highlighted by the fact that 1/3 of injury-related deaths are caused by TBI. The next imperative, following effective strategies to prevent TBI from concussive and blast assaults, is to acquire effective neuroprotective measures to salvage potentially surviving neurons from progressive damage and cell death. Earlier *in vitro* work on brain slice preparations showed significant neuroprotection with administered xenon (Table 3).[103] A recent *in vivo* study of a TBI model in rats[104] showed significant reduction in contusion volume, improved neurological outcome scores, and enhanced long-term locomotor function.

Stroke (Table 2)

Following the demonstration that intervention for a pre-hospital stroke can be delivered in the field,[26] the stage is set for extending xenon's use to a "field setting". Members of emergency medical services are quite skilled at securing the necessary airway to administer inhalation therapy at the site where the patient is first encountered, long before admission to the emergency department. It would not be necessary to rule out intracranial hemorrhage as a cause for the stroke. According to the best available evidence to date, xenon appears to be equally effective and non-toxic for both hemorrhagic and non-hemorrhagic causes of stroke.[105]

Cost

In studies using efficient closed systems to recirculate the non-biotransformable xenon, no more than 2 $L \cdot hr^{-1}$ is required. While the production costs of xenon may be below $\$50 \cdot L^{-1}$, the price for xenon produced under current Good Manufacturing Practices (cGMP) is likely to be considerably higher. Pricing a new therapy may be subject to strict cost-benefit analysis as performed by regulatory entities such as the National Institute for Health and Care Excellence in the UK. In other healthcare environments, incremental cost-effectiveness ratios are used to indicate whether the health benefits in quality-adjusted life years exceeds the overall costs (price of the product as well as the additional care costs). The estimated healthcare costs to provide one additional quality-adjusted year of life to a neurologically injured patient has been estimated to be > $850,000.[106] The cost of a 24-hr treatment with xenon could be compared with the use of nitric oxide over the same time period. Therefore, under the above circumstances, efficacious neuroprotection with xenon could be considered cost-effective.

Conclusion

Many preclinical neuroprotectants have failed to materialize into clinically effective treatments.[1] While there may be "many a slip twixt cup and lip",[E] the wealth

[D] *Centers for Disease Control and Prevention.* Injury Prevention & Control. Traumatic Brain Injury. Available from URL: http://www.cdc.gov/TraumaticBrainInjury/ (accessed August 2015).

[E] *The Free Dictionary by Farlex.* There's many a slip twixt cup and lip. https://idioms.thefreedictionary.com/There%27s+many+a+slip+twixt+cup+and+lip (accessed August 2015).

of positive preclinical studies appears to be translating into clinical utility. When the dust settles, we will know whether we are riding a horse or a donkey.

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Conflict of interest Mervyn Maze is a founder, director, and owner of equity in NeuroproteXeon, a company whose mission is to develop and market xenon-based products for use against acute ongoing neurologic injury.

References

1. *Sacchetti ML*. Is it time to definitely abandon neuroprotection in acute ischemic stroke? Stroke 2008; 39: 1659-60.
2. *Lapchak PA*, *Zhang JH*, *Noble-Haeusslein LJ*. RIGOR guidelines: escalating STAIR and STEPS for effective translational research. Transl Stroke Res 2013; 4: 279-85.
3. *Gilman DC, Peck TH, Colby FM* . "Xenon". *In:* Gilman DC, Peck TH, Colby FM (Eds). New International Encyclopaedia (Vol 17). New York: Dodd, Mead & Co.; 1902-1904: 906.
4. *Ramsay W*. An attempt to estimate the relative amounts of krypton and of xenon in atmospheric air. Proc R Soc Lond 1902; 71: 421-6.
5. *Patel CK*, *Bennett WR Jr*, *Faust WL*, *McFarlane RA*. Infrared spectroscopy using stimulated emission techniques. Phys Rev Lett 1962; 9: 102-4.
6. *Shuaibov AK*, *Shimon LL*, *Grabovaya A*. A xenon-iodine electric discharge bactericidal lamp. Tech Phys Lett 2003; 29: 871-2.
7. *Marin R*. Plasma TV: That New Object of Desire. The New York Times, Mar 25, 2001, Style Section. Available from URL: http://www.nytimes.com/2001/03/25/style/plasma-tv-that-new-object-of-desire.html (accessed August 2015).
8. *Glenn Research Center, NASA*. Fact Sheet: Innovative Engines: Glenn Ion Propulsion Research Tames the Challenges of 21st Century Space Travel - Updated May 20, 2008]. Available from URL: http://www.nasa.gov/centers/glenn/about/fs08grc.html (accessed August 2015).
9. *Marcucci C*, *Leopold DA*, *Cullen M*, *Zinreich SJ*, *Simon BA*. Dynamic assessment of paranasal sinus ventilation using xenon-enhanced computed tomography. Ann Otol Rhinol Laryngol 2001; 110: 968-75.
10. *Lawrence JH*, *Loomis WF*, *Tobias CA*, *Turpin FH*. Preliminary observations on the narcotic effect of xenon with a review of values for solubilities of gases in water and oils. J Physiol 1946; 105: 197-204.
11. *Cullen SC*, *Gross EG*. The anesthetic properties of xenon in animals and human beings, with additional observations on krypton. Science 1951; 113: 580-2.
12. *Ma D*, *Williamson P*, *Januszewski A*, *et al*. Xenon mitigates isoflurane-induced neuronal apoptosis in the developing rodent brain. Anesthesiology 2007; 106: 746-53.
13. *Cattano D*, *Williamson P*, *Fukui K*, *et al*. Potential of xenon to induce or to protect against neuroapoptosis in the developing mouse brain. Can J Anesth 2008; 55: 429-36.
14. *Sabir H*, *Bishop S*, *Cohen N*, *et al*. Neither xenon nor fentanyl induces neuroapoptosis in the newborn pig brain. Anesthesiology 2013; 119: 345-57.
15. *Franks NP*, *Dickinson R*, *de Sousa SL*, *Hall AC*, *Lieb WR*. How does xenon produce anaesthesia? Nature 1998; 396: 324.
16. *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: 756-67.
17. *Armstrong SP*, *Banks PJ*, *McKitrick TJ*, *et al*. Identification of two mutations (F758W and F758Y) in the N-methyl-D-aspartate receptor glycine-binding site that selectively prevent competitive inhibition by xenon without affecting glycine binding. Anesthesiology 2012; 117: 38-47.
18. *Dinse A*, *Fohr KJ*, *Georgieff M*, *Beyer C*, *Bulling A*, *Weigt HU*. Xenon reduces glutamate-, AMPA-, and kainate-induced membrane currents in cortical neurones. Br J Anaesth 2005; 94: 479-85.
19. *Gruss M*, *Bushell TJ*, *Bright DP*, *Lieb WR*, *Mathie A*, *Franks NP*. Two-pore-domain K+ channels are a novel target for the anesthetic gases xenon, nitrous oxide, and cyclopropane. Mol Pharmacol 2004; 65: 443-52.
20. *Olney JW*. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 1969; 164: 719-21.
21. *Choi DW*, *Koh JY*, *Peters S*. Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. J Neurosci 1988; 8: 185-96.
22. *Wilhelm S*, *Ma D*, *Maze M*, *Franks NP*. Effects of xenon on in vitro and in vivo models of neuronal injury. Anesthesiology 2002; 96: 1485-91.
23. *Petzelt C*, *Blom P*, *Schmehl W*, *Muller J*, *Kox WJ*. Prevention of neurotoxicity in hypoxic cortical neurons by the noble gas xenon. Life Sci 2003; 72: 1909-18.
24. *David HN*, *Leveille F*, *Chazalviel L*, *et al*. Reduction of ischemic brain damage by nitrous oxide and xenon. J Cereb Blood Flow Metab 2003; 23: 1168-73.
25. *Haley EC Jr*, *Thompson JL*, *Levin B*, *et al*. Gavestinel does not improve outcome after acute intracerebral hemorrhage: an analysis from the GAIN International and GAIN Americas studies. Stroke 2005; 36: 1006-10.
26. *Saver JL*, *Starkman S*, *Eckstein M*, *et al*. Prehospital use of magnesium sulfate as neuroprotection in acute stroke. N Engl J Med 2015; 372: 528-36.
27. *Ma D*, *Wilhelm S*, *Maze M*, *Franks NP*. Neuroprotective and neurotoxic properties of the 'inert' gas, xenon. Br J Anaesth 2002; 89: 739-46.
28. *Nagata A*, *Nakao Si S*, *Nishizawa N*, *et al*. Xenon inhibits but N(2)O enhances ketamine-induced c-Fos expression in the rat posterior cingulate and retrosplenial cortices. Anes Analg 2001; 92: 362-8.
29. *Sakamoto S*, *Nakao S*, *Masuzawa M*, *et al*. The differential effects of nitrous oxide and xenon on extracellular dopamine levels in the rat nucleus accumbens: a microdialysis study. Anesth Analg 2006; 103: 1459-63.
30. *Parsons CG*, *Gruner R*, *Rozental J*, *Millar J*, *Lodge D*. Patch clamp studies on the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). Neuropharmacology 1993; 32: 1337-50.
31. *Emnett CM*, *Eisenman LN*, *Mohan J*, *et al*. Interaction between positive allosteric modulators and trapping blockers of the NMDA receptor channel. Br J Pharmacol 2015; 172: 1333-47.
32. *de Sousa SL*, *Dickinson R*, *Lieb WR*, *Franks NP*. Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. Anesthesiology 2000; 92: 1055-66.
33. *Honore E*. The neuronal background K2P channels: focus on TREK1. Nat Rev Neurosci 2007; 8: 251-61.
34. *Heurteaux C*, *Guy N*, *Laigle C*, *et al*. TREK-1, a K+ channel involved in neuroprotection and general anesthesia. EMBO J 2004; 23: 2684-95.
35. *Young C*, *Tenkova T*, *Dikranian K*, *Olney JW*. Excitotoxic versus apoptotic mechanisms of neuronal cell death in perinatal hypoxia/ischemia. Curr Mol Med 2004; 4: 77-85.

36. *Ma D*, *Hossain M*, *Chow A*, *et al*. Xenon and hypothermia combine to provide neuroprotection from neonatal asphyxia. Ann Neurol 2005; 58: 182-93.
37. *Zhuang L*, *Yang T*, *Zhao H*, *et al*. The protective profile of argon, helium, and xenon in a model of neonatal asphyxia in rats. Crit Care Med 2012; 40: 1724-30.
38. *Ishikawa M*, *Zhang JH*, *Nanda A*, *Granger DN*. Inflammatory responses to ischemia and reperfusion in the cerebral microcirculation. Front Biosci 2004; 9: 1339-47.
39. *Arvin B*, *Neville LF*, *Barone FC*, *Feuerstein GZ*. The role of inflammation and cytokines in brain injury. Neurosci Biobehav Rev 1996; 20: 445-52.
40. *Han Z*, *Li L*, *Wang L*, *Degos V*, *Maze M*, *Su H*. Alpha-7 nicotinic acetylcholine receptor agonist treatment reduces neuroinflammation, oxidative stress and brain injury in mice with ischemic stroke and bone fracture. J Neurochem 2014; 131: 498-508.
41. *Han Z*, *Shen F*, *He Y*, *et al*. Activation of alpha-7 nicotinic acetylcholine receptor reduces ischemic stroke injury through reduction of pro-inflammatory macrophages and oxidative stress. PLoS One 2014; 9: e105711.
42. *Degos V*, *Vacas S*, *Han Z*, *et al*. Depletion of bone marrow-derived macrophages perturbs the innate immune response to surgery and reduces postoperative memory dysfunction. Anesthesiology 2013; 118: 527-36.
43. *Zhao H*, *Huang H*, *Ologunde R*, *et al*. Xenon treatment protects against remote lung injury after kidney transplantation in rats. Anesthesiology 2015; 122: 1312-26.
44. *Jia P*, *Teng J*, *Zou J*, *et al*. Xenon protects against septic acute kidney injury via miR-21 target signaling pathway. Crit Care Med 2015; 43: e250-9.
45. *Vizcaychipi MP*, *Lloyd DG*, *Wan Y*, *Palazzo MG*, *Maze M*, *Ma D*. Xenon pretreatment may prevent early memory decline after isoflurane anesthesia and surgery in mice. PLoS One 2011; 6: e26394.
46. *Bessiere B*, *Laboureyras E*, *Laulin JP*, *Simonnet G*. Xenon prevents inflammation-induced delayed pain hypersensitivity in rats. Neuroreport 2010; 21: 1167-71.
47. *Ma D*, *Lim T*, *Xu J*, *et al*. Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1alpha activation. J Am Soc Nephrol 2009; 20: 713-20.
48. *Merelli A*, *Czornyj L*, *Lazarowski A*. Erythropoietin: a neuroprotective agent in cerebral hypoxia, neurodegeneration, and epilepsy. Curr Pharm Des 2013; 19: 6791-801.
49. *Pfeffer MA*, *Burdmann EA*, *Chen CY*, *et al*. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. N Engl J Med 2009; 361: 2019-32.
50. *Zhao H*, *Watts HR*, *Chong M*, *et al*. Xenon treatment protects against cold ischemia associated delayed graft function and prolongs graft survival in rats. Am J Transplant 2013; 13: 2006-18.
51. *Goetzenich A*, *Hatam N*, *Preuss S*, *et al*. The role of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in late-phase preconditioning with xenon, isoflurane and levosimendan in rat cardiomyocytes. Interact Cardiovasc Thorac Surg 2014; 18: 321-8.
52. *Limatola V*, *Ward P*, *Cattano D*, *et al*. Xenon preconditioning confers neuroprotection regardless of gender in a mouse model of transient middle cerebral artery occlusion. Neuroscience 2010; 165: 874-81.
53. *Sun HS*, *Xu B*, *Chen W*, *et al*. Neuronal K(ATP) channels mediate hypoxic preconditioning and reduce subsequent neonatal hypoxic-ischemic brain injury. Exp Neurol 2015; 263: 161-71.
54. *Qu YY*, *Yuan MY*, *Liu Y*, *Xiao XJ*, *Zhu YL*. The protective effect of epoxyeicosatrienoic acids on cerebral ischemia/reperfusion injury is associated with PI3K/Akt pathway and ATP-sensitive potassium channels. Neurochem Res 2015; 40: 1-14.
55. *Li D*, *Huang B*, *Liu J*, *Li L*, *Li X*. Decreased brain K(ATP) channel contributes to exacerbating ischemic brain injury and the failure of neuroprotection by sevoflurane post-conditioning in diabetic rats. PLoS One 2013; 8: e73334.
56. *Bantel C*, *Maze M*, *Trapp S*. Neuronal preconditioning by inhalational anesthetics: evidence for the role of plasmalemmal adenosine triphosphate-sensitive potassium channels. Anesthesiology 2009; 110: 986-95.
57. *Bantel C*, *Maze M*, *Trapp S*. Noble gas xenon is a novel adenosine triphosphate-sensitive potassium channel opener. Anesthesiology 2010; 112: 623-30.
58. *Martin JL*, *Ma D*, *Hossain M*, *et al*. Asynchronous administration of xenon and hypothermia significantly reduces brain infarction in the neonatal rat. Br J Anaesth 2007; 98: 236-40.
59. *Thoresen M*, *Hobbs CE*, *Wood T*, *Chakkarapani E*, *Dingley J*. Cooling combined with immediate or delayed xenon inhalation provides equivalent long-term neuroprotection after neonatal hypoxia-ischemia. J Cereb Blood Flow Metab 2009; 29: 707-14.
60. *Sabir H*, *Walloe L*, *Dingley J*, *Smit E*, *Liu X*, *Thoresen M*. Combined treatment of xenon and hypothermia in newborn rats-additive or synergistic effect? PLoS One 2014; 9: e109845.
61. *Chakkarapani E*, *Dingley J*, *Liu X*, *et al*. Xenon enhances hypothermic neuroprotection in asphyxiated newborn pigs. Ann Neurol 2010; 68: 330-41.
62. *Faulkner S*, *Bainbridge A*, *Kato T*, *et al*. Xenon augmented hypothermia reduces early lactate/N-acetylaspartate and cell death in perinatal asphyxia. Ann Neurol 2011; 70: 133-50.
63. *Fries M*, *Coburn M*, *Nolte KW*, *et al*. Early administration of xenon or isoflurane may not improve functional outcome and cerebral alterations in a porcine model of cardiac arrest. Resuscitation 2009; 80: 584-90.
64. *Fries M*, *Brucken A*, *Cizen A*, *et al*. Combining xenon and mild therapeutic hypothermia preserves neurological function after prolonged cardiac arrest in pigs. Crit Care Med 2012; 40: 1297-303.
65. *Newman MF*, *Kirchner JL*, *Phillips-Bute B*, *et al*. Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. N Engl J Med 2001; 344: 395-402.
66. *Lynch DR*, *Guttmann RP*. Excitotoxicity: perspectives based on N-methyl-D-aspartate receptor subtypes. J Pharmacol Exp Ther 2002; 300: 717-23.
67. *Lockwood GG*, *Franks NP*, *Downie NA*, *Taylor KM*, *Maze M*. Feasibility and safety of delivering xenon to patients undergoing coronary artery bypass graft surgery while on cardiopulmonary bypass: phase I study. Anesthesiology 2006; 104: 458-65.
68. *Reinelt H, Schirmer U, Marx T, Topalidis P, Schmidt M.* Diffusion of xenon and nitrous oxide into the bowel. Anesthesiology 2001; 94: 475-7; discussion 6A.
69. *Arola OJ*, *Laitio RM*, *Roine RO*, *et al*. Feasibility and cardiac safety of inhaled xenon in combination with therapeutic hypothermia following out-of-hospital cardiac arrest. Crit Care Med 2013; 41: 2116-24.
70. *Langhelle A*, *Nolan J*, *Herlitz J*, *et al*. Recommended guidelines for reviewing, reporting, and conducting research on post-resuscitation care: the Utstein style. Resuscitation 2005; 66: 271-83.
71. *Neumar RW*, *Nolan JP*, *Adrie C*, *et al*. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the International Liaison Committee on Resuscitation (American Heart Association, Australian and New Zealand Council on Resuscitation, European Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart

Foundation, Resuscitation Council of Asia, and the Resuscitation Council of Southern Africa); the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; and the Stroke Council. Circulation 2008; 118: 2452-83.
72. *Perlman JM*, *Wyllie J*, *Kattwinkel J*, *et al*. Part 11: Neonatal resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation 2010; 122(16 Suppl 2): S516-38.
73. *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.
74. *Higgins RD*, *Raju TN*, *Perlman J*, *et al*. Hypothermia and perinatal asphyxia: executive summary of the National Institute of Child Health and Human Development workshop. J Pediatr 2006; 148: 170-5.
75. *Higgins RD*, *Raju T*, *Edwards AD*, *et al*. Hypothermia and other treatment options for neonatal encephalopathy: an executive summary of the Eunice Kennedy Shriver NICHD workshop. J Pediatr 2011; 159(851-8): e1.
76. *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: e1135-42.
77. *Wu YW*, *Bauer LA*, *Ballard RA*, *et al*. Erythropoietin for neuroprotection in neonatal encephalopathy: safety and pharmacokinetics. Pediatrics 2012; 130: 683-91.
78. *Azzopardi D*, *Robertson NJ*, *Kapetanakis A*, *et al*. Anticonvulsant effect of xenon on neonatal asphyxial seizures. Arch Dis Child Fetal Neonatal Ed 2013; 98: F437-9.
79. *Miller SP*, *Weiss J*, *Barnwell A*, *et al*. Seizure-associated brain injury in term newborns with perinatal asphyxia. Neurology 2002; 58: 542-8.
80. *Glass HC*, *Glidden D*, *Jeremy RJ*, *Barkovich AJ*, *Ferriero DM*, *Miller SP*. Clinical neonatal seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. J Pediatr 2009; 155: 318-23.
81. *Dingley J*, *Tooley J*, *Liu X*, *et al*. Xenon ventilation during therapeutic hypothermia in neonatal encephalopathy: a feasibility study. Pediatrics 2014; 133: 809-18.
82. *Jevtovic-Todorovic V*, *Hartman RE*, *Izumi Y*, *et al*. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003; 23: 876-82.
83. *Yon JH*, *Daniel-Johnson J*, *Carter LB*, *Jevtovic-Todorovic V*. Anesthesia induces neuronal cell death in the developing rat brain via the intrinsic and extrinsic apoptotic pathways. Neuroscience 2005; 135: 815-27.
84. *Twaroski DM*, *Yan Y*, *Olson JM*, *Bosnjak ZJ*, *Bai X*. Down-regulation of microRNA-21 is involved in the propofol-induced neurotoxicity observed in human stem cell-derived neurons. Anesthesiology 2014; 121: 786-800.
85. *Wise-Faberowski L*, *Zhang H*, *Ing R*, *Pearlstein RD*, *Warner DS*. Isoflurane-induced neuronal degeneration: an evaluation in organotypic hippocampal slice cultures. Anesth Analg 2005; 101: 651-7.
86. *Spahr-Schopfer I*, *Vutskits L*, *Toni N*, *Buchs PA*, *Parisi L*, *Muller D*. Differential neurotoxic effects of propofol on dissociated cortical cells and organotypic hippocampal cultures. Anesthesiology 2000; 92: 1408-17.
87. *Kodama M*, *Satoh Y*, *Otsubo Y*, *et al*. Neonatal desflurane exposure induces more robust neuroapoptosis than do isoflurane and sevoflurane and impairs working memory. Anesthesiology 2011; 115: 979-91.
88. *Satomoto M*, *Satoh Y*, *Terui K*, *et al*. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. Anesthesiology 2009; 110: 628-37.
89. *Fredriksson A*, *Ponten E*, *Gordh T*, *Eriksson P*. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits. Anesthesiology 2007; 107: 427-36.
90. *Bercker S*, *Bert B*, *Bittigau P*, *et al*. Neurodegeneration in newborn rats following propofol and sevoflurane anesthesia. Neurotox Res 2009; 16: 140-7.
91. *Gentry KR*, *Steele LM*, *Sedensky MM*, *Morgan PG*. Early developmental exposure to volatile anesthetics causes behavioral defects in Caenorhabditis elegans. Anesth Analg 2013; 116: 185-9.
92. *Schubert H*, *Eiselt M*, *Walter B*, *Fritz H*, *Brodhun M*, *Bauer R*. Isoflurane/nitrous oxide anesthesia and stress-induced procedures enhance neuroapoptosis in intrauterine growth-restricted piglets. Intensive Care Med 2012; 38: 1205-14.
93. *Zou X*, *Liu F*, *Zhang X*, *et al*. Inhalation anesthetic-induced neuronal damage in the developing rhesus monkey. Neurotoxicol Teratol 2011; 33: 592-7.
94. *Paule MG*, *Li M*, *Allen RR*, *et al*. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol 2011; 33: 220-30.
95. *Creeley CE*, *Dikranian KT*, *Dissen GA*, *Back SA*, *Olney JW*, *Brambrink AM*. Isoflurane-induced apoptosis of neurons and oligodendrocytes in the fetal rhesus macaque brain. Anesthesiology 2014; 120: 626-38.
96. *Creeley C*, *Dikranian K*, *Dissen G*, *Martin L*, *Olney J*, *Brambrink A*. Propofol-induced apoptosis of neurones and oligodendrocytes in fetal and neonatal rhesus macaque brain. Br J Anaesth 2013; 110(Suppl 1): i29-38.
97. *Brambrink AM*, *Evers AS*, *Avidan MS*, *et al*. Ketamine-induced neuroapoptosis in the fetal and neonatal rhesus macaque brain. Anesthesiology 2012; 116: 372-84.
98. *Ing CH*, *DiMaggio CJ*, *Malacova E*, *et al*. Comparative analysis of outcome measures used in examining neurodevelopmental effects of early childhood anesthesia exposure. Anesthesiology 2014; 120: 1319-32.
99. *Sprung J*, *Flick RP*, *Katusic SK*, *et al*. Attention-deficit/hyperactivity disorder after early exposure to procedures requiring general anesthesia. Mayo Clin Proc 2012; 87: 120-9.
100. *Flick RP*, *Katusic SK*, *Colligan RC*, *et al*. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 2011; 128: e1053-61.
101. *Shu Y*, *Patel SM*, *Pac-Soo C*, *et al*. Xenon pretreatment attenuates anesthetic-induced apoptosis in the developing brain in comparison with nitrous oxide and hypoxia. Anesthesiology 2010; 113: 360-8.
102. *Brosnan H*, *Bickler PE*. Xenon neurotoxicity in rat hippocampal slice cultures is similar to isoflurane and sevoflurane. Anesthesiology 2013; 119: 335-44.
103. *Coburn M*, *Maze M*, *Franks NP*. The neuroprotective effects of xenon and helium in an in vitro model of traumatic brain injury. Crit Care Med 2008; 36: 588-95.
104. *Campos-Pires R*, *Armstrong SP*, *Sebastiani A*, *et al*. Xenon improves neurologic outcome and reduces secondary injury following trauma in an in vivo model of traumatic brain injury. Crit Care Med 2015; 43: 149-58.
105. *Sheng SP*, *Lei B*, *James ML*, *et al*. Xenon neuroprotection in experimental stroke: interactions with hypothermia and intracerebral hemorrhage. Anesthesiology 2012; 117: 1262-75.

106. *Merchant RM*, *Becker LB*, *Abella BS*, *Asch DA*, *Groeneveld PW*. Cost-effectiveness of therapeutic hypothermia after cardiac arrest. Circ Cardiovasc Qual Outcomes 2009; 2: 421-8.
107. *Ma D*, *Hossain M*, *Pettet GK*, *et al*. Xenon preconditioning reduces brain damage from neonatal asphyxia in rats. J Cereb Blood Flow Metab 2006; 26: 199-208.
108. *Dingley J*, *Tooley J*, *Porter H*, *Thoresen M*. Xenon provides short-term neuroprotection in neonatal rats when administered after hypoxia-ischemia. Stroke 2006; 37: 501-6.
109. *Rajakumaraswamy N*, *Ma D*, *Hossain M*, *Sanders RD*, *Franks NP*, *Maze M*. Neuroprotective interaction produced by xenon and dexmedetomidine on in vitro and in vivo neuronal injury models. Neurosci Lett 2006; 409: 128-33.
110. *Dingley J*, *Hobbs C*, *Ferguson J*, *Stone J*, *Thoresen M*. Xenon/hypothermia neuroprotection regimes in spontaneously breathing neonatal rats after hypoxic-ischemic insult: the respiratory and sedative effects. Anesth Analg 2008; 106: 916-23.
111. *Cattano D*, *Valleggi S*, *Ma D*, *et al*. Xenon induces transcription of ADNP in neonatal rat brain. Neurosci Lett 2008; 440: 217-21.
112. *Valleggi S*, *Cavazzana AO*, *Bernardi R*, *et al*. Xenon up-regulates several genes that are not up-regulated by nitrous oxide. J Neurosurg Anesthesiol 2008; 20: 226-32.
113. *Luo Y*, *Ma D*, *Ieong E*, *et al*. Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology 2008; 109: 782-9.
114. *Chakkarapani E*, *Thoresen M*, *Hobbs CE*, *Aquilina K*, *Liu X*, *Dingley J*. A closed-circuit neonatal xenon delivery system: a technical and practical neuroprotection feasibility study in newborn pigs. Anesth Analg 2009; 109: 451-60.
115. *Jawad N*, *Rizvi M*, *Gu J*, *et al*. Neuroprotection (and lack of neuroprotection) afforded by a series of noble gases in an in vitro model of neuronal injury. Neurosci Lett 2009; 460: 232-6.
116. *Yang T*, *Zhuang L*, *Rei Fidalgo AM*, *et al*. Xenon and sevoflurane provide analgesia during labor and fetal brain protection in a perinatal rat model of hypoxia-ischemia. PLoS One 2012; 7: e37020.
117. *Chakkarapani E*, *Thoresen M*, *Liu X*, *Walloe L*, *Dingley J*. Xenon offers stable haemodynamics independent of induced hypothermia after hypoxia-ischaemia in newborn pigs. Intensive Care Med 2012; 38: 316-23.
118. *Chakkarapani E*, *Dingley J*, *Aquilina K*, *Osredkar D*, *Liu X*, *Thoresen M*. Effects of xenon and hypothermia on cerebrovascular pressure reactivity in newborn global hypoxic-ischemic pig model. J Cereb Blood Flow Metab 2013; 33: 1752-60.
119. *Homi HM*, *Yokoo N*, *Ma D*, *et al*. The neuroprotective effect of xenon administration during transient middle cerebral artery occlusion in mice. Anesthesiology 2003; 99: 876-81.
120. *David HN*, *Haelewyn B*, *Risso JJ*, *Colloc'h N*, *Abraini JH*. Xenon is an inhibitor of tissue-plasminogen activator: adverse and beneficial effects in a rat model of thromboembolic stroke. J Cereb Blood Flow Metab 2010; 30: 718-28.
121. *Harris K*, *Armstrong SP*, *Campos-Pires R*, *Kiru L*, *Franks NP*, *Dickinson R*. Neuroprotection against traumatic brain injury by xenon, but not argon, is mediated by inhibition at the N-methyl-D-aspartate receptor glycine site. Anesthesiology 2013; 119: 1137-48.
122. *Cattano D*, *Valleggi S*, *Cavazzana AO*, *et al*. Xenon exposure in the neonatal rat brain: effects on genes that regulate apoptosis. Minerva Anestesiol 2011; 77: 571-8.
123. *Schmidt M*, *Marx T*, *Gloggl E*, *Reinelt H*, *Schirmer U*. Xenon attenuates cerebral damage after ischemia in pigs. Anesthesiology 2005; 102: 929-36.
124. *Fries M*, *Nolte KW*, *Coburn M*, *et al*. Xenon reduces neurohistopathological damage and improves the early neurological deficit after cardiac arrest in pigs. Crit Care Med 2008; 36: 2420-6.
125. *Hobbs C*, *Thoresen M*, *Tucker A*, *Aquilina K*, *Chakkarapani E*, *Dingley J*. Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. Stroke 2008; 39: 1307-13.