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Anesthesia in Experimental Stroke Research

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Abstract

Anesthetics have enabled major advances in development of experimental models of human stroke. Yet their profound pharmacologic effects on neural function can confound the interpretation of experimental stroke research. Anesthetics have drug and dose-specific effects on cerebral blood flow and metabolism, neurovascular coupling, autoregulation, ischemic depolarizations, excitotoxicity, inflammation, neural networks, and numerous molecular pathways relevant for stroke outcome. Both pre- and post-conditioning properties have been described. Anesthetics also modulate systemic arterial blood pressure, lung ventilation, and thermoregulation, all of which may interact with the ischemic insult as well as the therapeutic interventions. These confounds present a dilemma. Here, we provide an overview of the anesthetic mechanisms of action and molecular and physiologic effects on factors relevant to stroke outcomes that can guide the choice and optimization of the anesthetic regimen in experimental stroke.

Introduction

There is strong evidence that sufficient overlap exists between the neurobiology of disease in laboratory animals and humans to justify definition of therapeutic safety and efficacy parameters in preclinical models to inform rational clinical trial design [1]. Surgically created disease models allow study of injury mechanisms and identification of therapeutic targets. *In vitro* and invertebrate systems can provide critical information regarding molecular mechanisms of injury, but cannot substitute for *in vivo* models. We still need to investigate these phenomena and confirm their relevance in intact mammalian systems where interactions between local, remote, and humoral factors contribute to the outcome of central nervous system (CNS) injury. Acute human CNS injury, such as ischemic or

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hemorrhagic stroke, trauma, and global hypoxic ischemic injury, largely occur in the absence of anesthesia. Anesthesia and analgesia are necessary to assure animal welfare while reproducing brain injury in the laboratory, but the implications of anesthetic/analgesic use in experimental CNS injury are often ignored or misunderstood. The impact of anesthetic/analgesic use on model outcomes may substantially confound our understanding of the neurobiology of acute brain injury, posing a significant dilemma. Conversely, some treatments for acute human stroke may involve anesthesia (e.g., hemicraniectomy, carotid endarterectomy or stenting, cerebral aneurysm clipping/coiling, mechanical thrombolysis). The extent to which clinical anesthetic exposure alters the course of disease and how this should be modeled experimentally has long been investigated, providing a wealth of information of relevance to model non-operative disease [2]. There also is strong evidence that neurobiological effects of anesthetics are not transient. Potent effects on pre- and post-conditioning, excitotoxicity, apoptosis, neurogenesis, gene-regulation, neurodevelopment, and short- and long-term outcome from ischemic insults have been widely reported. Here, we aim to provide a review of anesthetic modulation of CNS and systemic physiology relevant to experimental stroke to help define potential confounders of model outcomes in clinical translation.

Resting cerebral blood flow and metabolism

Historically, as a result of interest in aviation physiology, methods were developed in the 1940s to measure cerebral blood flow (CBF) and metabolic rate (CMR) in humans [3], followed by exploration of anesthetic effects on these measures [4]. First, barbiturates were found to decrease CMR. Further work indicated CMR reduction was maximal with onset of an isoelectric encephalogram (EEG) [5]. The mechanism of CMR reduction was attributed to inhibition of synaptic neurotransmission. Conservation of the energy required to support neurotransmission was postulated to increase tolerance to ischemia [6]. Extensive research has since been conducted across the spectrum of anesthetics for effects on CMR in both humans and laboratory animals. A consistent pattern is observed. Isoflurane [7], sevoflurane [8], propofol [9], benzodiazepines [10], etomidate [11], lidocaine [12], fentanyl [13], and dexmedetomidine [14] cause a dose-dependent generalized decrease in CMR. Maximal anesthetic-mediated CMR suppression ranges from 30-60% of baseline.

In contrast, ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, causes heterogeneous CMR changes. In humans, CMR is increased in the frontomedial and anterior cingulate cortex, but decreased in other regions [15]. Similar region-specific effects of ketamine have been reported in the rat [16]. Nitrous oxide (N₂O) can cause a major CMR increase when given alone, although this may be dose and species dependent [17, 18]. Like ketamine, the effect of N₂O on CMR is regionally heterogeneous [19]. When combined with other anesthetics, N₂O produces variable CMR effects [20, 21]. Lastly, high doses of alfentanil and fentanyl (typical of most opioids) can precipitate status epilepticus and markedly increase CMR [22].

Differential effects on CMR may be related to anesthetic mechanism of action. While most all anesthetics have a measurable effect on both GABA_A-ergic and glutamatergic systems, the spectrum of potency for individual anesthetics across neurotransmitter systems varies

markedly. Volatile anesthetics, and most hypnotics (e.g., propofol, barbiturates), exhibit more potent effects at the GABA_A receptor [23] with only weak inhibition of glutamatergic activity [24], and cause a dose-dependent decrease in CMR generalized across brain regions. In contrast, ketamine and N₂O are predominantly non-competitive NMDA receptor channel blockers but negligible effects on GABA_A receptors [25-28], and both induce regionally heterogeneous increases and decreases in CMR. Xenon differs, perhaps because it serves as a NMDA receptor antagonist by competitively inhibiting the glycine site [29]. While this likely contributes to improved stroke outcome [30], unlike the heterogeneous CMR effects of ketamine and N₂O, xenon causes a consistent decrease in CMR across a broad range of brain regions [31].

Besides indirectly influencing CBF via altered CMR, anesthetics can exert vasomotor effects through endothelial and smooth muscle mechanisms [32-34]. For example, in humans subjected to propofol-induced EEG isoelectricity, where anesthetic-mediated CMR suppression would be maximal [35], addition of equivalent doses of halothane, isoflurane, and desflurane all increased CBF, indicating direct pharmacologic effects on cerebral vascular tone. N₂O also increases CBF whether used alone or in combination with other anesthetics [36, 37]. Further, anesthetics may differentially alter CBF/CMR coupling. Volatile anesthetics decrease CMR but increase CBF, while most intravenous anesthetics decrease both. Although it is tempting to consider an uncoupling of flow and metabolism by volatile anesthetics, several studies have shown that coupling is preserved, although the ratio of flow to metabolism is altered in a dose-dependent fashion [38, 39]. Both direct and indirect vascular effects may be important considerations when selecting anesthetics in various experimental (or clinical) conditions.

Cerebral blood flow autoregulation

Autoregulation maintains CBF within physiological limits across a wide range of cerebral perfusion pressures. In focal ischemia, autoregulation is a critical vasodilator reflex that helps augment collateral blood supply when the occlusion of a cerebral artery creates low perfusion pressure state within and surrounding the ischemic brain. Volatile anesthetics impair autoregulation. Halothane abolished autoregulation at 1 MAC (minimum end-tidal alveolar concentration at which 50% of subjects continue to respond to a noxious stimulus with a motor response) in the goat [40], whereas 2 MAC was required for isoflurane and sevoflurane to abolish autoregulation in the dog and rat, respectively [41, 42]. Similar effects for sevoflurane, isoflurane, and desflurane have been observed in humans [43, 44]. N₂O also impairs autoregulation [45]. Little or no work has been performed to assess effects of inhalational anesthetics on autoregulation in ischemic brain. Intravenous anesthetics have less effect on autoregulation. Propofol does not impair autoregulation in normal baboon or human brain [9, 43]. However, propofol doses approaching those used for surgical anesthesia abolish residual autoregulation in traumatized human brain [46]. Racemic ketamine does not abolish porcine autoregulation [47], as is also true of the S(+)-ketamine enantiomer in rats [48] and humans [49]. Human dynamic autoregulation is improved by midazolam [50], but dampened by dexmedetomidine [51]. Again, little or no exploration of the effects of these drugs on autoregulation has been made in ischemic brain.

Thus, use of inhaled anesthetics (N₂O, isoflurane, sevoflurane, and desflurane) can produce an abnormal cerebrovascular responsivity to perfusion pressure changes in normal brain, which is dose-dependent and likely relevant at surgical concentrations. Intravenous anesthetics are more likely to preserve autoregulatory responses. While effects of anesthetics on CBF have typically been measured under steady state conditions, dynamic changes in flow are likely to induce variability in severity of an ischemic insult if the surgical preparation is hemodynamically unstable and autoregulation is impaired. If this is of importance to the investigator, use of an intravenous anesthetic or low dose volatile anesthetic would seem most likely to diminish perfusion artifacts. Should a volatile anesthetic be preferred, doses sufficient to provide surgical anesthesia could be decreased upon completion of surgery, after instillation of local anesthetic into the surgical wound, to maintain appropriate immobilization and minimize disruption of cerebrovascular responsivity to changes in perfusion pressure.

Ischemic depolarizations

Ischemic (i.e., anoxic) depolarization is arguably the most important and often terminal step in the progression of acute ischemic injury, marking the depletion of ATP stores and loss of transmembrane ionic gradients. Numerous anesthetics have been tested to delay the time to depolarization, because their inhibitory effect on CMR is expected to slow ATP depletion in ischemic tissue. Using either *in vivo* or *in vitro* preparations, lidocaine [52], thiopental [53], pentobarbital [54], propofol [55], isoflurane [56], desflurane [57], and sevoflurane [58] have all been reported to delay depolarization onset.

Whether CMR reduction alone is sufficient to explain anesthetic-induced delay in ischemic depolarization is unclear. However, when rats were subjected to severe global ischemia, onset of depolarization occurred 1.5 min slower if isoflurane was used compared to halothane at equi-anesthetic doses [59]. This is consistent with the known greater CMR suppression provided by isoflurane. Such differences appear to translate to outcome as well. For example, rats subjected to 6 min of severe forebrain ischemia had less hippocampal CA1 damage with isoflurane compared to an equi-anesthetic halothane dose. Increasing ischemia duration to 7.5 min during isoflurane anesthesia produced the same magnitude of CA1 damage seen with 6 min ischemia during halothane anesthesia [60].

Additionally, in focal cerebral ischemia recurrent spreading depolarizations (SD) occur in peri-infarct tissue and expand the infarct in a stepwise fashion over time [61]. SD frequency is anesthetic dependent. In experimental preparations, pentobarbital, urethane and propofol do not substantively suppress SD frequency [62], whereas inhalational anesthetics (e.g., isoflurane, halothane, N₂O) and dexmedetomidine do [63, 62, 64-66]. In humans, ketamine, N₂O, and isoflurane suppress peri-infarct SD frequency compared with propofol, opioids or midazolam [67, 68].

Ischemic Outcome

Historically, the translational pathway anesthetic neuroprotection has gone from bedside to bench because anesthetics are clinically available. Early studies were driven by the question

of which anesthetic might do the least harm, or even provide benefit if given to patients with acute or potential CNS injury. It has been difficult to predict the net effect of anesthetics on stroke outcome, given the heterogeneous effects on resting CMR and CBF, autoregulation, and peri-infarct SDs. Although one would expect the anesthetic-induced decrease in CMR to afford neuroprotection [69, 70], there is negligible clinical evidence to support this claim. Human trials to test this have been mostly negative and those few that were positive could not be reproduced [71]. However, the majority of these clinical trials would not meet current standards of design and execution, and some have been simply comparative studies of one anesthetic versus another. Therefore, lack of clinical evidence should be interpreted cautiously, as laboratory data suggest otherwise.

Volatile Anesthetics

Isoflurane has been shown to be protective against excitotoxicity. For example, primary mixed neuronal/glia cell cultures exposed to NMDA exhibited less LDH release in the presence of volatile anesthetics [72]. This was consistent with *in vivo* data indicating isoflurane ameliorates excitotoxicity induced by micro-injection of either NMDA or AMPA into the cortex of rats [73, 74]. In cortical brain slices subjected to anoxia, glutamate release was decreased by isoflurane, the magnitude of which was similar to hypothermia (28°C) [75]. When organotypic hippocampal slices were exposed to oxygen-glucose deprivation, delayed neuronal necrosis was inhibited by isoflurane, which was as effective as moderate hypothermia (34°C) [76]. Isoflurane neuroprotection was later attributed to a GABA_A-ergic mechanism and regulation of intracellular calcium [77, 78].

For *in vivo* studies, volatile anesthetic efficacy is model dependent. Rats anesthetized with isoflurane demonstrated major histologic protection (by more than 50%) and improved 8-week functional outcome in a filament MCAO model compared to rats that were un-anesthetized for the majority of the duration of ischemia, while controlling for the pericranial temperature and arterial perfusion pressure during MCAO and reperfusion [79]. Sevoflurane also improves focal ischemia outcome [80]. In hemispheric ischemia, rats had improved long-term functional outcome when anesthetized with sevoflurane when compared to fentanyl/ N₂O [81]. In a severe forebrain ischemia model, isoflurane was found to only transiently improve functional and histologic outcome [82]. This is consistent with other work in focal ischemia that identified inhibition of necrosis by isoflurane, but failure to inhibit apoptotic injury most likely generated from ongoing ischemia [83]. It is upon anesthetic backgrounds similar to these that stroke therapeutics are often evaluated for preclinical efficacy. The interaction between anesthetic neuroprotection and the purported stroke therapeutic is typically not considered. Although volatile anesthetics will likely continue to be favored in experimental stroke research for their ease of use and rapid reversibility, these results suggest that their use is likely to introduce major confounds in study of experimental stroke mechanisms and outcome.

Nitrous Oxide

N₂O is an NMDA receptor antagonist [84], and has received some attention as a neuroprotective agent. However, in a filament MCAO model, intra-ischemic N₂O did not alter histologic or neurologic outcome when compared directly to the same fractional

inspiratory concentration of nitrogen (70%) [85]. But, when given after MCAO reperfusion, N₂O has been reported to improve outcome, but only a narrow therapeutic window of less than 3h was evident and long-term outcome was not studied [86, 87]. In contrast, 70% N₂O worsened outcome when administered during an intermediate duration of global forebrain ischemia in gerbils (5 min), while no effect was observed in either brief (3 min) or prolonged (7 min) ischemic insults [88]. In a large human trial involving temporary vessel occlusion during cerebral aneurysm clipping, N₂O increased the frequency of delayed ischemic deficits, but an effect on long-term neurologic or neuropsychiatric outcome was not detected [89]. Altogether these data do not support a substantive neuroprotective effect of N₂O in cerebral ischemia.

Intravenous Anesthetics

Barbiturates have been reported to ameliorate focal ischemic injury [90]; however, the overall effect appears to be modest in thermo-regulated animals [91]. Effects in global ischemia are inconsistent and likely model dependent [92, 93]. The efficacy is often attributed solely to CMR reduction, although this has been disputed [91]. Barbiturates constitute a family of compounds, for which free radical scavenging [94], anticonvulsant properties [95], and attenuation of glutamate accumulation [54] have been reported. Ketamine has been widely studied as a non-competitive NMDA receptor antagonist with potential to limit excitotoxicity [96], but effects on ischemic outcome have been inconsistent [97-100], in part due to lack of brain temperature control [101, 102]. Nevertheless, ketamine is protective against ischemia *in vitro* as well [103], suggesting potential temperature independent effects. Propofol decreases CMR and CBF, but also offers other properties including mitigation of oxidative and excitotoxic stress [104, 105], apoptosis [106], inflammation [107], and autophagy [108]. Results of preclinical efficacy studies have been mixed. Propofol has been reported to be beneficial in focal [109, 110], hemispheric [111], and global [112-114] ischemic insults. These positive results have been countered by other work, which failed to detect an effect on outcome *in vivo* [115-117] or *in vitro* [53]. α -2-agonists are a class of compounds including dexmedetomidine, clonidine, and xylazine. α -2-agonists are commonly employed in veterinary anesthesia across a broad range of species. Although analgesic properties are present, there is a ceiling effect. Hence, surgical anesthesia typically requires use of a supplemental analgesic such as ketamine or an opioid. The most extensive investigation for α -2-agonist effects in experimental stroke has been on dexmedetomidine, possibly because it is also commonly used for human anesthesia. Dexmedetomidine has generally been reported to improve ischemic outcome across a range of species [118, 119], although adverse effects of dexmedetomidine-induced hypotension on intra-ischemic CBF have been noted [120].

As with any pharmacological intervention, conflicting results are likely due to the presence of multiple mechanisms of action often counteracting each other, and the outcomes depend on the experimental model and study design. Lack of a systematic dose-response analysis further complicates interpretation.

Other Anesthetics and Analgesics

Desflurane, etomidate, urethane, alpha-chloralose, chloral hydrate, and opioids and their combinations have also been investigated for neuroprotective properties or interactions with molecular and cellular responses to ischemia [121, 122, 2]. As a generalization, desflurane appears protective, μ -opioid agonists have little effect, and etomidate may be adverse. Urethane, alpha-chloralose, and chloral hydrate remain insufficiently investigated. Post-operative analgesics and sedatives can also potentially confound preclinical research. For example, common analgesics (e.g., lidocaine [123], bupivacaine [124], non-steroidal anti-inflammatory drugs [125, 126], and buprenorphine [127]) can modulate neural activity, inflammation, CBF, and apoptosis. Despite this, post-ischemic use of the partial μ -opioid agonist buprenorphine has been reported to have no effect on MCAO outcome in mice or rats [128, 129].

Anesthetic preconditioning

Models of experimental brain injury may require anesthetic exposure for instrumentation prior to administration of the primary insult. Research has consistently shown that anesthetic exposure (with or without surgical procedures) induces a complex response that can be highly protective against a subsequent primary CNS insult. Rats subjected to 1h of halothane anesthesia, followed by 2h MCAO had markedly smaller infarcts than those receiving propofol for the same duration [130]. In contrast, there was no benefit from an 8 h pre-ischemic exposure to either halothane or propofol. That a brief anesthetic exposure improves the outcome of a cerebral ischemic insult has been extended to isoflurane [131], sevoflurane [132], dexmedetomidine [133] and chloral hydrate [134], including long term (2-4 weeks) outcome [135, 136]. In contrast, alpha-chloralose may be devoid of this property [65], and ketamine may indeed block ischemic preconditioning mediated by activation of the NMDA receptor [137]. Anesthetic preconditioning is effective if employed when given immediately prior to, or as early as 24h before an ischemic insult [132]. Similarly, exposure to various anesthetics after the ischemic insult may confer sustained protection (anesthetic post-conditioning) [138, 139]. Mechanisms of anesthetic pre- and post-conditioning are numerous and remain under investigation. The extent to which this impacts studies designed to advance our understanding of the neurobiology of stroke or efficacy of purported interventions must be considered, particularly in the context of preclinical drug development.

Systemic Physiology And Other Factors

Most anesthetics depress myocardial contractility, systemic vascular resistance, and hence arterial blood pressure, which can be detrimental to ischemic tissue perfusion both at rest and during peri-infarct SDs [140-142]. Moreover, anesthetics are often respiratory depressants and promote airway obstruction, which may superimpose hypoxemia and hypercapnia on the ischemic insult. Finally, most anesthetics perturb thermoregulation and blood glucose concentration. Seemingly trivial changes in any of these variables can have major impact on ischemic outcome. Therefore, systemic physiological monitoring is critical

to avoid the confounding effects of anesthesia on ischemic outcomes and to reduce variation within and across laboratories.

Conclusions

Anesthesia is necessary to ensure animal welfare in experimental stroke models. Yet it has profound and variable effects on neurobiology and systemic physiology. Anesthesia can be neuroprotective in part through modulation of ion channels and neural activity, metabolism, inflammation, apoptosis and autophagy, and peri-infarct SDs. Conversely, anesthesia can worsen ischemic outcome indirectly by causing, for example, hypotension or impaired autoregulation. As such, anesthesia has been among the confounders haunting translational stroke research. For example, if an anesthetic has already decreased cerebral infarct volume by direct neuroprotection, the efficacy of another drug tested in such a model can be masked. Or using an anesthetic that inhibits peri-infarct SDs can mask the effect of a potential therapeutic intended to suppress SDs. It is unrealistic to formulate a set of rules in recommending a fixed anesthetic regimen in all experimental stroke research. Instead, reproducing a therapeutic effect of a novel intervention under different anesthetic regimens would help reduce the confound. Investigators must be aware of potential CNS and systemic anesthetic effects and tailor the anesthetic choice, combination and dosing based on the hypotheses being tested (Table 1). Finally, when reporting results from a stroke preparation, clear definition of the anesthetic management employed will allow others to consider anesthetic confounds inherent in the experiment. Continued efforts to understand mechanisms of anesthetic action in normal and diseased brain will serve to improve our use of experimental stroke models to advance patient care.

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References

1. Warner DS, James ML, Laskowitz DT, Wijdicks EF. Translational research in acute central nervous system injury: lessons learned and the future. *JAMA Neurol.* 2014; 71(10):1311–8. [PubMed: 25111291]
2. Schifilliti D, Grasso G, Conti A, Fodale V. Anaesthetic-related neuroprotection: intravenous or inhalational agents? *CNS Drugs.* 2010; 24(11):893–907. [PubMed: 20932063]
3. Kety SS, Schmidt CF. The effects of active and passive hyperventilation on cerebral blood flow, cerebral oxygen consumption, cardiac output, and blood pressure of normal young men. *J Clin Invest.* 1946; 25:107–19.
4. Himwich WA, Homburger E, et al. Brain metabolism in man; unanesthetized and in pentothal narcosis. *Am J Psychiatry.* 1947; 103(5):689–96. [PubMed: 20295286]
5. Michenfelder JD. The interdependency of cerebral functional and metabolic effects following massive doses of thiopental in the dog. *Anesthesiology.* 1974; 41(3):231–6. [PubMed: 4854910]
6. Michenfelder JD, Theye RA. The effects of anesthesia and hypothermia on canine cerebral ATP and lactate during anoxia produced by decapitation. *Anesthesiology.* 1970; 33(4):430–9. [PubMed: 5512331]
7. Todd MM, Drummond JC. A comparison of the cerebrovascular and metabolic effects of halothane and isoflurane in the cat. *Anesthesiology.* 1984; 60(4):276–82. [PubMed: 6703382]

8. Scheller MS, Tateishi A, Drummond JC, Zornow MH. The effects of sevoflurane on cerebral blood flow, cerebral metabolic rate for oxygen, intracranial pressure, and the electroencephalogram are similar to those of isoflurane in the rabbit. *Anesthesiology*. 1988; 68(4):548–51. [PubMed: 3354892]
9. Van Hemelrijck J, Fitch W, Mattheussen M, Van Aken H, Plets C, Lauwers T. Effect of propofol on cerebral circulation and autoregulation in the baboon. *Anesth Analg*. 1990; 71(1):49–54. [PubMed: 2363528]
10. Nugent M, Artru AA, Michenfelder JD. Cerebral metabolic, vascular and protective effects of midazolam maleate: comparison to diazepam. *Anesthesiology*. 1982; 56(3):172–6. [PubMed: 7059025]
11. Renou AM, Vernhiet J, Macrez P, Constant P, Billerey J, Khadaroo MY, et al. Cerebral blood flow and metabolism during etomidate anaesthesia in man. *Br J Anaesth*. 1978; 50(10):1047–51. [PubMed: 708546]
12. Post RM, Kennedy C, Shinohara M, Squillace K, Miyaoka M, Suda S, et al. Metabolic and behavioral consequences of lidocaine-kindled seizures. *Brain Res*. 1984; 324(2):295–303. [PubMed: 6543325]
13. Carlsson C, Smith DS, Keykhah MM, Englebach I, Harp JR. The effects of high-dose fentanyl on cerebral circulation and metabolism in rats. *Anesthesiology*. 1982; 57(5):375–80. [PubMed: 7137618]
14. Drummond JC, Dao AV, Roth DM, Cheng CR, Atwater BI, Minokadeh A, et al. Effect of dexmedetomidine on cerebral blood flow velocity, cerebral metabolic rate, and carbon dioxide response in normal humans. *Anesthesiology*. 2008; 108(2):225–32. DOI: 10.1097/01.anes.0000299576.00302.4c [PubMed: 18212567]
15. Vollenweider FX, Leenders KL, Scharfetter C, Antonini A, Maguire P, Missimer J, et al. Metabolic hyperfrontality and psychopathology in the ketamine model of psychosis using positron emission tomography (PET) and [18F]fluorodeoxyglucose (FDG). *Eur Neuropsychopharmacol*. 1997; 7(1):9–24. [PubMed: 9088881]
16. Oguchi K, Arakawa K, Nelson SR, Samson F. The influence of droperidol, diazepam, and physostigmine on ketamine-induced behavior and brain regional glucose utilization in rat. *Anesthesiology*. 1982; 57(5):353–8. [PubMed: 7137616]
17. Pelligrino DA, Miletich DJ, Hoffman WE, Albrecht RF. Nitrous oxide markedly increases cerebral cortical metabolic rate and blood flow in the goat. *Anesthesiology*. 1984; 60(5):405–12. [PubMed: 6424512]
18. Dashdorj N, Corrie K, Napolitano A, Petersen E, Mahajan RP, Auer DP. Effects of subanesthetic dose of nitrous oxide on cerebral blood flow and metabolism: a multimodal magnetic resonance imaging study in healthy volunteers. *Anesthesiology*. 2013; 118(3):577–86. DOI: 10.1097/ALN.0b013e3182800d58 [PubMed: 23324809]
19. Reinstrup P, Ryding E, Ohlsson T, Sandell A, Erlandsson K, Ljunggren K, et al. Regional cerebral metabolic rate (positron emission tomography) during inhalation of nitrous oxide 50% in humans. *Br J Anaesth*. 2008; 100(1):66–71. [PubMed: 18037671]
20. Reasoner DK, Warner DS, Todd MM, McAllister A. Effects of nitrous oxide on cerebral metabolic rate in rats anaesthetized with isoflurane. *Br J Anaesth*. 1990; 65(2):210–5. [PubMed: 2223338]
21. Kaisti KK, Langsjo JW, Aalto S, Oikonen V, Sipila H, Teras M, et al. Effects of sevoflurane, propofol, and adjunct nitrous oxide on regional cerebral blood flow, oxygen consumption, and blood volume in humans. *Anesthesiology*. 2003; 99(3):603–13. [PubMed: 12960544]
22. Kofke WA, Garman RH, Tom WC, Rose ME, Hawkins RA. Alfentanil-induced hypermetabolism, seizure, and histopathology in rat brain. *Anesth Analg*. 1992; 75(6):953–64. [PubMed: 1443714]
23. Jones MV, Brooks PA, Harrison NL. Enhancement of gamma-aminobutyric acid-activated Cl⁻ currents in cultured rat hippocampal neurones by three volatile anaesthetics. *The Journal of physiology*. 1992; 449:279–93. [PubMed: 1326046]
24. Yang J, Zorumski CF. Effects of isoflurane on N-methyl-D-aspartate gated ion channels in cultured rat hippocampal neurons. *Ann N Y Acad Sci*. 1991; 625:287–9. [PubMed: 1711810]

25. Irifune M, Sato T, Kamata Y, Nishikawa T, Dohi T, Kawahara M. Evidence for GABA(A) receptor agonistic properties of ketamine: convulsive and anesthetic behavioral models in mice. *Anesth Analg.* 2000; 91(1):230–6. [PubMed: 10866918]
26. Wakita M, Kotani N, Yamaga T, Akaike N. Nitrous oxide directly inhibits action potential-dependent neurotransmission from single presynaptic boutons adhering to rat hippocampal CA3 neurons. *Brain Res Bull.* 2015; 118:34–45. [PubMed: 26343381]
27. Yamakura T, Mori H, Masaki H, Shimoji K, Mishnina M. Different sensitivities of NMDA receptor channel subtypes to non-competitive antagonists. *NeuroReport.* 1993; 4:687–90. [PubMed: 8347808]
28. Mennerick S, Jevtovic-Todorovic V, Todorovic SM, Shen W, Olney JW, Zorumski CF. Effect of nitrous oxide on excitatory and inhibitory synaptic transmission in hippocampal cultures. *J Neurosci.* 1998; 18(23):9716–26. [PubMed: 9822732]
29. Dickinson R, Peterson BK, Banks P, Simillis C, Martin JC, Valenzuela CA, 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(5):756–67. [PubMed: 18073551]
30. Sheng SP, Lei B, James ML, Lascola CD, Venkatraman TN, Jung JY, et al. Xenon neuroprotection in experimental stroke: interactions with hypothermia and intracerebral hemorrhage. *Anesthesiology.* 2012; 117(6):1262–75. DOI: 10.1097/ALN.0b013e3182746b81 [PubMed: 23143806]
31. Rex S, Schaefer W, Meyer PH, Rossaint R, Boy C, Setani K, et al. Positron emission tomography study of regional cerebral metabolism during general anesthesia with xenon in humans. *Anesthesiology.* 2006; 105(5):936–43. [PubMed: 17065887]
32. Jensen NF, Todd MM, Kramer DJ, Leonard PA, Warner DS. A comparison of the vasodilating effects of halothane and isoflurane on the isolated rabbit basilar artery with and without intact endothelium. *Anesthesiology.* 1992; 76(4):624–34. [PubMed: 1550288]
33. Gelb AW, Zhang C, Hamilton JT. Propofol induces dilation and inhibits constriction in guinea pig basilar arteries. *Anesth Analg.* 1996; 83(3):472–6. [PubMed: 8780265]
34. Fukuda S, Murakawa T, Takeshita H, Toda N. Direct effects of ketamine on isolated canine cerebral and mesenteric arteries. *Anesth Analg.* 1983; 62(6):553–8. [PubMed: 6846876]
35. Matta BF, Mayberg TS, Lam AM. Direct cerebrovasodilatory effects of halothane, isoflurane, and desflurane during propofol-induced isoelectric electroencephalogram in humans. *Anesthesiology.* 1995; 83(5):980–5. discussion 27A. [PubMed: 7486184]
36. Hansen TD, Warner DS, Todd MM, Vust LJ. Effects of nitrous oxide and volatile anaesthetics on cerebral blood flow. *Br J Anaesth.* 1989; 63(3):290–5. [PubMed: 2803887]
37. Pelligrino D, Miletich D, Hoffman W, Albrecht R. Nitrous oxide markedly increases cerebral cortical metabolic rate and blood flow in the goat. *Anesthesiology.* 1984; 60:405–12. [PubMed: 6424512]
38. Lenz C, Frietsch T, Futterer C, Rebel A, van Ackern K, Kuschinsky W, et al. Local coupling of cerebral blood flow to cerebral glucose metabolism during inhalational anesthesia in rats: desflurane versus isoflurane. *Anesthesiology.* 1999; 91(6):1720–3. [PubMed: 10598615]
39. Franceschini MA, Radhakrishnan H, Thakur K, Wu W, Ruvinskaya S, Carp S, et al. The effect of different anesthetics on neurovascular coupling. *Neuroimage.* 2010; 51(4):1367–77. [PubMed: 20350606]
40. Miletich DJ, Ivankovich AD, Albrecht RF, Reimann CR, Rosenberg R, McKissic ED. Absence of autoregulation of cerebral blood flow during halothane and enflurane anesthesia. *Anesth Analg.* 1976; 55(1):100–9. [PubMed: 942820]
41. McPherson RW, Traystman RJ. Effects of isoflurane on cerebral autoregulation in dogs. *Anesthesiology.* 1988; 69(4):493–9. [PubMed: 3177910]
42. Werner C, Lu H, Engelhard K, Unbehaun N, Kochs E. Sevoflurane impairs cerebral blood flow autoregulation in rats: reversal by nonselective nitric oxide synthase inhibition. *Anesth Analg.* 2005; 101(2):509–16. [PubMed: 16037169]

43. Strebel S, Lam AM, Matta B, Mayberg TS, Aaslid R, Newell DW. Dynamic and static cerebral autoregulation during isoflurane, desflurane, and propofol anesthesia. *Anesthesiology*. 1995; 83(1): 66–76. [PubMed: 7605020]
44. Goettel N, Patet C, Rossi A, Burkhart CS, Czosnyka M, Strebel SP, et al. Monitoring of cerebral blood flow autoregulation in adults undergoing sevoflurane anesthesia: a prospective cohort study of two age groups. *J Clin Monit Comput*. 2016; 30(3):255–64. [PubMed: 26285741]
45. Girling KJ, Cavill G, Mahajan RP. The effects of nitrous oxide and oxygen on transient hyperemic response in human volunteers. *Anesth Analg*. 1999; 89(1):175–80. [PubMed: 10389799]
46. Steiner LA, Johnston AJ, Chatfield DA, Czosnyka M, Coleman MR, Coles JP, et al. The effects of large-dose propofol on cerebrovascular pressure autoregulation in head-injured patients. *Anesth Analg*. 2003; 97(2):572–6. [PubMed: 12873955]
47. Schmidt A, Ryding E, Akeson J. Racemic ketamine does not abolish cerebrovascular autoregulation in the pig. *Acta Anaesthesiol Scand*. 2003; 47(5):569–75. [PubMed: 12699515]
48. Engelhard K, Werner C, Lu H, Mollenberg O, Kochs E. Effect of S-(+)-ketamine on autoregulation of cerebral blood flow. *Anesthesiol Intensivmed Notfallmed Schmerzther*. 1997; 32(12):721–5. [PubMed: 9498088]
49. Engelhard K, Werner C, Mollenberg O, Kochs E. S-(+)-ketamine/propofol maintain dynamic cerebrovascular autoregulation in humans. *Can J Anaesth*. 2001; 48(10):1034–9. [PubMed: 11698326]
50. Ogawa Y, Iwasaki K, Aoki K, Gokan D, Hirose N, Kato J, et al. The different effects of midazolam and propofol sedation on dynamic cerebral autoregulation. *Anesth Analg*. 2010; 111(5):1279–84. [PubMed: 20881283]
51. Ogawa Y, Iwasaki K, Aoki K, Kojima W, Kato J, Ogawa S. Dexmedetomidine weakens dynamic cerebral autoregulation as assessed by transfer function analysis and the thigh cuff method. *Anesthesiology*. 2008; 109(4):642–50. [PubMed: 18813043]
52. Ayad M, Verity MA, Rubinstein EH. Lidocaine delays cortical ischemic depolarization: relationship to electrophysiologic recovery and neuropathology. *J Neurosurg Anesthesiol*. 1994; 6(2):98–110. [PubMed: 8012179]
53. Sasaki R, Hirota K, Roth SH, Yamazaki M. Anoxic depolarization of rat hippocampal slices is prevented by thiopental but not by propofol or isoflurane. *Br J Anaesth*. 2005; 94(4):486–91. [PubMed: 15708869]
54. Nakashima K, Todd MM. Effects of hypothermia, pentobarbital, and isoflurane on postdepolarization amino acid release during complete global cerebral ischemia. *Anesthesiology*. 1996; 85(1):161–8. [PubMed: 8694362]
55. Kobayashi M, Takeda Y, Taninishi H, Takata K, Aoe H, Morita K. Quantitative evaluation of the neuroprotective effects of thiopental sodium, propofol, and halothane on brain ischemia in the gerbil: effects of the anesthetics on ischemic depolarization and extracellular glutamate concentration. *J Neurosurg Anesthesiol*. 2007; 19(3):171–8. [PubMed: 17592348]
56. Verhaegen MJ, Todd MM, Warner DS. A comparison of cerebral ischemic flow thresholds during halothane/N₂O and isoflurane/N₂O anesthesia in rats. *Anesthesiology*. 1992; 76(5):743–54. [PubMed: 1575343]
57. Wang J, Cottrell JE, Kass IS. Effects of desflurane and propofol on electrophysiological parameters during and recovery after hypoxia in rat hippocampal slice CA1 pyramidal cells. *Neuroscience*. 2009; 160(1):140–8. [PubMed: 19236906]
58. Wang J, Meng F, Cottrell JE, Kass IS. The differential effects of volatile anesthetics on electrophysiological and biochemical changes during and recovery after hypoxia in rat hippocampal slice CA1 pyramidal cells. *Neuroscience*. 2006; 140(3):957–67. [PubMed: 16580780]
59. Verhaegen M, Todd MM, Warner DS. Ischemic depolarization during halothane-nitrous oxide and isoflurane-nitrous oxide anesthesia. An examination of cerebral blood flow threshold and times to depolarization. *Anesthesiology*. 1994; 81(4):965–73. [PubMed: 7943848]
60. Nellgård B, Mackensen GB, Pineda J, Wellons JC 3rd, Pearlstein RD, Warner DS. Anesthetic effects on cerebral metabolic rate predict histologic outcome from near-complete forebrain ischemia in the rat. *Anesthesiology*. 2000; 93(2):431–6. [PubMed: 10910493]

61. Hartings JA, Shuttleworth CW, Kirov SA, Ayata C, Hinzman JM, Foreman B, et al. The continuum of spreading depolarizations in acute cortical lesion development: Examining Leao's legacy. *J Cereb Blood Flow Metab.* 2016
62. Kudo C, Nozari A, Moskowitz MA, Ayata C. The impact of anesthetics and hyperoxia on cortical spreading depression. *Exp Neurol.* 2008; 212(1):201–6. [PubMed: 18501348]
63. Saito R, Graf R, Hubel K, Fujita T, Rosner G, Heiss WD. Reduction of infarct volume by halothane: effect on cerebral blood flow or perifocal spreading depression-like depolarizations. *J Cereb Blood Flow Metab.* 1997; 17(8):857–64. [PubMed: 9290583]
64. Takagaki M, Feuerstein D, Kumagai T, Gramer M, Yoshimine T, Graf R. Isoflurane suppresses cortical spreading depolarizations compared to propofol--implications for sedation of neurocritical care patients. *Exp Neurol.* 2014; 252:12–7. [PubMed: 24246282]
65. Zhao L, Nowak TS Jr. Preconditioning cortical lesions reduce the incidence of peri-infarct depolarizations during focal ischemia in the Spontaneously Hypertensive Rat: interaction with prior anesthesia and the impact of hyperglycemia. *J Cereb Blood Flow Metab.* 2015; 35(7):1181–90. [PubMed: 25757750]
66. Kudo C, Toyama M, Boku A, Hanamoto H, Morimoto Y, Sugimura M, et al. Anesthetic effects on susceptibility to cortical spreading depression. *Neuropharmacology.* 2013; 67:32–6. [PubMed: 23147413]
67. Hertle DN, Dreier JP, Woitzik J, Hartings JA, Bullock R, Okonkwo DO, et al. Effect of analgesics and sedatives on the occurrence of spreading depolarizations accompanying acute brain injury. *Brain : a journal of neurology.* 2012; 135(Pt 8):2390–8. [PubMed: 22719001]
68. Sakowitz OW, Kiening KL, Krajewski KL, Sarrafzadeh AS, Fabricius M, Strong AJ, et al. Preliminary evidence that ketamine inhibits spreading depolarizations in acute human brain injury. *Stroke.* 2009; 40(8):e519–22. [PubMed: 19520992]
69. Michenfelder JD, Theye RA. Cerebral protection by thiopental during hypoxia. *Anesthesiology.* 1973; 39(5):510–7. [PubMed: 4746056]
70. Newberg LA, Michenfelder JD. Cerebral protection by isoflurane during hypoxemia or ischemia. *Anesthesiology.* 1983; 59(1):29–35. [PubMed: 6859609]
71. Ishida K, Berger M, Nadler J, Warner DS. Anesthetic neuroprotection: antecedents and an appraisal of preclinical and clinical data quality. *Curr Pharm Des.* 2014; 20(36):5751–65. [PubMed: 24502575]
72. Kudo M, Aono M, Lee Y, Massey G, Pearlstein RD, Warner DS. Effects of volatile anesthetics on N-methyl-D-aspartate excitotoxicity in primary rat neuronal-glia cultures. *Anesthesiology.* 2001; 95(3):756–65. [PubMed: 11575551]
73. Kimbro JR, Kelly PJ, Drummond JC, Cole DJ, Patel PM. Isoflurane and pentobarbital reduce AMPA toxicity in vivo in the rat cerebral cortex. *Anesthesiology.* 2000; 92(3):806–12. [PubMed: 10719959]
74. Harada H, Kelly PJ, Cole DJ, Drummond JC, Patel PM. Isoflurane reduces N-methyl-D-aspartate toxicity in vivo in the rat cerebral cortex. *Anesth Analg.* 1999; 89(6):1442–7. [PubMed: 10589624]
75. Eilers H, Bickler PE. Hypothermia and isoflurane similarly inhibit glutamate release evoked by chemical anoxia in rat cortical brain slices. *Anesthesiology.* 1996; 85(3):600–7. [PubMed: 8853091]
76. Popovic R, Liniger R, Bickler PE. Anesthetics and mild hypothermia similarly prevent hippocampal neuron death in an in vitro model of cerebral ischemia. *Anesthesiology.* 2000; 92(5):1343–9. [PubMed: 10781280]
77. Elersy H, Mixco J, Sheng H, Pearlstein RD, Warner DS. Selective gamma-aminobutyric acid type A receptor antagonism reverses isoflurane ischemic neuroprotection. *Anesthesiology.* 2006; 105(1):81–90. [PubMed: 16809998]
78. Gray JJ, Bickler PE, Fahlman CS, Zhan X, Schuyler JA. Isoflurane neuroprotection in hypoxic hippocampal slice cultures involves increases in intracellular Ca²⁺ and mitogen-activated protein kinases. *Anesthesiology.* 2005; 102(3):606–15. [PubMed: 15731600]
79. Sakai H, Sheng H, Yates RB, Ishida K, Pearlstein RD, Warner DS. Isoflurane provides long-term protection against focal cerebral ischemia in the rat. *Anesthesiology.* 2007; 106(1):92–9. discussion 8–10. [PubMed: 17197850]

80. Warner DS, McFarlane C, Todd MM, Ludwig P, McAllister AM. Sevoflurane and halothane reduce focal ischemic brain damage in the rat. Possible influence on thermoregulation. *Anesthesiology*. 1993; 79(5):985–92. [PubMed: 8239017]
81. Pape M, Engelhard K, Eberspacher E, Hollweck R, Kellermann K, Zintner S, et al. The long-term effect of sevoflurane on neuronal cell damage and expression of apoptotic factors after cerebral ischemia and reperfusion in rats. *Anesth Analg*. 2006; 103(1):173–9. [PubMed: 16790648]
82. Elersy H, Sheng H, Lynch JR, Moldovan M, Pearlstein RD, Warner DS. Effects of isoflurane versus fentanyl-nitrous oxide anesthesia on long-term outcome from severe forebrain ischemia in the rat. *Anesthesiology*. 2004; 100(5):1160–6. [PubMed: 15114213]
83. Inoue S, Davis DP, Drummond JC, Cole DJ, Patel PM. The combination of isoflurane and caspase 8 inhibition results in sustained neuroprotection in rats subject to focal cerebral ischemia. *Anesth Analg*. 2006; 102(5):1548–55. [PubMed: 16632840]
84. Jevtovic-Todorovic V, Todorovic S, Mennerick S, Powell K, Dikranian K, Benshoff ND, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. *Nature Med*. 1998; 4:460–3. [PubMed: 9546794]
85. Yokoo N, Sheng H, Mixco J, Homi HM, Pearlstein RD, Warner DS. Intraischemic nitrous oxide alters neither neurologic nor histologic outcome: a comparison with dizocilpine. *Anesth Analg*. 2004; 99(3):896–903. [PubMed: 15333428]
86. David HN, Leveille F, Chazalviel L, MacKenzie ET, Buisson A, Lemaire M, et al. Reduction of ischemic brain damage by nitrous oxide and xenon. *J Cereb Blood Flow Metab*. 2003; 23(10):1168–73. [PubMed: 14526227]
87. Haelewyn B, David HN, Rouillon C, Chazalviel L, Lecocq M, Risso JJ, et al. Neuroprotection by nitrous oxide: facts and evidence. *Crit Care Med*. 2008; 36(9):2651–9. [PubMed: 18679119]
88. Taninishi H, Takeda Y, Kobayashi M, Sasaki T, Arai M, Morita K. Effect of nitrous oxide on neuronal damage and extracellular glutamate concentration as a function of mild, moderate, or severe ischemia in halothane-anesthetized gerbils. *Anesthesiology*. 2008; 108(6):1063–70. [PubMed: 18497607]
89. Pasternak JJ, McGregor DG, Lanier WL, Schroeder DR, Rusy DA, Hindman B, et al. Effect of nitrous oxide use on long-term neurologic and neuropsychological outcome in patients who received temporary proximal artery occlusion during cerebral aneurysm clipping surgery. *Anesthesiology*. 2009; 110(3):563–73. [PubMed: 19212259]
90. Nehls DG, Todd MM, Spetzler RF, Drummond JC, Thompson RA, Johnson PC. A comparison of the cerebral protective effects of isoflurane and barbiturates during temporary focal ischemia in primates. *Anesthesiology*. 1987; 66(4):453–64. [PubMed: 3565810]
91. Warner DS, Takaoka S, Wu B, Ludwig PS, Pearlstein RD, Brinkhous AD, et al. Electroencephalographic burst suppression is not required to elicit maximal neuroprotection from pentobarbital in a rat model of focal cerebral ischemia. *Anesthesiology*. 1996; 84(6):1475–84. [PubMed: 8669689]
92. Gisvold SE, Safar P, Hendrickx HH, Rao G, Moossy J, Alexander H. Thiopental treatment after global brain ischemia in pigtailed monkeys. *Anesthesiology*. 1984; 60(2):88–96. [PubMed: 6696250]
93. Bleyaert AL, Nemoto EM, Safar P, Stezoski SM, Mickell JJ, Moossy J, et al. Thiopental amelioration of brain damage after global ischemia in monkeys. *Anesthesiology*. 1978; 49(6):390–8. [PubMed: 103466]
94. Smith DS, Rehncrona S, Westerberg E, Akesson B, Siesjo BK. Lipid peroxidation in brain tissue in vitro: antioxidant effects of barbiturates. *Acta Physiol Scand*. 1979; 105(4):527–9. [PubMed: 452928]
95. Markowitz GJ, Kadam SD, Smith DR, Johnston MV, Comi AM. Different effects of high- and low-dose phenobarbital on post-stroke seizure suppression and recovery in immature CD1 mice. *Epilepsy Res*. 2011; 94(3):138–48. DOI: 10.1016/j.epilepsyres.2011.01.002 [PubMed: 21481568]
96. Hudetz JA, Pagel PS. Neuroprotection by ketamine: a review of the experimental and clinical evidence. *J Cardiothorac Vasc Anesth*. 2010; 24(1):131–42. [PubMed: 19640746]
97. Jensen ML, Auer RN. Ketamine fails to protect against ischaemic neuronal necrosis in the rat. *Br J Anaesth*. 1988; 61(2):206–10. [PubMed: 3415892]

98. Ridenour TR, Warner DS, Todd MM, Baker MT. Effects of ketamine on outcome from temporary middle cerebral artery occlusion in the spontaneously hypertensive rat. *Brain Res.* 1991; 565(1): 116–22. [PubMed: 1773349]
99. Engelhard K, Werner C, Eberspacher E, Bachl M, Blobner M, Hildt E, et al. The effect of the alpha 2-agonist dexmedetomidine and the N-methyl-D-aspartate antagonist S(+)-ketamine on the expression of apoptosis-regulating proteins after incomplete cerebral ischemia and reperfusion in rats. *Anesth Analg.* 2003; 96(2):524–31. [PubMed: 12538207]
100. Winkelheide U, Lasarzik I, Kaepfel B, Winkler J, Werner C, Kochs E, et al. Dose-dependent effect of S(+)-ketamine on post-ischemic endogenous neurogenesis in rats. *Acta Anaesthesiol Scand.* 2009; 53(4):528–33. [PubMed: 19317867]
101. Nurse S, Corbett D. Neuroprotection after several days of mild, drug-induced hypothermia. *J Cereb Blood Flow Metab.* 1996; 16(3):474–80. [PubMed: 8621752]
102. Zhang L, Mitani A, Yanase H, Kataoka K. Continuous monitoring and regulating of brain temperature in the conscious and freely moving ischemic gerbil: effect of MK-801 on delayed neuronal death in hippocampal CA1. *J Neurosci Res.* 1997; 47(4):440–8. [PubMed: 9057137]
103. Pohorecki R, Becker GL, Reilly PJ, Landers DF. Ischemic brain injury in vitro: protective effects of NMDA receptor antagonists and calmidazolium. *Brain Res.* 1990; 528(1):133–7. [PubMed: 2147119]
104. Cai J, Hu Y, Li W, Li L, Li S, Zhang M, et al. The neuroprotective effect of propofol against brain ischemia mediated by the glutamatergic signaling pathway in rats. *Neurochem Res.* 2011; 36(10): 1724–31. [PubMed: 21556843]
105. Basu S, Miculescu A, Sharma H, Wiklund L. Propofol mitigates systemic oxidative injury during experimental cardiopulmonary cerebral resuscitation. *Prostaglandins Leukot Essent Fatty Acids.* 2011; 84(5-6):123–30. [PubMed: 21316209]
106. Engelhard K, Werner C, Eberspacher E, Pape M, Blobner M, Hutzler P, et al. Sevoflurane and propofol influence the expression of apoptosis-regulating proteins after cerebral ischaemia and reperfusion in rats. *Eur J Anaesthesiol.* 2004; 21(7):530–7. [PubMed: 15318464]
107. Wang W, Lu R, Feng DY, Liang LR, Liu B, Zhang H. Inhibition of microglial activation contributes to propofol-induced protection against post-cardiac arrest brain injury in rats. *J Neurochem.* 2015; 134(5):892–903. [PubMed: 26016627]
108. Cui D, Wang L, Qi A, Zhou Q, Zhang X, Jiang W. Propofol prevents autophagic cell death following oxygen and glucose deprivation in PC12 cells and cerebral ischemia-reperfusion injury in rats. *PLoS One.* 2012; 7(4):e35324. [PubMed: 22509406]
109. Pittman JE, Sheng H, Pearlstein R, Brinkhous A, Dexter F, Warner DS. Comparison of the effects of propofol and pentobarbital on neurologic outcome and cerebral infarct size after temporary focal ischemia in the rat. *Anesthesiology.* 1997; 87(5):1139–44. [PubMed: 9366466]
110. Wang H, Luo M, Li C, Wang G. Propofol post-conditioning induced long-term neuroprotection and reduced internalization of AMPAR GluR2 subunit in a rat model of focal cerebral ischemia/reperfusion. *J Neurochem.* 2011; 119(1):210–9. [PubMed: 21790606]
111. Kochs E, Hoffman WE, Werner C, Thomas C, Albrecht RF, Schulte am Esch J. The effects of propofol on brain electrical activity, neurologic outcome, and neuronal damage following incomplete ischemia in rats. *Anesthesiology.* 1992; 76(2):245–52. [PubMed: 1736701]
112. Ito H, Watanabe Y, Isshiki A, Uchino H. Neuroprotective properties of propofol and midazolam, but not pentobarbital, on neuronal damage induced by forebrain ischemia, based on the GABAA receptors. *Acta Anaesthesiol Scand.* 1999; 43(2):153–62. [PubMed: 10027021]
113. Yamasaki T, Nakakimura K, Matsumoto M, Xiong L, Ishikawa T, Sakabe T. Effects of graded suppression of the EEG with propofol on the neurological outcome following incomplete cerebral ischaemia in rats. *Eur J Anaesthesiol.* 1999; 16(5):320–9. [PubMed: 10390668]
114. Ichinose K, Okamoto T, Tanimoto H, Taguchi H, Tashiro M, Sugita M, et al. A moderate dose of propofol and rapidly induced mild hypothermia with extracorporeal lung and heart assist (ECLHA) improve the neurological outcome after prolonged cardiac arrest in dogs. *Resuscitation.* 2006; 70(2):275–84. [PubMed: 16806640]

115. Tsai YC, Huang SJ, Lai YY, Chang CL, Cheng JT. Propofol does not reduce infarct volume in rats undergoing permanent middle cerebral artery occlusion. *Acta Anaesthesiol Sin.* 1994; 32(2): 99–104. [PubMed: 8038982]
116. Young Y, Menon DK, Tisavipat N, Matta BF, Jones JG. Propofol neuroprotection in a rat model of ischaemia reperfusion injury. *Eur J Anaesthesiol.* 1997; 14(3):320–6. [PubMed: 9202922]
117. Lasarzik I, Winkelheide U, Stallmann S, Orth C, Schneider A, Tresch A, et al. Assessment of postischemic neurogenesis in rats with cerebral ischemia and propofol anesthesia. *Anesthesiology.* 2009; 110(3):529–37. [PubMed: 19212268]
118. Zeng X, Wang H, Xing X, Wang Q, Li W. Dexmedetomidine Protects against Transient Global Cerebral Ischemia/Reperfusion Induced Oxidative Stress and Inflammation in Diabetic Rats. *PLoS One.* 2016; 11(3):e0151620. [PubMed: 26982373]
119. Maier C, Sun G, Kunis D, Giffard R, Steinberg G. Neuroprotection by the N-methyl-D-aspartate receptor antagonist CGP 40116: In vivo and in vitro studies. *J Neurochem.* 1995; 65(2):652–9. [PubMed: 7616221]
120. Nakano T, Okamoto H. Dexmedetomidine-induced cerebral hypoperfusion exacerbates ischemic brain injury in rats. *J Anesth.* 2009; 23(3):378–84. [PubMed: 19685118]
121. Soonthon-Brant V, Patel PM, Drummond JC, Cole DJ, Kelly PJ, Watson M. Fentanyl does not increase brain injury after focal cerebral ischemia in rats. *Anesth Analg.* 1999; 88(1):49–55. [PubMed: 9895065]
122. Drummond JC, Cole DJ, Patel PM, Reynolds LW. Focal cerebral ischemia during anesthesia with etomidate, isoflurane, or thiopental: a comparison of the extent of cerebral injury. *Neurosurgery.* 1995; 37(4):742–8. discussion 8-9. [PubMed: 8559304]
123. Yuan T, Li Z, Li X, Yu G, Wang N, Yang X. Lidocaine attenuates lipopolysaccharide-induced inflammatory responses in microglia. *J Surg Res.* 2014; 192(1):150–62. [PubMed: 24952412]
124. Block L, Jorneberg P, Bjorklund U, Westerlund A, Biber B, Hansson E. Ultralow concentrations of bupivacaine exert anti-inflammatory effects on inflammation-reactive astrocytes. *Eur J Neurosci.* 2013; 38(11):3669–78. [PubMed: 24083665]
125. Upton RN, Rasmussen M, Grant C, Martinez AM, Cold GE, Ludbrook GL. Pharmacokinetics and pharmacodynamics of indomethacin: effects on cerebral blood flow in anaesthetized sheep. *Clin Exp Pharmacol Physiol.* 2008; 35(3):317–23. [PubMed: 17973935]
126. Park EM, Cho BP, Volpe BT, Cruz MO, Joh TH, Cho S. Ibuprofen protects ischemia-induced neuronal injury via up-regulating interleukin-1 receptor antagonist expression. *Neuroscience.* 2005; 132(3):625–31. [PubMed: 15837124]
127. Cho HJ, Staikopoulos V, Furness JB, Jennings EA. Inflammation-induced increase in hyperpolarization-activated, cyclic nucleotide-gated channel protein in trigeminal ganglion neurons and the effect of buprenorphine. *Neuroscience.* 2009; 162(2):453–61. [PubMed: 19409968]
128. Jacobsen KR, Fauerby N, Raida Z, Kalliokoski O, Hau J, Johansen FF, et al. Effects of buprenorphine and meloxicam analgesia on induced cerebral ischemia in C57BL/6 male mice. *Comp Med.* 2013; 63(2):105–13. [PubMed: 23582417]
129. Yulug B, Cam E, Yildiz A, Kilic E. Buprenorphine does not aggravate ischemic neuronal injury in experimental focal cerebral ischemia. *J Neuropsychiatry Clin Neurosci.* 2007; 19(3):331–4. [PubMed: 17827420]
130. Bhardwaj A, Castro IA, Alkayed NJ, Hurn PD, Kirsch JR. Anesthetic choice of halothane versus propofol: impact on experimental perioperative stroke. *Stroke.* 2001; 32(8):1920–5. [PubMed: 11486126]
131. Kapinya KJ, Lowl D, Futterer C, Maurer M, Waschke KF, Isaev NK, et al. Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. *Stroke.* 2002; 33(7):1889–98. [PubMed: 12105371]
132. Payne RS, Akca O, Roewer N, Schurr A, Kehl F. Sevoflurane-induced preconditioning protects against cerebral ischemic neuronal damage in rats. *Brain Res.* 2005; 1034(1-2):147–52. [PubMed: 15713266]

133. Ding XD, Zheng NN, Cao YY, Zhao GY, Zhao P. Dexmedetomidine preconditioning attenuates global cerebral ischemic injury following asphyxial cardiac arrest. *Int J Neurosci*. 2016; 126(3): 249–56. [PubMed: 25565380]
134. Liu JH, Feng D, Zhang YF, Shang Y, Wu Y, Li XF, et al. Chloral Hydrate Preconditioning Protects Against Ischemic Stroke via Upregulating Annexin A1. *CNS Neurosci Ther*. 2015; 21(9):718–26. [PubMed: 26205642]
135. Li L, Zuo Z. Isoflurane preconditioning improves short-term and long-term neurological outcome after focal brain ischemia in adult rats. *Neuroscience*. 2009; 164(2):497–506. [PubMed: 19679170]
136. Ye R, Yang Q, Kong X, Li N, Zhang Y, Han J, et al. Sevoflurane preconditioning improves mitochondrial function and long-term neurologic sequelae after transient cerebral ischemia: role of mitochondrial permeability transition. *Crit Care Med*. 2012; 40(9):2685–93. [PubMed: 22732280]
137. Li C, Han D, Zhang F, Zhou C, Yu HM, Zhang GY. Preconditioning ischemia attenuates increased neurexin-neuroigin1-PSD-95 interaction after transient cerebral ischemia in rat hippocampus. *Neurosci Lett*. 2007; 426(3):192–7. [PubMed: 17904739]
138. Zhao P, Ji G, Xue H, Yu W, Zhao X, Ding M, et al. Isoflurane postconditioning improved long-term neurological outcome possibly via inhibiting the mitochondrial permeability transition pore in neonatal rats after brain hypoxia-ischemia. *Neuroscience*. 2014; 280:193–203. [PubMed: 25241064]
139. Lai Z, Zhang L, Su J, Cai D, Xu Q. Sevoflurane postconditioning improves long-term learning and memory of neonatal hypoxia-ischemia brain damage rats via the PI3K/Akt-mPTP pathway. *Brain Res*. 2016; 1630:25–37. [PubMed: 26541582]
140. Cole DJ, Drummond JC, Shapiro HM, Zornow MH. Influence of hypotension and hypotensive technique on the area of profound reduction in cerebral blood flow during focal cerebral ischaemia in the rat. *Br J Anaesth*. 1990; 64(4):498–502. [PubMed: 2334626]
141. Sukhotinsky I, Dilekoz E, Moskowitz MA, Ayata C. Hypoxia and hypotension transform the blood flow response to cortical spreading depression from hyperemia into hypoperfusion in the rat. *J Cereb Blood Flow Metab*. 2008; 28(7):1369–76. [PubMed: 18446167]
142. Sukhotinsky I, Yaseen MA, Sakadzic S, Ruvinskaya S, Sims JR, Boas DA, et al. Perfusion pressure-dependent recovery of cortical spreading depression is independent of tissue oxygenation over a wide physiologic range. *J Cereb Blood Flow Metab*. 2010; 30(6):1168–77. [PubMed: 20087371]

Table 1
Practical considerations for the choice of anesthetic regimen in experimental stroke

Anesthetic	rCMR	rCBF	AR	NVC	Other CNS effects	Cardiovascular	Respiratory	Other considerations
Volatile Anesthetics	↓	↑	↓	↓	↑ GABA _A receptor activation Improved Ca ²⁺ homeostasis ↓ Spreading depolarizations	↓	↓ ^b	Easy titration Rapid anesthesia onset and recovery Requires exhaust system
Nitrous Oxide	↑↓ ^a	↑	↓	↓	↓ NMDA receptor activation ↓ Spreading depolarizations	Myocardial ↓ Sympathetic tone ↑	Negligible ↓	Additional hypnotic/analgesic required Accumulates in closed gas spaces
Propofol	↓	↓	↔	↔	↑ GABA _A receptor activation Antioxidant	↓	↓ ^c	i.v. only Continuous infusion required Prolonged anesthesia emergence
Ketamine	↑↓ ^a	↑↓ ^a	↔	↓	↓ NMDA receptor activation ↓ Spreading depolarizations	Myocardial ↓ Sympathetic tone ↑	Minimal ↓ ^b	Repeated dosing required Adjunct anesthetics required (opioid, benzodiazepine)
Urethane	↑↓ ^a	↓	↔	↔	↑ Spreading depolarization frequency	↓	↓ ^b	i.p./i.v./p.o. Unpredictable and long duration Hyperglycemia Mutagenic and carcinogenic Metabolic acidosis at high doses Suitable for non-survival experiments
α-Chloralose	↓	↓	↔	↔/↓	↔ Spreading depolarization frequency	↓	↓	i.p./i.v./p.o. i.p. injection might cause ileus Adjunct surgical analgesic required
Barbiturates	↓	↓	↔	↔	↑ GABA _A receptor activation ↔ Spreading depolarization frequency Antioxidant	↓	↓ ^b	i.p./i.v. Painful injection, irritation at injection site Repetitive injections accumulate Unpredictable anesthesia duration
Dexmedetomidine	Modest ↓	↓	↓	↔	α-2 agonist, ↓ Spreading depolarizations	↓	Minimal ↓ ^b	i.p./i.v. May require continuous infusion Additional surgical analgesic required Transient hypertension on induction
Opioids (Fentanyl)	Modest ↓	Modest ↓	↔	↔	↔ Spreading depolarization frequency	Negligible ↓	↓ ^c	Additional surgical hypnotic required Rodents require high doses

rCMR = resting cerebral metabolic rate; rCBF = resting cerebral blood flow; AR= CBF autoregulation; NVC = neurovascular coupling; CNS = central nervous system;

^a regionally heterogeneous;

^b spontaneous ventilation possible;

^c mechanical ventilation necessary for surgical depth of anesthesia.