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### Authors

Niquet, Jerome  
Baldwin, Roger  
Gezalian, Michael  
et al.

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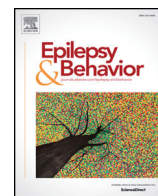
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## Deep hypothermia for the treatment of refractory status epilepticus

Jerome Niquet<sup>a,b,\*</sup>, Roger Baldwin<sup>b</sup>, Michael Gezalian<sup>b</sup>, Claude G. Wasterlain<sup>a,b,c</sup><sup>a</sup> Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA<sup>b</sup> Epilepsy Research Laboratory (151), Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA<sup>c</sup> Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

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## ABSTRACT

In a rat model of status epilepticus (SE) induced by lithium and pilocarpine and refractory to midazolam, deep hypothermia (20 °C for 30 min) reduced EEG power over 50-fold, stopped SE within 12 min, and reduced EEG spikes by 87%. Hypothermia deserves further investigation as a treatment of last resort for refractory SE.

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## 1. Introduction

Refractory and super-refractory status epilepticus (RSE, SRSE) are an increasingly common therapeutic challenge in our intensive care units (ICUs), at enormous financial and human costs. This increase may be due in part to greater availability of EEG monitoring, to increased recognition of “subtle” SE, to improvements in ICU care, and to the aging of the population, since SE and RSE are common in the elderly. Drugs fail to stop SE in 31–53% of cases [1–3]. In the VA Cooperative Study, 47% of patients with SE had RSE [3]. New methods for treating RSE and SRSE are clearly needed.

During SE, pharmacoresistance develops progressively. All currently available drugs display this phenomenon in experimental SE, with the possible exception of NMDA antagonists in some models [4]. In humans, early treatment of SE is much more effective than late treatment, suggesting that pharmacoresistance may be present as well. In the VA Cooperative Study [3], four treatments were randomly rotated. The first treatment (regardless of which one of the 4 was selected) was successful in 53% of patients. The third treatment given was successful in 2% of patients. Time-dependent pharmacoresistance is the most likely explanation for these results. We need alternative treatments for RSE/SRSE and a way to overcome pharmacoresistance. Here, we suggest that hypothermia, which acts by completely different mechanisms

than anticonvulsant drugs, may be able to stop the pharmacoresistant seizures of RSE/SRSE.

Hypothermia activates many anticonvulsant and neuroprotective mechanisms. It reduces the cerebral metabolic rate by 6–7% per degree Celsius, so that at 20 °C, the human cerebral oxygen consumption was measured at one-fifth of normothermic values [5]. It alters the function of ion pumps [6,7], intrinsic membrane properties, and voltage-gated ion channels [8–10]. It slows the release of excitatory neurotransmitters [11,12] and modifies gene expression [13].

These actions reduce excitatory drive and would be expected to inhibit seizure activity. They also activate several neuroprotective mechanisms: reduction of the cerebral demand for oxygen and glucose [14]; preservation of ATP, energy stores, and tissue pH; reduction of the release of excitotoxic amino acids [15] and of calcium influx into neurons [16,17]; inhibition of early gene expression and stress response; induction of the expression of heat shock and other stress proteins [18,19]; and inhibition of early molecular cascades involved in neuronal apoptosis.

Mild to moderate hypothermia has been shown to reduce seizure activity in experimental animals and in humans [20,21], although seizures frequently recur upon rewarming. Maeda et al. [22] induced SE with intraamygdalar kainic acid injection and found that mild hypothermia (30 °C) reduced seizure frequency and severity. Schmitt et al. [23] used mild hypothermia ( $\geq 29$  °C) to treat SE induced by perforant path stimulation. Hypothermia alone reduced motor but not EEG seizures. The combination of mild hypothermia with low-dose diazepam reduced all measures of seizure activity, but seizures returned upon rewarming. Liu et al. [24] induced SE with kainate in rats kept hypothermic for 4 h and in normothermic controls. Seizure reduction

\* Corresponding author at: Epilepsy Research Laboratory, Veterans Affairs Greater Los Angeles Healthcare System, 11301 Wilshire Boulevard, Building 114, Room 139, West Los Angeles, CA 90073, USA. Tel.: +1 310 478 3711x41974; fax: +1 310 268 4856.

E-mail address: [jniquet@ucla.edu](mailto:jniquet@ucla.edu) (J. Niquet).

was much greater during hypothermia at 23 °C than at 28 °C, suggesting that the depth of hypothermia increases its efficacy [25].

Anecdotal reports of successful treatment of clinical SE with hypothermia have been published [21,26]. Recooling stopped seizure activity which developed upon rewarming in an infant treated for hypoxic-ischemic encephalopathy [27]. Cold saline perfusion suppressed interictal spike foci during electrocorticography [20] and was used to stop seizures triggered by intraoperative cortical stimulation.

The neuroprotective role of hypothermia has been well-documented in focal hypoxia-ischemia, in traumatic brain injury (TBI), and in global cerebral ischemia. It has also been seen with seizure-associated neuronal injury [28], seizure-associated leaks in the blood-brain barrier (BBB) [29], and other conditions [30].

In neonatal hypoxic-ischemic encephalopathy, hypothermia improves developmental and radiological outcome [31–33]. Bernard et al. [34] and the Hypothermia after Cardiac Arrest Study Group [35] showed benefits of hypothermia after cardiac arrest due to ventricular fibrillation. In focal ischemia, many animal models showed improved outcome after hypothermia [36], but adverse effects of deep hypothermia have been reported [37]. Hypothermia reverses many ischemia-induced changes in gene expression and alters the expression of genes involved in protein synthesis, in cell cycle and cell division, and in apoptotic pathways [38].

Hypothermia protects from TBI-induced neuronal injury [39–41] and changes in gene expression [42]. It reduces BBB disruption and cell-mediated inflammation [43,44]. Hypothermia decreases TBI-induced inflammatory cytokines, cell-mediated immune responses, and activation of immune transcription factors [45,46]. Mild hypothermia after TBI mitigated the TBI-associated reduction in pentylentetrazol seizure threshold 12 weeks later [47].

Recent developments in ICU technology have reduced the complications of hypothermia. Mild hypothermia has become routine treatment for neonatal hypoxic-ischemic encephalopathy [48,49], for TBI [50], and for postcardiac arrest encephalopathy [34] and may have potential for stroke [51]. Deep hypothermia is routinely used to protect the brain or spinal cord when circulatory arrest is needed in cardiac surgery [52], vascular surgery [53], and neurosurgery [54]. Most of the complications reported after deep hypothermia are the result of induced circulatory arrest, not the result of hypothermia itself [55,56], although the potential for adverse effects, especially for very deep hypothermia, is significant [57].

Our results suggest that deep hypothermia is quite effective in stopping seizures in this experimental model of RSE and may deserve further study.

## 2. Materials and methods

Status epilepticus was induced with lithium (3 mEq/kg ip 16 h prior) and pilocarpine (60 mg/kg ip) + methylscopolamine (1 mg/kg ip) in adult male Wistar rats (200–250 g) previously implanted with skull screw electrodes. Control animals were given an equal volume of saline ip. Only lithium/pilocarpine-treated rats displaying behavioral/EEG manifestations of seizures were used. Electroencephalography was recorded from skull-implanted electrodes. “Brain surface” temperature was recorded from a probe preimplanted near the surface of the parietal cortex. When full-blown SE was established (12 min after the 2nd stage  $\geq 3$  seizure), midazolam (3 mg/kg ip) was injected. The second stage  $\geq 3$  seizure is a discrete and easily recognizable event. The time between the second stage 3 or higher seizure and the onset of continuous polyspikes is both short and reproducible ( $1.28 \pm 1.1$  min,  $n = 16$ ). Choosing the second stage 3 seizure as our time anchor and recording 12 min of EEG before treating guarantees that all our rats are in full-blown SE when treatment begins. In this study, we initiated cooling right after completing the midazolam injection. In later experiments (not reported here), we introduced an additional 15 min delay between midazolam and the initiation of hypothermia, in order to eliminate animals which respond to midazolam from the study, but in the current study, we

cannot rule out a midazolam contribution to the behavioral or EEG response to treatment.

All animals received scopolamine (2 mg/kg) at the same time as midazolam. One of the problems of chemical models of SE is the difficulty of obtaining clean results because of the presence of a convulsant in the animal. A specific treatment might stop the seizures, but the convulsant could immediately restart them if it is not neutralized. Morrissett et al. [58] were the first to show that an amount of atropine or scopolamine which blocked SE as pretreatment was unable to stop SE after seizures became established. The seizures had become independent of their original trigger (probably because of receptor trafficking). We took advantage of this feature to block the effects of pilocarpine without altering the course of SE. In preliminary experiments, we studied the amount of antimuscarinic agent which stops 100% of seizures when given as pretreatment but blocks 0% of seizures when given after seizure onset. Scopolamine 2 mg/kg reached that goal.

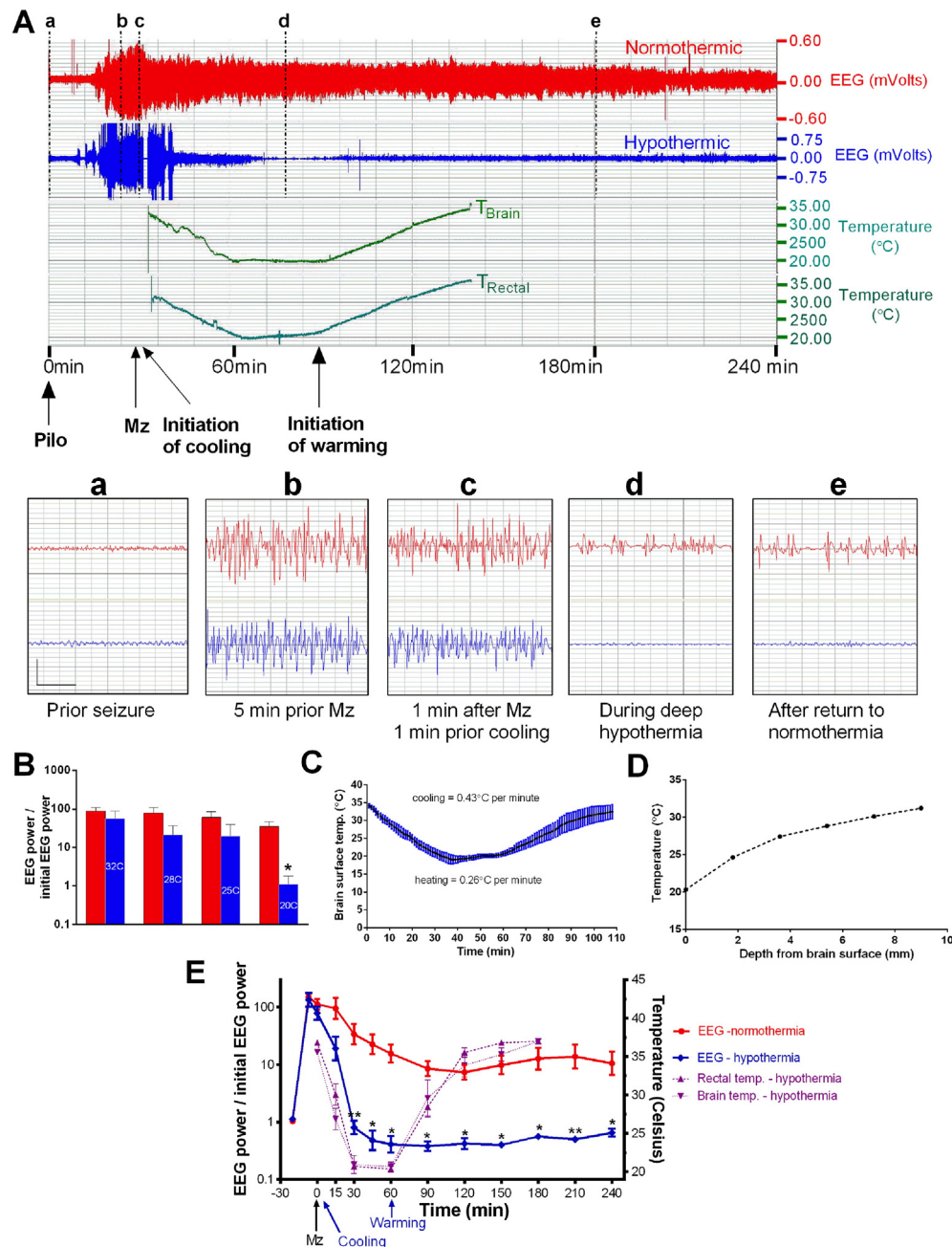
Cooling was then started with whole body ice packs. Rectal temperature closely approximated “brain surface” temperature. When rectal temperature reached 20 °C, it was held for 30 min at that temperature  $\pm 1$  °C, then ice packs were replaced by a warming blanket with circulating 37 °C water, until rectal temperature reached 36 °C. Mean cooling time from 37 °C to 20 °C was 39.5 min. Mean rewarming time from 20 °C to 36 °C was 61.5 min. In “cool cap” animals, only the head and neck of the animals were packed with ice.

Acute seizures were monitored by video/EEG for 24 h. The video/EEGs were analyzed using our standard methods for quantifying many components of seizure severity [59,60]. Relative EEG power was the ratio of EEG power at the time of measurement to EEG power before seizure induction in the same rat. It increased approximately 100-fold during early SE, then declined as a function of time, treatment, and temperature.

Analysis of variance was used for statistical testing if the data were normally distributed with similar variance; otherwise, either data transform to improve normality or nonparametric tests such as the Kruskal–Wallis test were used. P values were considered significant at the 0.05 level. Post hoc tests consisted of Scheffe's or Tukey's in the case of ANOVA and Dunn's in the case of Kruskal–Wallis. The Graph-Pad/Prism statistical package was used for these tests.

## 3. Results

Pilocarpine injection was followed by an increase in EEG power and in behavioral activity, then by the appearance of EEG spikes, followed by individual EEG seizures with behavioral arrest, twitching of face and vibrissae, or clonic forelimb activity. The EEG seizures (Fig. 1A) merged rapidly into nearly continuous polyspikes, while behavioral seizures increased in frequency, severity, and duration but remained intermittent. The injection of midazolam ( $41 \pm 19$  min after pilocarpine) was followed by a mild decrease in EEG power in both groups, but SE was not interrupted until animals became hypothermic. Hypothermia stopped seizures after approximately 25 min of SE ( $1483 \pm 291$  s), or 12 min ( $761 \pm 243$  s,  $n = 5$ ) after initiation of hypothermia (Fig. 1A and E), while SE continued for  $17.9 \pm 4.6$  h after initiation of cooling in paired normothermic animals ( $n = 5$ ,  $p < 0.0001$ ). The average brain and rectal temperatures at the time of seizure termination was  $31 \pm 0.6$  °C (Fig. 1C and E). In hypothermic animals, EEG power decreased with cooling (Fig. 1B), and by the time they reached 20 °C, relative power in hypothermic animals was 3% of that of the normothermic group measured at the same time ( $p < 0.01$ ). It was below prepilocarpine baseline and showed no seizure or residual paroxysmal activity. It remained below pre-seizure baseline through rewarming and in most animals, stayed close to baseline values for the remaining of the 24 h, although some late spikes and sharp wave activity did return. Manual review of EEGs confirmed the absence of seizures after rewarming in most rats. In one animal, 12 late seizures were observed 8.2–11.9 h after the initiation of rewarming. Many of these seizures were brief (mean duration:



**Fig. 1.** Deep cooling durably reduces relative EEG power. (A) The upper panel shows the compressed 4-hour EEG of a normothermic (red) and a hypothermic rat (blue, 20 °C for 30 min) with brain and rectal temperature. Both rats received midazolam 3 mg/kg ip (arrow), and cooling was initiated 2 min later. The lower panel shows the magnified 4-second EEG traces marked by vertical lines (a–d) (vertical bar = 0.75 mV, horizontal bar = 1 s). (B) Relative EEG power (EEG power / baseline EEG power) in normothermic (red bars) and hypothermic rats (blue bars, temperature in degrees Celsius) at the same time points. Relative EEG power was reduced by hypothermia in a dose-dependent fashion. The 20 °C point and the slope were significantly different from normothermics ( $*p < 0.01$ ). (C) “Brain surface” temperature during cooling and rewarming. (D) Brain temperature as a function of electrode depth with the “cool cap” method. Steep temperature gradients suggest that deep structures such as the hippocampus may remain warmer than the cortical surface. (E) The left y-axis of this graph shows the ratio of EEG power at each time point to initial EEG power at baseline, before pilocarpine injection. Note the logarithmic scale. Every time point beyond 30 min showed a significant difference between normothermic and hypothermic rats ( $**p < 0.01$ ,  $*p < 0.05$ ). The right y-axis shows the rectal and brain temperature scales. Note that the temperature of a probe positioned at the surface of the parietal cortex was close to the rectal temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

45 ± 6 s), and SE did not recur. In normothermic rats, EEG power decreased from pretreatment SE (probably in part due to midazolam) but remained significantly above baseline for many hours, reflecting continuation of SE (Fig. 1E). In most normothermic rats, semiperiodic spike bursts which slowly decreased in amplitude over time were separated by stretches of low voltage background activity which increased progressively in duration (Fig. 1A), and this pattern often continued for the whole 24 h of recording.

Most measures of the severity of SE were reduced in the hypothermic group compared to their normothermic counterparts. The number of EEG spikes per 24 h, including those which occurred before the initiation of cooling, was 7440 ± 1999 in the normothermic group (N) and 1004 ± 296 in the hypothermic group (H) ( $p < 0.01$ ). The time it took for EEG power to decline to twice prepilocarpine values was 4.97 ± 1.89 h after pilocarpine injection in the normothermic group against 0.8 ± 0.13 h in the hypothermic group ( $p < 0.05$ ). Hjorth function



integral [61] for the first 6 h after pilocarpine injection was  $9879 \pm 1939$  in normothermics versus  $3990 \pm 618$  in hypothermics ( $p = 0.01$ ).

#### 4. Discussion

These results suggest that deep hypothermia is a powerful tool for seizure termination in this animal model and deserves further evaluation as a potential treatment of last resort for RSE. In the current study, hypothermia reduced EEG power over 100-fold and reduced the duration of SE from a mean of 17.9 h (median: 24 h) to a mean of 0.2 h (median: 0.16 h). It terminated RSE in all animals. Seizures stopped well before reaching our target temperature (range: 29–33 °C) and returned in only one rat, many hours after the completion of rewarming. Our experiments were designed to mimic the clinical situation where hypothermia is likely to be used only after drug treatment. We induced SE with lithium and pilocarpine and treated with midazolam when SE was well established. Midazolam reduced EEG power and seizure severity but did not stop SE in normothermic animals. In this study, hypothermia was applied just after midazolam injection. Pilot experiments showed that the temperature of a probe positioned on the surface of the parietal cortex was close to rectal temperature, so the latter was used in most experiments. Temperature was brought down and maintained near 20 °C for the relatively short period of 30 min. Warming was then initiated by wrapping the rats in an electrical heating pad. Mean cooling time (39.5 min) was shorter than mean rewarming time (61.5 min). All animals tolerated hypothermia well.

Deep hypothermia (13–20 °C) is used in vascular and cardiac surgeries and neurosurgery, with good results for periods of circulatory arrest of 25–50 min [62], and the equipment and expertise needed are available in most large surgical centers. Its potential for treating RSE has never been evaluated, although results in experimental SE were encouraging [23,25]. Our results suggest that cooling to 20 °C can be very effective in stopping seizures. While surgical applications use hypothermia as a method to allow circulatory arrest during repair of aortic or cerebral aneurysms or congenital heart disease [63,64], its use as a treatment for RSE would be free of the complications of circulatory arrest. It would add a nonpharmacological option to our treatment regimens for RSE as well as for cholinergic seizures induced by nerve agents or organophosphate insecticides and deserves further study.

Computer-generated seizure counts were reviewed manually, to eliminate frequent duplicate counts, but underestimate seizure activity since the computer counts long periods of high-amplitude polyspikes (frequently seen in the early phase of SE) as single seizures and does not count as seizures the long periods of semiperiodic bursts on a low-amplitude background which is seen in most normothermic animals in the late phase of SE. Preliminary experiments using “Cool Cap” hypothermia were disappointing. The depression of seizure activity by head cooling was transient, and in these adult rats, “cool cap” hypothermia did not block seizures from returning upon rewarming. In “cool cap” animals, we found steep temperature gradients between the parietal cortex and deep brain, as shown in Fig. 1D. At a depth of 5 mm from the cortical surface, brain temperature was over 8 °C warmer than cortex temperature. This result is not surprising since the adult brain tissue has a high fat content and would be expected to be an excellent thermal insulator, and steep temperature gradients after local cooling have been observed in the mature primate brain [65]. This could result in inadequate cooling of key brain regions, such as the ventral hippocampus, where seizure activity could persist during cortical cooling and from which it could spread (and restart SE) upon rewarming.

The rationale for injecting scopolamine in all animals at the time of treatment was that two components are involved in chemically induced SE: the initial pilocarpine stimulation of muscarinic receptors, and a later component of self-sustaining seizures which are independent of the original trigger. Since the dose of scopolamine used here blocks all seizure activity when used as pretreatment, it should be sufficient to prevent residual pilocarpine from restarting the seizures if a treatment

stops self-sustaining SE. It enables us to study self-sustaining SE without the confusing presence of a chemical convulsant on board [60,66].

#### 5. Conclusion

This present study shows that deep hypothermia (20 °C for 30 min) is an efficient treatment to stop RSE. Hypothermia is very neuroprotective in animal models of ischemic or traumatic brain injury [67], and our preliminary data (not shown) indicate a strong reduction of neuronal injury in RSE as well. Further studies of the risk/benefit ratio of hypothermia in RSE and SRSE seem to be worth undertaking.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

#### Disclosure of conflicts of interest

None of the authors has any conflict of interest to disclose.

#### References

- [1] Holtkamp M, Othman J, Buchheim K, Meierkord H. Predictors and prognosis of refractory status epilepticus treated in a neurological intensive care unit. *J Neurol Neurosurg Psychiatry* 2005;76:534–9.
- [2] Mayer SA, Claassen J, Lokin J, Mendelsohn F, Dennis LJ, Fitzsimmons BF. Refractory status epilepticus: frequency, risk factors, and impact on outcome. *Arch Neurol* 2002;59:205–10.
- [3] Treiman DM, Meyers PD, Walton NY, Collins JF, Colling C, Rowan AJ, et al. A comparison of four treatments for generalized convulsive status epilepticus. Veterans Affairs Status Epilepticus Cooperative Study Group. *N Engl J Med* 1998;339:792–8.
- [4] Mazarati AM, Wasterlain CG. N-methyl-D-aspartate receptor antagonists abolish the maintenance phase of self-sustaining status epilepticus in rat. *Neurosci Lett* 1999;265:187–90.
- [5] McCullough JN, Zhang N, Reich DL, Juvonen TS, Klein JJ, Spielvogel D, et al. Cerebral metabolic suppression during hypothermic circulatory arrest in humans. *Ann Thorac Surg* 1999;67:1895–9 [discussion 1919–21].
- [6] Aihara H, Okada Y, Tamaki N. The effects of cooling and rewarming on the neuronal activity of pyramidal neurons in guinea pig hippocampal slices. *Brain Res* 2001;893:36–45.
- [7] Volgushev M, Vidyasagar TR, Chistiakova M, Yousef T, Eysel UT. Membrane properties and spike generation in rat visual cortical cells during reversible cooling. *J Physiol* 2000;522(Pt 1):59–76.
- [8] Schiff SJ, Somjen GG. The effects of temperature on synaptic transmission in hippocampal tissue slices. *Brain Res* 1985;345:279–84.
- [9] Thompson SM, Masukawa LM, Prince DA. Temperature dependence of intrinsic membrane properties and synaptic potentials in hippocampal CA1 neurons in vitro. *J Neurosci* 1985;5:817–24.
- [10] Shen KF, Schwartzkroin PA. Effects of temperature alterations on population and cellular activities in hippocampal slices from mature and immature rabbit. *Brain Res* 1988;475:305–16.
- [11] Volgushev M, Kudryashov I, Chistiakova M, Mukovski M, Niesmann J, Eysel UT. Probability of transmitter release at neocortical synapses at different temperatures. *J Neurophysiol* 2004;92:212–20.
- [12] Yang XF, Ouyang Y, Kennedy BR, Rothman SM. Cooling blocks rat hippocampal neurotransmission by a presynaptic mechanism: observations using 2-photon microscopy. *J Physiol* 2005;567:215–24.
- [13] Fuller BJ. Gene expression in response to low temperatures in mammalian cells: a review of current ideas. *Cryo Letters* 2003;24:95–102.
- [14] Soukup J, Zauner A, Dopperberg EM, Menzel M, Gilman C, Young HF, et al. The importance of brain temperature in patients after severe head injury: relationship to intracranial pressure, cerebral perfusion pressure, cerebral blood flow, and outcome. *J Neurotrauma* 2002;19:559–71.
- [15] Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 1989;20:904–10.
- [16] Nilsson P, Laursen H, Hillered L, Hansen AJ. Calcium movements in traumatic brain injury: the role of glutamate receptor-operated ion channels. *J Cereb Blood Flow Metab* 1996;16:262–70.
- [17] Colbourne F, Grooms SY, Zukin RS, Buchan AM, Bennett MV. Hypothermia rescues hippocampal CA1 neurons and attenuates down-regulation of the AMPA receptor GluR2 subunit after forebrain ischemia. *Proc Natl Acad Sci U S A* 2003;100:2906–10.
- [18] Liu L, Yenari MA. Therapeutic hypothermia: neuroprotective mechanisms. *Front Biosci* 2007;12:816–25.

- [19] Yenari MA, Han HS. Influence of hypothermia on post-ischemic inflammation: role of nuclear factor kappa B (NFkappaB). *Neurochem Int* 2006;49:164–9.
- [20] Sartorius CJ, Berger MS. Rapid termination of intraoperative stimulation-evoked seizures with application of cold Ringer's lactate to the cortex. Technical note. *J Neurosurg* 1998;88:349–51.
- [21] Karkar KM, Garcia PA, Bateman LM, Smyth MD, Barbaro NM, Berger M. Focal cooling suppresses spontaneous epileptiform activity without changing the cortical motor threshold. *Epilepsia* 2002;43:932–5.
- [22] Maeda T, Hashizume K, Tanaka T. Effect of hypothermia on kainic acid-induced limbic seizures: an electroencephalographic and <sup>14</sup>C-deoxyglucose autoradiographic study. *Brain Res* 1999;818:228–35.
- [23] Schmitt FC, Buchheim K, Meierkord H, Holtkamp M. Anticonvulsant properties of hypothermia in experimental status epilepticus. *Neurobiol Dis* 2006;23:689–96.
- [24] Liu Z, Gatt A, Mikati M, Holmes GL. Effect of temperature on kainic acid-induced seizures. *Brain Res* 1993;631:51–8.
- [25] Kowski AB, Kanaan H, Schmitt FC, Holtkamp M. Deep hypothermia terminates status epilepticus—an experimental study. *Brain Res* 2012;1446:119–26.
- [26] Orłowski JP, Erenberg G, Lueders H, Cruse RP. Hypothermia and barbiturate coma for refractory status epilepticus. *Crit Care Med* 1984;12:367–72.
- [27] Kendall GS, Mathieson S, Meek J, Rennie JM. Recooling for rebound seizures after rewarming in neonatal encephalopathy. *Pediatrics* 2012;130:e451–5.
- [28] Lundgren J, Smith ML, Blennow G, Siesjö BK. Hyperthermia aggravates and hypothermia ameliorates epileptic brain damage. *Exp Brain Res* 1994;99:43–55.
- [29] Oztas B, Kaya M. The effect of profound hypothermia on blood–brain barrier permeability during pentylenetetrazol-induced seizures. *Epilepsy Res* 1994;19:221–7.
- [30] Elmas I, Kucuk M, Kalayci RB, Cevik A, Kaya M. Effects of profound hypothermia on the blood–brain barrier permeability in acute and chronically ethanol treated rats. *Forensic Sci Int* 2001;119:212–6.
- [31] Eicher DJ, Wagner CL, Katikaneni LP, Hulsey TC, Bass WT, Kaufman DA, et al. Moderate hypothermia in neonatal encephalopathy: efficacy outcomes. *Pediatr Neurol* 2005;32:11–7.
- [32] Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365:663–70.
- [33] Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic–ischemic encephalopathy. *N Engl J Med* 2005;353:1574–84.
- [34] Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002;346:557–63.
- [35] Hypothermia after Cardiac Arrest Study G. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med* 2002;346:549–56.
- [36] Polderman KH. Induced hypothermia and fever control for prevention and treatment of neurological injuries. *Lancet* 2008;371:1955–69.
- [37] Weinrauch V, Safar P, Tisherman S, Kuboyama K, Radvovsky A. Beneficial effect of mild hypothermia and detrimental effect of deep hypothermia after cardiac arrest in dogs. *Stroke* 1992;23:1454–62.
- [38] Nagel S, Papadakis M, Pflieger K, Grond-Ginsbach C, Buchan AM, Wagner S. Microarray analysis of the global gene expression profile following hypothermia and transient focal cerebral ischemia. *Neuroscience* 2012;208:109–22.
- [39] Fay T. Observations on generalized refrigeration in cases of severe cerebral trauma. *Assoc Res Nerv Ment Dis Proc* 1945;24:611–9.
- [40] Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, et al. Treatment of traumatic brain injury with moderate hypothermia. *N Engl J Med* 1997;336:540–6.
- [41] Dietrich WD, Bramlett HM. The evidence for hypothermia as a neuroprotectant in traumatic brain injury. *Neurotherapeutics* 2010;7:43–50.
- [42] Truettner JS, Alonso OF, Bramlett HM, Dietrich WD. Therapeutic hypothermia alters microRNA responses to traumatic brain injury in rats. *J Cereb Blood Flow Metab* 2011;31:1897–907.
- [43] Lotocki G, de Rivero Vaccari JP, Perez ER, Sanchez-Molano J, Furones-Alonso O, Bramlett HM, et al. Alterations in blood–brain barrier permeability to large and small molecules and leukocyte accumulation after traumatic brain injury: effects of post-traumatic hypothermia. *J Neurotrauma* 2009;26:1123–34.
- [44] Oda Y, Gao G, Wei EP, Povlishock JT. Combinational therapy using hypothermia and the immunophilin ligand FK506 to target altered pial arteriolar reactivity, axonal damage, and blood–brain barrier dysfunction after traumatic brain injury in rat. *J Cereb Blood Flow Metab* 2011;31:1143–54.
- [45] Truettner JS, Suzuki T, Dietrich WD. The effect of therapeutic hypothermia on the expression of inflammatory response genes following moderate traumatic brain injury in the rat. *Brain Res Mol Brain Res* 2005;138:124–34.
- [46] Tomura S, de Rivero Vaccari JP, Keane RW, Bramlett HM, Dietrich WD. Effects of therapeutic hypothermia on inflammasome signaling after traumatic brain injury. *J Cereb Blood Flow Metab* 2012;32:1939–47.
- [47] Atkins CM, Truettner JS, Lotocki G, Sanchez-Molano J, Kang Y, Alonso OF, et al. Post-traumatic seizure susceptibility is attenuated by hypothermia therapy. *Eur J Neurosci* 2010;32:1912–20.
- [48] Azzopardi D, Strohm B, Edwards AD, Halliday H, Juszczak E, Levene M, et al. Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch Dis Child Fetal Neonatal Ed* 2009;94:F260–4.
- [49] Shankaran S, Pappas A, McDonald SA, Vohr BR, Hintz SR, Yolton K, et al. Childhood outcomes after hypothermia for neonatal encephalopathy. *N Engl J Med* 2012;366:2085–92.
- [50] Kamps M, Bisschops LA, van der Hoeven JG, Hoedemaekers CW. Hypothermia does not increase the risk of infection: a case control study. *Crit Care* 2011;15:R48.
- [51] De Georgia MA, Krieger DW, Abou-Chebl A, Devlin TG, Jauss M, Davis SM, et al. Cooling for Acute Ischemic Brain Damage (COOL AID): a feasibility trial of endovascular cooling. *Neurology* 2004;63:312–7.
- [52] Gega A, Rizzo JA, Johnson MH, Tranquilli M, Farkas EA, Eleftheriades JA. Straight deep hypothermic arrest: experience in 394 patients supports its effectiveness as a sole means of brain preservation. *Ann Thorac Surg* 2007;84:759–66 [discussion 766–7].
- [53] Rubens FD, Nathan H. Lessons learned on the path to a healthier brain: dispelling the myths and challenging the hypotheses. *Perfusion* 2007;22:153–60.
- [54] Todd MM, Hindman BJ, Clarke WR, Torner JC. Mild intraoperative hypothermia during surgery for intracranial aneurysms. *N Engl J Med* 2005;352:135–45.
- [55] Fleck TM, Czerny M, Hutschala D, Koinig H, Wolner E, Grabenwoger M. The incidence of transient neurologic dysfunction after ascending aortic replacement with circulatory arrest. *Ann Thorac Surg* 2003;76:1198–202.
- [56] Parissis H, Hamid U, Soo A, Al-Alao B. Brief review on systematic hypothermia for the protection of central nervous system during aortic arch surgery: a double-sword tool? *J Cardiothorac Surg* 2011;6:153.
- [57] Bickler PE, Warren DE, Clark JP, Gabatto P, Gregersen M, Brosnan H. Anesthetic protection of neurons injured by hypothermia and rewarming: roles of intracellular Ca<sup>2+</sup> and excitotoxicity. *Anesthesiology* 2012;117:280–92.
- [58] Morrisett RA, Jope RS, Snead III OC. Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. *Exp Neurol* 1987;97:193–200.
- [59] Mazarati AM, Baldwin RA, Sankar R, Wasterlain CG. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. *Brain Res* 1998;814:179–85.
- [60] Suchomelova L, Baldwin RA, Kubova H, Thompson KW, Sankar R, Wasterlain CG. Treatment of experimental status epilepticus in immature rats: dissociation between anticonvulsant and antiepileptogenic effects. *Pediatr Res* 2006;59:237–43.
- [61] Hjorth B. Principles for transformation of scalp EEG from potential field into source distribution. *J Clin Neurophysiol* 1991;8:391–6.
- [62] Prêtre R. Deep hypothermic circulatory arrest. In: Cohn L, Edmunds L, editors. *Cardiac surgery in the adult*. New York: McGraw-Hill; 2003. p. 401–12.
- [63] Bellinger DC, Newburger JW, Wypij D, Kuban KC, duPlessis AJ, Rappaport LA. Behaviour at eight years in children with surgically corrected transposition: the Boston Circulatory Arrest Trial. *Cardiol Young* 2009;19:86–97.
- [64] Wypij D, Newburger JW, Rappaport LA, duPlessis AJ, Jonas RA, Wernovsky G, et al. The effect of duration of deep hypothermic circulatory arrest in infant heart surgery on late neurodevelopment: the Boston Circulatory Arrest Trial. *J Thorac Cardiovasc Surg* 2003;126:1397–403.
- [65] King C, Robinson T, Dixon CE, Rao GR, Larnard D, Nemoto CE. Brain temperature profiles during epidural cooling with the ChillerPad in a monkey model of traumatic brain injury. *J Neurotrauma* 2010;27:1895–903.
- [66] Brandt C, Tollner K, Klee R, Broer S, Loscher W. Effective termination of status epilepticus by rational polypharmacy in the lithium–pilocarpine model in rats: window of opportunity to prevent epilepsy and prediction of epilepsy by biomarkers. *Neurobiol Dis* 2015;75:78–90.
- [67] Darwazeh R, Yan Y. Mild hypothermia as a treatment for central nervous system injuries: positive or negative effects. *Neural Regen Res* 2013;8:2677–86.