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Transcortical Cooling Inhibits Hippocampal-kindled Seizures in the Rat

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Summary: *Purpose:* When epileptogenic regions encroach on eloquent brain, surgery may incur unacceptable deficits. Reversible cooling may control seizures while preserving function. We describe the effects of cooling kindled seizures in awake, freely moving rats.

Methods: We kindled rats after placement of a bipolar electrode and a copper cooling coil in dorsal hippocampus. Fully kindled animals (three consecutive grade 5 seizures) were cooled to one of two target temperatures (24° or 27°C) for 3 min preceding a kindling stimulation and 2 minutes after. We compared seizure score (0–5) and afterdischarge duration (ADD) with and without cooling. Target temperatures were confirmed in identical animals by using a needle thermocouple advanced to the kindling target while circulating coolant.

Results: Circulation of 16°C and 8°C coolant reliably achieved transcortical cooling of the hippocampal target to 27.0 ± 1.2°C and 23.8 ± 2.0°C, respectively, by 180 s. Cooling with 16°C coolant (n = 5) significantly reduced seizure scores from 5 to 2.57 ± 1.56, and ADD from 142 ± 94.5 s to 45.7 ± 20.5 s. Cooling with 8°C coolant (n = 5) reduced seizure scores from 5 to 2.0 ± 0.42, and ADD from 132.3 ± 29.6 s to 55.5 ± 25.9 s. In 33.3% of all cooled stimulations, grade 0 seizures resulted; grade 5 seizures recurred during subsequent stimulations when cooling was withheld.

Conclusions: Fully kindled, tonic–clonic seizures can be suppressed or aborted with periictal cooling of the kindling target. Anticonvulsant activity occurred at temperatures well above those known to result in tissue injury or inhibition of normal neurologic function. These findings have important implications for the potential use of implantable cooling devices in humans with refractory epilepsies in or near eloquent cortex or dominant hippocampal formations. **Key Words:** Kindled seizures—Cooling—Rat—Hippocampus.

When epileptogenic regions encroach on or involve eloquent cortex, surgical options are limited and may incur unacceptable neurologic deficits. Resection of mesial temporal lobe structures (selective amygdalohippocampectomy or anterior temporal lobectomy) has neuropsychological sequelae that, although often acceptable given the benefits of seizure freedom, still remain a significant liability in terms of surgical treatment of epilepsy (1–3). A means of reversibly deactivating epileptogenic regions, instead of permanently resecting brain structures, would be a very attractive method of relieving intractable epilepsy.

Cooling has been shown to suppress reversibly normal central nervous system activity (4–12). Several in vitro and in vivo studies have demonstrated that focal brain cooling also reduces epileptiform activity in seizure models (13–18) and in humans (19–21). As such, direct brain cooling may represent a viable treatment modality for refractory seizure disorders, particularly from seizure foci not amenable to surgical resection. The development of an implantable, electrically driven cooling device, potentially coupled with closed-loop seizure-detection software, may result in a useful treatment for refractory epilepsy. This technology has been applied to an animal model of acute seizures by our group [topically applied 4-amino-pyridine (16,17)], but has yet to be implemented in a reliable chronic seizure model. This step is a necessary precursor to the development of an implantable system for use in humans.

Amygdaloid/hippocampal kindling models have been used for decades to investigate epileptogenesis and responses to therapeutic interventions (22–24). The hippocampal kindling model provides the capability to initiate robust electrographic and behavioral seizures in a specific region of hippocampus in a controlled, reproducible fashion. An additional advantage of the hippocampal

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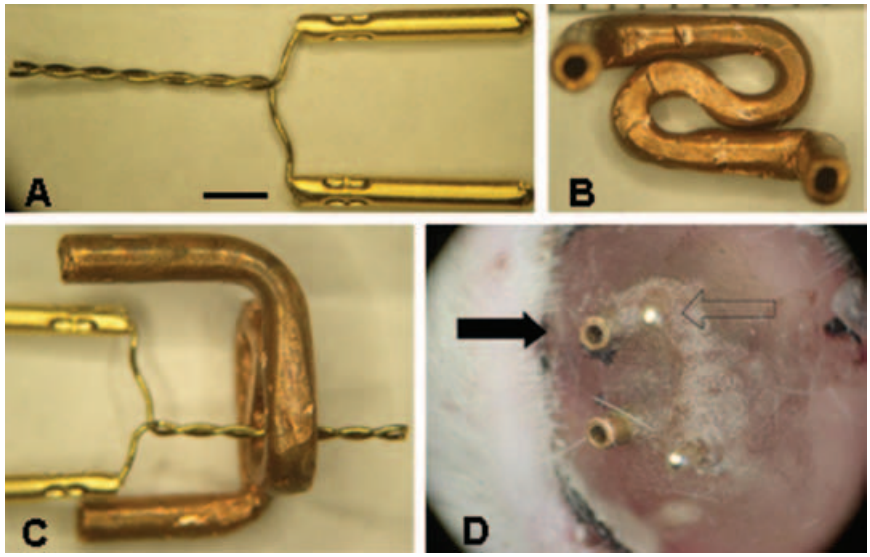

FIG. 1. Custom copper cooling coil implant. **A:** Custom twisted bipolar stainless steel insulated kindling electrode with gold male connector pins. **B:** Implant comprised of pure copper tubing (99.9%,.876 mm ID, 1.59 mm OD), machined in a tight, flat coil contacting the dorsal brain surface. Ingress/egress ports extend superficially for attachment to plastic tubing circulating coolant. **C:** The anteromedial bend of the implant encircles the perpendicular kindling electrode, allowing direct cooling of brain tissue for several millimeters around the kindling electrode entry point. **D:** Skull surface showing implant with surrounding acrylic cement (*open arrow*, male electrode pin; *solid arrow*, ingress/egress cooling coil port). Scale bar in **A**, 2 mm, applies to **A–C**.

kindling model for evaluating prolonged brain cooling for epilepsy is the ability to initiate multiple, recurrent seizures at the time of the investigator's choosing. This obviates the need for seizure-detection algorithms (17,25–27) to test the effect of cooling on randomly occurring seizures.

Ultimately to advance the use of cooling in selected patients, we have developed a model that allows us to investigate the effect of focal cooling on kindled seizures in awake, freely moving rats.

METHODS

Animal use

Experiments were performed on male Sprague–Dawley rats weighing 250–350 g by using a protocol approved by the Washington University Animal Studies Committee. Animals were housed in a vivarium with a 12-h light/12-h dark cycle and access to food and water ad lib. Animals with permanent implants had trimethoprim-sulfamethoxazole (Bactrim; 4 ml/L) added to their water supply. The animals were sacrificed with intraperitoneal injections of pentobarbital (Nembutal; 125 mg/kg) at the completion of the kindling/cooling paradigm or before histologic study.

Long-term implantation procedure

The rats were anesthetized with 1.5–2.5% halothane and placed in a stereotaxic frame with a nosepiece (David Kopf Instruments, Tujunga, CA, U.S.A.). Body temperature was maintained at 34.5 to 35.5°C with a radiant heating pad regulated by feedback from a rectal thermocouple. The scalp was clipped of hair and prepared with povidine-iodine (Betadine) before midline incision. By using a high-speed rotary drill, a right parietal craniectomy was performed, based on the midline sagittal suture, and extending from the coronal suture to the lambdoid suture. Venous bleeding was controlled with absorbable gelatin foam (Gelfoam). Next, a twisted bipolar Teflon-coated stainless steel kindling electrode (0.2 mm diameter; California Fine Wire Company, Grover Beach, CA, U.S.A.) attached to gold male connector pins (MRO Electronic Supply, Ltd., Vancouver, B.C., Can.) was placed stereotaxically into the dorsal hippocampus (Fig. 1A). Target coordinates measured from bregma were A-P, –3.0 mm; M-L, +2.0 mm; and depth, 3.3 mm (28). After stereotaxic placement of the kindling electrode, a custom copper cooling coil (Fig. 1B and C) was placed on the surface of the hemisphere, encircling the entry point of the bipolar kindling electrode. The implants were then secured into place with acrylic dental cement with the electrode pins and ingress/egress ports of the copper cooling coil exposed above the cement for later access (Fig. 1D). Animals were allowed to recover for 1 week after electrode placement, before initiating afterdischarge (AD) threshold testing, kindling stimulation, and cooling paradigms.

AD threshold testing and kindling acquisition

The AD threshold and duration were determined for each animal by stimulating at 60 Hz with 1-ms square-wave pulses for 1 s. The initial 100-μA current amplitude was increased in 100-μA steps every 60 s until an AD was observed (Fig. 2). Rats with an AD threshold >500 μA were excluded. Bipolar EEG was digitized (200 Hz) and stored by using PC-based commercial hardware (Biopac MP150) and software (AcqKnowledge 3.7.3; Biopac Information Systems, Goleta, CA, U.S.A.) and a custom-made stimulation/monitor toggle switch. Animals were stimulated twice daily, $\geq$6 h apart, at 60 Hz with 1-ms, 400-μA square-wave pulses for 1 s until three consecutive grade 5 seizures were observed. AD duration and behavioral seizure score [Racine seizure classification (24)] were measured after each kindling stimulation. Seizures were classified as follows: grade 1, behavior arrest; grade

FIG. 2. Representative afterdischarge recording. This is a 14-s grade 2 seizure recorded during the kindling-acquisition phase, before the animal progressed to fully kindled grade 5 seizures.

2, facial twitching or clonus; grade 3, unilateral forelimb twitching or clonus; grade 4, bilateral forelimb clonus and rearing; and grade 5, generalized tonic–clonic activity with loss of posture. The frequency of AD failure and the total number of kindling stimulations were measured for each animal during the kindling acquisition phase.

Seizure cooling

Once each animal achieved three consecutive grade 5 seizures, periictal cooling was initiated. Each animal underwent two trials of cooling with one of two coolant temperatures (8 or 16°C H_2O). For each trial, implanted hippocampi were cooled to one of two target temperatures (24 or 27°C) by connecting the copper coil to water cooled to 8 or 16°C and flowing at 10 ml/min for 3 min before, and 2 min after, three consecutive ictal events (standard kindling stimuli, 60 Hz with 1-ms, 400-μA square-wave pulses for 1 s). Similar to the kindling acquisition phase, cooled ictal events were initiated twice daily, separated by ≥6 h. Seizure scores (0–5) and afterdischarge durations (ADDs) were recorded with and without cooling, immediately after kindling stimulation delivery. Between the first and second cooling trials, standard kindling stimulations were administered until three consecutive grade 5 seizures were confirmed (Fig. 3). Coolant temperatures were regulated by using a bipolar temperature controller (CL-100; Warner Instruments, Hamden, CT, U.S.A.) and in-line solution cooler (SC-20; Warner Instruments).

Target temperature confirmation

Although it would have been preferable to record directly the brain temperatures of the main group of experimental animals undergoing kindling acquisition and cooling trials, it was not feasible to measure brain temperatures with a stereotactically placed thermocouple at multiple depths in a freely moving rat also implanted with a kindling electrode, anchor screws, and a cooling coil implant. Instead, we performed a series of experiments in an identical group of animals by using similar experimental conditions. We performed right parietal hemicraniectomies on eight rats exactly as described earlier. Next, a needle

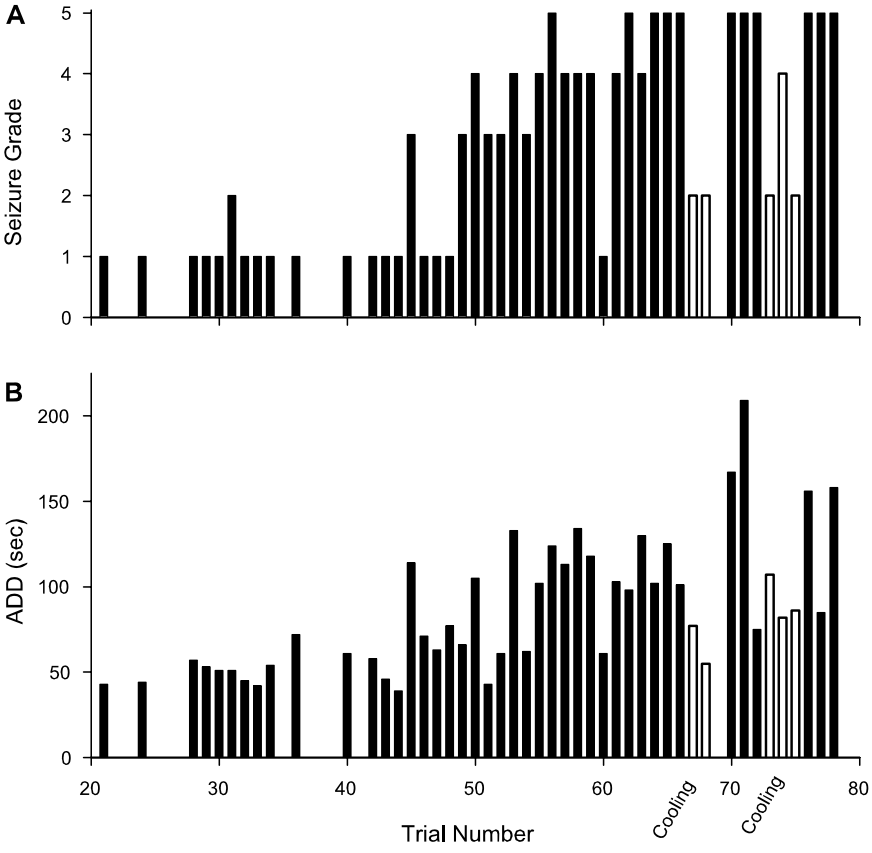

FIG. 3. Sample kindling and cooling protocol. **A:** Bar histogram showing Racine seizure grade during kindling-acquisition phase and two cooling trials in a representative animal. **B:** Bar histogram showing afterdischarge duration during kindling-acquisition phase and two cooling trials in a representative animal. Stimulations were given twice daily, separated by ≥6 h.

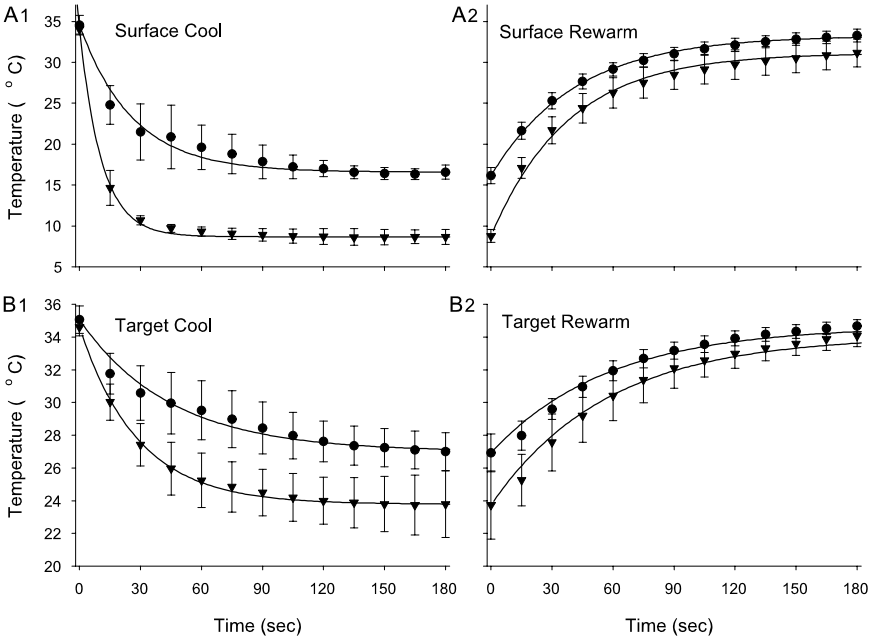

FIG. 4. Effect of cooling on brain temperature. **A_1:** Plot of surface temperature over time for perfusion with 16°C (*circles*) and 8°C (*triangles*) water. **A_2:** Rate of rewarming after discontinuing cold-water perfusion. **B_1, B_2:** Hippocampal target temperatures during identical paradigm. Note the smaller relative temperature difference in **B_1** at the target compared with **A_1**. All eight curves were fit to single exponentials.

thermocouple (HYPO-33–1-T-G-60-SMP-M; Omega Instruments, Stamford, CT, U.S.A.) connected to a temperature controller (Omega CN1001TC) was placed stereotaxically above the dorsal hippocampal target (A-P, –3.0 mm; M-L, +2.0 mm) at the dura. The needle thermocouple was placed inside an insulating plastic sheath (PVC tubing, ID 0.5 mm) and encircled by a custom copper cooling coil also placed directly on the exposed dural surface. The implants were then secured into place with acrylic dental cement with the PVC insulating sheath and ingress/egress ports of the copper cooling coil exposed above the cement. Once the rat core temperature and brain surface temperatures stabilized, the needle thermocouple was advanced stereotaxically, while circulating coolant at either 8 or 16°C. We measured temperature at the dural surface and at 0.5-mm intervals to a depth of 6 mm, including an additional measurement at the target depth of 3.3 mm. Measurements at the dural surface and the target were recorded during 3-min periods of coolant circulation and rewarming (Fig. 4). An additional temperature gradient-versus-depth curve (Fig. 5) was generated by circulating coolant (8 or 16°C) for 3 min before stereotactically advancing the needle thermocouple to, and beyond, the target in eight animals.

RESULTS

Target temperature confirmation

Circulation of 16°C and 8°C coolant reliably achieved transcortical cooling of the hippocampal target to 27.0 ± 1.2°C and 23.8 ± 2.0°C at 180 s, respectively (n = 8 animals) (see Figs. 4 and 5). Temperature stabilization occurred mostly by 120 s. Cooling was not instantaneous because of perfusion dead time and the specific heat of the copper tubing.

Kindling acquisition

Three consecutive grade 5 seizures were achieved after a mean of 42.1 ± 13.8 SD (n = 10 animals) kindling stimulations. The rare inability to elicit consistent grade 5 seizures was attributed to electrode failure or infection, and those animals were not analyzed. In total, 10 animals with dorsal hippocampal kindling electrodes and copper

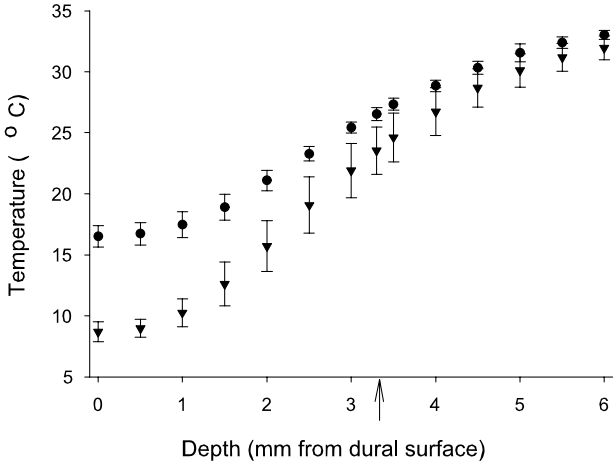

FIG. 5. Brain temperature gradient as a function of distance from dural surface/implant produced after 3 min of continuous perfusion with 16°C (*circles*) and 8°C *(triangles*) water. Kindling target depth was 3.3 mm (*arrow*). Note that the temperature differential between coolant temperatures becomes negligible beyond 3.5 mm and approaches core temperature beyond 5 mm. Circulation of 16°C coolant (*circles*) yields target temperature of 27.0°C (ΔT = 8.0°C). Circulation of 8°C coolant (*triangles*) yields target temperature of 23.8°C (ΔT = 11.2°C).

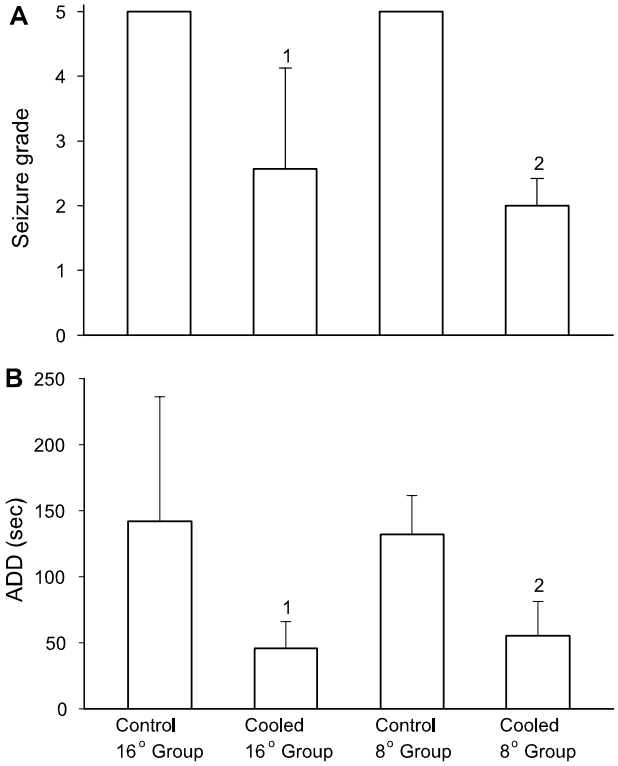

FIG. 6. Effect of cooling on seizure grade and afterdischarge duration (ADD). **A:** Cooling with 16°C and 8°C coolant significantly reduced seizure grade from corresponding controls (1, 2: $p < 0.001$; analysis of variance (ANOVA) followed by Student–Newman–Keuls method). No significant difference in seizure score was found between the two coolant temperatures ($p = 0.28$). No error bars are present for seizure grade in controls because all seizures, by our criteria, were grade 5 before initiating cooling. **B:** Cooling with 16°C and 8°C coolants significantly reduced ADD from corresponding controls (1, 2: $p < 0.05$; ANOVA followed by Student–Newman–Keuls method). No significant difference in ADD was found between the two coolant temperatures ($p = 0.77$).

cooling coil implants with consistent grade 5 seizures were subjected to a cooling paradigm (see Fig. 3). The average grade 5 ADD before initiating cooling was 128.1 s ± 13.8 SD (range, 39–420 s).

Cooling effect on fully kindled seizures

Cooling of the target by using 16°C coolant (n = 5) significantly reduced seizure scores from 5 to 2.57 ± 1.56 and ADD from 142 ± 94.5 s to 45.7 ± 20.5 s; cooling using 8°C coolant (n = 5) significantly reduced seizure scores from 5 to 2.0 ± 0.42 and ADD from 132.3 ± 29.6 s to 55.5 ± 25.9 s (paired Student's *t* test; Fig. 6). Nineteen of the total 57 cooled stimulations were Racine grade 0 (33.3%), without electrographic afterdischarge. No significant difference was found between the two cooled groups for seizure score or ADD (Fig. 6, ANOVA followed by Spearman–Newman–Keuls method), likely because of the small temperature difference ($\Delta T = 3°C$) at the hippocampal cooling target. Grade 5 seizures recurred for subsequent kindling stimulations with cooling withheld in all animals, except one case, in which the electrode and cooling coil implant became loosened during the second cooling trial. Only data from the first trial were analyzed for that animal.

DISCUSSION

Selection of kindling target and cooling device

Methods previously described for focal brain cooling include cooling coils (29) and thermoelectric Peltier devices (30,31). Our earlier attempts to implant a small Peltier chip permanently with the necessary heat-sink and power-supply wires, in addition to hippocampal kindling electrodes, proved difficult because of the bulk of the implant and the relatively long time course (≤4 weeks) of the experiments needed to kindle fully and then subsequently cool seizures. However, we found that our copper cooling coil implant (see Fig. 1) was robust and stable, and for the purposes of brief periods of surface cooling was quite reliable. In between cooling sessions, the animals were able to move freely without any tethering wires or tubing. The implants were well tolerated by the animals; the only complications identified were occasional infections or loosening of the implant over time. Ideally we would have liked to record data from a larger group of animals to reduce the variability in the results; however, the complexity of each implant procedure and the time required to kindle each animal fully made implanting additional animals prohibitive. One of the greatest challenges we encountered in developing this model was miniaturizing the cooling and thermocouple elements—as such, modifying these or other cooling devices for larger animal models or human use may actually be less technically challenging.

Although we would have preferred direct cooling of the kindled hippocampus, bringing any type of permanently implanted cooling device into direct contact with the hippocampus is significantly less practicable and might have disrupted kindling acquisition. With only 2.5 mm of intervening cortex between the surface and the dorsal hippocampus, we hypothesized that transcortical cooling of the hippocampus would prove to be a reliable method to achieve hippocampal cooling. Our temperature-confirmation experiments demonstrated that decreases in temperature from core body temperature to 23.8–27.0°C ($\Delta T = 8.5–11.5°C$) at the target were consistently attainable with the coolant temperatures used (see Fig. 4).

Because ΔT beyond a depth of 4 mm is diminishingly small (Fig. 5), we selected the dorsal hippocampus as the kindling target for these experiments. It lies within 2.5 mm of the parietal cortical surface (28), making this region technically accessible to transcortical cooling methods. Ventral hippocampus and basolateral amygdala, although robust kindling targets, would be more difficult to cool because of their distance from the cortical surface and were not chosen for this reason.

It should also be emphasized that we selected the hippocampal kindling seizure model to test the effect of cooling on epileptiform activity because fully kindled hippocampal seizures are exceedingly robust. These seizures involve generalized tonic–clonic activity that lasts ~2 min, and represent a "worst-case" candidate seizure for a therapeutic cooling device. Given the severity of these seizures, it is gratifying that focal cooling near the initial kindling target had such potent anticonvulsant activity. In most cases, cooled seizures did not fully generalize, resulting only in facial twitching, behavior arrest, or no visible seizure activity, although grade 4 or 5 seizures were occasionally observed during cooling trials. Average seizure scores decreased from consistent grade 5 seizures to grade 2.6 and 2 with a nonsignificant trend ($p = 0.28$; ANOVA) for a temperature-dependent effect (16°C and 8°C coolant, respectively). ADD was reduced by nearly two thirds for both temperatures tested, but a similar temperature-dependent effect was not evident.

Selection of cooling temperatures

The temperatures required to inhibit or abort ictal activity have not been firmly determined and may vary depending on the seizure type and location. However, based on current data, it would seem likely that a temperature range exists in which ictal activity can be abolished without significant loss of neurologic function or permanent tissue injury. Prior animal (16,31,32) and hippocampal slice (15,18) experiments have shown that temperatures in the range of 20–28°C may have anticonvulsant activity without limiting normal electrical activity. Moreover, prolonged cooling of cat cerebral cortex to temperatures in this range did not result in permanent structural, metabolic, or functional changes after ≤2 years of prolonged cryoloop implantation. However, at <24°C, function was disrupted in the cat cortex (29,33). Given the constraints of the transcortical cooling model, we selected coolant temperatures of 8°C and 16°C to achieve target temperatures at a depth of 3.3 mm between 20 and 28°C.

Clinical considerations

Many patients with pharmacologically resistant seizures have lesional epilepsy arising from regions of cortical dysplasia (34,35), or medial temporal lobe epilepsy (TLE) (36,37). Other patients with nonlesional epilepsy frequently have seizure foci colocalizing with eloquent cortex (38,39). These patients are frequently referred to epilepsy centers for localization of foci and resection of epileptogenic cortex. Unfortunately, many of these patients are not good surgical candidates because the focus resides in or near eloquent cortex, and surgical resection carries significant morbidity (40,41). Surgical options for these patients include partial resection (unsatisfying with a lower chance of seizure freedom), multiple subpial transections (controversial but beneficial in some cases) (42,43), and vagal nerve stimulation (also controversial) (44,45). Techniques that preserve the anatomic and functional integrity of cerebral cortex while disrupting synchronized epileptogenic discharges may offer a better surgical solution for selected patients with epileptogenic foci colocalizing with eloquent cortex. Two such techniques under investigation include direct neurostimulation (46) and direct cooling.

Direct cortical cooling offers the potential advantages of broad adaptability to nearly any superficial or deep-brain structure, and the ability to adjust temperature to achieve seizure control while preserving normal function. Direct cortical or mesial temporal cooling could be used to abort or prevent seizures and may have a number of other uses in functional neurosurgery in place of currently used ablative procedures (such as pallidotomy for movement disorders and cingulotomy for affective/behavioral disorders). For a focal cooling system to provide anticonvulsant activity without impairing normal function, cooling may have to be intermittently administered at regular intervals or combined with seizure-detection algorithms and closed-loop monitoring systems. Although these goals may appear overly optimistic, currently extensive research activity is occurring in the fields of seizure prediction and thermoelectric devices, so clinical devices could be available by the end of the decade (47,48).

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