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RESEARCH ARTICLE

Focal cooling: An alternative treatment for drug-resistant epilepsy in a mesial temporal lobe epilepsy primate model—A preliminary study

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Abstract

Objective: Focal cooling is emerging as a relevant therapy for drug-resistant epilepsy (DRE). However, we lack data on its effectiveness in controlling seizures that originate in deep-seated areas like the hippocampus. We present a thermoelectric solution for focal brain cooling that specifically targets these brain structures.

Methods: A prototype implantable device was developed, including temperature sensors and a cannula for penicillin injection to create an epileptogenic zone (EZ) near the cooling tip in a non-human primate model of epilepsy. The mesial temporal lobe was targeted with repeated penicillin injections into the hippocampus. Signals were recorded from an sEEG (Stereoencephalography) lead placed 2 mm from the EZ. Once the number of seizures had stabilized, focal cooling was applied, and temperature and electroclinical events were monitored using a customized detection algorithm. Tests were performed on two *Macaca fascicularis* monkeys at three temperatures.

Results: Hippocampal seizures were observed 40–120 min post-injection, their duration and frequency stabilized at around 120 min. Compared to the control condition, a reduction in the number of hippocampal seizures was observed with cooling to 21°C (Control: 4.34 seizures, SD 1.704 per 20 min vs Cooling to 21°C: 1.38 seizures, SD 1.004 per 20 min). The effect was more pronounced with cooling to 17°C, resulting in an almost 80% reduction in seizure frequency. Seizure duration and number of interictal discharges were unchanged following focal cooling. After several months of repeated penicillin injections, hippocampal sclerosis was observed, similar to that recorded in humans. In addition, seizures were

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identified by detecting temperature variations of 0.3°C in the EZ correlated with the start of the seizures.

Significance: In epilepsy therapy, the ultimate aim is total seizure control with minimal side effects. Focal cooling of the EZ could offer an alternative to surgery and to existing neuromodulation devices.

KEYWORDS

hippocampal sclerosis, implantable device, neuromodulation, seizure detection, seizure reduction, sensors

1 | INTRODUCTION

Epilepsy affects ~1% of the global population, with ~30% being drug-resistant epilepsy (DRE).¹⁻³ Surgical resection may represent an effective solution, but only ~50% of patients achieve long-term seizure freedom (Engel Class 1a or International League Against Epilepsy [ILAE] 1 outcome scale).⁴⁻⁶ Only a small minority of patients are suitable candidates. For the others, neuromodulation techniques may provide a viable alternative.

Three neuromodulation techniques currently exist. The first is vagus nerve stimulation.⁷ Randomized controlled trials have demonstrated reductions in seizure frequency of 16% to 22%, but only 5.1% of patients achieve complete freedom from seizures. The second is responsive neurostimulation, which has demonstrated a median reduction in seizure frequency of 37.9% compared to 17.3% in the control group. Unfortunately, no patient was free from seizures during the 6-year follow-up.^{8,9} The third technique is deep brain stimulation of the anterior nucleus of the thalamus. A controlled trial showed a median seizure frequency reduction of 40.4% after 3 months,¹⁰ but again only a small proportion of patients (5.5%) achieved a seizure-free interval.¹¹

Although these alternative treatments offer benefits to patients with DRE, they still leave the ultimate goal of a seizure-free life unmet. For instance, no long-term study has demonstrated seizure freedom for the current neuromodulation techniques.¹² Therefore, there is a pressing need for new therapies for patients who are ineligible for surgical procedures. In our efforts to assist this patient group, we explored cooling as a therapy for a deep-seated epileptic focus in a non-human primate (NHP) model of mesial temporal lobe epilepsy (MTLE). Cooling, primarily applied to the brain's surface,¹³ has been used for decades to control seizures.¹⁴ Animal studies have shown its effectiveness in halting seizures and its advantages compared to electrical currents.¹⁵⁻¹⁷ However, the effects of focal cooling in deep brain regions have not been studied extensively. Therefore, we explored cooling applied to hippocampal epileptogenic zone (EZ) using an implantable

Key points

- An implantable cooling lead equipped with temperature sensors and sEEG (Stereoelectroencephalography) electrodes treats deep-seated structures in an animal model of drug-resistant epilepsy (DRE).
- Cooling to 21°C significantly reduced seizure frequency in the hippocampus in a non-human primate model of epilepsy.
- Effects were most noticeable when cooling to 17°C, leading to an almost 80% reduction in seizure frequency.
- Seizure detection was improved by monitoring focal temperature changes in the epileptogenic zone (sclerotic hippocampus) during seizures.

device in a penicillin-induced MTLE model in NHP. Here, we present the results obtained with our prototype device.

2 | METHODS

2.1 | Description of the experiment

2.1.1 | Animals

The study was conducted using two *Macaca fascicularis* (Noveprim; Mauritius Island): one female, 10 years old, weighing 7 kg (monkey A); and one male, 7 years old, weighing 9.6 kg (monkey B).

2.1.2 | Description of the cooling device

A complete cooling system was developed consisting of an implanted part and an external part. The implanted part included a cooling lead, sEEG (Stereoelectroencephalography) electrode (Medtronic DBS lead 3389), and integrated

thermal sensors (IT-24P Ultra Fine Flexible Microprobe, Physitemp Instruments, LLC; USA). The external part, contained within a chamber screwed to the skull, included a Peltier system (Ferrotec GmbH, Germany), thermal diffuser, pipes connected to an external thermal bath (Huber Minichillers 300 OLE, Germany), and a central cannula integrated into the cooling lead. This cannula facilitates precise targeting of penicillin injection at depth, ensuring that it is delivered near the cooling tip, to create an EZ in proximity (<0.5 mm). The cooling lead consists of a stainless-steel vacuum chamber with a copper core; the semi-spherical shape of its tip minimizes the risk of trauma during insertion. The device was coated with biocompatible epoxy (Epo-Tek 301, Epotek, USA) and a layer of Parylene C (Comelec, Switzerland) to improve biocompatibility (Figure 1C). The probe has a diameter of 3 mm, and the thermal sensor diameter is 230 μm . Details can be found in the following French patent <https://data.inpi.fr/brevets/FR3075034> and in [Supplementary Material](#).

LabVIEW-based software (National Instruments, USA) was developed to control the implant, operate the syringe

pump, supply power, and record temperature. The device was connected to a Video-EEG System (BRAIN QUICK, Micromed, Italy).

2.1.3 | Surgery

After a period of habituation to the environment and the primate chair, the NHPs underwent scheduled surgery. The procedure was brief (~4 h) and conducted using ketamine and xylazine (induction: 7 mg/kg ketamine and 0.6 mg/kg xylazine; maintenance: 3.5 and 0.3 mg/kg IM hourly, or adjusted to the depth of anesthesia). Anesthesia monitoring included respiratory and cardiovascular functions (using Draeger Infinity Delta monitors), rectal temperature, and clinical neurological assessments. Ringer's lactate was administered IV at 5–10 mL/kg/h via the saphenous vein. Before the skin incision, 2% xylocaine was applied. Analgesics (buprenorphine 10 $\mu\text{g}/\text{kg}$ before surgery and ketoprofen 1.5 mg/kg postoperatively) and prophylactic antibiotics were administered. Intraoperative

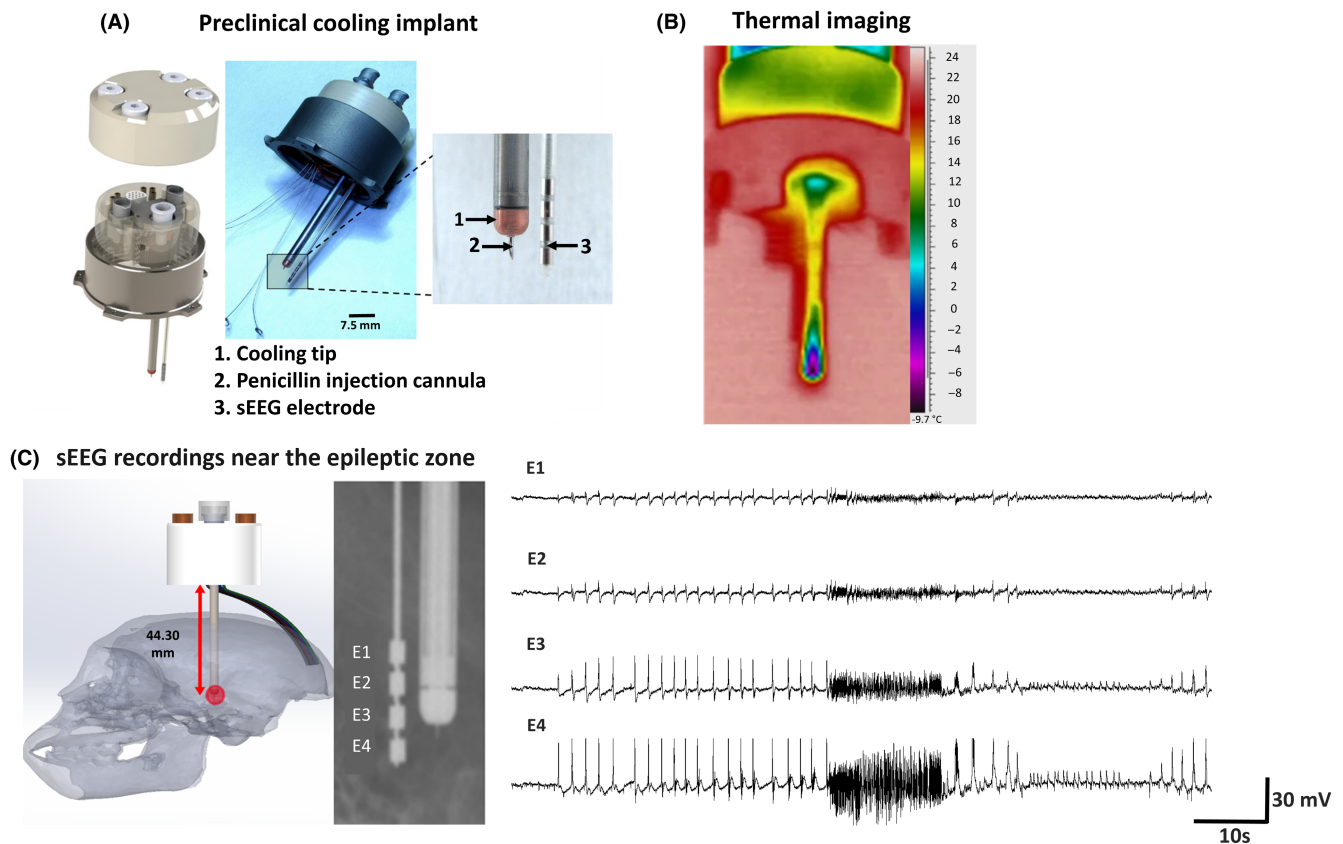


FIGURE 1 (A) Preclinical Cooling Implant: 1. Cooling lead, 2. Penicillin injection cannula, 3. sEEG electrode. The implant used a commercially available Peltier chip to induce the thermoelectric effect. A copper thermal diffuser was attached to the cold side of the chip, connected to the cooling lead. The hot interface was connected to a chamber receiving cold fluid (water block) from an external thermal bath. The external chamber was covered with a PEEK (polyetheretherketone) lid. (B) Thermogram image depicts the cooling lead, which is in an air, with an electric current of 2.6 amperes running through a Peltier chip. (C) sEEG signal during penicillin-induced seizures: contacts are located in the hippocampus, 2 mm from the cooling tip and injection cannula.

electrode impedance measurements ensured proper device function and placement. Recovery was completed in heated cages under staff surveillance. Details of the perioperative management have been described previously.^{18,19}

A cooling lead and sEEG electrodes were unilaterally implanted into the hippocampus (Figure 1C). Prior to the surgery, magnetic resonance imaging (MRI) and ventriculography images were fused to localize the implantation zone within the hippocampus (Figure 2A). The target coordinates were determined before the intervention: −5 mm posterior to the anterior commissure for the cooling lead, and −7 mm for the sEEG lead.²⁰ After placement of the lead and electrodes, the preclinical cooling device and a head-holder were secured to the skull using titanium screws and dental cement.

2.1.4 | Seizure induction

The NHP was positioned on a primate chair (Primate Instruments, USA) allowing free movement and feeding. The implant was connected to an experimental test bench (Figure 2B). Before inducing seizures, hippocampal activity was recorded for 10–20 min. Penicillin G sodium (Sigma-Aldrich) was diluted in sterile water for injection (1000 IU/μL). Using a sterile technique, penicillin (5000–8000 IU) was injected into the left hippocampus 32 mm from the dura and 0.5 mm from the cooling tip, at a rate of 1–1.5 μL/min (total 5–8 μL). Brain activity was recorded for 5–7 h, taking care that the NHP remained contained and comfortable. Post-experiment, the NHPs were observed for a further 3 h. Injections were performed weekly for a total of 203 days (29 weeks). After each experiment, a single dose of diazepam (0.5 mg/kg) was administered intra-rectally before returning the NHPs to their housing.

2.2 | Description of the detection algorithm

sEEG signals (Figure 1C) were processed using MATLAB 2018 software (MathWorks Inc). Signal pre-processing involved applying a 50-Hz notch filter and a low-pass filter at 1020 Hz, and then downsampled to 256 Hz. The software detected two phenomena: seizures and interictal epileptiform discharges (IEDs) or spikes. Seizures were defined as events lasting ≥10 s, separated by ≥3 s. IEDs were short, intermittent electrophysiological events, either monophasic or polyphasic, observed between seizures.²¹

Seizures were detected using a 360-tree random forest classifier, using features extracted from 1-s epochs with

75% overlap. For IED classification, a 160-tree random forest classifier was used, with features extracted from 0.8-s epochs with 75% overlap.

To train our models, a 356-min pre-study data set was acquired across 4 days using signal coming from monkey A. We optimized epoch lengths and overlaps for optimal detection. This final model was applied to our study signals without further updates (see [Supplementary Material](#)).^{22,23}

2.3 | Spatial distribution of temperature

To analyze the temperature distribution surrounding the cooling probe, a Finite Element Model for Multiphysics simulations was used, developed with COMSOL Multiphysics simulation software v 6.0 (Comsol Inc., USA).

Penne's equation

$$\rho_b c_b \frac{\partial T}{\partial t} = \nabla \cdot (k_b) \nabla T + \rho_b c_b \omega_b \cdot (T_b - T) + q_{m,b}$$

And

$$\omega_b = \omega_{b,0} \cdot 3^{\frac{T-T_b}{10}}$$

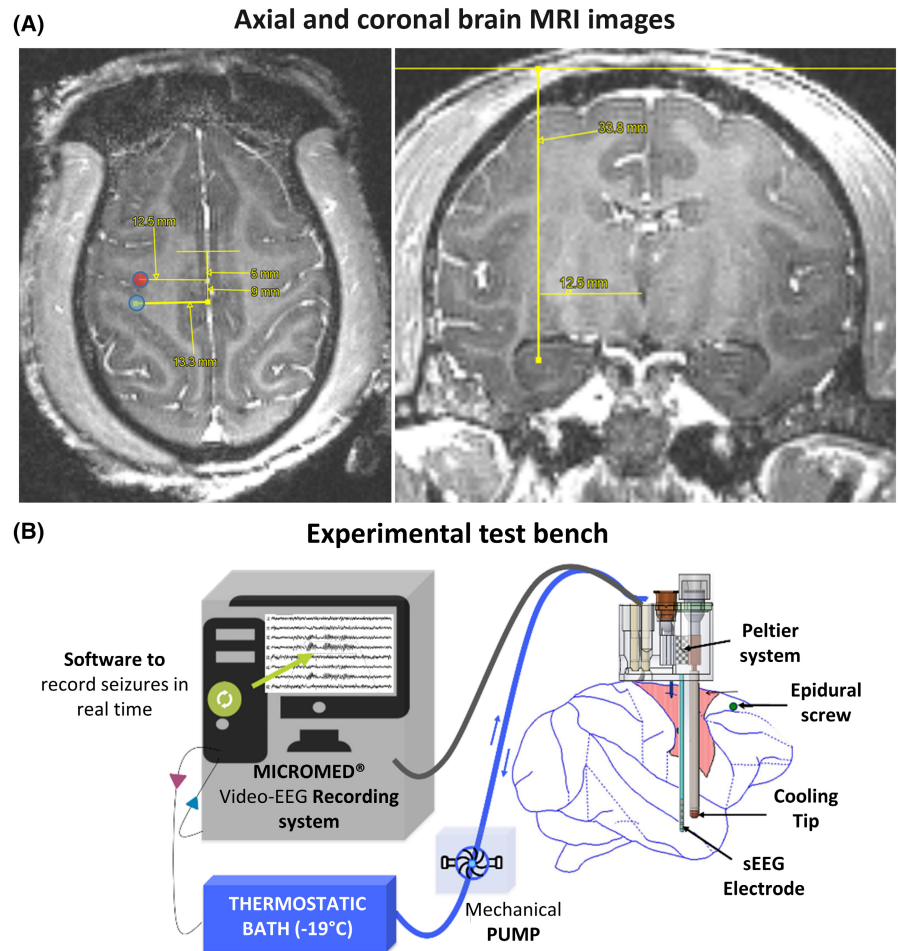
To account for the metabolism and perfusion of brain tissue, Penne's equation was incorporated into the simulation software. Brain bioheat coefficients were defined ($\omega_{b,0} = 7.85 \text{ E-}3 \text{ s}^{-1}$, $k_b = .536 \text{ W/m/K}$, $\rho_b = 1080 \text{ kg/m}^3$, $c_b = 3800 \text{ J kg}^{-1} \text{ K}^{-1}$, $T_b = 37^\circ\text{C}$, $q_{m,b} = .025 \text{ W/cm}^3$).²⁴ In addition, a temperature dependency was introduced into the perfusion coefficient.²⁵

To simplify, assumptions were made about the Peltier element and circulation of the cooling liquid. The temperature in the proximal part of the cooling probe was set to the temperature measured at the distal part. An axisymmetric model was applied, considering the cooling probe's geometry. The simulation returns an isothermal distribution in °C around the device's cooling tip (Figure 5; see [Supplementary Material](#)).

2.4 | Immunohistological studies

At the end of the experimental period, brains were removed and post-fixed overnight, as described previously.¹⁹ Images were recorded 3.9 mm anterior to and 8.7 mm posterior to the injection site. Thirty symmetrical sections were imaged from the ipsilateral and contralateral dentate gyrus (DG) of each section. The mean and standard deviation (SD) of the 30 measurements were calculated for each DG (see [Supplementary Material](#)).

FIGURE 2 (A) Axial and coronal brain MRI images showing the final position of the cooling lead. The red dot in the axial MRI indicates the location of the cooling lead, whereas the blue dot represents the final placement of the sEEG electrode. (B) Experimental test bench: schematic representation of the experimental setup, which includes a Micromed® video-EEG recording system, a thermostatic bath (-19°C) for heat evacuation, a pump connected to the thermostatic bath, and an injection system to administer penicillin.



2.5 | Statistical analysis

Data were analyzed using GraphPad Prism 7.04 software (GraphPad Software, La Jolla, CA, USA). Parameters considered included seizure count, duration, IED, and seizure power for each frequency band. To determine statistical significance, a multiple comparison non-parametric two-way analysis of variance (ANOVA) was applied followed by a Kruskal–Wallis test with Dunn's multiple comparison correction. The threshold for statistical significance was set at $p < .05$.

3 | RESULTS

3.1 | Animal A

3.1.1 | Control session

The MLTE model was characterized over the course of several control sessions. During these sessions, a baseline for seizure discharges following penicillin injection was established for subsequent comparisons. Control sessions were distributed randomly between cooling sessions, and numbered five in total ($n = 5$). Each session lasted 310 ± 51.62 min

on average, with an interval between the first and last seizure of 275.77 ± 42.21 min. The total number of seizures per session was 44.6 ± 20.72 . The number of seizures per 20-min period in control conditions was 3.31 ± 1.39 (median 3.67; interquartile range [IQR] 2.47). Time-frequency analysis of these seizures revealed a predominance of the 1–5 Hz component followed by the 10–15 Hz component. A typical seizure discharge was accompanied by clinical signs of focal hippocampal involvement, such as chewing movements, head and eyes turning contralateral to the discharges, and salivation. When contralateral motor symptoms were observed, the injection was considered to have missed the target, and the sessions were discarded (only 2–3 occurrences). The detection software also identified IEDs. A mean of 5269.40 ± 512.32 IEDs was identified per session, which is equivalent to 19.41 ± 3.12 IED/min (median 20.02; IQR 3.17). Brain temperature recorded at the tip of the lead was $33.43 \pm 1.55^{\circ}\text{C}$.

3.1.2 | Cooling sessions

Cooling sessions were applied once seizure discharges had stabilized. The temperature was measured at the

tip of the cooling lead after applying optimal voltage to the thermoelectric Peltier device. Temperature variations due to residual seizures during cooling were excluded. Two cooling temperatures were obtained: 21°C and 23°C, with five sessions each. Each session lasted an average of 349.35 ± 59.54 min (298.03 ± 72.90 min from the first seizure to the end). The total cooling time was 198.61 ± 26.96 min. Cooling to 21°C reduced the total number of hippocampal seizures recorded during the session compared to control conditions. For instance, the number of seizures per 20-min period was 1.36 ± 0.84 (median 1.31; IQR 0.58) vs 3.31 ± 1.39 in control conditions ($p < .05$). A total of 5001.40 ± 45.14 IEDs were identified, which is equivalent to 14.47 ± 1.87 IED/min (median 15.81; IQR 1.48) vs 19.41 ± 3.12 IED/min in control sessions. During cooling sessions performed at 23°C, no reduction in the number of seizures was observed (5.28 ± 2.92 per 20 min; median 6.68; IQR 4.98). The number of IEDs recorded during cooling to 23°C was 14.33 ± 4.38 /min (median 14.27; IQR 5.15) vs 19.41 ± 3.12 IED/min in control sessions. Seizure duration was not significantly different at the three temperatures tested (control: 36.9 ± 8.75 s, median 32.56; IQR 14.35); cooling to 23°C: 41.5 ± 14.92 s (median 38.13; IQR 19.82); cooling to 21°C: 48.62 ± 18.93 s (median 41.17; IQR 10.45) (Figure 3).

3.2 | Animal B

With monkey B, only a single session was performed. The total duration of the session was 421.40 min (7h 4 min), divided into a control part (339.8 min) and a cooling part (80.57 min). The temperature applied during the cooling part was 17°C; thus this experiment was more severe than that performed with monkey A. A mean of 15.29 seizures/20 min was recorded during the pre-cooling control periods, compared to 3.25 seizures/20 min with cooling to 17°C (78.73% reduction). As with monkey A, no difference in the number of IEDs was observed with cooling to 17°C (6.78 vs 6.09 IED/min). Surprisingly, seizure duration showed a non-significant increase from an average of 16.7 ± 67.47 s during the control periods to 30.44 ± 7.05 s when cooling was applied. However, it should be remembered that only a single session was performed with monkey B, and this result will need to be confirmed.

3.3 | Does focal cooling affect residual seizures?

To accurately analyze the power changes on the sEEG trace, we used fast Fourier transformation to calculate power for

the different frequency bands. Raw and electronically filtered signals (notch filter 50 Hz) were analyzed across the 1–250 Hz range using a MATLAB script. Seizures that occurred during cooling were compared to seizures during the control sessions in the two animals. For monkey A, a significant power reduction was observed in the 1–5-Hz band at 21°C ($p < .0001$), with a non-significant trend for power reduction from 50 to 250 Hz. Of interest, power increased significantly in the 1–5-Hz band when the cooling temperature was 23°C, with a trend for increasing power from 50 to 250 Hz. Thus a mild temperature reduction was not effective, inducing a reverse effect: an increase in ictal activity. In contrast, in monkey B, when a cooling temperature of 17°C was applied, power across all frequency bands was decreased significantly. These results confirm that effective cooling reduces power during seizures, and affects all frequency bands. The extent of temperature reduction correlated directly with the power recorded in each frequency band (Figure 4A).

3.4 | Seizures affect intracerebral temperature

We analyzed temperature recordings at 0.5 mm from the penicillin injection site, using two thermocouples located at the tip of the cooling lead. Variations in temperature were recorded during seizures and classified depending on whether the brain was in a baseline steady state (33.5°C) or had been subjected to cooling (to 21°C, 23°C, or 17°C). We compared this classification with the temperatures recorded during non-seizure periods before injection. Animals had a baseline brain temperature of $33.50^\circ\text{C} \pm 1.85^\circ\text{C}$ and a stable body temperature, between 36°C and 37°C. Brain temperature variability was observed in both animals over multiple 2-min intervals without seizures and before penicillin injections. In these settings, temperature varied by $0.05^\circ\text{C} \pm 0.02^\circ\text{C}$ ($n = 10$, two animals). During penicillin-generated seizures, a temperature increase of $0.29^\circ\text{C} \pm 0.2^\circ\text{C}$ was measured relative to baseline brain temperature (33.5°C). More interestingly, when we measured temperature variation during seizures ($\Delta T^\circ\text{S}$) occurring during focal cooling of the EZ, we found a $\Delta T^\circ\text{S}$ of $0.35^\circ\text{C} \pm 0.05^\circ\text{C}$ with cooling to 23°C, $0.35^\circ\text{C} \pm 0.19^\circ\text{C}$ with cooling to 21°C, and $1.60^\circ\text{C} \pm 0.34^\circ\text{C}$ with cooling to 17°C. Thus, there was a significant increase (Kruskal–Wallis test, $p < .0001$) in $\Delta T^\circ\text{S}$ in the EZ relative to the surrounding brain temperature. The difference was best identified when the brain temperature in the EZ was cooled to the lowest temperature tested: 17°C. No correlation was found between seizure duration and $\Delta T^\circ\text{S}$ (Figure 4B and Supplementary Material).

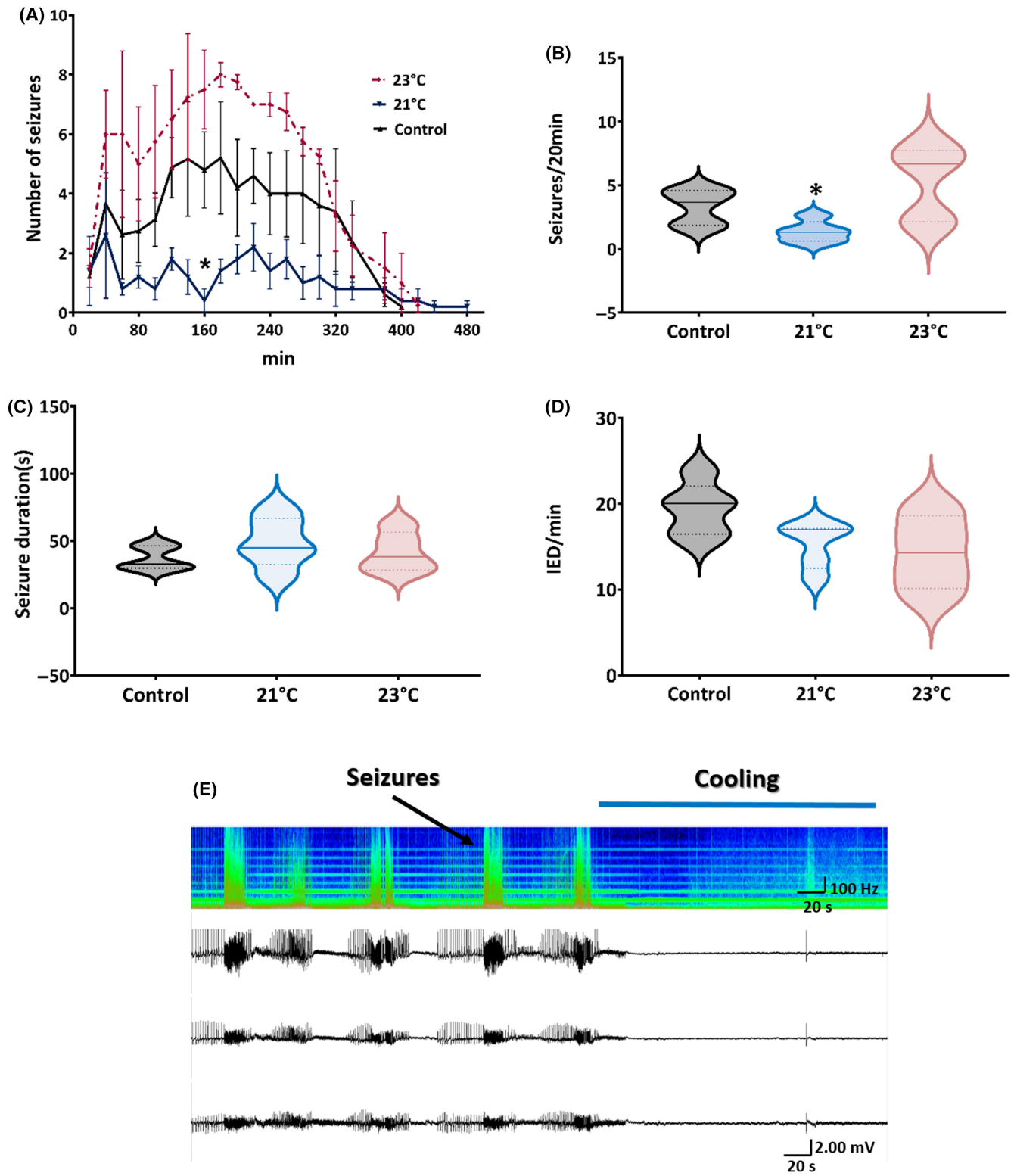


FIGURE 3 Cooling the EZ to 21°C resulted in a reduction in the total number of seizures, but not their duration. (A) The graph shows the number of seizures over time after hippocampal penicillin injection in control conditions (black line), after cooling to 21°C (blue line) and to 23°C (red line). Data were derived from five sessions ($n=5$) ($p=.014$, two-way ANOVA with Kruskal–Wallis test with Dunn's multiple comparison correction). (B) Violin plot (median and IQR) showing the number of seizures occurring per 20-min period in the three conditions (control, 21°C, 23°C; $p<.05$). (C) Violin plot (median and IQR) showing seizure duration in seconds for all three conditions. (D) Violin plot (median and IQR) illustrating the influence of cooling on the number of IEDs per minute. Differences in seizure duration or number of IEDs/min were not statistically significant. (E) Representative image of hippocampal sEEG displaying four channels: the top channel is adjacent to the epileptogenic zone (2 mm). The top channel is represented in power spectrum in a time display or sonographic display. A Hanning window was applied, 96 dB/Pixel, block size 256 (Spike2 v6.11 CED, Cambridge, UK). Cooling was set to 17°C. All results are for monkey A except for (E) for Monkey B.

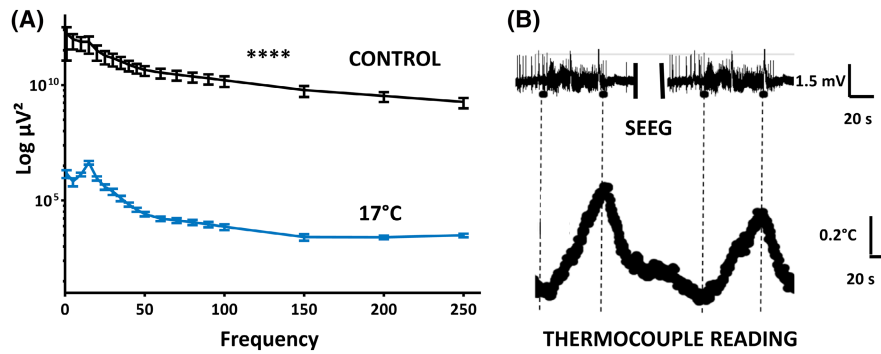


FIGURE 4 Focal cooling affects the intensity of power spectrum of seizures. (A) Power spectral analysis of seizures during the no-cooling period (control) and seizures during the 17°C cooling period (monkey B). Residual seizures at 17°C showed significantly reduced power across all frequencies ($p < .0001$). (B) Temperature variation relative to the onset of the seizure: Images showing changes in thermocouple readings related to seizures (monkey A). Thermocouple sensors in the lead recorded the temperature of the surrounding brain tissue. Temperature variations ($\Delta T^{\circ}S$) associated with seizures were more pronounced during cooling. When the brain tissue was at 21°C, $\Delta T^{\circ}S$ was $\sim 0.4^{\circ}C$. However, with cooling to 17°C, $\Delta T^{\circ}S$ increased to $\sim 1.6^{\circ}C$.

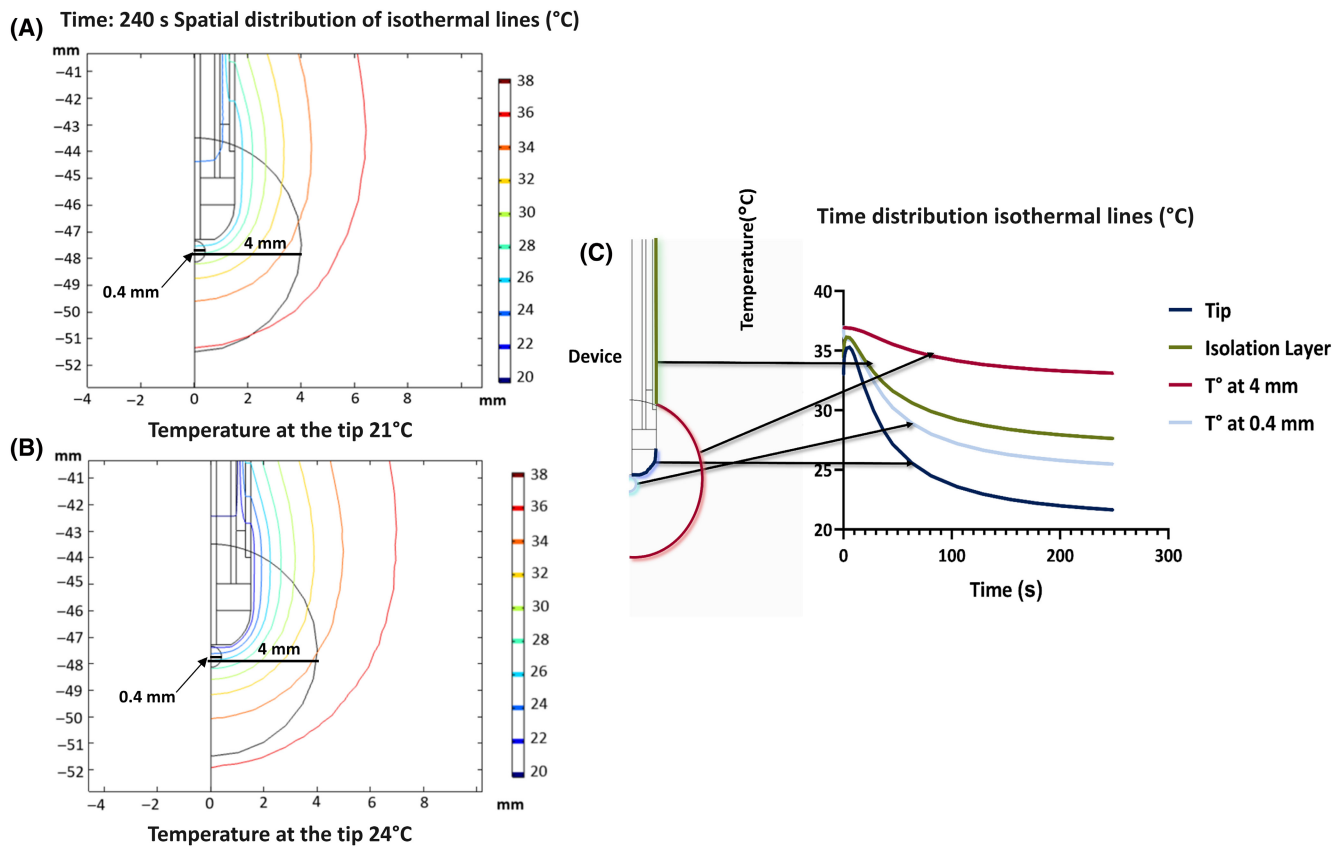


FIGURE 5 Simulation of spatiotemporal temperature distribution. Axisymmetric mode showing isothermal lines around the cooling lead and tip. Through a combination of tissue conduction and perfusion, the temperature distribution reaches a new equilibrium, and the steady state is achieved at 240 s. (A) Distal temperature targeted 24°C. (B) Distal temperature targeted 21°C. The Y-axis in (A) and (B) represents the distance in millimeters from the brain's surface, whereas the X-axis represents the distance from the center of the lead. (C) Graphical representation of temperature changes ($^{\circ}C$) simulated for each isothermal line over time (s).

3.5 | Simulations of the spatial distribution of temperature

A small half-circle (0.4 mm radius) was defined to account for the penicillin injection sphere, and a large half-circle

(4 mm radius) was plotted to determine average temperature on spheres at various distances from the tip of the probe. Analysis of this model revealed an increase in brain temperature in proportion to the distance from the cooling site, with temperature increases of approximately

+3°C at 0.4 mm, and +16°C at a distance of 4 mm from the injection site (Figure 5B,C).

3.6 | Repeated penicillin injection generates hippocampal sclerosis

Hippocampal sclerosis was observed in the area where the penicillin injections were administered. An expanded granular layer in the dentate gyrus and a reduction in neurons in the CA4 region (Figure 6) characterized this sclerosis. These alterations were identified around the injection site (1.3 mm anterior to 6.7 mm posterior).

4 | DISCUSSION

This study provides the first experimental evidence that cooling deep-seated structures can reduce the frequency of seizures in an NHP model of temporal lobe epilepsy. Previous research focused on cooling the brain's surface, but DRE typically affects the temporal lobe and hippocampus, presenting accessibility challenges for the delivery of cooling via a long cannula or lead. To address these challenges, we developed an insulated lead designed specifically to cool the hippocampal area without major heat dissipation. Another challenge was to control the distance between the cooling tip and the EZ. Indeed, according to our simulation studies, brain temperature increases rapidly with distance from the cooling site. To ensure precise control, our cooling lead incorporated a central cannula for insertion of an infusion needle. This structure allowed us to accurately determine the distance between the cooling tip and the EZ, which was 0.5 mm for all experiments.¹⁷

The results of the experiments described here indicate that cooling the hippocampal area to 21°C can reduce the number of seizures in a penicillin-induced NHP model of MLTE. This temperature may represent the threshold for seizure reduction. In monkey B, a more significant reduction in seizure frequency was achieved by applying a temperature of 17°C. At this temperature, a drastic reduction in power spectra was also observed across all frequency bands compared to the control sessions. This finding suggests that significant seizure reduction in this model was achieved within a temperature range of 21°C–17°C.

The temperature threshold for seizure reduction may differ from that required to stop seizures when cooling is applied to the brain's surface. Ren et al.¹⁶ reported a threshold of 16°C for controlling subcortical seizures using a 15 mm diameter cooling ring placed in direct contact with the surface of the cortex in cynomolgus monkeys. The main difference with respect to our study is the distance between the cooling source and the zone

where seizures originate. Thus, in Ren et al.,¹⁶ the injection site was ~3 mm from the cooling device, compared to 0.5 mm here. Our results show that the effectiveness of cooling directly correlates with distance, diminishing rapidly after just a few millimeters. In addition, the area cooled by their source would resemble a plate rather than a sphere as with our probe. Tanaka et al.¹⁷ also applied cooling using a probe to control seizures induced in the rat hippocampus using 4-aminopyridine. The tip of their lead dropped below 20°C within 1 min. Like us, in these conditions, they found a reduction in the amplitude of epileptic discharge. However, they do not mention whether the number of seizures was also reduced. Considering these previous studies and our own results, we can conclude that the temperature threshold necessary to induce an effect on seizures in this model is at least 21°C.

We also observed a non-significant trend toward increased seizure frequency at 23°C. This result corroborated observations from Gotoh et al.,²⁶ who reported a nonlinear biphasic response in evoked potentials following cooling, with increasing amplitude above 17°C and a decreasing amplitude at lower temperatures. This phenomenon may be explained by the higher temperature sensitivity of inhibitory inputs.

Our results show a significant reduction in seizure power spectra with cooling. This power reduction is notable in the lower frequency bands (1–5 Hz) at 21°C, and across all frequency bands at 17°C. This progressive reduction in power spectra with decreasing temperatures aligns with similar experiments conducted in a neocortical seizure model, with measurements taken at the brain's surface in humans or rodents.¹⁶ Kida et al.²⁷ observed a gradual decrease in spectral power when applying temperatures of 25°C, 20°C, and 15°C, with 15°C resulting in a complete suppression of the delta to beta bands. In both cases, this reduction in spectral power was observed for motor cortex seizures. This consistent effect may offer insights into the mechanisms behind brain cooling's antiepileptic effects.

Of interest, our results indicated that effective cooling had no impact on seizure duration or interictal spikes, whatever the temperature tested. In contrast, Ablah et al.²⁸ reported a reduction in the number of IEDs in patients undergoing cortical irrigation with chilled (4°C) saline solutions during epilepsy surgery. Others have also reported evidence of reduced numbers of IEDs during surgery when irrigating the EZ with chilled saline solution.^{29,30} In our conditions, despite a clear reduction in seizure frequency, we observed no decrease in IEDs during cooling. One possible explanation for this apparent discrepancy is that the clinical studies used colder temperatures (4°C) applied to a larger cortical area. Our relatively mild and

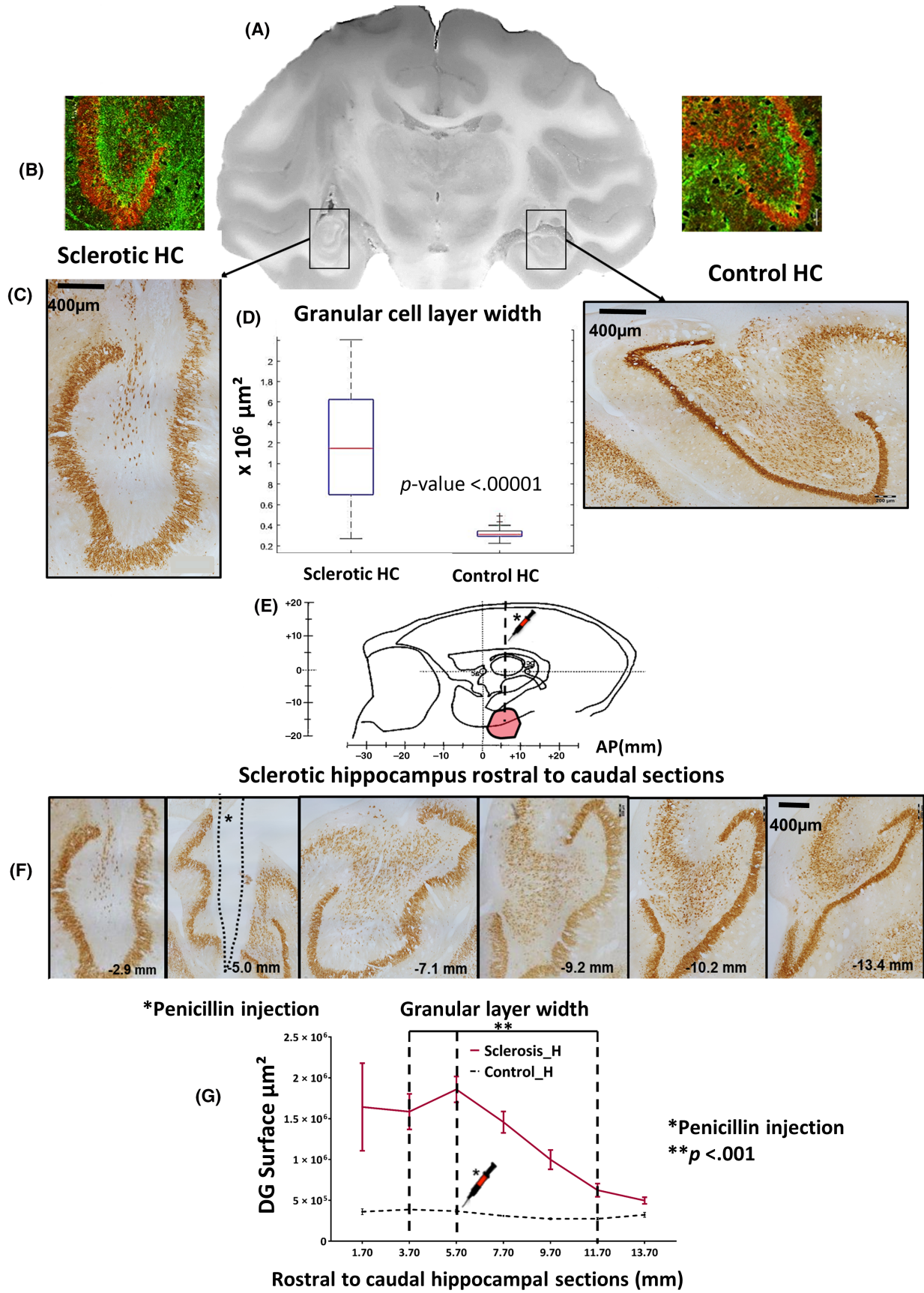


FIGURE 6 Penicillin injection induces histological alterations in hippocampus. Control (right) and penicillin-injected sclerotic (left) hippocampus (HP) from monkey A following 16 penicillin injections. Injections administered at -5.0 mm posterior to the anterior commissure (AC) led to an expanded granular layer in the dentate gyrus (DG) and neuronal loss in the CA4 region. These changes extended from -3.7 mm AC (1.3 mm rostral to the injection site) to -11.5 mm AC (6.5 mm caudal to the injection site). (A) Coronal brain section at -5 mm from AC. (B) Detailed view of the granular layer of HP in control (right) and sclerotic (left) HP, immunostained with antibodies against NeuN (red) and GFAP (green). (C) NeuN-stained HP control and sclerotic (neurons in brown) showing reduced neuron counts and increased DG width. (D) Box plot comparison of DG surface width between sclerotic and control HP ($p < .00001$). (E) Atlas sagittal view of the relative position of the injection site in the *Macaca* brain. (F) NeuN-stained sections of the sclerotic (injected) HP showing the rostro-caudal extent of the changes. (G) Curves showing the spatial distribution from rostral to caudal of the surface increase of the DG in the sclerotic HP vs the control HP.

highly localized cooling may not have been sufficient to generate a significant reduction in the number of IEDs in the EZ. A similar argument can be made with regard to seizure duration. Studies in other animals have shown a correlation between seizure duration and temperature.³¹ However, in our study, seizure duration was unaffected by cooling. It is possible that even colder temperatures are required to achieve a reduction in seizure duration or number of IEDs.

During this study, we observed that seizures affected brain temperature at the EZ. The two thermocouples in the cooling lead were initially intended to monitor brain temperature during cooling, but they also detected seizure-related temperature changes at the injection site. In rats, Yang et al.³² also found that seizures caused a temperature increase— $\sim 0.3^\circ\text{C}$ —that correlated with seizure duration and was preceded by increased blood flow. Likewise, in humans, Dymond and Crandall³³ observed changes to focal temperature in the anterior hippocampus and hippocampal gyrus during seizures using modified sEEG leads with thermal sensors. This contributed to the success of the surgical resection in that study.³³

Cooling of the EZ also resulted in increased temperature variations during seizures at the injection site. These larger temperature variations during seizures are indicative of a hyperactive EZ, possibly associated with increased requirements for cellular energy production and blood flow. Human studies with imaging have shown a direct relationship between elevated cerebral blood flow and metabolism during seizures.^{34,35} Federico et al.³⁵ reported significant pre-seizure changes corresponding to hyperfusion zones observed in single-photon emission computerized tomography (SPECT) analysis. The temperature increase could be explained by an influx of warmer systemic blood to the EZ. Our results indirectly confirmed this relationship, potentially paving the way for future advancements to delimit an EZ as part of pre-surgical evaluation, based on the use of thermographic maps.

Furthermore, in the area surrounding the injection site in the penicillin-induced NHP model of epilepsy, we observed hippocampal sclerosis, in line with previous

reports.³⁶ This sclerosis resembles the EZ found in DRE MTLE. The profile of this sclerosis showed similarities to hippocampal sclerosis ILAE type 1, which is characterized by predominant neuronal cell loss and gliosis in the CA1 and CA4 regions.³⁷ We also discovered changes at the surface of the DG following penicillin injection over several months. These findings are significant for the model's future evolution from an acute MTLE model to a potential chronic model that closely mimics DRE neuropathology with spontaneous seizures.

4.1 | Study limitations

The main challenge with any cooling method is to determine the volume effectively reached. To determine the cooling area, we defined the EZ as a spherical zone around the tip of the lead (radius: 1.19 mm) with a 7- μL penicillin injection. However, histological changes surrounding the injection site suggest that a potentially larger area may be affected, and should therefore be cooled. The zone affected may be even greater than that reported here, as we did not extend our histological analysis beyond the temporal lobe. Consequently, cooling the EZ in the human hippocampus may require a thicker lead or multiple leads. Reducing the temperature of the cooling tip below 20°C could also help enhance coverage.

The time required to attain the target temperature must be minimized. In our study, we observed a cooling rate of approximately 0.30°C/s in air and 3.70°C/min in contact with tissue. Motamedi et al.³⁸ highlighted that with slower cooling rates (0.11°C/s to 1°C/s), a more significant temperature reduction is needed to suppress epileptic discharges compared to faster cooling rates (2°C/s to 5°C/s).

In addition, we did not conduct a separate analysis of the rewarming period, as our objective was to identify the effective temperature that leads to seizure reduction. The rewarming phase, occurring hours after penicillin injection, cannot be studied precisely due to the spontaneous reduction in seizure frequency as the effect of penicillin wears off. An accurate evaluation of the rewarming impact requires a dedicated protocol.

A final limitation of our study is the potential overestimation of the cooling required due to the severity of the penicillin model.

5 | CONCLUSION

Our findings demonstrate the potential of focal cooling to reduce penicillin-induced hippocampal seizures, and thus represents a novel approach to DRE management. The results presented here underscore the efficacy of focal brain cooling, confirming it as a viable treatment option. Further research will focus on examining the lesional effects of cooling as well as understanding its spatial and temporal distribution in the brain. We aim to compare intermittent cooling with continuous methods, and study rewarming processes. To improve efficiency, we are developing new technologies to increase cooling speeds beyond the current rates, aiming to develop a future clinical device.

AUTHOR CONTRIBUTIONS

Napoleon Torres designed the study from an original idea proposed by Stephan Chabardes. Napoleon Torres, Etienne De Montalivet, Quentin Borntrager, Nicolas Aubert, Jenny Molet, and Thomas Costecalde performed the preclinical experiments. Fabien Sauter-Starace and Claude Chabrol designed and manufactured all the devices, Quentin Borntrager and Selimen Benahmed developed the thermal model in COMSOL simulation software. Selimen Benahmed developed the experimental chain set up in the LABVIEW environment. Antoine Legrain has significantly improved the detection algorithm and performed calculations to assess the accuracy of the software's detection capabilities. Vincent Auboiroux contributed to all MRI acquisitions. Napoleon Torres, Etienne De Montalivet, Fabien Sauter-Starace, Thomas Costecalde, Felix Martel, and Tetiana Aksenova performed the literature search. Eleonora Adesso has enriched the manuscript through drafting, revising, and conducting comprehensive bibliographic research. Brigitte Piallat contributed with expertise in the penicillin epilepsy model. David Ratel and Christophe Gaude performed the histological analysis. All authors contributed to the revision and critical review of the manuscript, based on their specific professional knowledge and background. The original draft and subsequent versions of the manuscript were written by Napoleon Torres and reviewed by all authors. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The project was authorized by the regional ethics committee (383_Clinatéc-NTM-11) and the French Ministry for Research and Higher Education (2017041910245906), and procedures were conducted in line with the guidelines set down by the European parliament in September 2010 related to the respect and safety of the animals used (directive 2010/63/UE).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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