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Review

Recent antiepileptic and neuroprotective applications of brain cooling

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ABSTRACT

Hypothermia is a widely used clinical practice for neuroprotection and is a well-established method to mitigate the adverse effects of some clinical conditions such as reperfusion injury after cardiac arrest and hypoxic ischemic encephalopathy in newborns. The discovery, that lowering the core temperature has a therapeutic potential dates back to the early 20th century, but the underlying mechanisms are actively researched, even today. Especially, in the area of neural disorders such as epilepsy and traumatic brain injury, cooling has promising prospects. It is well documented in animal models, that the application of focal brain cooling can effectively terminate epileptic discharges. There is, however, limited data regarding human clinical trials. In this review article, we will discuss the main aspects of therapeutic hypothermia focusing on its use in treating epilepsy. The various experimental approaches and device concepts for focal brain cooling are presented and their potential for controlling and suppressing seizure activity are compared.

1. Characteristics of epilepsy

Epilepsy is a heterogeneous group of chronic neurological disorders that are characterized by recurrent unprovoked seizures. Epileptic seizures occur when neurons are intermittently activated in an abnormally excessive and highly synchronous manner. Focal and generalized seizures are distinguished according to the localization of activated neurons. The onset of a focal seizure is limited to a part of one hemisphere, during generalized seizures, the initial activation of neurons happen throughout both hemispheres [1].

Interictal - meaning between seizures - epileptiform activity can also occur in epilepsy patients. It takes the form of epileptiform discharges (EDs), which are simply transients with a characteristic spiky morphology. EDs are important in the diagnosis of epilepsy [2].

Primarily, epilepsy is treated with pharmacological interventions such as administration of antiepileptic drugs (AEDs, but even the best medications cannot control the seizures of over 30 % of epilepsy patients. These patients are considered to have refractory epilepsy (SE) [3].

Surgical resection can only be performed if the epileptogenic foci are not located in eloquent areas such as the motor and speech cortices, and is not always successful [4].

One potential evidence based alternative to AEDs and surgical resection is neuromodulation by focal stimulation in the central nervous system, like the anterior thalamus deep brain stimulation [5] or stimulation of the vagus nerve on the neck [6].

The third option would be the application of focal brain cooling in cases of focal epilepsies where the epileptogenic zone is in an eloquent area. Numerous animal studies show the efficacy of focal cooling in terminating ongoing seizures, reducing seizure frequency and the amplitude of epileptic discharges [7]. Human trials with the use of therapeutic hypothermia were also conducted, investigating its beneficial effects on other disorders as well, such as traumatic brain injury [8] or ischemic stroke [9].

2. Historical background of therapeutic hypothermia

The earliest descriptions on the use of therapeutic hypothermia date back to the late 19th century. By the 1870s, researchers concluded, that local hypothermia could reduce neurologic functions at a systemic level. At this time, only global conclusions were drawn without any insight regarding the cellular mechanisms underlying hypothermia [10].

The potential clinical use of cooling has been discussed since the 1940s. In 1959, Fay suggested that implanting a metal capsule, through which chilled fluid is circulated, has promising effects in treating head

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trauma [10]. Baldwin was the first to demonstrate that systemic hypothermia can suppress EDs in the epileptic primate brain [11].

It was reported in 1969 that grand mal seizures stop when rectal temperature reaches 36 °C in human patients with epilepsy. Also, focal cooling in cats below 26.9 °C terminated penicillin G induced seizures in the visual area, which reappeared after rewarming [12].

Sourek and colleagues used a device called the Autohypotherm for general hypothermia combined with local extravascular irrigation with iced saline in their 1970 study. Core temperatures of human patients could be dropped below 30 °C, while final local brain temperature dropped below 24 °C in 21 patients and below 20 °C in 10 patients. They were also able to measure the temperature gradient in the brain tissue during irrigation: the temperature of deeper structures were always higher than that of the surface and rose about 2 °C for each additional 10 mm of depth. During a 1 year post-surgery period, seizures were reduced or completely eliminated in 60 % of the patients [13].

Focal cooling was intensely researched in the 1960s and 1970s, but later the interest in the field dropped. The number of published studies rose again in the late 1990 and early 2000s and since then it has remained an area of active research.

An important observation was in the 1980s that the mild to moderate range (reducing brain temperature to 31 °C–35 °C) yielded the best results in terms of neuroprotection [14].

Insights gained through experiments with acutely perfused brain slice preparations carried out from the 1980s up until today further confirmed the neuroprotective functions of lowered temperature: excessive release of acetylcholine [15] and ischemia-induced release of monoamines are decreased by hypothermia [16]. Anticonvulsant effect of cooling is apparent as it is able to terminate seizure-like activity in rodent slices within seconds [17]

Nowadays, therapeutic hypothermia is one of the most important methods of neuroprotection: cooling the body to 32–35 °C improves neurological outcome in scenarios such as post-cardiac arrest [18], traumatic brain injury [19], spinal cord injury [20] and controlled temporary arterial ligation during microsurgical neurovascular interventions [21]

3. Physiological effects of hypothermia and its use in treating epilepsy

Hypothermia causes reduced neurologic functions through a variety of mechanisms: rapid reduction in neurotransmitter release [22], alternation of kinetics in voltage-gated ion channels, decreased cerebral metabolism and reversible disruption of the network synchrony [23]. Other important ways in which hypothermia affects central neuronal excitability include increased input resistance, reduced synaptic transmission, decreased amplitude in population spikes and an increased spike duration [24–26].

The evoked potential of the cat somatosensory cortex is recognizably changed by focal cooling: irrigating the cortex with iced fluid for 30 s caused the complete disappearance of evoked waves, the alterations depended not only on the degree, but also on the rate of cooling [27]. Cooling the rat visual cortex from 35 °C to 7 °C increased input resistance and decreased K^+ conductance, but had no effect on Na^{2+} current or its activation threshold [28]. The glutamate binding affinity of neurons is significantly affected by temperature change, and the speed of diffusion may also be altered [29]. Even structural changes of the cerebral cortex can occur: the density of synaptic terminals and the number of microtubuli in the preterminal axon both increased in response to cooling to 8−10 °C [30]. All these changes are reversible and normal neurologic functions are restored upon warming up [31].

Therapeutic hypothermia has anti-inflammatory, anti-apoptotic and anti-epileptic effects as well. The beneficial effects of hypothermia may affect pathophysiologic cascades initiated by brain injuries, that can cause edema, seizures, and apoptosis [23]. The neuroprotective consequences of cooling are summarized in Table 1.

Table 1
Physiological effects of hypothermia (based on [36]).

Condition, process	Neuroprotective effect of hypothermia	Reference
Ischemia	Reduced extracellular levels of excitatory neurotransmitters	[37]
	Decreased brain glycine levels	[38]
	Decreased release of monoamines and their toxic metabolites into the extracellular space	[16]
	Increased levels of BDNF and other neurotrophins	[39]
	Reduced proliferation, migration, transformation, and activation of astroglial cells	[40]
Apoptosis	Decreased p53 protein levels in the brain	[41]
	Altered levels of proteins Bcl-2 and cytochrome C	[42]
	Blocked TNF pathway of apoptosis	[43]
	Stress-activated signaling pathways are affected avoiding cell apoptosis	[44]
	Apoptosis is prevented by inhibiting the caspase pathway	[45]
	Blocked proteins responsible for mediating the caspase-independent apoptosis	[46]
	Blocked delta-PKC after ischemia	[47]
	Reinforced Akt pathway and structural alterations in PTEN	[48]
	Induced formation of cold shock proteins	[49]
Metabolic, oxidative, inflammatory and electrical damages	Lowered lactate levels from anaerobic metabolism	[50]
	Decreased concentrations of thromboxane A2	[51]
	Reduced free radical levels after neuronal damage	[52]
	Decreased production of inflammatory cytokines	[53]
	Reduced disruption of the blood–brain barrier	[54]
	Suppressed epileptogenic electrical activity	[55]

3.1. Epilepsy treatment with hypothermia

There are several possible mechanisms suggested by which focal cooling exerts its antiepileptic effects, however, the exact mechanisms are poorly characterized. Some of the mechanisms are reduction in neurotransmitter release, alternation of activation-inactivation kinetics in voltage-gated ion channels, and slowing of catabolic processes [4]. Evoked synaptic activity and synchronized spontaneous epileptiform activity are both affected by hypothermia. Ion channels possess a key role in synaptic transmission and epileptiform discharges suggesting that hypothermia could affect ion channel gating properties [3].

Effects of hypothermia on epileptic brain activities are extensively researched in animal models. Injection of kainate into specific areas of the brain is a common way to induce spontaneous epileptic seizures in animals.

Kainic acid-induced seizures in rats treated with hypothermia at core temperatures of 28 °C reduced ictal discharges by 50 %. Reaching a core temperature of 23 °C by further cooling almost completely eliminated the epileptiform discharges, without any hippocampal cell loss. On the other hand, animals treated with 42 °C hyperthermia had significant cell loss, and died within 2 h after severe tonic seizures [32]. Cooling to 30 °C can result in longer latency, shorter duration, and less frequent seizures even when it is applied before a kainate injection [33]. Nitric oxide production and hippocampal cell loss were both reduced during kainate induced seizures in immature rabbits [34]. Beside reducing seizures, cooling can also decrease brain edema and improve cognitive functions [35].

In animals, hypothermia may be achieved by forced swimming in cold water. Rats with lithium-pilocaprine seizures forced to swim for at least 5 min in 20 °C showed anticonvulsant and neuroprotective effects of lowered temperature, including a significantly reduced hippocampal cell loss compared to controls. No tolerance was developed even after repeated swimming [56].

In human patients with intractable epilepsy, focal brain cooling affected glutamate and GABA concentrations, cerebral blood flow and glucose metabolism. Nomura et al. induced focal cooling limited to the epileptogenic zone, where brain temperature immediately reached 15 °C and was maintained at this temperature for 30 min. Cooling reduced amplitude of ECoG and cerebral blood flow was significantly decreased. Extracellular glutamate was significantly reduced to 51.2 %, while cortical GABA levels were reduced to 35.1 % of control levels during brain cooling; however this reduction did not correlate with the reduction in ECoG and was not significant. Glycerol and lactate levels decreased during cooling, while glucose and pyruvate levels were maintained throughout the procedure. After cooling was stopped, brain temperature returned to normal within 30 min. No patients experienced adverse effects relevant to the measurements [57,58].

4. Approaches to cooling the body and brain

General hypothermia or systemic cooling refers to cooling the whole organism to a target temperature by means of a cooling chamber or ice bath. With this method mild to moderate hypothermia can be induced. It was applied mostly in early human trials, but for certain clinical conditions such as ischemia or post cardiac arrest, it is still in use today.

Focal cooling offers more specificity to cool the brain than general hypothermia. A cooling helmet or cap is a noninvasive approach, besides that, several practices exist for the invasive cooling of the brain. Invasive focal cooling methods offer the greatest temperature reduction. Some invasive techniques utilize a special device for the purpose of cooling.

Among the various methods of local hypothermia, the simplest and most prevalent in clinical scenarios is epidural, subdural or subarachnoid irrigation of the brain with cold saline [59].

The most commonly used cooling device *in vitro* and *in vivo* studies is the Peltier chip [60]. Another common way of inducing local hypothermia is placing a device of plastic or metal tubing in contact with the brain, through which chilled fluid is circulated. Table 2 shows the various cooling methods used in animal models and human patients with typical cooling ranges.

4.1. General hypothermia

General hypothermia means that the whole organism is cooled to a specific temperature to mitigate local adverse effects of diseases. The degrees of cooling have been previously classified as mild (34–35.9 °C), moderate (32–33.9 °C), moderate-deep (30–31.9 °C), and deep (<30 °C) hypothermia [14].

General hypothermia is becoming a standard practice for adults resuscitated after cardiac arrest and for infants treated for perinatal hypoxic ischemic encephalopathy. In these cases, core body temperature is usually lowered to 32–35 °C meaning a state of moderate hypothermia. Systemic hypothermia showed beneficial effects in neonates diagnosed with perinatal stroke and encephalopathy. Seizures did not develop in neonates treated with hypothermia, while the untreated ones had seizures and worse cognitive outcome [61].

Regarding animal experiments, electrical stimulation induced self-sustaining status epilepticus (SSSE) was completely suppressed, when animals were cooled to 20 °C using ice packs only (Fig. 1). This effect was sustainable after rewarming with no mortality or discernible morbidity [67].

In another experiment, researchers induced status epilepticus by electrical stimulation using implanted electrodes in the perforant paths of rats to show the anticonvulsant effects of hypothermia. Treatments included external systemic cooling for 3 h (temperature minimum was 29 °C), administration of diazepam or both. The severity of motor seizures and frequency and amplitude of spontaneous seizure discharges were compared among the groups. In diazepam treated animals, seizure activity didn't reduce, except for the later stages of motor seizures and only a slight decrease in the amplitude of the discharges was observed. Cooling only significantly reduced discharges without affecting the EEG discharges. Animals treated with both cooling and low-dose diazepam showed a significant drop in frequency and amplitude of EEG discharges [68].

General hypothermia can also be induced by infusing chilled saline into the vasculature. In one example, 4 patients with intractable status epilepticus underwent vascular cooling to 31 °C–35 °C. Despite being on intravenous drug therapy that could not address their seizure severity, the application of this modest cooling dramatically reduced seizures in all 4 patients. However, some adverse effect occurred including shivering and two patients died. The deaths were not directly related to cooling, but hypothermia may have contributed to a fatal sepsis in one patient [55].

Legriel et al. also perfomed vascular cooling for 24 h reaching core body temperatures between 32 °C and 34 °C. They recruited 270 critically ill patients with convulsive status epilepticus and randomly assigned them to standard care or standard care with hypothermia. They found no significant improvements in 90-day functional outcomes associated with hypothermia added to standard care, and adverse effects were more frequent in the hypothermia group [69].

Besides the beneficial effects of systemic hypothermia on neurologic function, whole body cooling also carries the risk of infection, coagulopathy and cardiopulmonary compromise. These in addition to the risks of rewarming set back the clinical utility of systemic therapeutic hypothermia, suggesting that localized cooling may be a better approach in treating neurologic diseases [70,71].

Table 2
Comparison of the various cooling methods in terms of temperature reduction, cooling time, area and effect on epileptic conditions.

Principle	Organism	Cooling parameters	Cooling effect	Reference
General hypothermia	human	35−31 °C core temp. for 24 h.	Dramatic reduction in seizures, two of four remained seizure free after rewarming.	[55]
	rat	28 °C and 23 °C core temp., each for 30 min.	50 % reduction of ictal discharges at 28 °C, nearly abolished discharges at 23 °C.	[32]
External head-neck cooling	human	Focal temp. reduced by 12.2 °C, core temp. by 1.7 °C for 1 h, once a week.	Weekly seizures decreased from 2.7 to 1.7 events	[62]
Chilled saline irrigation	human	Cortex irrigation for 30 s.	Cessation of spiking for 6 min.	[59]
Passive focal cooling	rat	Focal temp. reduced by 2 °C for 5.5 weeks	Ictal activity abolished for ten weeks after cooling cessation	[63]
Peltier-effect	human	15 °C focal temp. for 30 min.	Reduced ECoG power, Glu and GABA concentrations	[57,58]
	rat	25−10 °C focal temp.	EDs were completely attenuated at 15 °C along with sensorimotor function deterioration	[64]
Liquid coolant	human	Focal temp. reduced by 0.6−2 °C for 5 min.	–	[65]
	rat	27 °C and 24 °C focal temp.	Both reduced seizure score and after discharge duration	[66]

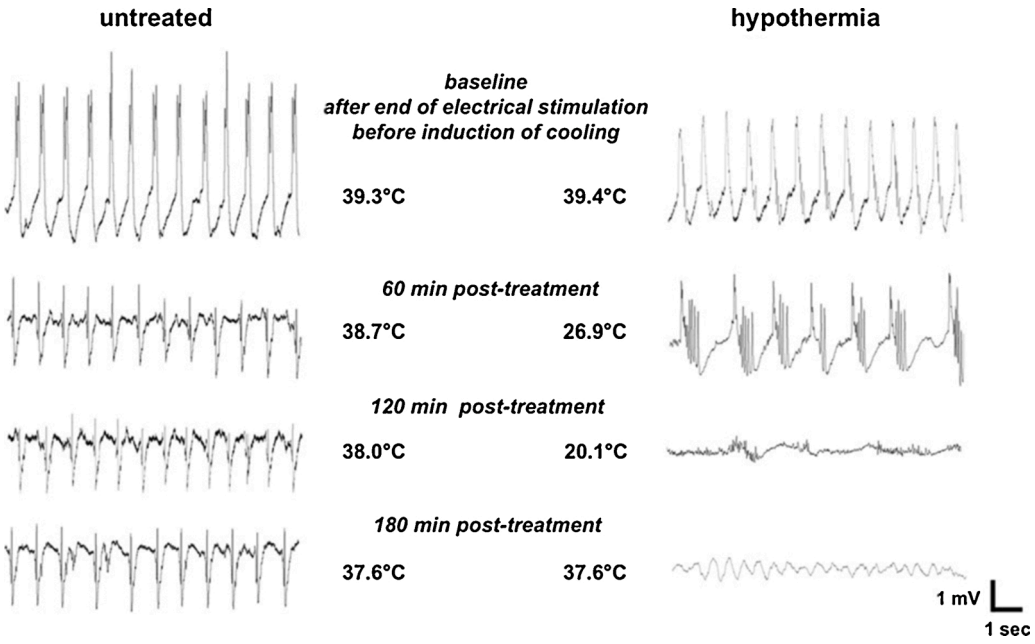

Fig. 1. After 60 min of cooling discharge frequency is significantly decreased and by reaching target temperature of 20 °C, SSSE is completely terminated. Spontaneous discharges do not reoccur upon rewarming [67].

4.2. External head cooling

Researchers report on the trial of a cooling helmet or cap to induce brain hypothermia in volunteers and patients with stroke, head injury or medically refractory epilepsy. The non-invasive device features a head/neck liner made of a lightweight, thin, flexible, laminated nylon urethane fabric and a conditioning unit (temperature control, liquid pump and pressure control, air pump).

In one case, brain temperatures were continuously monitored for a mean of 48–72 h. Warming blankets were used to maintain a core temperatures at safe levels. Temperature data of a Neurotrend sensor (0.8 cm below the cortical surface) was compared with the patients' bladder (core) temperatures. Brain temperature reduction of 1.84 °C (range 0.9–2.4 °C) was observed within 1 h of helmet application (Fig. 2). It took 3.4 h (range 2–6 h) to achieve a brain temperature lower than 34 °C, and 6.67 h (range 1–12 h) before systemic hypothermia (<36 °C) occurred. Use of the helmet resulted in no significant complications. A mean 0.63 °C/hour (range 0.15–1.45 °C/hour) passive rewarming rate was observed [72].

With the same construct, the lowest temperature that the cooling device can produce was used. Volunteers had 30 and 60 min long cooling sessions, 3 days separately. Patients had four 60 min long sessions, once a week. Shivering was prevented by keeping the torso warm during cooling. Temperature measurements were taken every 5 min. Tympanic and intestinal measurements (temperature sensor pill, swallowed by subjects) served as core temperatures. External sensors were placed at multiple sites. Seizure counts over 4-week precooling, treatment and follow-up phases were compared. At the end of 60 min of cooling, scalp temperature fell by 12.2 °C, tympanic temperature by 1.67 °C (both significant), and intestinal temperature by 0.12 °C (not significant). There were no changes in the patients' physical examination after cooling. Average weekly seizure frequency significantly decreased from 2.7 to 1.7 events per patient per week [62].

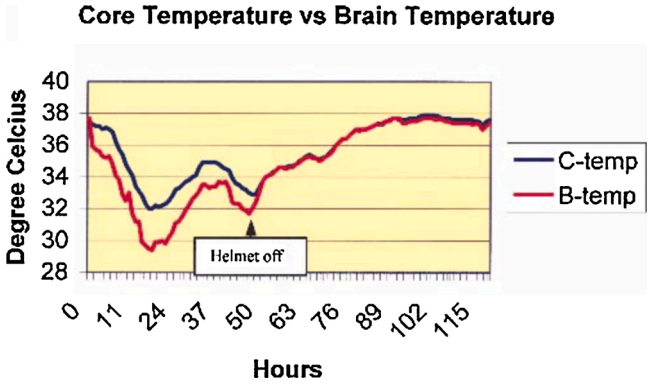

Fig. 2. Brain and core temperatures during cooling and rewarming [72].

A cooling cap and neckband was utilized in a larger trial among 90 patients with traumatic brain injury. The use of the cooling cap circulating 4 °C water in it was an addition to standard TBI care in half of the patients. With the cooling cap, mildly hypothermic parenchymal temperatures of 33−35 °C was achieved after four hours of cooling per day for three days. The cooling group had a significantly lower intracranial pressure 24, 48 and 72 h after injury, than the control group. There were no major differences in adverse outcomes [8].

A forced air-cooling helmet was used in an animal trial, for 16 anesthetized dogs. Wass et al. found an average drop of 0.5 °C in intraparenchymal temperature at a depth of 2 cm with 2 °C being the biggest reduction [73].

To sum up the experiences in head cooling, this method is noninvasive, yet offers local cooling. Its beneficial effects on seizure frequency may last for weeks post treatment. However, the area to be cooled cannot be specified, and not as focal as invasive methods. Brain tissue is only slightly cooled despite using the maximum capacity of the devices. Some torso warming must be used to counteract the systemic effects of cooling, which might cause discomfort in the long run.

4.3. Chilled saline irrigation

Irrigating the exposed brain surface with ice cold saline is a widespread method used by neurosurgeons during operations. The lowered cortical temperature terminates paroxysmal discharges as confirmed by studies of human brain cooling during operative neurosurgical mapping [74]. In these cases, the application of iced saline to the neocortex abruptly terminated focal spiking (Fig. 3). Cortical stimulation mapping potentially induces focal seizure activity that can be rapidly halted by

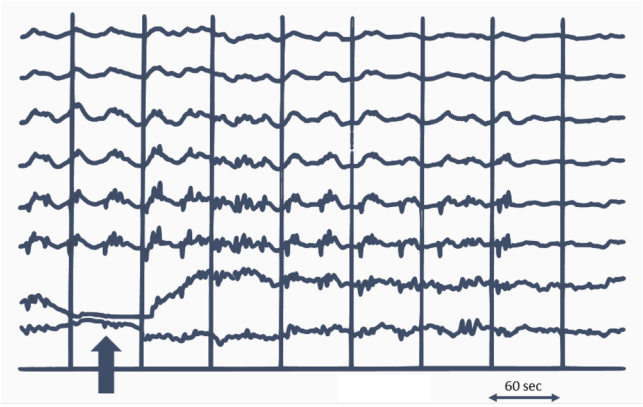

Fig. 3. Electrical after-discharge terminates within seconds after the application of cold Ringer's lactate to the cortex, signified by the arrow. Redrawn based on [75].

irrigating the brain surface with cold Ringer's solution [75].

The effect of cold saline cooling on epileptiform discharges was investigated in a 41 year-old human patient with focal seizures. Cold saline at 4 °C was applied to the interictal spike focus for 30 s, and its effect on the epileptiform discharges was observed. Cortical stimulation was performed after cooling along with ECoG before resection of a right frontal tumor. Application of cold saline to the spike focus resulted in a transient, complete cessation of spiking for 6 min, while the motor threshold for electrical stimulation remained unchanged [59].

Ablah et al. aimed to determine if applying chilled solution to the exposed cerebral cortex can reduce interictal epileptiform activities in 12 patients undergoing cortical mapping and resection surgery. They applied 150 cm^3 of chilled (4 °C) or room temperature normal saline solution to the cortex. Interictal spikes were counted at baseline and compared with spikes after applying saline solutions. Chilled saline reduced the number of interictal spikes from 11.46 to 4.87 spikes per minute, while room temperature saline had no significant effect on the epileptic spike frequency [76].

In an animal study of a porcine model, epidural temperatures of 13 °C, subdural temperatures of 19 °C and parenchymal temperatures of 28 °C were achieved by continuous epidural irrigation with chilled saline administered via burr holes. After five minutes of irrigation, local hypothermia took place and it was maintained for 6 h without difficulty [77].

Chilled saline irrigation is the most practical and immediate intervention during surgery. Rapid and effective local cooling is achieved, although cooled area and degree of cooling cannot be controlled. It is not suitable for long term solutions for epilepsy patients, because the effects are temporal - lasting for minutes - even when cooling induced changes outlast the application of chilled liquid.

4.4. Passive focal cooling

A cooling device can be as simple as a metal plate in contact with the brain surface that aids in dissipating the heat from the tissue, thereby achieving focal cooling.

In the study of D'Ambroso et al., the effect of passive focal cooling on the development of neocortical epileptic seizures after head injury in the rat was studied. ECoG headsets were equipped with metal plates for calibrated passive heat dissipation and placed on the exposed cortex for several weeks post-injury. Electrical activity was monitored by 5-electrode video-electrocorticography 2–16 weeks post-injury. Cooling by 0.5–2 °C inhibited the onset of epileptic seizures. Cooling by 2 °C for 5.5 weeks beginning 3 days after injury virtually abolished ictal activity. This effect persisted over ten weeks after cessation of cooling. This study suggests a potential for hypothermia to not only act as an anti-convulsant but also to block epileptogenesis [63].

Even though the temperature reduction in the above study proved to be adequate to inhibit epileptic seizures, the degree of cooling possible with passive heat dissipation is only very slight and uncontrolled. Also, passive focal cooling cannot differentiate between normal brain states and seizures, preventing targeted intervention in patients with epilepsy.

4.5. Thermoelectric devices

Thermoelectric devices use Peltier's 1834 observation that a temperature gradient develops at the junction between two dissimilar conductors when an electric current is applied across them. Thermoelectric devices are composed of arrays of crystalline semiconductors sandwiched between metallized ceramic plates to create a wafer. One of the plates is rapidly cooled while the other is heated by current flowing through them. The hot side should be connected to a heat sink to ensure effective cooling (Fig. 4). Modern Peltier devices are only a few millimeters in length and width and about 1.5 mm thick and are typically made of alloys of bismuth, tellurium, selenium, and antimony [78].

Peltier devices are widely used tools in animal studies to induce focal cooling in a specific area of the brain. An array of such devices can be fabricated permitting adjacent areas to be cooled independently or simultaneously, as demonstrated by Reed et al. in 1978. Awake cebus monkeys and anesthetized cats were used for the experiment. An array of 12 small Peltier modules of 4 mm x 4 mm x 2.5 mm was fabricated, through which heat could be individually removed or applied to restrict cooling to a desired region. A copper baseplate for the Peltier modules was shaped to fit the curvature of the area to be implanted. A copper pipe for circulation of cold water was soldered to the upper surface of the baseplate to function as a heat-sink. For measurements in anesthetized cats the module was cooled to 1 °C and temperature was measured several distances from the device. 30 min long cooling trials were performed during chronic implantation in monkeys. Temperature change underneath one module was approximately 8 °C. The addition of a second module also cooled to 1 °C at a distance 2 mm from the first caused the 20 °C isotherm to shift from a maximum depth of 1.5 mm–2.3 mm. Cooling the sensorimotor cortex produced postural and behavioral deficits in the contralateral arm of one test monkey. Deficits appeared within the first few minutes of the 30 min long cooling trial and persisted for 30−60 min following termination of cooling [79].

The first study to investigate the ability of thermoelectric devices to abort 4-aminopyridine–induced ictal activity was carried out using hippocampal slices. The slices were placed on the surface of a Peltier module attached to the base of a perfusion chamber. Seizures were terminated in 8 s in slices cooled to 21 °C, whereas in the control slices, ictal events lasted 35 s. The authors also developed a thresholding algorithm, that automatically detected seizures and triggered cooling. They observed that cooling terminated seizures only when the thermoelectric device was in direct contact with the slices, so the effect was not attributed to the electrical field generated by the unit [17].

One study described the design, optimization and implementation of a lightweight Peltier thermoelectric device for modulating temperature in brain structures of zebra finch songbirds. The authors provided a detailed mathematical description of heat transfer and temperature changes in the Peltier device. The device performance was optimized based on the derived equations using the construction parameters: semiconductor geometry, number of semiconductor junctions, heat sinking options and probe dimensions. Two types of miniature devices were fabricated. One device was used for cooling a superficial region (nucleus HVC, located between 100 μm and 600 μm of the brain surface) and thus uses a plate positioned over HVC. The other device was used for cooling nucleus LMAN (lateral magnocellular nucleus of the anterior nidopallium), which is located 2 mm deep in the brain and was cooled by inserting a thin cylindrical wire into the nucleus. Both devices had a convective heat sink made of 8 mm long copper wire mesh and a body coupled heat sink made of silver sheet. The amount of current passing

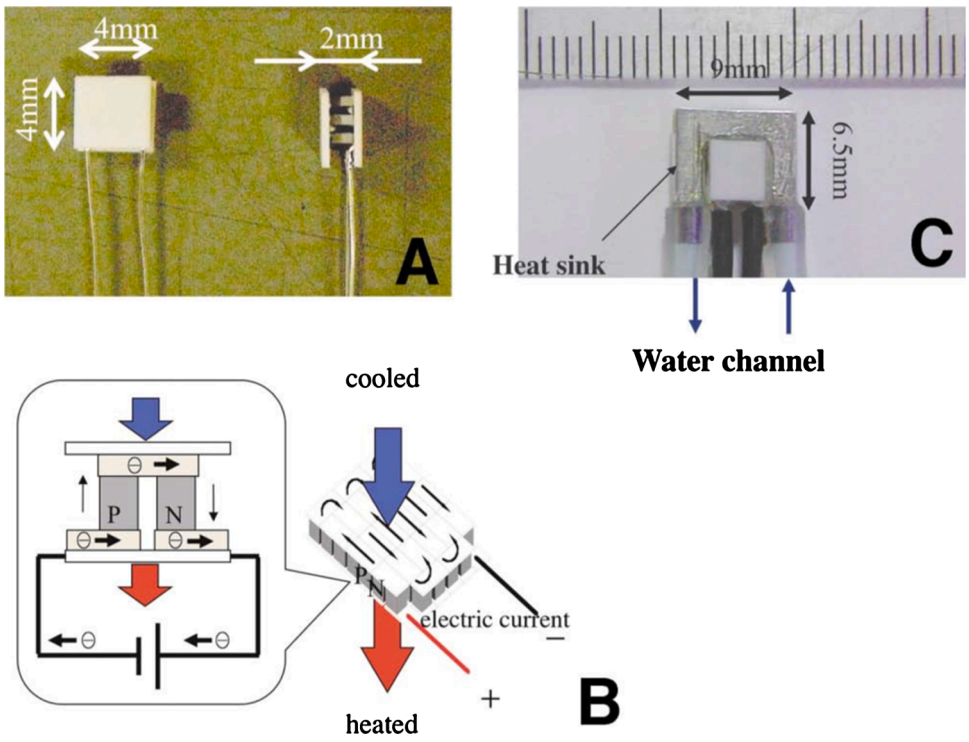

Fig. 4. A Peltier device with heat sink: **(a)** Front and side view of a typical Peltier cell; **(b)** Dimensions of an attached aluminum heat sink; **(c)** Schematics of the current flow through the cell [4].

through the devices was changed every 100 s decreasing from 1 A to -2.5 A in 0.5 steps. After a change in current, temperatures reached steady state in 10–20 s. Maximum amount of cooling at the HVC device plate was 10 °C at -1.5 A current, at the HVC center cooling was 7 °C. For the LMAN device, maximum cooling at the plate was 15 °C at -1.5 A, at the probe tip 11 °C, and 4 °C 500 μm from probe surface [80].

A Peltier chip is also suitable for cooling deeper structures, such as the hippocampus (Fig. 5). In anesthetized rats, the inhibitory effect of cooling on experimental hippocampal seizures was investigated. A 6 mm x 6 mm x 2 mm Peltier chip was used for cooling with a copper plate and a 6 mm long silicone coated copper needle attached to it. A copper heat sink with water channel circulating 4 °C water and a thermometer was attached. The cooling needle, a thermocouple, and a needle electrode were inserted into the left hippocampus at a depth of 4 mm, and kainic acid was injected into the right hippocampus to provoke EDs. Temperature dropped at the needle tip from 33.1 ± 0.7 °C to 14.5 ± 1.3 °C, below 20 °C within a 1.6 mm radius, and below 25 °C within a 2.4 mm radius from the cooling center. The needle tip temperature decreased below 20 °C within 1 min. The amplitude of EDs was suppressed to 68.1 ± 4.8 % of the precooling value. No histological damage was observed [81].

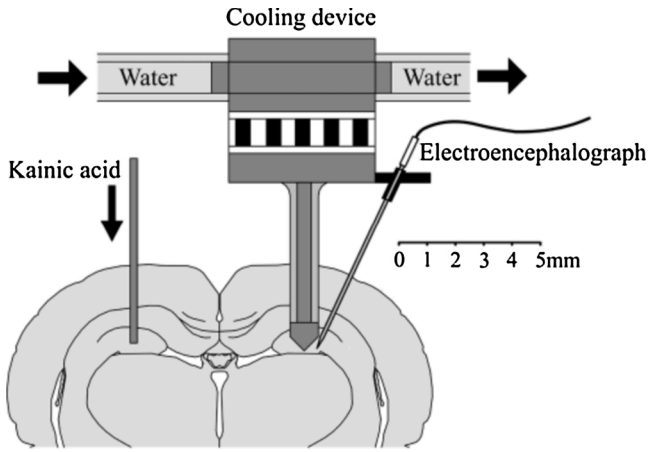

Fig. 5. Thermoelectric chip for cooling the hippocampus [81].

The cooling effect seems to be localized to just a small region of neocortex in proximity of the cooling device. With a small thermocouple inserted into a 30-guage needle, the cortex was mapped below the thermoelectric device showing that cooling extends only approximately 4 mm below the surface [82].

Yang et al. explored focal cooling with a thermoelectric device in anesthetized rats as a potential therapy for neocortical epilepsy. Artificial cerebrospinal fluid containing 4-aminopyridine (4-AP) was injected to provoke EDs, then focal cooling was applied to the cortex with a Peltier device. Within 30 min of 4-AP injection, animals developed recurrent seizures lasting 85.7 ± 26.2 s that persisted for 2 h. Cooling to 20–25 °C at seizure onset reduced the seizure duration to 8.4 ± 5.0 s. There was no effect on seizure duration when the Peltier device was not allowed to make physical contact with the brain (Fig. 6). The duration of seizures remained reduced even after rewarming from prior cooling. Histopathologic examination of these animals has not revealed any abnormalities after 2 h long exposure to as low as 5 °C. There was no neuronal loss or activation of apoptotic pathways, and dendritic beading and spine loss were reversible [83].

The same group observed that the threshold temperature required for any reduction in seizure duration was 24 °C. A sharp temperature gradient developed across the cooled neocortex: 31 °C at 4 mm below the Peltier, which was cooled to the lowest temperature of 20 °C [84].

Regarding threshold temperature, different results were gained by a study group investigating a Peltier chip in rats having epileptic discharges induced by kainic acid injection in the neocortex. The Peltier chip was attached to a heat sink with a water channel. Two silicon tubes were connected to the heat sink, and water at 37 °C was circulated in the channel. The epileptic cortex was cooled to 30 °C, 28 °C and 25 °C. Within 30 s, brain surface temperature decreased to 14.8 ± 1.5 °C and at 2 mm depth temperature decreased to 27.1 ± 3.1 °C. Epileptic activity started to decrease immediately after cooling started. Cooling to 30 °C,

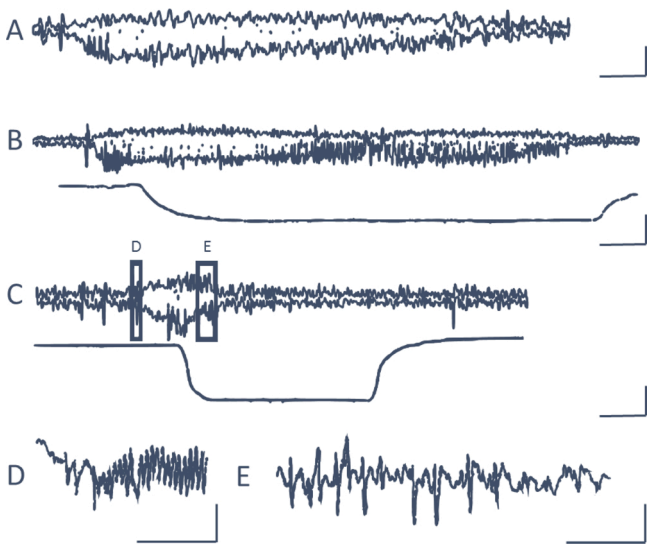

Fig. 6. Examples of 4-AP-induced neocortical seizures: **(a)** 90 s long control seizure; **(b)** The course of a seizure is unchanged, when the Peltier device is not in direct contact with the brain; **(c)** A seizure is terminated by direct cortical cooling within 9 s; **(d)** and **(e)** are start and end of the seizure in (c). Signal amplitude and time scale are 0.3 mV and 10 s, respectively. Redrawn based on [83].

28 °C and 25 °C resulted in a gradual activity reduction and complete cessation at 25 °C. The temperature of the hot side of the chip was maintained at approximately 36.9 °C [85].

A possible lower limit for the therapeutic temperature was described by Fuji et al. upon investigating focal cooling on penicillin G induced epileptic seizures in a freely-moving rat. They used a PID-controlled Peltier device chronically implanted on the epileptic focus. Cooling to 20 °C, 15 °C, and 10 °C was performed, while monitoring seizure frequency and neurologic changes. Cooling down from 20 °C to 15 °C significantly improved both seizure frequency and neurologic functions. Cooling to 10 °C also suppressed seizures, but with no further improvement in neurologic function. Subsequent investigation of sensorimotor function revealed significant deterioration in foot-fault tests and the receptive field size at 15 °C, suggesting that this temperature may be the safety limit [86].

Investigating local hypothermia in the same temperature range, Kida et al. also noted, that different frequency ranges were sensitive to particular temperatures, that is, 25 °C, 20 °C, and 15 °C attenuated beta, alpha to beta, and delta to beta frequency discharges, respectively [64].

A low temperature limit also exists for irreversible histological change and motor dysfunction. Oku and colleagues performed cooling to 20 °C, 15 °C, 10 °C, 5 °C, 0 °C, and −5 °C for 1 h using a 6 mm x 6 mm x 2 mm thermoelectric chip placed on the sensorimotor cortex of anesthetized rats. Natural rewarming was allowed thereafter. Motor function was evaluated using the beam-walking scale (BWS) every day for 7 days after cooling. Temperature values in the cooled groups of 5.0 °C, 0.0 °C and -5.0 °C were at 1 mm depth 8.4 °C, 7.0 °C and 3.9 °C, at 3 mm depth 18.0 °C, 17.6 °C and 14.8 °C, respectively. BWS score decreased in the -5 °C group on the day after cooling, other temperature groups were not affected. Histologically, cryo-injury was observed only in the -5 °C group. Focal cooling of the cortex for 1 h above the temperature of 0 °C did not induce any irreversible histological change or motor dysfunction [87].

Cooling itself may not be responsible for any additional foreign body response. Although there is minimal gliosis close to the region of cortical contact with the Peltier, it is similar to the response provoked by any foreign body and cannot be attributed to cooling [88]. In vivo experiments in rats and cats using Peltier device lasting 10 months long resulted in minimal gliosis near the area of cortical contact with the device, which changes were also seen in sham-operated rat cortex [82].

The effect of cooling can be evaluated through nociceptive pain tests. In one case hot plate tests and thermal withdrawal tests at 52 °C were conducted, latency in escape behavior was measured in restrained, awake rats. The cooling component was a 6 mm x 6 mm, PID-controlled Peltier chip, with an attached thermocouple probe and a copper heat sink. Heat was transferred via 5 °C Ringer's solution. Brain surface temperature was controlled and cooled to 20 °C, 15 °C and 10 °C. In the thermal withdrawal tests, 20 °C cooling resulted no latency in escape behaviour, however, significant latency occurred at 15 °C. Histological examination showed no neuronal damage after 2 weeks of implantation. This study showed, for the first time, that focal cooling of the sensorimotor cortex at 15 °C was able to suppress nociceptive pain with a minimal influence on sensorimotor functions [89].

It should be noted that experimental epilepsies in rat models typically result in 60–80 s long seizures every 2−3 min, which is an unrealistically high frequency for almost all human epilepsies. This suggests that induced focal seizures in rodents would require a much larger temperature reduction than epileptic seizures in humans, meaning that in human cases the current to power the device and the amount of heat that needs to be dissipated would possibly be lower, too, since these are directly linked to the necessary degree of cooling [82].

Thermoelectric cooling is possibly the most extensively researched approach to focal cooling. It offers specific, targeted cooling of selected brain areas, whether it is a superficial or a deeper structure. Most Peltier devices used in animal studies are very similar in size, performance and construction materials, which makes the results easily comparable. The possible degree of cooling is large enough to reach temperatures that also inhibit normal neural functions so a safety limit can be established while ensuring maximum cooling efficiency at the same time to cancel epileptic discharges. A major downside to this method is the inevitable activation of astroglial cells at sites close to or in contact with the device. Although cooling does not induce additional astrogliosis on top of the normal foreign body response, this phenomenon can hinder the performance of devices equipped with additional sensors, such as temperature measurement or EEG.

4.6. Liquid coolant

Similarly to irrigation with iced saline, focal cooling devices can utilize some sort of liquid as their cooling medium. In most cases, a metal tubing shaped as a loop or a coil serves as the cooling surface through which chilled saline, ethanol or methanol is circulated. Usually, the degree of cooling is manipulated through the adjustment of the liquid flow rate that allows for precise target temperature control. In vitro studies with brain slices are also conducted in this manner, in this case, the chilled perfusate itself functions as the cooling medium.

In experiments with rat brain slices exposed to 4-AP, 4-AP plus bicuculline, and Mg^{2+}-free artificial cerebrospinal fluid (aCSF), field excitatory synaptic potentials as well as spontaneous epileptiform activity were consistently aborted, when the aCSF was cooled to 28–34 °C. By adjusting the flow rate of cold aCSF, slow and rapid cooling paradigms were studied, both of which completely, but reversibly, aborted epileptiform discharges. Interestingly, only a slight temperature drop of 1–2 °C was needed to terminate the discharges with rapid cooling, while cooling slower gradually decreased the amplitude of EDs, requiring a much longer time before complete cessation. Slow cooling reduced discharge frequency by 90 % at absolute temperature drops to 21–23 °C and completely terminated them at 14–15 °C. Hypothermia seemed safe in these models, because slices could tolerate as long as 2 h of cooling at temperatures as low as 8 °C [90].

Slowly cooling the perfusate bathing hippocampal slices from 34 °C to 20 °C can reversibly abolish the rapid gamma oscillations of approximately 40 Hz, while preserving normal evoked excitatory synaptic potentials. The observation, that evoked synaptic responses were maintained but gamma activity disappeared, suggests that cooling may

limit paroxysmal activity (EDs) without shutting down normal brain electrical activity [91].

Using circulation of chilled fluid, Burton et al. studied the effects of cooling kindled seizures in awake, freely moving rats. The fabricated implant comprised of a pure copper tubing flat coil contacting the dorsal brain surface. Cooling to target temperatures of 24 °C and 27 °C was achieved by circulating 8 °C and 16 °C water, respectively, at a flow rate of 10 mL/min. Both target temperatures were reached by 180 s of cooling. After discharge duration and seizure score (0–5) were significantly reduced compared to controls, but no significant difference was found between the two cooled groups for these parameters [66].

A microfluidic device, termed the cooling chip was fabricated by Cooke and colleagues for reversible deactivation of the neocortex to examine its functional macro-circuitry, behavioral and cortical plasticity in rodents, ferrets and primates (Fig. 7). The device, termed the cooling chip consisted of a thin silicone tubing (through which chilled ethanol was circulated) embedded in mechanically compliant polydimethylsiloxane (PDMS). PDMS was tailored to compact device dimensions (as small as 21 mm^3), MR compatibility was ensured due to the absence of metal parts. The device was shaped to two different forms: a gyral design for contacting the brain surface, and a sulcal design for insertion into a sulcus. The silicone tubing for the gyral design was shaped as a coil embedded in PDMS, the sulcal design tubing was forming a loop with variable length to be able to cool deeper sulcii. After implantation, localized cortical temperature drop from 37 °C to 20 °C could be achieved in less than 3 min and target temperature could be maintained indefinitely. Cooling to 20 °C and below abolished sensory activity in the species investigated and spontaneous activity was frequently aborted too [92].

An earlier construct was the so-called cryoloop developed by Lomber et al. The device consisted of straight 23 gauge hypodermic stainless steel tubing and cooling was effected by passing chilled methanol through its lumen. The circulated methanol was cooled by passing it through a bath of methanol and dry ice pellets reaching a temperature as low as −75 °C. The cryoloops were implanted in cats and monkeys and were maintained there for periods in excess of 2 years. Even for this long duration, researchers observed no structural, metabolic of functional changes in the deactivated tissue, and full reversibility of cooling-induced effects was maintained. Cooling the cryoloop and cortex from 38 °C to 8 °C silenced visually evoked activity in vigorously responding neurons. During cooling of cryoloops to even 0 °C afferent axons remained active and 20 °C was identified as the critical temperature below which neurons fail to be activated. Temperatures above 24 °C showed little diminution in evoked activity. There is a very steep gradient between 24 °C and 20 °C along which activity progressively decreases. Effect of the cooling was restricted to 1.5–2.5 mm distance from the cryoloop even when cryoloop temperature was reduced to 1 °C. Positions 2 mm distant from the cryoloop are cooled only slightly, and positions 5.6 mm distant are completely unaffected by the cooling. Cortical tissue adjacent to the cryoloop reaches a deactivating temperature of 20 °C within 10 s. For deep cortical layers at 1.3 mm, the deactivating temperature lags by approximately 1−2 min. 2−3 min is required for temperatures at all distances from the cryoloop to return to normal body temperature [93].

Coomber et al. induced focal hypothermia in the guinea pig auditory cortex with a cooling loop to determine the extent to which reversible cooling deactivation spreads throughout the brain. Dry ice cooled ethanol was circulated in a 4 mm diameter cooling loop made of a stainless steel tube with 0.25 mm lumen diameter. Cortex temperature could be manipulated by altering the speed of the pump. Auditory "click"-evoked potentials were recorded before cooling, and then at progressively lower cooling loop temperatures (20 °C, 15 °C, 10 °C, and 2 °C), and finally after a 20 min recovery period. Cooling the surface of auditory cortex to 2 °C reduced the temperature in all layers of primary auditory cortex below 20 °C. Temperature gradients in the cortex ranging from 10 °C to 20 °C up to a depth of 2000 μm were observed. Cooling the surface of the cortex to 2 °C abolished or significantly attenuated auditory-evoked field potentials in all cortical layers [94]. The liquid coolant approach was tested in human clinical trials. In one study, researchers implanted eighteen patients scheduled to undergo surgery for medically intractable epilepsy with a cooling probe. It consisted of a stainless steel chamber infused with chilled saline, cooling the device close to, but not below, 0 °C (Fig. 8). The thermal gradients created under the cooling probe during active cooling were examined

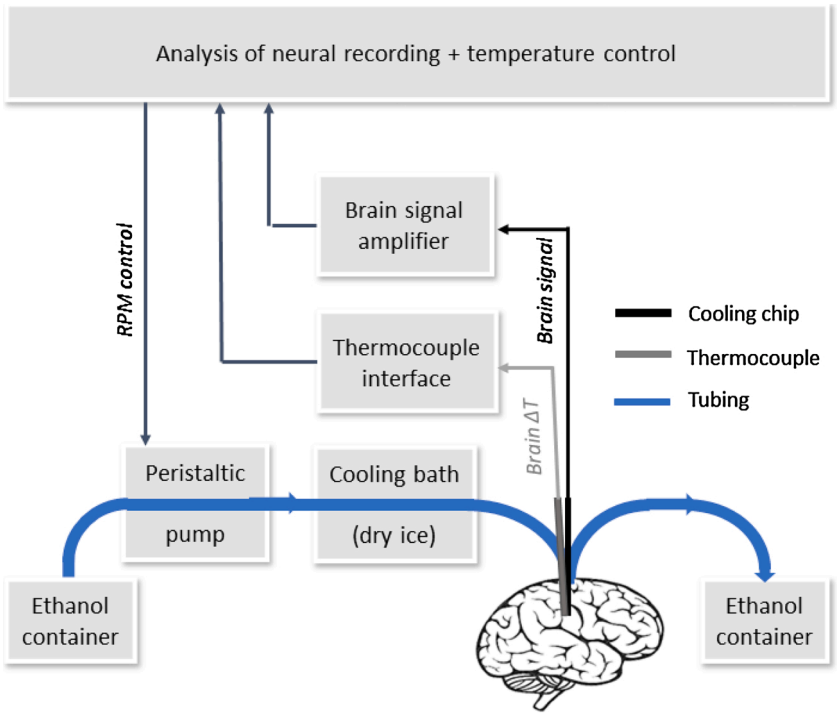

Fig. 7. Schematic layout of the experimental design for the cooling chip. Redrawn based on [92].

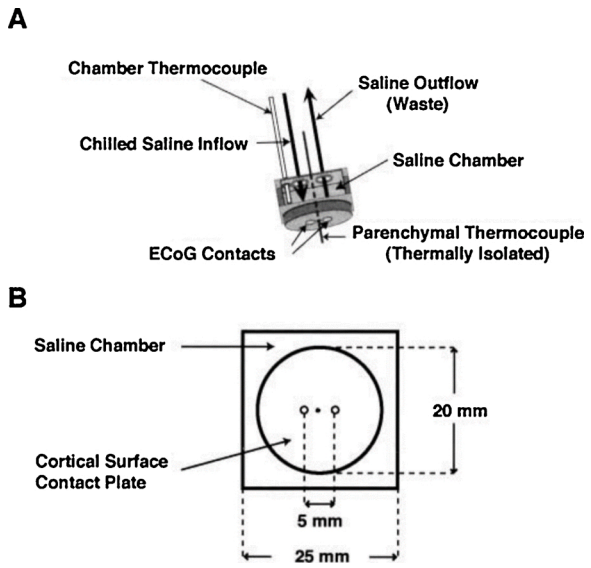

Fig. 8. Design of the cooling chamber for human trials: **(a)** The arrows show the direction of saline inflow and outflow, there are separate thermocouples for chamber and parenchymal temperature measurement; **(b)** The probe from below indicating the two silver contacts spaced 5 mm apart [94].

along with the effects of cooling on spontaneous and stimulus-evoked local EEG activity. Also, critical sites of language in the left frontal lobe were cooled in two patients. In all cases it was possible to cool the chamber temperature to between 0 °C and 3 °C. Temperatures that disrupt local synaptic activity (below 20 °C) were achieved throughout the gray matter directly below the cooling probe (<4 mm thickness). Tissue temperatures at brain sites farther than 6 mm from the probe surface do not reach this threshold. In the verbal counting task, when the temperature of the cooling probe chamber had been decreased and maintained within a range of 1 °C–3 °C, both patients demonstrated qualitatively similar speech dysfunction. Following rewarming, both patients performed the counting task in a normal fashion [94].

An active cooling device using chilled liquid can be fabricated from a standard ECoG grid (Fig. 9). Smyth et al. integrated a custom made liquid cooling system circulating 4 °C saline on an ECoG grid to examine the thermal characteristics of canine and human brain. The grid was made in two sizes: 4cm × 4cm = 1600 mm^2 and 2cm × 4cm = 800 mm^2 for humans and dogs, respectively. Cooling experiments took place before the resection of a neocortical epileptogenic focus in human patients. Dogs were implanted with perfused grids and actively cooled for 2 h. Cooling of 0.6–2 °C was achieved both actively and passively to a depth of 10–15 mm from the cortical surface. A stable 14-18 °C decrease in temperature was measured in dogs after closing the craniotomy, however, no effect on brain activity was observed [65].

Circulating chilled liquid can result in temperature drops similar to that of a Peltier device, depending on the coolant used. Geometries and size of these devices are much more easily tailored. This might also be a downside regarding the comparability of the results. Because the liquid needs continuous refrigeration and circulation, some additional equipment is necessary, such as cooling reservoirs and pumps, making it less mobile than a Peltier device. However, cooling with liquid does not need any heat sinks, that are a must for thermoelectric cooling.

5. Conclusion

Therapeutic hypothermia has various applications, in both systemic and focal ways. Its beneficial effects in a number of neurologic diseases have been researched and recognized for over several decades.

Historically, general or systemic cooling was studied first in humans with disorders such as epilepsy or TBI, and it remained in clinical practice to aid in treating patients suffering from hypoxic ischemic encephalopathy or post cardiac arrest. Systemic hypothermia is however not well suited for epilepsy treatment, because the prolonged cooling required could raise risks of cardiopulmonary compromise and other side effects.

Localized cooling is much more researched recently as it offers important advantages over systemic hypothermia: cooling affects only the targeted area, temperature reductions can exceed that of reached with systemic cooling, generalized side effects are avoided, devices used are much smaller and practical and are easier to control.

Among these, the Peltier device and cooled fluid circulating devices are the most promising approaches. Both offer significant temperature reduction in the brain tissue and exert their effects only focally. Numerous studies show that these devices are highly effective in reducing the amplitude of epileptic discharges and terminating ongoing seizures while preserving normal brain activity, though inactivating temperatures of 15 °C or below are readily reached, too. A Peltier device seems more adequate to long term implantation, as it needs fewer accessories than a liquid circulating implant.

However, long-term clinical application of such devices needs to be considered with extra care regarding the materials used for contacting the human brain. Biocompatibility must be ensured by encapsulation of device surfaces. Good candidates for soft encapsulation are silicon,

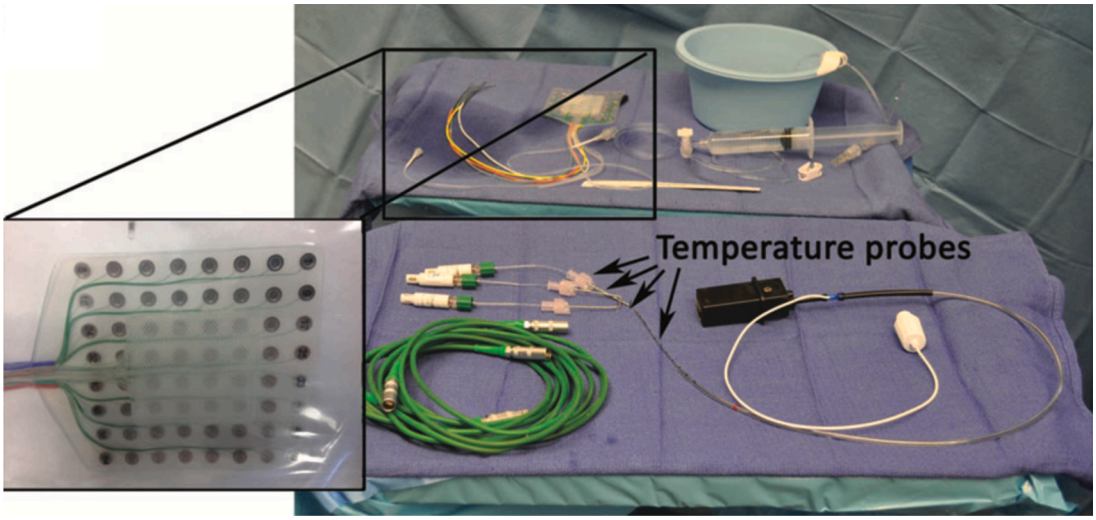

Fig. 9. The sterile cooling grid for measurements in humans with temperature probes [65].

polyimides, liquid crystal polymers, to name a few [96]. Ethical questions of long-term brain machine interfaces should also be clarified before the translational potential of these cooling devices could be realized.

Another obstacle to overcome in the future is the induced foreign body response by implanted devices, which is rather related to the device itself, and not cooling effect it exerts on the nearby tissue.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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