Brain surface temperature under a craniotomy

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Kalmbach AS, Waters J. Brain surface temperature under a craniotomy. J Neurophysiol 108: 3138–3146, 2012. First published September 12, 2012; doi:10.1152/jn.00557.2012.—Many neuroscientists access surface brain structures via a small cranial window, opened in the bone above the brain region of interest. Unfortunately this methodology has the potential to perturb the structure and function of the underlying brain tissue. One potential perturbation is heat loss from the brain surface, which may result in local dysregulation of brain temperature. Here, we demonstrate that heat loss is a significant problem in a cranial window preparation in common use for electrical recording and imaging studies in mice. In the absence of corrective measures, the exposed surface of the neocortex was at -28°C, -10°C below core body temperature, and a standing temperature gradient existed, with tissue below the core temperature even several millimeters into the brain. Cooling affected cellular and network function in neocortex and resulted principally from increased heat loss due to convection and radiation through the skull and cranial window. We demonstrate that constant perfusion of solution, warmed to 37°C, over the brain surface readily corrects the brain temperature, resulting in a stable temperature of 36–38°C at all depths. Our results indicate that temperature dysregulation may be common in cranial window preparations that are in widespread use in neuroscience, underlining the need to take measures to maintain the brain temperature in many physiology experiments.

craniotomv; δ-wave; neocortex; slow wave; temperature

THE DEVELOPMENT OF IMAGING techniques that allow resolution of cells and subcellular structures deep in brain tissue (e.g., Denk et al. 1990; Svoboda et al. 1997) together with increasing use of whole-cell recording techniques in vivo have contributed to a recent surge in the popularity of anesthetized and awake animal preparations, particularly in cellular neuroscience. Key advantages of anesthetized and awake animal preparations include the preservation of synaptic connections, which would be cut during the preparation of brain slices or dissociated cultures, and the maintenance of the extracellular milieu by the intact circulatory system.

Superficial structures, such as neocortex and cerebellum, are often the structures of interest in in vivo studies. Superficial structures are relatively accessible in vivo, being exposed by the removal of skin and a small area of bone overlying the region of interest. No brain tissue needs to be removed, and this approach might therefore be considered minimally invasive. Nonetheless, blood vessels may connect the brain surface, dura, and bone, and the local circulatory system might be disrupted, perhaps compromising its ability to maintain the normal extracellular milieu and a stable and physiologically appropriate temperature. The removal of bone and skin might further limit temperature regulation by reducing thermal insulation above the structure of interest.

Brain temperature has been measured in several species, often with thermocouple or thermistor probes and occasionally with thermal imaging techniques. In large mammals, including primates, the temperatures of deep-brain structures and of the scalp, subarachnoid space, and cortical surface are all similar to the core body temperature, at approximately 38–39°C (Hayward and Baker 1968, 1969), but deep anesthesia can suppress brain temperature by several degrees (Hayward and Baker 1968). In awake rats, deep-brain structures are typically at approximately 37–40°C, with temperature changes of up to -3°C during activity, probably due to muscular activity and brain activation (Abrams and Hammel 1965; Moser et al. 1993). In rats, the superficial layers of neocortex are several degrees cooler than core body temperature, with the temperature 400 μm below the pial surface of neocortex being approximately 3–4°C cooler than the core body temperature (Bindman et al. 1962; Zhu et al. 2006), consistent with scalp measurements, which also suggest a cooler surface temperature (Barone et al. 1997). Hence, in an anesthetized rodent with a core body temperature of 37–38°C, one might expect the temperature at the surface of neocortex to be approximately 33–35°C. In an awake rodent, the surface temperature is likely to be similar or slightly warmer, probably approximately 33–36°C.

Here, we show that the surface of the neocortex immediately below a small cranial window in the anesthetized mouse can be up to 10°C colder than the core body temperature. We study the network and cellular consequences of this failure to maintain the brain surface temperature. We identify the main routes of heat loss from the brain surface and show that constant perfusion of warmed solution across the surface of the brain ensures that neocortex is maintained at physiological temperature during experiments that require exposure of the brain surface via a craniotomy.

METHODS

All experiments and procedures were approved by the Northwestern University Institutional Animal Care and Use Committee. Anesthetized mouse preparation. Postnatal days 19-30 C57BL/6 mice were anesthetized with 1.5–2% isoflurane (inhaled). Depth of anesthesia was sufficient to eliminate pinch withdrawal, corneal reflex, and vibrissal movements. The skin and scalp on both sides of the midline were removed, and a small steel plate was attached to the bone using dental acrylic (Stoelting, Wood Dale, IL). A 2- x 2-mm craniotomy was opened over barrel cortex (centered 1 mm posterior to bregma, 3 mm lateral). The craniotomy was covered with agarose (1–1.5%, type III-A; Sigma, St. Louis, MO) in artificial cerebrospinal fluid (ACSF): 135 mM NaCl, 5.4 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, and 5 mM HEPES. A glass coverslip was positioned over the agarose and clamped in place using steel springs, which were attached...
to the steel plate. Gentle downward pressure was applied to the coverslip to reduce heartbeat and breathing-induced motion of the cortex. The core temperature of the mouse was measured using a rectal probe and maintained at 37–38°C with a heating pad and temperature controller (FHC, Bowdoin, ME) throughout the surgery and subsequent experiment.

Awake mouse preparation. We measured the brain surface temperature from four awake mice. All other experiments were performed on anesthetized mice. For awake measurements, a craniotomy was first opened under anesthesia. The mouse was anesthetized using isoflurane (2–4%, inhaled). Depth of anesthesia was sufficient to eliminate pinch withdrawal, palpebral reflex, and vibrissal movements. The mouse was placed in a stereotaxic frame, and the skin and scalp on both sides of the midline between ~7 mm posterior and ~3 mm anterior to bregma were removed. A 200-mg aluminum plate was attached to the animal’s head using dental acrylic. This plate contained a 7-mm diameter hole, which was centered over barrel cortex (centered 1 mm posterior to bregma, 3 mm lateral). The mouse was allowed to recover for 2–3 days, during which it was conditioned to head restraint while on an exercise ball (20–30 min conditioning a day). On the day of the experiment, the mouse was reanesthetized, and a 2- × 2-mm craniotomy was opened over barrel cortex and covered with 1% agarose in ACSF. After a brain surface temperature measurement in the anesthetized condition, isoflurane was withdrawn, waking the mouse. A second temperature reading was made while the mouse was stationary or running on an exercise ball.

Temperature measurements. All temperature measurements were made with a BAT-12 Microprobe Thermometer (Physitemp Instruments, Clifton, NJ) and a 24-gauge polyurethane-coated wire probe with polyester-insulated thermocouple bead (IT-24P). The probe was attached to an acrylic post and mounted on a micromanipulator. The tip of the probe (maximum diameter 230 μm) was then positioned on the surface of the neocortex.

Accuracy of the thermometer/thermocouple probe was verified by measuring the temperature of a beaker of water with the thermocouple probe and comparing the result to the temperature measured by a standard liquid-filled laboratory thermometer (61019-010; VWR International, Radnor, PA). We were also concerned that the thermocouple probe might report the temperature averaged over some length of probe near the tip rather than the temperature at the tip of the probe. We therefore placed only the tip of the probe against a heating block, which was at 37°C. The measured temperature was 37 ± 1°C, indicating that the temperature reported was that at the tip of the probe.

We imaged neurons by two-photon microscopy (below) using a ×40/0.8-numerical aperture (NA) water-immersion objective (Carl Zeiss). The temperature of this objective was controlled with a microscope objective warmer custom-fit to the objective (OW-1 and TC-124 controller; Warner Instruments, Hamden, CT). In control experiments, we attached the tip of the temperature probe to the barrel of the objective and measured the temperature of the objective when the heater was switched on. We found that the temperature changed slowly, with a time constant of 6–7 min, although the heater controller indicated that the command temperature was achieved a few seconds after switching on the controller. The measured temperature was also up to 10°C cooler than the command temperature despite the controller reporting that the command temperature had been achieved.

To warm the surface of the brain, warmed ACSF was perfused across the craniotomy with a thermistor-based in-line solution heater placed a few inches from the craniotomy (SH-27B; Warner Instruments). The temperature of the solution was monitored a few millimeters from the craniotomy and maintained at 37°C by a feedback circuit and temperature controller (TC-324B). Flow rate was ~3 ml/min.

Whole-cell electrophysiology. Whole-cell patch-clamp recordings were obtained using a “blind” technique as described previously (Margrie et al. 2002; Waters et al. 2003). Recordings were obtained using pipettes with 4- to 6-MΩ tip resistance when filled with an intracellular solution containing: 135 mM K-glucoside, 4 mM KCl, 10 mM HEPES, 10 mM Na2-phosphocreatine, 4 mM Mg-ATP, 300 μM Na-GTP, 20 μM Alexa 594, and 0.2% (w/vol) biocytin, pH 7.2, 291–293 mOsM. Positive pressure (200 mbar) was applied to the pipette as it was inserted through the agarose, dura, and pial surface of the cortex. The positive pressure was reduced to 25–30 mbar when the tip was at approximately the upper border of layer 2/3 and the pipette was then advanced in 2-μm steps. Voltage pulses were applied to the pipette, and the current response was monitored. Positive pressure was relieved when the series resistance of the electrode abruptly increased immediately after a step, indicating that the tip of the pipette may have been pushed against a neuronal plasma membrane. Gentle suction (up to 100 mbar) was applied where necessary to obtain a gigahm seal. All recordings were obtained using an AxoClamp-2B amplifier (Molecular Devices, Sunnyvale, CA). Layer 2/3 pyramidal neurons were identified in somatic recordings by 1) their regular firing pattern, 2) their characteristic apical dendrite and distal tuft, and 3) the depth of the soma below the pia (150–400 μm), measured by two-photon microscopy.

In the two-photon microscope, tissue was illuminated with 830-nm light from a Ti:Sapphire Laser (Chameleon Ultra; Coherent, Santa Clara, CA) through a ×40/0.8-NA water-immersion objective (Carl Zeiss). Emitted light was collected in the epifluorescence configuration via a 680-nm dichroic reflector and infrared blocking emission filter (ET070sp-2P, Chroma Technology, Bellows Falls, VT). Alexa 594 fluorescence was detected using a 575- to 645-nm band-pass filter (Chroma Technology) and photomultiplier tube (R6357; Hamamatsu Photonics). Scanning and acquisition were controlled by software written in LabVIEW (National Instruments, Austin, TX) by J. Waters.

Data analysis. Analysis was performed using IGOR Pro 6.0 (WaveMetrics, Lake Oswego, OR) and MATLAB (The MathWorks, Natick, MA). Physiological properties were measured in bridge mode, using 300-ms square current injections. Voltage-current (V-I) relationships were derived from the steady-state voltage during 300-ms current steps and were fit with the following relationship (Waters and Helmchen 2006):

$$\Delta V = R_N \Delta I + C_{AR} \Delta I^2$$

where $R_N$ is the resting input resistance (slope at $I = 0$) and $C_{AR}$ is the coefficient of anomalous rectification, which describes curvature of the V-I relationship.

Action potential threshold was defined as the point at which the first temporal derivative of the voltage (dV/dt) first exceeded 55 mV/ms. Action potential amplitude was measured from threshold. Action potential half width was measured at half the amplitude. Action potential rise and decay times were measured as the time intervals between 10 and 90% of the voltage difference between threshold and peak. Rheobase was defined as the minimum amount of current required to evoke an action potential using a 300-ms current pulse.

Mean membrane potentials during Down and Up states were defined as the modal values of the two peaks in the membrane potential frequency histogram (e.g., Fig. 2D). For calculations of percentage time in Up and Down states and the durations of Up and Down states, Up and Down states were again distinguished using membrane potential frequency histograms, the dividing voltage being defined as the least frequent membrane potential between the Up and Down state peaks.

Multitaper spectral analysis was performed on 20-s segments of membrane potential using the Chronux toolbox for MATLAB (Mitra and Pesaran 1999), averaging power in the 0- to 1.5-Hz frequency band.
RESULTS
Like many investigators who study neocortex in vivo, we access the neocortex through a small (2 × 2 mm) craniotomy over the brain region of interest. In such experiments, we commonly cover the exposed brain surface with agarose and a coverslip and then record from and image neurons in the surface layers of neocortex using a water-immersion microscope objective. To facilitate these experiments, we first remove the skin and scalp over the skull and attach a small steel plate to the animal’s head with dental acrylic (Fig. 1A). In this preparation, the potential routes of heat transfer to/from the brain surface include 1) convection in the brain (principally by circulating blood), in the ACSF, and in the air surrounding the craniotomy, 2) conduction through the dental acrylic, steel plate, and microscope objective, and 3) radiation through the craniotomy or through the dental acrylic, steel plate, and exposed skull.

In this preparation, the temperature at the surface of neocortex, measured with a small-diameter thermocouple probe, was 29.6 ± 0.3°C (n = 8 mice; range 28.6–30.7°C). This is cooler than we had expected and almost 10°C below the core body temperature, which was maintained at 37–38°C. Replacing the ACSF over the craniotomy with fresh ACSF warmed to 38°C resulted in an almost instant rise in temperature, which was maintained at 37–38°C. Replacing the artificial cerebrospinal fluid (ACSF) with fresh ACSF warmed to 38°C. ACSF was replaced twice, indicated by the arrowheads. The temperature decaying rapidly and approximately exponentially back to its initial value with a time constant of 40 ± 2 s (n = 3 mice; Fig. 1B).

Some anesthetics can alter brain temperature by several degrees (e.g., Baker et al. 1973). To determine whether the cool surface temperature was the result of anesthesia, in four mice we measured the surface temperature when the mouse was anesthetized (1–1.5% isoflurane) and again 12–35 min later after the anesthetic had been withdrawn and the mouse had recovered consciousness. The cortical surface temperature was 28.4 ± 0.6°C under anesthesia and 29.4 ± 0.7°C when awake (not significantly different, paired t-test). Hence, in both awake and anesthetized mice, heat was lost from the brain surface, resulting in the brain surface near the craniotomy being significantly below core body temperature.

Effects of brain temperature on network activity and the cellular properties of superficial pyramidal neurons. The published literature suggests that the temperature at the surface of neocortex should be at least several degrees warmer than 28–31°C, suggesting that the low surface temperature in our experiments was an artifact of the preparation. We determined how this lower surface temperature affects neocortical network activity and cellular function in anesthetized mice by manipulating the surface temperature, raising the temperature by perfusing across the craniotomy ACSF warmed to 37°C. To monitor network activity and cellular function, we changed the surface temperature while recording membrane potential from a layer 2/3 pyramidal neuron (Fig. 2). As expected, the membrane potential fluctuated between a relatively hyperpolarized Down state and a more depolarized Up state. This pattern of periodic depolarization is observed under many anesthetics, Up

Fig. 1. Rapid cooling of the brain surface in an in vivo mouse preparation. A: schematic representation of a cranial window during recording of temperature and single-cell activity in the anesthetized mouse. The main potential routes of heat transfer are indicated. B: brain surface temperature measured with the thermocouple during replacement of the artificial cerebrospinal fluid (ACSF) with fresh ACSF warmed to 38°C. ACSF was replaced twice, indicated by the arrowheads.

Fig. 2. Example of effects of surface temperature on ongoing synaptic activity. A: 2-photon maximum intensity fluorescence side-projection of a layer 2/3 pyramidal neuron in barrel cortex during somatic whole-cell recording. The neuron was filled with indicator (Alexa 594) through the recording pipette, which is visible to the right of the image. The pial surface of the cortex is visible as punctate staining near the top of the image. B: surface temperature (temp.) and intracellular membrane potential (Vm.) of the neuron in A. The brain surface was warmed by perfusion with ACSF at 38°C. The brain surface was initially at ~28°C with no perfusion. The perfusion began after almost 10 min of recording. After the surface temperature warmed to ~36°C, the heater was switched off, and the brain surface cooled once more to ~29°C. Heating the perfusate a 2nd time had a similar effect. The membrane potential of the neuron was recorded throughout these manipulations. C: membrane potential recordings from 5 periods at ~28°C (blue) and ~36°C (red) at the times indicated in B. Dashed horizontal lines mark ~70 mV. D: membrane potential frequency histograms for the traces in C.
states resulting from waves of synaptic activity propagating through cortex (Cowan and Wilson 1994; Petersen et al. 2003; Steriade et al. 1993). Warming the brain surface resulted in a decrease in the frequency of Up states (Fig. 2, C and D) and a hyperpolarization of the Down state membrane potential (Fig. 2D). The effects of warming on membrane potential and network activity were reversible and reproducible (Fig. 2).

On average, warming the surface of neocortex from 28–30°C to 36–37°C resulted in ~30% increase in the mean duration of Down states and ~30% decrease in the mean duration of Up states, such that layer 2/3 pyramidal neurons shifted from spending 50% of their time in each state to spending ~65% of their time in Down states and only ~35% in Up states (Table 1; Fig. 3). Spectral analysis of our recordings revealed that warming the brain surface resulted in an increase in low-frequency power in four of eight recordings (Fig. 4). Hence, network activity in neocortex is sensitive to temperature in the 28–37°C range.

Warming the brain surface also changed some of the intrinsic properties of layer 2/3 pyramidal neurons (Fig. 5). At warmer temperatures, the membrane potential of the Down state was ~5 mV more hyperpolarized than at cooler temperatures, the Down state input resistance was ~30% lower, outward rectification in the Down state was decreased (Fig. 5; Table 2), and the neuron spiked less readily in response to somatic current injection (Fig. 5; Table 3). In contrast, none of these four intrinsic properties was sensitive to temperature in the Up state (Fig. 5; Tables 2 and 3). During both Up and Down states, action potential amplitude, half width, rise time, and decay time, but not threshold, all decreased with temperature (Table 4). Decreasing temperature also increased the decay time constants of putative excitatory postsynaptic potentials (EPSPs), measured during the Down state (Table 4).

We conclude that both network activity in neocortex and the cellular properties of layer 2/3 pyramidal neurons change with temperature. Temperature loss results in more prolonged Up states and layer 2/3 pyramidal neurons that are more depolarized and spike more readily in the absence of background synaptic activity.

**Routes of heat loss.** What are the main routes by which heat is lost from the surface of neocortex? We investigated whether conduction through the microscope objective or the dental acrylic and steel plate resulted in cooling of the brain surface.

To eliminate the potential temperature gradient between the brain and the microscope objective, we used a microscope objective warmer. Warming the objective to a command temperature of 37°C had no effect on the surface temperature of the cortex (Fig. 6A). Since we found that the temperature of the objective could be colder than the command temperature (see METHODS), to ensure that the objective was at ≥37°C, we then warmed the objective to 50°C. Again, this had no effect on the surface temperature of the cortex (Fig. 6B). Hence, heat loss from the brain by conduction through the microscope objective is negligible.

Next, we measured the surface temperature of the cortex without the dental acrylic and steel plate. The head of the mouse was held in a stereotaxic frame. The size of the incision in the skin was minimized (approximately 5 × 5 mm), and the craniotomy was covered in agarose and ACSF. In this prepa-

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**Table 1. Effects of temperature on Up and Down states**

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Low Temperature</th>
<th>High Temperature</th>
<th>Number of Recordings</th>
<th>P, Paired t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SE</td>
<td>Mean SE</td>
<td></td>
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<tr>
<td>Temperature, °C</td>
<td>29.4 0.3</td>
<td>36.5 0.3</td>
<td>6</td>
<td>&lt;0.0001*</td>
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<tr>
<td>V_m, Down state, mV</td>
<td>−69.8 4.6</td>
<td>−74.6 3.6</td>
<td>6</td>
<td>0.014*</td>
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<tr>
<td>V_m, Up state, mV</td>
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<td>−58.7 3.2</td>
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<td>0.32</td>
</tr>
<tr>
<td>%Down state</td>
<td>50.9 5.3</td>
<td>63.6 3.9</td>
<td>7</td>
<td>0.025*</td>
</tr>
<tr>
<td>%Up state</td>
<td>49.1 5.3</td>
<td>36.4 3.9</td>
<td>7</td>
<td>0.025*</td>
</tr>
<tr>
<td>Down state duration, ms</td>
<td>508 68</td>
<td>668 96</td>
<td>7</td>
<td>0.019*</td>
</tr>
<tr>
<td>Up state duration, ms</td>
<td>353 121</td>
<td>367 53</td>
<td>7</td>
<td>0.069</td>
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</table>

V_m, membrane potential. *P < 0.05.

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Fig. 3. Effects of temperature on cortical Up and Down states. The effects of temperature on the mean membrane potentials of Up and Down states, percentage of time spent in each state, and the duration of Up and Down states are shown. Each pair of points (connected by a line) shows the mean ± SE values for a single neuron. n = 7 Recordings from 6 mice. Temperature was manipulated by perfusion of warmed ACSF across the brain surface. Down state membrane potential, percentage of time in Up and Down states, and Down state duration each changed significantly (P < 0.05, paired t-test) with temperature.

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Innovative Methodology

BRAIN SURFACE TEMPERATURE UNDER A CRANIOTOMY

Between skin and skull. With the tip of the thermocouple temperature under the skin by pushing the thermocouple probe through the dental acrylic and steel plate is negligible. Was 33.2 cm from the incision, the temperature between skull and skin was 31.4 °C (Fig. 7A). In this preparation, the surface temperature of the brain was 29°C (Fig. 7B). Hence, heat loss from the brain by conduction through the exposed bone (Fig. 7A). The main barriers to heat loss are the skin and hair overlying the skull, both of which must be removed to open a craniotomy.

Maintenance of physiological temperature in the brain. We tested whether providing additional heat, by raising the core temperature of the mouse via the heating pad and temperature controller, would warm the brain surface to near-physiological temperatures. On increasing the core temperature, the brain surface temperature increased by approximately the same amount after a delay of several minutes (Fig. 8A; mean increase in core temperature 4.4 ± 0.4°C, in surface temperature 3.2 ± 0.5°C, 3 mice). Since the brain surface under a craniotomy is generally at least 5°C too cold, raising the brain surface temperature to near-physiological temperatures requires an elevation in core temperature of ≥5°C. Unsurprisingly, this also resulted in an equivalent rise in temperature deep in the brain (Fig. 8B). Hence, increasing the core temperature main-

Fig. 4. Effects of temperature on low frequency power. A: example of the effect of temperature on mean power in the 0- to 1.5-Hz band for a voltage recording from layer 2/3. Trials classified as warm and cold are denoted by red and blue symbols, respectively. B: mean spectra for the recording in A at colder (blue; 9 trials) and warmer (red; 9 trials) temperatures. Shaded areas represent SE. Inset: plot of mean spectral power at 0–1.5 Hz as a function of temperature for the 18 trials shown in A: plot of mean spectral power at 0-1.5-Hz power for a voltage recording from layer 2/3. Trials classified as warm and cold are denoted by red and blue symbols, respectively. C: mean ± SE 0- to 1.5-Hz power at warm and cold temperatures for 6 recordings from 7 mice.

In the absence of dental acrylic and the steel plate, the incision in the skin was exposed, enabling us to measure the temperature under the skin by pushing the thermocouple probe between skin and skull. With the tip of the thermocouple ~1 cm from the incision, the temperature between skull and skin was 33.2 ± 0.05°C (n = 3), which is warmer than the brain surface. We reason that the temperature of the brain surface must therefore be ≥33°C when covered by skin and that removing the skin and cutting a craniotomy reduces the temperature of the brain surface by at least several degrees Celsius.

As the loss of heat from the brain surface due to conduction through the microscope objective, dental acrylic, and steel plate is negligible, we conclude that the main routes of heat loss from the brain surface are convection and radiation through the exposed bone (Fig. 7A). The main barriers to heat

Fig. 5. Effects of temperature on membrane properties of cortical pyramidal neurons. A: voltage-current (V-I) curves, sorted by state and temperature, for an example neuron. Temperature was manipulated by perfusion of warmed ACSF across the brain surface. B: effects of temperature on input resistance (Rn), the coefficient of anomalous rectification (cAR), and rheobase. n = 5 Recordings from 5 mice for input resistance and anomalous rectification, and rheobase. Asterisks denote significant differences (P < 0.05, paired t-test).

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ological temperature in brain tissue under a craniotomy. Simple and effective approach that readily restores the physiological temperature even several millimeters below the surface (Fig. 9A). When perfused with ACSF at 37.4°C, the brain surface remains the brain surface at a near-physiological temperature but at the cost that most of the brain and mouse is ~5°C warmer than the normal physiological temperature range. Obviously this rise in core and deep-brain temperature may have undesirable consequences for the physiology of the brain and other tissues.

A preferable solution would be to eliminate heat loss from the brain surface by replacing the skin and fur with a warm barrier between the exposed brain and cooler air. Warming the head plate might reduce radiative heat loss but would presumably still permit heat loss through the brain-agarose-ACSF pathway. A more direct solution to the problem is to perfuse warm ACSF constantly across the brain surface. We characterized the effect of constant perfusion on temperature up to 4.5 mm below the pial surface of the cortex. With no perfusion, the temperature increased steadily with depth but was below physiological temperature even several millimeters below the surface (Fig. 9A). When perfused with ACSF at 37.4 ± 1.3°C, the brain temperature was more uniform, between 37 and 39°C at all depths (Fig. 9A). Warming by surface perfusion was rapidly reversible (Fig. 9A). We conclude that surface perfusion is a simple and effective approach that readily restores the physiological temperature in brain tissue under a craniotomy.

DISCUSSION

We have shown that the temperature on the brain surface is reduced to 28–31°C by a small cranial window over the intact mouse neocortex. This reduction in temperature alters network activity in neocortex and causes depolarization and an increase in input resistance in layer 2/3 pyramidal neurons, reducing the current required to evoke spikes.

Mechanism by which temperature affects network activity. The cellular effects of cooling neocortex from physiological to room temperature have previously been studied in slice preparations but not, to our knowledge, in vivo. Cooling can cause depolarization and increase the input resistance of layer 2/3 pyramidal neurons in acute slices (Hedrick and Waters 2012; Trevelyan and Jack 2002; Volgushev et al. 2000b). Here, we have shown that temperature has similar effects on layer 2/3 pyramidal neurons in vivo. However, the relationship between temperature and cellular properties is complex. For example, cooling from physiological temperature to room temperature results in hyperexcitability of layer 2/3 pyramidal neurons, but

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**Table 2. Effects of temperature on input resistance and rectification**

<table>
<thead>
<tr>
<th>Temperature, °C</th>
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<td></td>
<td>Mean SE</td>
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<tr>
<td>29</td>
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<td>36.7 0.3</td>
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<td>30</td>
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<td>36.7 0.3</td>
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<tr>
<td>31</td>
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<tr>
<td>32</td>
<td>0.4 0.5</td>
<td>36.7 0.3</td>
<td>5</td>
<td>0.0001*</td>
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R_{in}, input resistance; C_{An}, coefficient of anomalous rectification. *P < 0.05.

**Table 3. Effects of temperature on rheobase**

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<th>Temperature, °C</th>
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<tr>
<td>29.1</td>
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<td>36.7 0.3</td>
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<td>Rheobase, Up state, pA</td>
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<td>139 32</td>
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*P < 0.05.

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**Table 4. Effects of temperature on action potential characteristics and EPSP decay time**

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<th>Temperature</th>
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<th>High Temperature</th>
<th>Number of Recordings</th>
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<td>Down states</td>
<td>Threshold, mV</td>
<td>-29.6 2.3</td>
<td>-31.3 2.9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Amplitude, mV</td>
<td>67.3 4.3</td>
<td>57.8 3.4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Half-width, ms</td>
<td>0.98 0.06</td>
<td>0.68 0.04</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10–90% Rise</td>
<td>0.25 0.02</td>
<td>0.20 0.01</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10–90% Decay</td>
<td>1.35 0.13</td>
<td>0.96 0.1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>EPSP decay time</td>
<td>17.0 1.6</td>
<td>10.2 0.9</td>
<td>5</td>
</tr>
<tr>
<td>Up states</td>
<td>Threshold, mV</td>
<td>-32.6 1.8</td>
<td>-34.8 2.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Amplitude, mV</td>
<td>71.3 3.5</td>
<td>62.3 1.3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Half-width, ms</td>
<td>0.9 0.04</td>
<td>0.64 0.04</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10–90% Rise</td>
<td>0.25 0.01</td>
<td>0.19 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10–90% Decay</td>
<td>1.23 0.11</td>
<td>0.87 0.06</td>
<td>8</td>
</tr>
</tbody>
</table>

EPSP, excitatory postsynaptic potential. *P < 0.05.

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Fig. 6. Warming the microscope objective has no effect on brain surface temperature. Examples of the lack of effect on brain surface temperature of warming of the microscope objective to 37°C (A) and 50°C (B). In each case, the objective warmer was switched on at $t = 1$ min and remained on thereafter.
excitability declines with further cooling toward 10°C (Volgushev et al. 2000b). The spiking patterns of neocortical pyramidal neurons (Hedrick and Waters 2011, 2012) and synaptic transmission are also temperature-sensitive (e.g., Hardingham and Larkman 1998; Volgushev et al. 2000a). The slowing of EPSP decay times at cooler temperatures, described here and previously (Volgushev et al. 2000a), may also contribute to the prominence of Up states at cooler temperatures. Hence, it is difficult to predict how temperature will affect network activity from studies of the temperature sensitivity of the many components of neocortical networks.

The effects of temperature on network activity have been studied in slices and in vivo. In anesthetized rats, a decrease in core body temperature promotes slow-wave activity in neocortex (Whitten et al. 2009). In slices, local field recordings of spontaneous slow rhythmic activity revealed a prolongation of both Up and Down states on cooling below physiological temperatures (Reig et al. 2010). With whole-cell recordings in vivo, we observed an increase in Up state duration but a decrease in the duration of Down states. The different effects on network activity observed in our study and in slices may result from altered propagation of activity through neocortical networks in slice preparations, in which many connections are cut, or from differences between the extracortical milieu in vivo and the ACSF bathing the slice since this rhythmic activity is sensitive to extracellular ion concentrations in vitro (Sanchez-Vives and McCormick 2000).

The cellular and synaptic basis of these changes in network activity is unclear in part because the origins of Up states and the determinants of their durations are uncertain. In addition, the effects of temperature on most neocortical cell types and synaptic connections are unknown. In layer 2/3 neurons, Up states reflect activation of synapses throughout the apical and probably basal dendritic trees (Milojkovic et al. 2007; Waters and Helmchen 2004). These synapses report to layer 2/3 pyramidal cells the activities of neurons in many cortical layers, and the activities of these different classes of neuron are synchronized during Up states (Lampl et al. 1999; Petersen et al. 2003). Hence, the changes in network activity with surface temperature, as reported here, probably involve changes in activity throughout the layers of neocortex. It is possible that the effects of temperature are mediated principally via a profound effect on one or more classes of neocortical neuron, but it is more likely that the effects of temperature result from more subtle effects on many different types of neuron in the neocortex, with the change in network activity being the aggregate of many cellular and synaptic effects.

Routes and prevention of heat loss. Conduction is unlikely to be a major source of heat loss from the brain in an intact animal in the absence of a craniotomy. Skin is a weak conductor of heat (thermal conductivity \( \sim 0.6 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1} \)), and air is an excellent insulator (thermal conductivity 0.024 \( \text{W} \cdot \text{m}^{-1} \cdot \text{K}^{-1} \)). Hence, an unstirred layer of air overlying the skin, trapped by the rodent’s hair, restricts conductive heat exchange between the brain surface and the surrounding environment. It appeared likely that conduction might become a significant source of heat loss once this skin and hair is removed, particularly with a small steel plate and water-immersion optics secured close to the animal’s head: thermal conductivities for steel, water, and glass are 43, 0.58, and 1.05 \( \text{W} \cdot \text{m}^{-1} \cdot \text{K}^{-1} \), respectively. However, we found that the steel plate and water-immersion optics have little effect on brain surface temperature. This is perhaps because water-immersion microscope objectives are designed to minimize thermal conductivity and the steel plate was

![Fig. 7. Brain surface temperature in the absence of conduction.](image)

A: schematic showing the likely routes of heat loss for a preparation without dental acrylic, steel plate, or microscope objective. B: temperature change on replacing the ACSF with ACSF warmed to 38°C in the preparation shown in A. The ACSF was replaced twice, as indicated by the arrowheads. C: overlaid temperature measurements from addition of warmed ACSF with (gray) and without (black) a head plate, illustrating the negligible effect of the head plate on heat loss.

Fig. 7. Brain surface temperature in the absence of conduction. A: schematic showing the likely routes of heat loss for a preparation without dental acrylic, steel plate, or microscope objective. B: temperature change on replacing the ACSF with ACSF warmed to 38°C in the preparation shown in A. The ACSF was replaced twice, as indicated by the arrowheads. C: overlaid temperature measurements from addition of warmed ACSF with (gray) and without (black) a head plate, illustrating the negligible effect of the head plate on heat loss.

![Fig. 8. Brain surface temperature during an increase in rectal temperature.](image)

A: example of the effect of raising core temperature on brain surface temperature. Heat was supplied to the mouse via a heating blanket with a rectal temperature probe and feedback control circuit. The command temperature was switched between 38 and 42°C. B: results from a similar experiment in which the thermocouple was inserted ~5 mm into the brain.

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attached to the mouse with dental acrylic. Dental acrylic is principally polymethyl methacrylate, which has a low thermal conductivity of \( \sim 0.2 \text{ W·m}^{-1} \cdot \text{K}^{-1} \) (Assael et al. 2005). Hence, the layer of dental acrylic probably provides substantial insulation, greatly reducing conductive heat transfer between the animal and the steel plate. These measurements suggest that warming the steel plate is unlikely to be an effective method of maintaining the physiological temperature of the brain surface.

With conductive heat loss contributing little to the lowering of brain surface temperature near a craniotomy, convection and radiation must be the main routes of heat loss from the brain surface. Convection includes bulk flow (e.g., blood flow) and evaporation. Potential routes of convective heat transfer include: the transfer of heat from deep within the brain to the surface via the blood; the transfer of heat through the agarose and ACSF overlaying the brain to the colder air and microscope objective; and the removal of heat from the ACSF by circulating air molecules. Radiative heat loss can presumably occur via the craniotomy or through the bone, dental acrylic, and steel plate.

We found that cutting a smaller craniotomy does little to limit the loss of heat from the brain surface. Reducing the size of the craniotomy would reduce convective heat transfer between the agarose, ACSF, and air and also radiative heat loss through the craniotomy. Hence, these routes are unlikely to be major routes of heat loss from the brain surface, suggesting that most heat loss occurs via radiation through the bone, acrylic, and steel plate.

Hair and skin presumably provide an insulating layer over the bone and brain surface as well as supporting constant perfusion with warmed blood. Hence, retraction of the skin allows pronounced cooling of the brain surface. In many preparations, skin and fur are removed over a much larger area than the craniotomy, and cooling of the brain surface may extend laterally beyond the craniotomy. Perfusion of the brain surface with warmed ACSF maintains the brain at physiological temperature, resolving the cooling problem. Although perfusion is simple to arrange, it suffers the inconvenience of additional elements that must be placed close to the craniotomy, including tubes for ACSF in- and outflow and a thermocouple probe to provide feedback to the electronics that control the ACSF temperature. Perfusion with ACSF may also perturb the extracellular concentrations of ions and metabolites near the surface of the neocortex.

One important question is whether perturbed blood flow in the neocortex in the vicinity of the craniotomy contributes to the failure to maintain a more normal surface temperature. Blood vessels may connect the brain surface, dura, and bone over neocortex, and removing the bone will necessarily damage these vessels. In addition, edema is a common problem in craniotomies, and severe edema can impede blood flow in surface vessels. Edema can be minimized by careful preparation of the craniotomy, and in our preparations little or no edema was visible. However, it is unclear to what extent local blood flow is perturbed near craniotomies even in the absence of visible edema. Whether possible perturbations in local blood flow contribute to the surface temperature loss is therefore unknown, but any such effect is likely to be common in craniotomy preparations, further emphasizing the need to measure and control brain surface temperature near a craniotomy.

**Physiological consequences of a reduction in brain surface temperature.** The change in temperature and resulting alteration in network activity that we have observed are likely to affect the functioning of neocortex under the craniotomy. For example, the responsiveness of neurons in primary sensory areas to sensory stimuli changes with ongoing activity in neocortex (Petersen et al. 2003; Sachdev et al. 2004) and temperature-dependent changes in network activity and cellular properties could therefore both affect sensory responses in cortex. In addition, brain surface temperature changes could have more widespread effects that extend beyond neocortex. In the songbird, localized cooling of the brain surface by just a few degrees affects the interaction of superficial and deep-brain structures (Long and Fee 2008). It is likely, therefore, that cooling of the neocortical surface under a craniotomy would affect activity in deeper structures that are connected to the cooled area of neocortex. Hence, cooling of the brain surface may have widespread effects throughout the brain.

In summary, we have shown that a cranial window over neocortex can have profound effects on the temperature of the brain surface. The preparation in which we have observed this loss of surface temperature is typical of preparations now widely employed in whole-cell recording and imaging studies in intact mice (e.g., Lee et al. 2006; Margrie et al. 2003; Stosiek et al. 2003), and craniotomies are routinely used in neuroscience to investigate activity in almost all regions of the brain. The extensive connectivity of neocortex with subcortical
areas means that changes in cortical activity with temperature can readily impact activity in subcortical structures. Clearly maintenance of the brain surface temperature is essential for the normal functioning of the brain, and temperature loss is likely to affect information processing in neocortex and perhaps other brain regions. Our results underline the need to measure the surface temperature of neocortex during experiments in intact rodents and, where necessary, to warm the brain surface.

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REFERENCES


Barone FC, Feuerstein GZ, White RF. Innovative Methodology


