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Potential for Thermal Damage to the Blood-Brain Barrier during Craniotomy Procedure: Implications for Intracortical Recording Microelectrodes

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Abstract

Objective: Our objective was to determine how readily blood-brain barrier (BBB) disruption occurred as a result of bone drilling during a craniotomy to implant microelectrodes in rat cortex. While the phenomenon of heat production during bone-drilling is well-known, practices to evade damage to the underlying brain tissue are inconsistently practiced and reported in the literature.

Approach: We conducted a review of intracortical microelectrode literature to summarize typical approaches to mitigate drill heating during rodent craniotomies. *Post mortem* skull-surface and transient brain-surface temperatures were experimentally recorded using an infrared camera and thermocouple, respectively. A number of drilling conditions were tested, including varying drill speed and continuous *vs* intermittent contact. *In vivo* BBB permeability was assayed 1-hour after the craniotomy procedure using Evans blue dye.

Main results: Of the reviewed papers that mentioned methods to mitigate thermal damage from the craniotomy, saline irrigation (6 of 7) was the most frequently cited. In *post mortem* tissues, we observed increases in skull-surface temperature ranging from +3°C to +21°C, dependent on drill speed. *In vivo*, pulsed-drilling (2s-on/2s-off) and slow-drilling speeds (1k RPM) were the most

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Author contributions:

A.J.S. drafted the majority of the manuscript and was involved with the design and implementation of all experiments

J.P. and D. Menendez drafted relevant portions of the manuscript and carried out experiments and analysis relating to Figure 4.

D. Miller and G.R. drafted relevant portions of the manuscript and conducted the literature review and analysis presented in Figure 1.

M.W. drafted relevant portions of the manuscript and conducted the infrared and transient heating experiments and analysis presented in Figures 2 and 3.

S.H. helped design the studies, contributed to data interpretation, and review and revised the manuscript. He also constructed the finite-element analysis provided in Supplementary Information.

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None of the authors have a conflict of interest

effective methods we studied to mitigate heating effects from drilling, while inconclusive results were obtained with saline irrigation.

Significance: Neuroinflammation, initiated by BBB damage and perpetuated by the foreign body response, is thought to play a key role in premature failure of intracortical recording microelectrodes. This study demonstrates the extreme sensitivity of the BBB to overheating caused by bone drilling. To avoid BBB damage, the authors recommend that craniotomies be drilled with slow speeds and/or with intermittent drilling with complete removal of the drill from the skull during “off” periods. While saline alone was ineffective to prevent overheating, its use is still recommended to remove bone dust from the surgical site and to augment other cooling methods.

Introduction

Intracortical recording microelectrodes have tremendous potential for use in both research and medical applications.(1–4) Intracortical microelectrodes act as an electrical interface that can map and record neural activity.(5, 6) The information captured can be processed to reveal how neuronal cells interact with one another in response to certain stimuli such as neurological diseases, neurorehabilitation and/or environmental factors.(6–10) The signals can also be decoded to reveal practical information for use in medical applications. Most prominently, signal decoding can yield intended movements or actions that can be used as controller input for prosthetic devices, cursors on a screen, or even to restore volitional control of a paralyzed patient’s own muscles via functional electrical stimulation.(2, 5, 6, 8, 11)

While the promise is great, widespread adoption is challenged by inconsistent device longevity and performance.(12–15) Loss of signal from intracortical microelectrodes is likely a result of a multitude of biological and material factors.(12) Many approaches have been tested to understand and modulate the mechanisms leading to microelectrode failure. (12) While many factors are likely intertwined and together propagate the neurodegenerative and corrosive responses to microelectrode implantation, the process is clearly initiated during device implantation.

Several groups have demonstrated a direct connection between the damage to the neural tissue and surrounding vasculature, and the performance of the implanted microelectrode. (16–18) In particular, damage to the blood-brain barrier (BBB) in the presence of a prolonged foreign body response appears to setup a self-perpetuating pro-inflammatory state.(19, 20) While preventative techniques have been developed, damage to the brain tissue during microelectrode insertion is inevitable.(21, 22) Trauma to the BBB allows blood proteins and cells that are not normally present into the immune-privileged brain tissues. Soluble factors that are released from the disrupted BBB play different roles in the inflammatory process with detrimental effects on neural cells and the electrode.(23–29) The breach allows macrophages to infiltrate the brain tissue and localize at the implanted device, (30) while microglia that are normally not-activated in the tissues become activated due to both the cellular and acellular changes in the microenvironment.(30–33) As a result, the activated monocytes release more soluble factors (including reactive oxygen species)(19,

34–37) at the implant site, further triggering BBB leakage and perpetuating a pro-inflammatory state.(20, 38) While the inflammatory cascade is complex and takes weeks to develop, trauma during the implantation marks its onset and may be a major determining factor in the magnitude of the ultimate response.

To attempt to mitigate the inflammatory response, we therefore focused our attention on the initial surgery to implant the intracortical devices. First and foremost, the procedure requires the removal of bone from skull to allow for access to the brain.(39) The microelectrode field commonly uses craniotomies produced by high-speed drills to insert devices into the rodent brain.(39) While many labs regularly take precautions against drill overheating, e.g., by using saline irrigation,(40) the heat of the drill is a potential source of damage that has not been extensively explored with relation to intracortical microelectrode implantation.

New evidence from our ongoing studies of neuroinflammatory response to implanted intracortical microelectrodes indicated that the BBB was disrupted at acute time points (less than 1-hour) as a result of the craniotomy procedure alone (i.e. “sham” rats). As part of our studies to investigate methods to reduce the neuroinflammatory response to chronically implanted intracortical microelectrodes, we injected Evans blue dye intravenously in rats prior to performing the craniotomy. Evans blue dye binds to circulating albumin in the bloodstream which cannot normally cross the BBB, therefore making it a sensitive marker of BBB permeability.(41–43) After 1-hour post-craniotomy, we observed a drastic increase in dye concentration in the brain compared to those that had not undergone the craniotomy procedure (i.e., naïve rats). We sought to better characterize the effects of and determine the cause of BBB permeability at acute time points. Since the dye extravasation was localized directly under the site of drilling, we hypothesized the effect was a result of local heating.

While it is generally understood that drill over-heating may cause tissue damage,(44–46) a phenomenon particularly well-documented in the orthopedics field,(47) the impact of craniotomies on highly vascularized brain tissue has not been adequately characterized in the field of intracortical microelectrode research (Figure 1). In their comprehensive review of drill bone heating in orthopedic fixation procedures, Pandey et al, identified a number of factors that influence the degree of drill heating.(47) These factors as well as others found from our literature review are shown in Table 1.

Since visible lesions and/or overt functional damage has not been observed in our study, nor reported in the literature associated with craniotomy procedures, the amount of heating caused by drilling is likely not sufficient to cause **permanent** thermal damage to the brain tissues. However, given our interest in investigating the neuroinflammatory response after device implantation, and the role that serum proteins may play in promoting inflammatory cascades, the increased permeability of the BBB may significantly influence or confound the results found in experimental models that require a craniotomy.(16, 17, 19)

In the current study we sought to: 1) review the literature for rodent craniotomy procedures for methods to prevent drill over-heating, 2) test our hypothesis that observed BBB damage was from drill overheating, 3) better characterize the temperatures and parameters that may

lead to drill over-heating and BBB damage, and 4) determine a drilling protocol that avoids damage to the BBB.

Methods

Literature review of rodent craniotomy methods, within the context of microelectrode implantation

An extensive review of rodent craniotomy methods described in the intracortical microelectrode literature was conducted using the PubMed database. We pulled references pertaining to craniotomy procedures in rodent models, or intracortical microelectrodes in rodent models.

PubMed Search string:

“(craniotomy OR (microelectrode AND intracortical)) AND (rat OR mouse OR rodent)”—The initial search yielded approximately 698 results. We then omitted all articles for which access to the full article was not available or the articles were published prior to January 1st, 2000 (N=219 results). The remaining 479 journal articles were then categorized into broad medical fields (e.g., oncology, orthopedics, and imaging). To reduce the scope of the search and hone-in on our particular interests, we then focused on the subset of papers related to the intracortical microelectrode field.

We systematically reviewed each paper to determine if the methods sections contained information pertaining to craniotomy methods (e.g. factors mentioned in Table 1) and/or the generation or prevention of heating. We then further analyzed the group of articles that referenced craniotomy methods for a number of other specific factors related to drill-heating and prevention, such as the use of saline irrigation as well as the speed, size and sharpness of a drill bit. It is important to note that review of specific methods sections was limited to the context of papers related to intracortical microelectrode implantation. The review of heat prevention methods, therefore, omitted other related results such as from the fields of *in vivo* optical imaging via cranial windows, implantation of fiber optic or other probes, traumatic brain injury, stroke and other fields, that may also produce craniotomies in rodent models for which the implications of our research findings may be equally relevant.

Materials: Evans blue dye and dimethylformamide (DMF), were purchased from Sigma Aldrich (St. Louis, MO). Evans blue solutions for *in vivo* infusion were prepared as 4% w/v % solution in 0.9% sterile saline. The solutions were then sterile filtered to remove precipitates with a 0.2µm syringe filter. All other reagents were used as received. The high-speed power drill was purchased from Pearson Dental, Osada Model EXL-M40 (Los Angeles, CA). Spherical drill bits were purchased from Stoelting (Wood Dale, IL) and were 1.75 mm in diameter (Stoelting Co. #514556).

Rat Surgical Procedures: All surgeries were performed acutely, i.e., with euthanasia of the animal prior to it waking up.(48) Briefly, rats were anesthetized with 4% isoflurane, shaved, and mounted in a stereotaxic frame equipped with blunt ear bars and an incisor bar. A dose of 0.2 ml of 0.25% Marcaine was administered subcutaneously at the incision site, followed

by cleaning of the skin with betadine and isopropyl alcohol. Anesthesia was maintained via the oxygen flow path on the stereotaxic frame using 0.5% - 2% isoflurane. Prior to surgery, the rat received 16 mg/kg (SQ) of Cefazolin as an antibiotic as an extra precaution to prevent intraoperative infection as well as 1 mg/kg (SQ) of Meloxicam for pain management.

To perform the craniotomy, a 1-inch incision down midline of the scalp (~2 cm) was followed by removal of superficial soft tissues such that the dry bone skull was exposed. All drilling was completed with a 1.75 mm diameter spherical drill bit (as identified above). While not specifically controlled, the angle was approximated at ~75° (in reference to the table) to allow for simultaneous drilling and visualization by the microscope. A single hole was created using a drill-press style (as opposed to tracing a circle or rectangular hole). After the drilling or heating of the skull was performed, and all measurements taken, rats were euthanized under deep anesthesia via cardiac perfusion with saline. All rat procedures were reviewed and approved by the Louis Stokes Cleveland Department of Veterans Affairs Medical Center Institutional Animal Care and Use Committee.

In vivo Evans blue BBB permeability experiment: Rats were anesthetized and prepared as described above. Once the skull bone was exposed, rat tails were catheterized with a 24g needle, and infused with sterile-filtered 4% Evans blue solution over the course of 1 minute at a dose of 2 ml/kg. Proper infusion was verified by the rat's ears, nose, and eyes turning blue. Evans blue was allowed to circulate for 5 minutes prior to commencement of drilling. Drilling was performed bilaterally (one hole on either side of midline, - 3mm AP, +/- 3mm ML). Craniotomies were performed at various speeds (1k, 5k, 10k, and 15k RPM) as well as either continuously or with intermittent application (15k RPM, 2s-on/2s-off) to allow the bone to dissipate heat between each application. During "off" periods, the drill bit was entirely removed from the skull surface to allow for heat dissipation with the air. Another cohort of rats was provided continuous saline irrigation at 10 ml/min from a calibrated syringe pump and suction setup (i.e., the continuous drilling group '15k Saline').

After drilling, the scalp was temporarily closed and the rat was maintained on isoflurane for 1-hour. After 1-hour, the rat was euthanized via cardiac perfusion with saline. The brains were harvested, coronally bisected through the area exposed by the craniotomy, and photographed to visualize the extent of blue dye extravasation. Small biopsies of the affected brain tissue were then collected using a 3 mm diameter biopsy punch and suspended in 400 µl DMF overnight. Affected tissue had easily identified margins of blue staining as seen in the inlaid picture in Figure 4. The 3mm biopsy punch sufficiently captured the entire margins.

After incubation at room temperature, 100µl of each sample was pipetted into a glass 96-well plate. An Evans blue standard curve was produced ranging from 25 pg/ml to ~0.2 pg/ml in DMF by 2x serial dilution. Samples were read on a plate reader (SpectraMax M3, Molecular Devices, Sunnyvale, CA) using Evans blue fluorescent spectra peak (620/680 excitation/emission). The results were compiled and graphed in Origin (North Hampton, MA).

Determination of typical drilling times and peak temperatures: Craniotomy holes were drilled with the power-drill at manufacturer-set speeds ranging from 1k, 5k, 10k, and 15k RPM. Maximum temperatures and temperature profiles were obtained with an infrared camera (FLIR model E6, Wilsonville, OR). Drilling times were recorded as the time required to perform the full craniotomy, i.e., expose but not disrupt the dura. At least 3 trials per speed combination were performed and representative infrared images are shown.

Post mortem measurement of transient increase in brain surface temperature from drilling: We sought to understand how the brain surface temperature was transiently affected throughout the drilling procedure. We used a *post mortem* preparation of rat skulls (post-euthanasia). A single hole was drilled in the skull, through which a temperature measurement probe (miniature type-T thermocouple, 0.02" diameter, TC-Direct, Hillside, IL.) was placed and tunneled on the surface of the brain, ~2–3 mm away. (Figure 3, **inlay**) The skull was then drilled directly over the thermocouple, continuously, pulsed for 2s-on/2s-off, or 2s-on/5s-off. Temperature changes were directly recorded via a National Instruments DAQ, USB-6008 (National Instruments, Austin, TX) and baseline temperature subtracted. Measurements were recorded in triplicate for each drilling method.

Statistics: One-way ANOVA was performed to compare the Evans blue dye concentration by drilling method. Tukey tests were used to make pairwise comparisons between groups with significance level set at $p < 0.05$. Statistics were performed in Minitab (City, ST). Graphs were produced in Origin.

Results

Literature review of rodent craniotomy methods

During an initial review of the intracortical microelectrode literature, we found a wide range and depth of reporting details regarding specific craniotomy methods. Details related to heat dissipation from bone drilling were absent in the majority of papers. As a result, it was often unclear if and how techniques were being applied. We therefore sought to more thoroughly review the currently available literature referencing the performance of rodent craniotomies. Of the 61 articles we categorized as related to the “intracortical microelectrode” field, 23 (37.7%) had little to no reference to specific techniques used to perform a craniotomy, and 31 (50.8%) referenced a craniotomy method in general terms but did not mention heat and/or methods to mitigate the effects of heat. Overall, only 7 (11.5%) referenced a specific method for prevention of thermal damage from drill overheating (Figure 1). Saline irrigation, either continuous or intermittent was used in 6 of the 7 papers that described specific methods to avoid drill overheating. An alternative drilling method, a biopsy punch, was used in 1 of the 7 papers. Relevant quoted text from the methods section of the 7 articles are provided (Table 2).

Typical drilling times and peak temperatures

To determine the magnitude of heating caused by drilling, we performed craniotomies on *post mortem* rat skulls, while simultaneously imaging the surgical field with a thermal infrared camera. Given the wide range of drilling methods we encountered during the

literature review, we tested various drill speeds, including 1k revolutions per minute (RPM), 5k RPM, 10k RPM, and 15k RPM. Pictures were recorded for each of the tested speeds at the maximum recorded temperatures. At each of the speeds, from slowest to fastest, temperatures increased by 3°C, 5°C, 8°C, and 21°C respectively (Figure 2). The fastest drill speed (15k RPM) reached a maximum temperature of 21°C above baseline, which was much higher than anticipated given the brevity of the procedure. In our experiments, we found that there was significant variability in time-to-produce-craniotomy. Typical drilling times ranged from 15 seconds to 30 seconds. Interestingly, there was only a mild increase in time to complete the craniotomy with 10k and 5k speeds compared to 15k (16.4 $\pm$ 3.8s and 17.0 $\pm$ 4.1s, versus 14.9 $\pm$ 1.6s respectively). Slow drilling with 1k RPM had the greatest impact on drill time, increasing the total time by nearly 2x (28.4 $\pm$ 1.6s) when compared to 15k RPM.

Transient increase in brain surface temperature from drilling

Three types of drilling were tested (continuous, Pulsed 2s-on/2s-off, and pulsed 2s-on/5s-off all at 15k RPM) in triplicate (Figure 3). A thermocouple was used to record the temperature under the skull over time while drilling (Figure 3, **schematic inset**). The continuous drilling had higher peak temperatures (+20.7 to +27.8°C) than any of the other drilling methods; pulsed 2s-on/2s-off drilling yielded a temperature range of (+9.1 to +13.6°C) and pulsed 2s-on/5s-off drilling yielded a range of (+3.1 to +7.9°C).

The heating rate and cooling rates were approximated by fitting the transient thermal curves with a linear model where the peak temperature value was used as a common point on either side, i.e. as the last point during the heating phase and the first point during the cooling phase. The average rate of heating for the continuous scenario (Figure 3A) was +2.7 ($\pm$ 2.2) °C/s, represented as mean ($\pm$ std. deviation). For the intermittent scenarios, pulsed 2s-on/2s-off (Figure 3B), and pulsed 2s-on/5s-off (Figure 3C), the heating rate was +0.5 ($\pm$ 0.1) °C/s and +0.3 ($\pm$ 0.3) °C/s, respectively. The average rate of cooling for the continuous, pulsed 2s-on/2s-off, and pulsed 2s-on/5s-off scenarios were -0.4 ($\pm$ 0.2) °C/s, -0.2 ($\pm$ 0.1) °C/s, and -0.1 ($\pm$ 0.02) °C/s, respectively.

The experiment was performed on *post mortem* tissue since placement of the thermocouple was impractical in a live rat preparation. Since the lack of perfusion would have decreased heat dissipation rates via fluid convection, the main heat transfer mode was by conduction through the tissues and air interface. We recapitulated these findings in a simple bio-heat finite element model (Supplementary Information), comparing our findings with theoretical spatial and temporal mechanics of heat transfer through the skull and brain tissue. As expected, while lack of perfusion was determined to have an effect on heat transfer, at the scale and magnitude relevant for the craniotomy, the impact on peak temperatures was projected to be minimal (dashed-line in Supplementary Figure 1). Both the infrared camera technique and the direct measurement of brain surface temperature with a thermocouple led to similar peak temperature measurements, with the peaks occurring directly below the site of the drill bit, suggesting rapid and efficient transfer of heat through the skull.

Drilling during rat craniotomy can quickly unintentionally increase BBB permeability

In order to investigate BBB damage caused during craniotomy, a variety of powered drill methods were explored. Rats were subjected to continuous drilling at different speeds (1k, 5k, 10k, 15k RPM), pulsed application (15k RPM, 2s-on/2s-off), or saline application (15k continuous), and compared to naïve rats (“no drill”) as control (Figure 4).

Among the drill speeds tested, only 1k RPM appeared to be slow enough to reliably avoid damage to the BBB. Drilling at 15k, 10k, and 5k RPM speeds led to significantly higher Evans blue concentration within the brain tissue than the “no drill” condition ($p < 0.001$, $p < 0.001$, $p < 0.05$, respectively). Furthermore drilling at 1k RPM speed led to a lower dye concentration compared to both 15k and 10k RPM speeds ($p < 0.01$ and $p < 0.05$, respectively), and did not significantly differ from the “no drill” condition. Drilling at 5k RPM led to inconsistent results, with different rats displaying either significant or insignificant BBB permeability, suggesting a potentially narrowly gated threshold for inducing BBB permeability. However, overall, drilling at 5k led to significantly higher dye concentration in the brain tissue than the “no drill” condition ($p < 0.05$).

A separate cohort of rats was subjected to pulsed drilling, 2s on and 2s off, allowing the drill and tissue to cool off in between drilling periods. Evans blue concentration from intermittent drilling was very similar to that of the “no drill” control tissue (n.s.) and was significantly lower than drilling at high speeds (10k and 15k RPM, $p < 0.01$ each). The concentration of Evans blue is visibly higher in the 15k-continuously drilled side as compared to 15k-pulsed drilling on the same rat, as shown in the inlaid picture in the top right corner of Figure 4. Importantly, the damage is not visible without the aid of the dye, and can therefore occur essentially invisibly during the course of a typical experiment.

Discussion

In our study, we examined the role of drill overheating damage caused during craniotomy on the BBB permeability. We found that the damage to the BBB was extraordinarily easy to reproduce with 15k RPM drill speeds and a typical commercially available drill bit size (1.75 mm). Furthermore, the damage occurred in under 15–30 seconds and achieved temperature increases of $>25^{\circ}\text{C}$ as measured at the brain surface. Intermittent drilling (2s on, 2s off), even without irrigation of saline (though not recommended as standard practice), was sufficient to prevent damage to the BBB from drill overheating.

The authors are not asserting that all craniotomies performed by the field have incurred BBB damage. In fact, one notable example publication describes robust methods to avoid thermal damage during drilling. Xu *et al.*, describes the use of intermittent drilling, where cooling of the drill bit with cold artificial cerebrospinal fluid is performed.⁽⁴⁹⁾ Furthermore, since visible lesions and/or overt functional damage has not been observed in our previous studies, nor reported in the literature associated with craniotomy procedures, the amount of heating caused by drilling is likely not sufficient to cause **permanent** thermal damage to the brain tissues. However, given how quickly BBB permeability changes can occur, without any visible damage to the surface of the brain (in the absence of a tracer such as Evans blue dye), it seemed appropriate to investigate and share with the research community.

A critical finding of our study is that methods for craniotomy are inconsistently reported in the field. We conducted a comprehensive review of intracortical microelectrode literature to evaluate the frequency and content of reported craniotomy methods. We found that very few published articles provided explicit details about their craniotomy methods and especially those relating to the prevention of heat: e.g. drill speed, drill bit sharpness, duration etc. Of the reviewed intracortical microelectrode literature, 7 of 61 papers described a craniotomy procedure that detailed a method to prevent overheating. While heat prevention during drilling is a regular practice by most groups, its use is inconsistently reported, and therefore assumed to be inconsistently performed with various parameters in the rat craniotomy and related electrophysiology recording literature. Equally important, some researchers opt to not use a power drill approach, and instead used a biopsy punch, manual low-speed hand-drill, or rongeurs for craniotomies to presumably avoid overheating of the brain tissues.(39, 48, 50–53) While seemingly mundane disclosures, craniotomy procedure parameters may have significant unintended confounding impacts on findings.

Of the papers reviewed, external saline irrigation was the most frequently cited method for prevention of heating.(17, 40, 54–56) While widely accepted practice in orthopedics, some have suggested that saline-alone may not be enough to prevent overheating since the drill bit may be buried deep in the tissue while the saline is only applied at the skull surface.(47) One group demonstrated that light application pressure was required in addition to saline coolant to sufficiently control temperatures.(57) Nonetheless, it would appear that the consensus opinion is that saline irrigation is effective as long as it is applied in the proper manner (e.g. cold temperature saline, applied at high enough rate).(47) From our experience, we discovered it to be very easy to accidentally overheat brain tissue with the power drill during craniotomy, even with the use of continuous saline irrigation at high flow rates (10 ml/min). Our findings are consistent with those found in the literature. Others in the TBI literature (for controlled cortical impact models) have previously suggested that the craniotomy only group may not be a “true sham” as it itself causes damage to the underlying tissues from heat, vibration and removal of a bone flap.(58) Similar results were found by Olesen *et al.*, who found that there was significant leakiness of vasculature after craniotomy.(59) Adelsen *et al.*, reported on increased Evans blue dye extravasation at 1-hour time points in their craniotomy-sham procedures alone.(60) Specifically, the craniotomy produced a 2-fold increase in Evans blue over un-operated controls after 1-hour. Importantly, Adelsen *et al.* also observed that BBB permeability returned to baseline after just 24-hours.(60) While the other groups indicated that their craniotomy procedure may have induced mild trauma, none had presented the potential cause as being related to thermal damage from drilling.

From our study, it is apparent the BBB is highly sensitive to temperature change, a finding that is consistent with other studies. In their paper, Kiyatkin *et al.*, showed that systemic hyperthermia conditions (36°C–42°C), induced mild-to-moderate increased BBB permeability, resulting in brain edema.(61) In a different study that chronically implanted a cranial window, the authors described heat loss which resulted in temperature dysregulation.(62) They showed that the brain temperature directly under the window was roughly 10°C cooler than the core temperature of mice.(62) The authors reported lasting effects on blood vessel diameters observed through the window. Similar effects on vasculature were recently observed using an optical coherence topography imaging method, and it is possible that

temperature effects played a role.(18) Kosaras *et al.*, described the vascular and innervation structures of the rat skull calvaria and showed the highly vascularized mesh network.(63) Disruption or structural changes to blood vessels in the brain may cause premature and additional activation of inflammatory pathways concurrently with the implantation of the intracortical devices, which already elicit a foreign body inflammatory response. Increasing the BBB permeability immediately prior to device insertion may act as a priming effect that exacerbates the inflammatory response.

Interestingly, during our transient response experiment, we found that the temperature was still significantly increased by 2s on/off intermittent application. However, from our *in vivo* experiments, we know that the method was sufficient to prevent BBB damage. Therefore, we believe that intermittent application, which yielded temperature increases of +9.1 to +13.6°C, was below the BBB damage threshold for the duration of application. Another potential explanation is that the perfusion of the highly vascularized brain cooled the affected area much more quickly than suggested by our *post mortem* transient response curves. Unfortunately, it was impractical to place our thermocouple in an *in vivo* preparation due to potential damage to the brain. Therefore, the brain surface temperature may have varied from the infrared camera measurements taken of the superficial skull surface. Together, the results seem expected since tissue damage is both a function of temperature magnitude and duration of the contact.(64, 65)

Since drilling times were not significantly altered when using different rotational speeds, it suggests that the feed-rate of the drill was held relatively constant compared between groups, and therefore not optimized for the particular rotational drill speed. In other words, at faster rotational speed cases, the drilling (or raking of bone bits) was essentially less efficient than at the slower speeds, where some of the additional work output was heat. In order to prevent this unnecessary heat build-up with faster rotational drilling speeds, it may be necessary to drill with faster feed rates. However, faster feed-rates to achieve the ‘optimal’ rate for a fast drill-speed is likely contraindicated in this particular application since severe damage may occur if the surgeon were to overshoot the margin of the skull and accidentally penetrate the brain tissues. It is likely better in most hand-driven applications to simply use both a slower rotational speed and feed-rate. We therefore reach a similar recommendation to that of Toews *et al.*, to use a slow drill speed combined with sufficient axial force to advance the drill as rapidly as possible through the bone.(66)

A limitation of our study was the significant trial-to-trial variability in both temperature and time, suggesting that force, feed-rate, angle of application, and other variables inherent in manual drilling inherently influenced the heating outcome in each trial. Applied force (and the proportionally related feed-rate parameter) increases the rate of heat generation, as does rotational speed. At the same time, increases in force, feed-rate, and rotational speed also decrease the duration of contact needed to penetrate the bone, which overall decreases the total temperature change of the bone.(66–69) The heat generated during bone drilling is complex and has been extensively studied and modeled in the orthopedics space.(67) Due to the competing effects of the different mechanical parameters, the impact of each one has a nonlinear relationship with maximum bone temperature during drilling. Surgeons most often use constant force drilling as opposed to constant feed rate(67), using the change in load as a

feedback detection for knowing when the boundaries of the bone have been reached. During our experiments, every attempt was made to use a consistent force applied to the drill bit, taking great care to not mechanically damage the brain surface after the skull had been breached. While we did not directly assess post-mortem tissue histopathology to assess the condition of the meninges, gross anatomy of the brain surfaces appeared intact. The same surgeon performed all procedures and in a similar manner across the study. While these factors were controlled as well as possible, future studies may find better repeatability and accuracy with automated drilling setups such as the one described by Pak *et al.*, which can tightly control feed-rate and/or force of application (as well as x-y controls).(70)

The use of more sophisticated technologies may be able to mitigate the issue of heating during bone drilling. Internally cooled drill bits have been used in the orthopedics space.(45, 47) While the use of such drilling apparatuses in research is currently limited due to added cost and complexity, it is worthwhile to note that they exist and may potentially alleviate heating issues in these applications if they can be adapted to the required size constraints for performing rodent craniotomies. Gupta *et al.* recently published an ultrasonic cortical drilling method to reduce the risk of heating.(46) The use of novel cutting methods like this may even further reduce risk to the underlying tissues.

As a final limitation to our study, we did not directly measure the effect of increased acute BBB permeability on the evolution of the chronic inflammatory cascade for implanted intracortical microelectrodes. Future work may be aimed at determining if there is a significant impact on the cellular immune/inflammatory response, based on the extent of initial BBB breach during craniotomy, or even device insertion. However, a main conclusion of the present study is that overheating prevention can be easily and unintentionally achieved. If the nuanced differences between in performing the same procedure indeed has an effect on the broader inflammatory response after microelectrode implantation, it may further contribute to the challenge of comparing results across different research groups and surgeons.

Conclusion

We hypothesized that overheating the brain tissues during typically-performed craniotomy drilling procedures may inadvertently increase BBB permeability, and that practices to avoid routine iatrogenic injury were inconsistently reported and practiced in the field. We confirmed that drilling was indeed a source of heat and that it was capable of producing temperature increases on the brain surface of $>25^{\circ}\text{C}$. We consequently determined the root cause of the effect and validated a method to avoid drill overheating in the future, i.e., pulsed drilling with intermittent or continuous irrigation with saline. While pulsed application still produced heat, even momentarily increasing surface brain temperatures to above $+13^{\circ}\text{C}$, it was apparently below a threshold to produce measureable damage to the BBB.

While the authors are not suggesting that every rat craniotomy method published in the field is confounded as a result of overheating, we are suggesting that it is rather easy to do accidentally, and it should be brought to the forefront of researchers minds as they are

studying the varying impacts of materials implanted in the brain on the neuroinflammatory response. To avoid a systematic and potentially confounding variable, the authors recommend that individual research labs validate their specific and potentially nuanced craniotomy procedures with the use of intravenously injected Evans blue dye, which provides a simple and rapid evaluation of BBB permeability. The authors additionally recommend that researchers use a slow drill speed combined with sufficient axial force to advance the drill as rapidly as possible through the bone, and in conjunction with other prevention methods such as intermittent (pulsed on/off) drilling with complete removal of the drill from the skull during “off” periods. While saline alone was ineffective to prevent overheating, its use is still recommended to remove bone dust from the surgical site and to augment other cooling methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Literature review of rodent craniotomy methods.

479 journal articles were identified from a PubMed database search that included a search for rodent (rat or mouse) craniotomy methods, as well as all papers pertaining to “intracortical microelectrodes”. *Column 1:* articles were categorized by their primary field of publication (e.g., “trauma” vs “orthopedics”). *Column 2:* of the subset of journal articles pertaining to “intracortical microelectrodes” (n=61), 89% did not specifically mention methods for the prevention of drill overheating during craniotomy; 38% provided no details regarding the craniotomy method whatsoever. *Column 3:* of the subset of intracortical microelectrode papers that described a method to prevent drill overheating (n=7), 4/7 described the use intermittent saline, 2/7 described the use of continuous saline irrigation, and 1/7 described the use of an alternative method for producing the craniotomy, i.e., a biopsy punch.

Figure 2: Maximum temperature recorded during drilling of rat craniotomy.

To quickly visualize whether we were overheating the skull during drilling, we captured thermal images of the drilling with an infrared camera. Maximum temperatures exceeded an increase of +21 °C from baseline for the 15k revolutions per minute (RPM) drill speed. The image shown was captured using a thermal infrared camera to show the temperature profile of the skull surface. A picture was taken for 1k, 5k, 10k, and 15k RPM, which increased the baseline temperature by +3°C, +5°C, +8°C, and +21°C, respectively.

Figure 3: Transient temperature changes at the brain surface during 15k RPM drilling (directly below the *post mortem* rat skull).

We placed a small thermocouple on the brain surface from a secondary hole previously drilled ~2 mm away from the site (figure inlay). The experiment was performed with *post mortem* tissue as placement of the thermocouple was likely to cause damage to the superficial surface of the brain during placement therefore making *in vivo* measurement impractical. Three types of drilling at 15k RPM were tested (continuous, pulsed 2s-on/2s-off, and pulsed 2s-on/5s-off) in triplicate (n=3 per condition). We observed that continuous application of the drill led to temperature increases greater than 25°C within 15–30s. Pulsed application still led to a significant increase in temperature (ranging from +9.1 to +13.6°C). Further extending the “off” period duration to 5s during pulsed application of the drill to allow for additional cooling resulted in (+3.1 to +7.9°C). In all tested cases, the brain cooled down back to baseline within 60s from peak temperature.

Figure 4: Evans blue dye concentration 1-hour after performing various powered drill craniotomy techniques.

We measured Evans blue concentrations after drilling at various speeds (15k, 10k, 5k, 1k RPM) as well as either continuously or pulsed 2s-on/2s off. There was a nearly 15-fold increase in dye concentration in the brain tissues that were exposed to continuous 10k or 15k speed drilling versus the “no drill” control. Slow speed (1k) and pulsed application appear to be the most effective means to reduce impacts on BBB permeability. Interestingly, saline application was not an effective or repeatable method to mitigate increases in BBB permeability from 15k continuous drilling. n=11 for 15k and “no drill” conditions; n=4 per group for 10k, 5k, 1k, “15k pulsed”, and “15k saline” conditions. Bars represent mean $\pm$ standard error of the mean. Analysis included ANOVA with pairwise comparisons with Tukey tests: *p<0.05; **p<0.01; ***p<0.001

Table 1:

Factors that Influence Bone Drill Overheating (44, 45, 47, 71, 72)

➤ Drill Speed
➤ Contact Time
➤ Feed Rate (Force of Application)
➤ Bit Age (Sharp/Dullness)
➤ Bit Size (Dimensions)

Table 2:

Methods to prevent drill overheating during rodent craniotomy procedures referenced from intracortical microelectrode literature

	Ref.
<i>"The biopsy punch prevented heat damage caused by motorized drills and provided a well-defined 3 mm opening. "</i>	(50)
<i>"A craniotomy (5 mm x 3mm) was made in the parietal bone to expose the somatosensory cortex. Sterile saline was applied during pauses while drilling to help dissipate any local heating and to clean the surfaces. "</i>	(55)
<i>"A single craniotomy, roughly 2.5 mm in diameter, was made centered at approximately -1.5 mm posterior to bregma and 2.1 mm lateral to midline using a dental drill (Z-35, Henry Schein Inc., Melville, NY). Sterile saline was applied during pauses while drilling to help dissipate any local heating. "</i>	(40)
<i>"A 3-4 mm diameter craniotomy was created over primary motor cortex using an air driven drill bit with sterile saline rinses to prevent excessive heating "</i>	(17)
<i>"A 4 mm by 6 mm craniotomy was made by thinning the skull over the somatosensory and visual cortex using a high speed dental drill and then removed with forceps. The skull was periodically bathed in saline to ensure that the underlying cortex did not experience thermal damage from drilling. "</i>	(56)
<i>"A small pinhole craniotomy was made over visual cortex with a high-speed dental drill and bone fragments were carefully removed with forceps and saline. Saline was applied continuously onto the skull to dissipate heat from the high-speed drill. "</i>	(54)
<i>"The top surface of the skull was exposed and a 3 mm by 3 mm craniotomy made over the primary visual cortex using a manual hand drill. Saline was applied onto the skull continuously to dissipate heat from the high-speed drill."</i>	(53)