Brief anesthesia, but not voluntary locomotion, significantly alters cortical temperature

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Brief anesthesia, but not voluntary locomotion, significantly alters cortical temperature. J Neurophysiol 114: 000–000, 2015. First published May 13, 2015; doi:10.1152/jn.00046.2015.—Changes in brain temperature can alter electrical properties of neurons and cause changes in behavior. However, it is not well understood how behaviors, like locomotion, or experimental manipulations, like anesthesia, alter brain temperature. We implanted thermocouples in sensorimotor cortex of mice to understand how cortical temperature was affected by anesthesia before experimental procedures. Analysis of the temperature changes revealed a large increase in vessel diameter that ceased before the cessation of anesthesia. Cortical temperature decreases were not accompanied by a concomitant decrease in the core temperature, indicating the inflow of blood cools the brain (Delgado and Hanai 1966; Hayward and Baker 1968; Kiyatkin et al. 2002). If the generated heat is not dissipated, it will increase neural excitability, potentially leading to runaway excitation (Holtzman et al. 1981; Dubé et al. 2007). A variety of behaviors (as reviewed in Kiyatkin 2007), as well as both noxious and arousing stimuli, have been observed to increase brain temperature across many species (Aronov and Fee 2012; Kiyatkin et al. 2002; Delgado and Hanai 1966; Baker et al. 1973), including forced locomotion (Caputa et al. 1983). Despite the potential role that neurally generated heat might play in modulating excitability, it is not well understood how voluntary locomotion impacts brain temperature.

Experimental setups where mice are head-fixed on a spherical treadmill are widely used (Dombeck et al. 2007; Huo et al. 2014 and 2015). Neural responses to sensory stimuli are typically heightened during locomotion (Niell and Stryker 2010; Polack et al. 2013; Lee et al. 2014). While these studies have shown that locomotion affects neural activity across the cortex, the potential role of cortical temperature changes due to locomotion modulating neural excitability has not been investigated. An additional methodological concern with awake recordings in mice is the need to anesthetize the animal to perform procedures to prepare it for imaging or electrophysiology (Drew et al. 2011; Gao and Drew 2014; Niell and Stryker 2010; Keller et al. 2012; Polack et al. 2013; Lee et al. 2014). General anesthesia diminishes neural activity and decreases both brain and body temperature (Kiyatkin and Brown 2005; Hayton et al. 1999; Hayward and Baker 1968; Zhu et al. 2009), but knowing the dynamics of the temperature recovery will inform the design of experiments utilizing anesthesia.

In the present study, we measured temperature changes in sensorimotor cortex of awake, behaving mice with chronically implanted thermocouples. We have previously shown that volitional locomotion drives large increases in cerebral blood volume and neural activity in forelimb/hind limb sensorimotor cortex, the potential role of cortical temperature changes due to locomotion affecting neural activity across the cortex, the potential role of cortical temperature changes due to locomotion modulating neural excitability has not been investigated. An additional methodological concern with awake recordings in mice is the need to anesthetize the animal to perform procedures to prepare it for imaging or electrophysiology (Drew et al. 2011; Gao and Drew 2014; Niell and Stryker 2010; Keller et al. 2012; Polack et al. 2013; Lee et al. 2014). General anesthesia diminishes neural activity and decreases both brain and body temperature (Kiyatkin and Brown 2005; Hayton et al. 1999; Hayward and Baker 1968; Zhu et al. 2009), but knowing the dynamics of the temperature recovery will inform the design of experiments utilizing anesthesia.

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NEURAL EXCITABILITY, RELEASE probability, and ion channel kinetics are drastically affected by temperature (Hodgkin and Katz 1949; Volgushev et al. 2000a, 2000b, and 2004; Hedrick and Waters 2012; Kalmbach and Waters 2012). Temperature affects neural dynamics and oscillations (Reig et al. 2010; Rinberg et al. 2013; Soofi et al. 2014). In homeothermic animals, fluctuations in temperature are buffered, but brain temperature can still fluctuate by several degrees (Delgado and Hanai 1966). Experimenter-induced and spontaneous brain temperature changes contribute to behavioral variability (Long and Fee 2008; Aronov and Fee 2012; Nybo 2012). Increases in neural activity lead to increases in local brain temperature (Yablonskiy et al. 2000; Baker et al. 1973; Trübel et al. 2006), partially due to changes in core body temperature that accompany behavior (De Castro 1980; Wanner et al. 2013). However, temperature increases in the brain precede and exceed those in the core (Kiyatkin et al. 2002), indicating a neural origin to this heating. Blood exiting the brain is of higher temperature than blood entering the brain, indicating the inflow of blood cools the brain (Delgado and Hanai 1966; Hayward and Baker 1968; Kiyatkin et al. 2002). If the heat generated by neural activity is not dissipated, it will increase neural excitability, potentially leading to runaway excitation (Holtzman et al. 1981; Dubé et al. 2007). A variety of behaviors (as reviewed in Kiyatkin 2007), as well as both noxious and arousing stimuli, have been observed to increase brain temperature across many species (Aronov and Fee 2012; Kiyatkin et al. 2002; Delgado and Hanai 1966; Baker et al. 1973), including forced locomotion (Caputa et al. 1983). Despite the potential role that neurally generated heat might play in modulating excitability, it is not well understood how voluntary locomotion impacts brain temperature.

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cortex (Huo et al. 2014 and 2015). Here, we found that locomotion drove small (~0.1°C) but reliable increases in cortical temperature. Surprisingly, brief bouts (~90 s) of isoflurane anesthesia drove large (~2°C) and prolonged (30-min) decreases in cortical temperature, despite recovery of a conscious state within a few minutes, as characterized by the return of volitional locomotion and recovery of neural activity to baseline. Even when body temperature was held constant, cortical temperature decreased during anesthesia. Anesthesia-induced decreases in temperature were also found in the subcortex, suggesting the cooling effects of anesthesia affect the entire brain. In vivo two-photon imaging of cortical arteriole diameters showed that these vessels only dilated briefly in response to isoflurane anesthesia, suggesting vasodilation was not the primary cause of heat loss. These results suggest that, under normal physiological conditions, cerebral temperature is well regulated but can be profoundly decreased by even short durations of anesthesia.

METHODS

All care and experimental manipulation of animals adhered to Institutional Animal Care and Use Committee of Pennsylvania State University regulations. Thirteen male and four female C57BL/6J mice (3–11 mo old at surgery, 29.9 ± 4.3 g; Jackson Laboratory), whose thermocouples (n = 11) or stereotrodes (n = 6) were localized to cortex, as confirmed histologically, were used in the final analysis. An additional four mice (3 male, 1 female, 3 mo old at surgery, 26.3 ± 3.7 g; Jackson Laboratory) had thermocouples implanted into the subcortex. An additional three mice (2 male, 1 female, 5–7 mo old at surgery, 29.8 ± 4.7 g; Jackson Laboratory) were implanted with polished and reinforced thinned-skull (PoRTS) windows over the somatosensory cortex (Drew et al. 2010). Mice were maintained on a 12:12-h light-dark cycle (lights on at 0700) in isolated cages.

Surgery. For thermocouple implants in cortex (n = 11), a small craniotomy and duratomy were performed over the forelimb/hind limb sensorimotor cortex in the left hemisphere. The thermocouple (see Fig. 1A) was inserted ~750 μm deep, at a 30° angle relative to the brain surface (Fig. 1C). For mice implanted with thermocouples in the subcortex (n = 4), the thermocouple was inserted 6 mm deep at a 25° angle relative to the brain surface. The thermocouple was held in place with cyanoacrylic cement (catalog no. 32002; Vibra-Tite) and dental acrylic (catalog no. 1530; Lang Dental Manufacturing).

Five of the mice with thermocouples were also implanted with electrocorticography (ECoG) electrodes in the contralateral hemisphere. Stainless steel self-tapping screws (no. 000/3/32 in.; J.I. Morris, Southbridge, MA) were implanted in the frontal and parietal cortex and connected to stainless steel wires (no. 7936000; A-M Systems) using colloidal silver (Pelco Colloidal Silver Liquid) for electrical contact between the wire and the screws. Cyanoacrylic cement was used to secure and insulate the screws and wire with the output secured over the head bar with dental acrylic.

In a separate group of mice (n = 6), tungsten stereotrodes were implanted into the infragranular layers (~0.8–1.0 mm below the
cortical surface) of the forelimb/hind limb somatosensory cortex (1.5–1.8 mm lateral and 0–0.5 mm rostral relative to bregma) in the left hemisphere to record local field potentials (LFP) and multiunit activity (MUA). Stereotrodes (impedance of 100 kΩ) were constructed by threading two Teflon-coated tungsten microwires (no. 795500; A-M Systems) through a piece of polymide tubing (no. 822200; A-M Systems). A small craniotomy was made over the chosen location, and the electrodes were fixed in place with cyanoacrylic cement and dental acrylic.

An additional group of mice (n = 3) had PoRTS windows installed in the right parietal bone over somatosensory cortex to allow imaging of individual blood vessels before and after exposure to isoflurane (Drew et al. 2010; Shih et al. 2012a,b). One no. 000 3/32-in. self-tapping screw was positioned in each of the left parietal and right frontal bones. The window region was thinned to a 10- to 15-μm thickness in the right parietal bone and polished using a 3F grit (Covington Engineering, Redlands, CA) slurry for 10 min. Cyanoacrylic was used to attach a no. 0 cover glass over the thinned and polished cranium and further sealed around the edges with dental acrylic.

In all animals, a custom-machined titanium head bar was cemented to the skull, caudal to lambda, for head fixation (Fig. 1D). Temperature, imaging, and electrophysiological measurements started 2–3 days after recovery from surgery. Mice were habituated to the spherical treadmill for one or two sessions to allow them to acclimate to the recording environment and be handled by investigators.

Histology. After experiments had concluded, mice were deeply anesthetized and transcardially perfused with heparinized saline, followed by 4% paraformaldehyde. The brain was removed and sunk in 4% paraformaldehyde with 30% sucrose. To verify the depth and location of the thermocouple in sensorimotor cortex, the left hemisphere was sectioned sagittally and stained for Nissl (Smith and Alloway 2013). Data were excluded if an implanted probe was not in the gray matter of cortex, or if extensive tissue damage was present. To determine stereotrode location and depth the left cortical hemisphere was flattened, sectioned tangentially, and stained for the presence of cytochrome oxidase (Drew and Feldman 2009).

Data acquisition. Mice were head fixed on a spherical treadmill (Gao and Drew 2014; Huo et al. 2014, 2015) covered with nonabrasive, antislip tape (Fig. 1D). A US Digital (E7PD-720-118) optical rotary encoder on the axle of the treadmill was used to measure locomotion. The velocity signal from the rotary encoder was low pass filtered at 10 Hz (Butterworth) and binarized as previously described (Huo et al. 2014) categorizing the mouse’s behavior in 1-s bins as “running” or “not running.” We use the term running colloquially, and no information about the mouse’s gait should be inferred from this term. Temperature data, ball velocity, and electrophysiological recordings (see below) were acquired using custom written LabView software (32-bit Windows XP; National Instruments). Recordings were performed in an enclosed experimental chamber with a measured temperature of −25°C (model INFCT-012B-1.2; Newport Electronics, Santa Ana, CA).

Temperature measurements. Temperature measurements were taken using 40-gauge K-type thermocouples (STC-TT-K-40-36; Omega Engineering) modified for chronic implantation (Fig. 1A) with the positive lead connected to the noninverting input terminal of a low-drift amplifier (Linear Technology LTC1050CS8) and the negative lead connected to a cold junction compensator (Linear Technology LT1025ACN8) (Arnon and Fee 2012). The width of the exposed thermocouple tip was ~150 μm. The transducer signal was low-pass filtered (20 Hz) in hardware (Brownlee 440). The relationship of circuit output voltage to temperature (0.0405 V/°C) was determined by placing a thermocouple in an insulated beaker of water at a range of known temperatures (Fig. 1B).

Temperature changes during locomotion. Trials measuring cortical temperature changes during locomotion were 2 h in duration. Temperature data were acquired at 250 Hz, digitally band-pass filtered between 0.003 and 0.25 Hz (Butterworth), downsampled to 1 Hz, and truncated by 15 min from the start and the end to remove boundary effects from filtering.

Cross-covariance. For calculating cross-covariance, the binarized locomotion and cortical temperature were normalized such that their autocorrelations were unity at zero time lag. The 95% confidence interval was determined by calculating the cross-covariance of shuffled locomotion and temperature measurements.

Linear convolution model. Given the strong correlation between locomotion and cortical temperature (Fig. 2, A and B), we treated temperature responses to locomotion events as a linear, time-invariant system. We quantified this relationship using a linear convolution model, similar to our previously described model for hemodynamic responses to locomotion (Huo et al. 2015). Briefly, the animal’s velocity was binarized into locomotion events. The impulse response (or kernel) characterized the change of cortical temperature (in °C) in response to a single locomotion event (with a 1-s temporal resolution), and was numerically solved assuming a linear relationship between the behavior (running or not running) and the cortical temperature. The average kernel was filtered with a Savitzky-Golay filter (4th-degree polynomial, frame size of 25 s). The goodness-of-fit was quantified as Pearson’s correlation coefficient between the measured cortical temperature change and the estimated data from convolving the locomotion events and the impulse response. To evaluate the repeatability of cortical temperature changes based on locomotion, within each animal, we predicted the temperature change for one trial by convolving the locomotion events with the impulse response calculated from the averaged kernel from all other trials from the same animal.

Cortical temperature and core temperature measurements under anesthesia with body warming. In the five mice with thermocouples and ECoG implants, measurements of cortical temperature and core temperature were taken at varying levels of isoflurane anesthesia. All measurements were done in the afternoon (1300–1700) in a 25°C environment. Temperature measurements taken with a rectal temperature probe (placed ~5 cm up the descending colon, juxtaposed to the liver, which should truly measure core temperature), the thermocouple used for cortical temperature measurements, and a thermocouple meter/controller measuring room air (INFCT012B; Newport Electronics) were all validated to be within a tenth of a degree Celsius of each other before the experimental procedure began. Initially, a cortical temperature measurement was taken while the mouse was awake. Next, the mouse was anesthetized with 5% isoflurane to allow insertion of a rectal temperature probe, and body temperature was actively maintained at 36.8°C using a homeothermic monitor, warming blanket, and rectal thermometer (Harvard Apparatus). A period of ~10 min at 1% isoflurane followed to stabilize body temperature (at 36.8°C), cortical temperature, and a lightly anesthetized ECoG state (Friedberg et al. 1999). After cortical and core temperatures were recorded, isoflurane levels were increased in 1% increments and allowed to stabilize for 1 min before recording cortical and core temperatures.

Anesthesia trial and control trial procedures. To measure changes in cortical temperature due to brief isoflurane anesthesia, mice were head-fixed on the spherical treadmill as before. After a 15-min baseline measurement period, a mixture of 5% isoflurane (E-Z Anesthesia) in air (anesthesia trials) or air alone (control trials) was administered at 1 l/min for 90 s via a nose cone positioned over the snout. The nose cone was promptly removed after the air/isoflurane exposure ended. Recording continued for another 55 min. Anesthesia and control trials were performed on separate days. All temperature measurements for anesthesia and control trials were digitally low-pass filtered at 0.01 Hz (Butterworth), downsampled to 1 Hz, and baseline subtracted. Temperature data were averaged into 5-min bins to test for statistical significance.
**ECoG measurements.** ECoG signals were acquired at 1 kHz. The ECoG signal was differentially amplified (DAM80; WPI) and bandpass filtered (0.1–300 Hz) in hardware (Brownlee 440). At least one control trial and one anesthesia trial of simultaneous ECoG and temperature measurements were taken in each of the five mice. The ECoG data were digitally bandpass filtered between 0.01 and 300 Hz (Butterworth). Spectrograms were computed with a frequency resolution of 2 Hz and temporal resolution of 5 s using the Chronux toolbox (Mitra and Bokil 2008). For behavioral ECoG data, a multitaper power spectrum was calculated for each second of ECoG data. For each trial, an average power spectrum for running and not running behaviors was calculated. A γ-band (40–100 Hz) power ratio to baseline (Pγ/P0) was then found for each behavior by dividing the average power spectrum in each respective time period (Pγ) by the baseline power spectrum (P0) and then averaging over the γ-band. Line noise was not present in ECoG recordings, likely due to the low impedance of the electrodes.

**LFP recordings.** LFP recordings were acquired at 30 kHz, differentially amplified (DAM80; WPI), and bandpass filtered (0.1–10 kHz) in hardware (Brownlee 440). MUA was acquired by further bandpass filtering in hardware between 300 Hz and 5 kHz (Brownlee 440). LFP recordings were conducted in six mice, with each mouse having one control trial and one anesthesia trial of simultaneous ECoG and temperature measurements. The LFP data were digitally bandpass filtered between 0.1 and 300 Hz (Butterworth) and downsampled to 300 Hz. Power in the 55- to 65-Hz range was excluded to remove the effects of line noise. For visualization, multitaper power spectrograms of the LFP data were calculated with a frequency resolution of 2 Hz and temporal resolution of 5 s using the same procedure as the ECoG spectrogram. The γ-band power ratio to baseline (Pγ/P0) (Huo et al. 2014) was calculated in the same manner as ECoG data.

**Multiunit firing rate.** The MUA discharges were obtained from mean subtracted data using a threshold of three standard deviations. These MUA spikes were binned into 1-s bins, and firing rates for periods of running and not running were calculated using the binned LFP data. The mean firing rates for running and not running were then found for baseline and various periods of time (30-s and 5-min periods). The firing rate was normalized by the preanesthesia firing rate.

**Two-photon microscopy.** A two-photon microscope, consisting of a MaiTai HP laser (Spectra-Physics, Santa Clara, CA), a Moveable Objective Microscope (Sutter Instruments, Novato, CA), and a ×20, 0.95 aperture water immersion objective (Olympus, Center Valley, PA), was used for single-vessel imaging in animals with installed PoRTS windows (n = 3). The system was controlled by MPScope software (Nguyen et al. 2006). Before imaging, the mouse was briefly anesthetized with 5% isoflurane in oxygen, and 0.05 ml FITC-dextran (5% wt/vol, FD70S; Sigma) in saline was injected retro-orbitally (Shih et al. 2012a). The mouse was then placed on a free-moving spherical treadmill and allowed to locomote voluntarily, and the head bar was secured to a stable fixture to prevent skull movement during image collection. A recovery period of at least 60 min passed between anesthesia for FITC-dextran injections and data collection. Locomotion data were collected using a US Digital (E5-720-118) optical rotary encoder.

**Diameter measurements.** The effects of isoflurane on individual penetrating arterioles were assessed using diameter measurements from movies collected using two-photon microscopy. Two experiments were performed on separate days for each vessel: anesthesia trials (5% isoflurane in air at 1 l/min) and control (air at 1 l/min). For anesthesia and control trials, a 3-min baseline movie was collected immediately before isoflurane or air exposure. Isoflurane (or air) was...
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then administered for 90 s, and imaging resumed immediately thereafter. Movies of vessel diameters were collected for approximately 3 min, with 5-min intervals between the collection periods for 60 min after the isoflurane (or air) was applied. Vessel diameters were quantified using the full-width at half-maximum method (Drew et al. 2011).

Results and statistical tests. All analyses were performed in Matlab (Mathworks) and Origin8 (Origin Lab). Independent 2-tailed t-tests and Student’s t-tests were used (t value, P value), and a Bonferroni correction was used to correct for multiple comparisons. Two-way ANOVA [F value(df between, df group), P value], and a paired two-tailed t-test with Bonferroni correction [t value, P value] were used to compare brain temperature and core body temperature. Power analyses were performed using G*Power (version 3.1, Faul et al. 2007).

Results

We recorded temperature in the sensorimotor cortex of mice during voluntary locomotion (n = 5), following brief anesthesia without body warming (n = 7) and sustained anesthesia with body warming (n = 5). A summary of the locations of thermocouple implants is shown in Fig. 1E. In five of the mice used for the anesthesia experiments, ECoG activity in the contralateral hemisphere was recorded. A second group of mice (n = 6) was implanted with stereotrodes in the infragranular layers of sensorimotor cortex to measure LFP and MUA in response to brief isoflurane anesthesia. A third group of mice (n = 4) was implanted with thermocouples in the subcortex, and temperatures were recorded during brief anesthesia without body warming and sustained anesthesia with body warming. A fourth group of mice (n = 3) was implanted with polished and reinforced thinned-skull windows (Drew et al. 2010), allowing measurement of individual blood vessel diameters in response to brief isoflurane anesthesia.

Cortical temperature increases were correlated with locomotion. To determine the effects of locomotion on cortical temperature, we recorded cortical temperature with implanted thermocouples and locomotion behavior via a rotary encoder on the axle of the spherical treadmill. The overall mean cortical temperature across all animals for trials where they were allowed to voluntarily locomote was 35.1 ± 0.54°C (0000–0600: 35.4 ± 0.49°C, n = 2; 0600–1200: 34.6 ± 1.1°C, n = 2; 1200–1800: 35.2 ± 0.47°C, n = 5; 1800–2400: 35.5 ± 0.21°C, n = 2). This cortical temperature was cooler than reported core temperatures (~36.9°C) in mice (Green et al. 1966), consistent with previous measures in rats showing cortical temperature being lower than core temperature (Bindman et al. 1963; Barone et al. 1997).

In response to bouts of volitional locomotion, we observed small, but consistent, increases in cortical temperature (~0.1°C), as shown in Fig. 2. These small changes in cortical temperature due to voluntary locomotion agree with anecdotal observations of locomotion in felines (Delgado and Hanai 1966) and somatosensory stimulation in rodents (Trübel et al. 2006). The representative temperature trace in Fig. 2A shows small increases in cortical temperature with locomotion events. During longer bouts, the cortical temperature increased more substantially, by as much as 0.2°C (Fig. 2A). The cross-covariance between binary locomotion events and temperature changes revealed a peak, corresponding to a temperature increase that lagged locomotion by about 6 s (Fig. 2B), and that significantly exceeded the 95% confidence interval of the cross-covariance obtained from shuffled data.

We calculated the impulse response function (kernel) between binary locomotion events and cortical temperature changes, assuming a linear, time-invariant system. This calculation revealed that cortical temperature has a biphasic response to 1-s locomotion impulses (Fig. 2C). The impulse response increases sharply after the initiation of locomotion and peaks 7 s later, similar to the cross-covariance results. Following the peak, cortical temperature steadily decreases and overshoots the baseline before returning to zero (Fig. 2C). Kernels were similar across all mice.

Predicting cortical temperature changes from locomotion. Next we assessed how well the linear model explained changes in cortical temperature due to voluntary locomotion. We used the impulse response function to predict cortical temperature based on locomotion and compared this prediction with the actual temperature changes. An example of actual and predicted temperature changes is shown in Fig. 3A and reveals that our model fits the actual data reasonably well during periods of locomotion. The linear correlation between actual and predicted cortical temperature changes in response to locomotion for each animal is shown in Fig. 3B (average Pearson’s correlation coefficient 0.63 ± 0.09, n = 5), and were similar across all animals. Given that the mice spent only a small fraction of their time moving (10.0 ± 5.2%), the model captures a sub-

Fig. 3. A linear convolution model captured cortical temperature changes driven by locomotion. A: predicted (gray) and actual (black) temperature changes from representative example in Fig. 2A. Black dots show binarized-locomotion. B: mean Pearson’s correlation coefficient (r) between actual and predicted temperature for each animal; error bars show 1 SD. C: predicted temperature changes for 30 s (top) and 60 s (bottom) of continuous locomotion.
stantial fraction of the variability in cortical temperature. Using the mean kernel across animals, we estimated temperature changes from sustained locomotion bouts to determine if they might be large enough to affect neural excitability. The predicted temperature change in response to 30 s of continuous locomotion was calculated using the average impulse response across all animals (Fig. 3C). The predicted maximum temperature increase was 0.10°C, reaching the peak immediately after locomotion ended. Predicted temperature changes for a continuous 60-s bout of locomotion (Fig. 3C, bottom) showed a maximum increase of 0.11°C occurring 44 s after initiation of running (Fig. 3C), comparable in magnitude to the predicted temperature peak for 30 s of continuous locomotion. These temperature changes were similar to those observed in our data (Fig. 2A) but would be unlikely to affect neural excitability due to their small magnitude.

Isoflurane anesthesia caused decreases in brain temperature despite stable body temperature. Our next experiments aimed to determine the effects of sustained isoflurane anesthesia on cortical and core temperature. Previous work has suggested that isoflurane anesthesia substantially decreases brain temperature relative to the core (Kalmbach and Waters 2012). However, these studies were done in animals with a cranial window, whereas our preparation was insulated. Another previous study found that brain temperature could be depressed even when the body is heated with a warming blanket (Kiyatkin and Brown 2005), but this study used pentobarbitol, which is not commonly used in mice. We wanted to see if decoupling between cortical and core temperature takes place under isoflurane anesthesia, we simultaneously recorded brain temperature and rectal temperature at varying concentrations of isoflurane anesthesia (Fig. 4). As before, we simultaneously recorded cortical temperature and rectal temperature at varying concentrations of isoflurane anesthesia (Fig. 4A) while maintaining core temperature with a homeothermic warming blanket. These experiments were done in a subset of mice that had both thermocouples and ECoG electrode implants ($n = 5$) and were all performed in the afternoon (1200–1800). The average cortical temperature in these mice when nonanesthetized was 35.6 ± 0.2°C, which is significantly lower than the core temperature of 36.8 ± 0.0°C at 1% isoflurane ($t_{(4)} = 13.4, P = 0.00018$; power = 0.95). The average awake cortical temperature was also lower than the accepted, normal physiological body temperature of 36.9°C (Green et al. 1966). The average cortical temperature in the awake mice was also significantly higher than the average cortical temperature at 1% isoflurane ($t_{(4)} = 9.5, P = 0.00069$). The location of the temperature measurement (cortex vs. core) and the level of isoflurane concentration both had significant differences between population means [2-way ANOVA: location: $F(1,4) = 1449.6, P = 0$; isoflurane concentration: $F(4,1) = 8.35, P = 0.000054$]. Despite actively maintaining core temperature with the warming blanket, higher concentrations of isoflurane caused cortical temperature to increasingly differ from core temperature [1% isoflurane: $t_{(4)} = 14.6, P = 0.00065$; 2% isoflurane: $t_{(4)} = 15.2, P = 0.00055$; 3% isoflurane: $t_{(4)} = 17.4, P = 0.00032$; 4% isoflurane: $t_{(4)} = 19.4, P = 0.00021$; 5% isoflurane: $t_{(4)} = 26.3, P = 0.000062$]. These results show that cortical temperature can uncouple from body temperature in the anesthetized mouse.

To assess whether the decrease in temperature due to isoflurane was occurring throughout the brain, we repeated this experiment in a separate set of mice with thermocouples implanted into the subcortex ($n = 4$). As before, we simultaneously recorded brain temperature and rectal temperature at varying concentrations of isoflurane anesthesia (Fig. 4B) while maintaining core temperature with a homeothermic warming blanket. The average subcortical temperature in these mice when nonanesthetized was 36.2 ± 0.2°C, which was significantly lower than the core temperature of 36.9 ± 0°C at 1% isoflurane ($t_{(3)} = 6.3, P = 0.008$). The subcortical temperature was also found to be significantly higher than our previous measurements of cortical temperature ($t_{(3)} = 4.0, P = 0.005$). The average subcortical temperature in awake mice was sig-

![Fig. 4. Brain temperature and core temperature were uncoupled under isoflurane anesthesia. A: average cortical temperature (gray) and core temperature (black) is shown vs. isoflurane concentration ($n = 5$). Error bars show SD. B: average subcortical temperature (gray) and core temperature (black) vs. isoflurane concentration ($n = 4$). Cortical and subcortical temperatures were significantly different from core temperature at all levels of isoflurane concentration (**P < 0.01, ***P < 0.001, and ****P < 0.0001).](image-url)
significantly higher than the average cortical temperature at 1% isoflurane \( t(3) = 6.6, P = 0.0007 \). The location of the temperature measurement (subcortex vs. core) and the level of isoflurane concentration both had significant differences between population means [2-way ANOVA: location: \( F(1,4) = 418.6, P = 0 \); isoflurane concentration: \( F(4,1) = 6.3, P = 0.0008 \)]. Despite actively maintaining core temperature with the warming blanket, higher concentrations of isoflurane caused subcortical temperature to increasingly differ from core temperature [1% isoflurane: \( t(3) = 9.0, P = 0.0015; 2\% \) isoflurane: \( t(3) = 9.8, P = 0.01; 3\% \) isoflurane: \( t(3) = 13.5, P = 0.0045; 4\% \) isoflurane: \( t(3) = 10.5, P = 0.001; 5\% \) isoflurane: \( t(3) = 11.1, P = 0.001 \). These results show that cortical and subcortical temperatures were significantly lower than in the awake animal, irrespective of core temperature.

**Long duration decrease in cortical temperature following brief isoflurane anesthesia.** Next, we tested whether brief exposure to isoflurane (5% in air, 90 s) through a nose cone significantly altered cortical temperature. This duration of anesthesia was long enough to cause loss of consciousness, indicated by lack of muscle tone and loss of withdrawal reflexes. An anesthetic regimen of this duration (or longer) is frequently employed for brief procedures such as retro-orbital injections, craniotomies, and other preparations necessary for physiological measurements in awake animals. We observed a large decrease in cortical temperature (~2°C) after anesthesia in all individual anesthesia trials, but cortical temperature did not decrease in control trials using air alone (Fig. 5, A and B), indicating that the cooling was due to isoflurane, and not from the flow of air. A clear, short-term loss of power in the ECoG signal, particularly in the \( \gamma \)-band (40–100 Hz), occurred during exposure to isoflurane (Fig. 5, A and C) that returned quickly after cessation of anesthesia. In control trials, exposure to just air flow from the nose cone caused no decrease in the ECoG power (Fig. 5D), but rather agitated the animals, causing them to increase their locomotion, increasing the \( \gamma \)-band power in the ECoG.

The maximal average change in cortical temperature was \(-2.00 \pm 0.69^\circ\text{C}\), occurring 11.2 min after exposure to isoflurane (Fig. 6A). The average change in cortical temperature after anesthesia was significantly different from baseline for 45 min after the beginning of anesthesia [0–5 min: \( t(6) = 7.4, P = 0.0045; 5–10 \) min: \( t(6) = 9.0, P = 0.0015; 10–15 \) min: \( t(6) = 7.7, P = 0.0035; 15–20 \) min: \( t(6) = 6.9, P = 0.0066; 20–25 \) min: \( t(6) = 5.6, P = 0.019; 25–30 \) min: \( t(6) = 4.9, P = 0.038; 30–35 \) min: \( t(6) = 5.4, P = 0.023; 35–40 \) min: \( t(6) = 5.3, P = 0.026; 40–45 \) min: \( t(6) = 5.7, P = 0.017 \). The mean temperature change across all animals for anesthesia was significantly different from control for 30 min after anesthesia began [0–5 min: \( t(6) = 6.5, P = 0.00068; 5–10 \) min: \( t(6) = 8.15, P = 0.00014; 10–15 \) min: \( t(6) = 6.7, P = 0.00083; 15–20 \) min: \( t(6) = 5.9, P = 0.0021; 20–25 \) min: \( t(6) = 5.0, P = 0.0074;
where the maximal average change in subcortical temperature in response to brief exposure to isoflurane (Fig. 7). As we had observed in cortex, brief anesthesia also caused a significant decrease in the subcortex, which the maximal average change in subcortical temperature was a decrease of $-1.68 \pm 0.46^\circ$C, which occurred 8.8 min after exposure to isoflurane (Fig. 7B). The average change in subcortical temperature after anesthesia was significantly different from baseline for 10 min after the beginning of anesthesia [0–5 min: $t_{(3)} = 8.2, P = 0.05$; 5–10 min: $t_{(3)} = 8.2, P = 0.05$]. The mean subcortical temperature change across all animals for anesthesia was significantly less than control for 10 min after anesthesia began [0–5 min: $t_{(3)} = 5.8, P = 0.017$; 5–10 min: $t_{(3)} = 8.0, P = 0.004$].

Neural activity was unaffected by decreased cortical temperature. ECoG and LFP recordings were conducted to understand the effect of brief exposure (90 s) to 5% isoflurane anesthesia on neural activity ($n = 5$ for ECoG, $n = 6$ for LFP; 1 control and 1 anesthesia trial/animal). We focused our analysis to the $\gamma$-band (40–100 Hz) because this range of frequencies has previously been shown to robustly increase power during locomotion (Polack et al. 2013; Huo et al. 2014) and decrease power in response to isoflurane anesthesia (Hudetz et al. 2011). Ratios of $\gamma$-band power relative to baseline for periods after anesthesia/air were found using pre- and postanesthesia/control power spectrums, as illustrated by a representative example in Fig. 6B. Some of the time bins had fewer observations than others because not every animal ran during every time bin. The average ECoG $\gamma$-band power was greatly decreased during isoflurane anesthesia, but quickly

Fig. 6. Brief exposure to isoflurane anesthesia caused a prolonged, significant decrease in cortical temperature, but only a transient decrease in ECoG $\gamma$-band power. A: average changes in cortical temperature for control (brown) and anesthesia (teal) trials across all animals (top). Cortical temperature after anesthesia was significantly different from control for 30 min after the start of anesthesia. Shaded areas enclose $1 SD$. Bottom: lomotion activity averaged across all animals with 95% confidence interval (dashed teal line) from preanesthesia locomotion. B: representative examples of ECoG power spectra for the anesthesia trial and a control trial for mouse MS-20 (shown in Fig. 5). The preanesthesia power spectrum (top left) and precontrol power spectrum (bottom left) represent the first 15 min of ECoG measurements for each trial that were used as baseline to find $\gamma$-band ratios to baseline. The postanesthesia power spectrum (top right) and postcontrol power spectrum (bottom right) represent the power in the ECoG signal for the first 15 min after anesthesia and control, respectively. The highlighted yellow region indicates the $\gamma$-band frequency range. C: the average ECoG $\gamma$-band power ratio relative to baseline across all animals for anesthesia trials and control segregated by behavior. The average $\gamma$-band power in the ECoG signal for anesthesia was significantly different from baseline only during exposure to isoflurane anesthesia (***$P < 0.001$).
recovered after cessation of anesthesia (Fig. 6C). The average ECoG γ-band power during quiescence was only significantly decreased from baseline for the duration of anesthesia \[ t(4) = 18.4, \, P = 0.0006 \]. The average ECoG γ-band power while the mice were not running for control trials was elevated from baseline during the full 90 s of air-only controls \[ t(4) = 9.7, \, P = 0.0075 \], possibly due to the stimulating effects of airflow over the face.

As with the ECoG, the γ-band power in the LFP recorded in the sensorimotor cortex (\( n = 6; \) 1 control and 1 anesthesia trial/animal) was drastically reduced by isoflurane, as demonstrated in the representative example shown in Fig. 8A. γ-Band power in the LFP signal fell dramatically during anesthesia, and recovered within 30 s of removal of anesthesia (Fig. 9B). In anesthesia trials, the average γ-band power in the LFP during times the animal was not running was significantly decreased from baseline for the last 60 s of anesthesia \[ 0–1.5 \text{ min during anesthesia}: t(5) = 14.4, \, P = 0.0003; \, 0.5–1 \text{ min from anesthesia start}: t(5) = 9.8, \, P = 0.0024; \, 1–1.5 \text{ min from anesthesia start}: t(5) = 105.7, \, P = 1.9 \times 10^{-8} \]. Control trials did not have any loss of power in the LFP signal, nor was the control average γ-band power significantly different from baseline at any time.

Isoflurane had similar effects on the MUA as on other measures of neural activity, causing the MUA firing rate to decrease to zero during anesthesia. MUA data were simulta-
neously recorded during LFP recordings discussed above. After cessation of anesthesia, the MUA underwent a slow recovery back to baseline over the course of several minutes (Fig. 9C). The MUA firing rate during not running was significantly different from baseline from 2 to 4 min after anesthesia ended [0–5 min after anesthesia: \( t_{(5)} = 9.9, P = 0.002, 0–5\) min after anesthesia: \( t_{(5)} = 10.8, P = 0.001 \)]. To more precisely determine the temporal duration of isoflurane on the MUA, we analyzed the time around anesthesia in 30-s time bins, shown in Fig. 9C, bottom. The MUA during not running was significantly depressed from baseline during the last 60 s of anesthesia and for 1.5 min after anesthesia ended \([0.5–1\) min during anesthesia: \( t_{(5)} = 7.55, P = 0.0084; 1–1.5\) min during anesthesia: \( t_{(5)} = 99.8, P = 2.5 \times 10^{-8}; 0–0.5\) min after anesthesia: \( t_{(5)} = 12.3, P = 0.00081\); 0.5–1 min after anesthesia: \( t_{(5)} = 6.35, P = 0.019; 1–1.5\) min after anesthesia: \( t_{(5)} = 6.2, P = 0.021 \)]]. During periods of running, the MUA was significantly suppressed from baseline from 2 to 4 min after anesthesia ended \([0–5\) min after anesthesia: \( t_{(5)} = 8.8, P = 0.004; 2–2.5\) min after anesthesia: \( t_{(5)} = 7.4, P = 0.0091; 2.5–3\) min after anesthesia: \( t_{(5)} = 9.0, P = 0.0037; 3–3.5\) min after anesthesia: \( t_{(5)} = 6.6, P = 0.015; 3.5–4\) min after anesthesia: \( t_{(5)} = 5.2, P = 0.045 \)]]. The average control MUA was never significantly different from baseline for periods of running or not running. Interestingly, the LFP recovered more quickly from anesthesia than the MUA, suggesting that these two indicators of neural activity can decouple from one another.

**Isoflurane-induced cerebral artery dilation recovers rapidly.** We assessed whether decreases in cortical temperature following isoflurane anesthesia could be due to dilation of cerebral blood vessels. Increases in arteriole diameter would cause an increase in blood flow, which could cause cooling of nearby neural tissue (Yablonskiy 2000). To test this possibility, we measured diameter changes of individual cerebral penetrating arterioles in response to brief anesthesia using two-photon imaging in mice fitted with cranial windows (3 mice, 11 vessels). For each vessel, after recording baseline vessel dynamics, animals were exposed to 5% isoflurane (anesthesia) or air (control), as done in previous experiments measuring temperature, and subsequently imaged for 1 h. Due to experimental constraints, there was a few second delay in starting imaging after the cessation of isoflurane. Figure 10A shows a representative example of vessel dynamics in an anesthesia trial. Immediately following exposure to isoflurane, the vessel was clearly larger in diameter compared with baseline, followed by a transient period of vessel constriction. Also apparent were clear increases in vessel diameter locked to locomotion events, as previously observed (Gao and Drew 2014; Huo et al. 2015).

**Exposure to isoflurane caused a potent dilation of all vessels measured.** To quantify this effect during periods of not running across all vessels measured, we first removed all video frames where the animal was locomoting, based on a binarization of the velocity encoder as done for temperature experiments above. Brief anesthesia caused an 18.8 ± 1.4% increase in vessel diameter over baseline, followed by a −21.6 ± 4.3% decrease in diameter (peak time \( \approx 3\) min after cessation of anesthesia). Isoflurane-induced diameter changes returned to baseline 6.5 min after cessation of anesthesia. Control experiments on all vessels yielded no significant changes in vessel
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blood vessels in response to isoflurane anesthesia were performed to determine if increased arteriole dilation could account for the prolonged decrease in cortical temperature. However, these experiments clearly demonstrated the isoflurane-induced vessel dilations return to baseline within minutes, and are in fact followed by an equal period of constriction, indicating that increased cerebral vessel dilation was not driving the delayed and prolonged cortical temperature decrease. These findings suggest that brief isoflurane can significantly reduce cortical temperature for a prolonged period following exposure to anesthesia, but has no long-term observable effect on neural activity, blood vessel dynamics, or locomotion behavior. A summary of these results can be seen in Fig. 11, illustrating the surprising effect that brain temperature was significantly reduced for an extended period of time (>30 min) despite neural activity, locomotion behavior, and blood vessel diameter returning to baseline a few minutes after cessation of anesthesia.

Cortical temperature in awake, behaving mice. We found the mean cortical temperature to be 35.1 ± 0.5°C in nonanesthetized mice, substantially lower than the canonical core temperature of ~36.9°C (Wanner et al. 2013). This finding is consistent with a previous report that the cortical temperature in rats is ~1.5°C cooler than the base of the brain (Andersen and Moser 1995), and with previous observations of a ventrodorsal temperature gradient in the mammalian brain (Hayward and Baker 1968; Zhu et al. 2006; Yablonskiy et al. 2000). Previous studies measuring temperature in the cortex of rodents, however, were all performed with the use of anesthesia, shortly after the cessation of anesthesia, or while the cortical surface was exposed (Barone et al. 1997; Trübel et al. 2006; Kalmbach and Waters 2012).

Regulation of cortical temperature during voluntary locomotion. Temperature fluctuations in various regions of the brain have been shown to be associated with functional activation. Brain temperature increases of up to 1–2°C have been observed in rats and birds following the presentation of noxious and arousing sensory stimuli (Aronov and Fee 2012; Kiyatkin et al. 2002) and forced locomotion in guinea pigs (Caputa et al. 1983). Smaller increases in temperature (0.1–0.5°C) have been reported in cats with stimuli such as awakening, petting, and paw stimulation (Delgado and Hanai 1966; Baker et al. 1973). To our knowledge, cortical temperature fluctuations due to completely voluntary movement in nonanesthetized mice have not been measured. We measured temperature changes in the forelimb/hind limb representation of sensorimotor cortex of awake, behaving mice with a chron-
ically implanted thermocouple and found that locomotion drove small (~0.1°C) but reliable increases in cortical temperature.

Small temperature increases following locomotion were likely due to multiple factors. Locomotion raises the core temperature (de Castro 1980; Wanner et al. 2013). Additionally, heat from increased metabolic activity in the local cortical neurons during locomotion (Huo et al. 2014) is likely to contribute substantially to brain temperature changes (Yablonskiy et al. 2000; Kiyatkin et al. 2002; Trübel et al. 2006). The cross-covariance plot and linear response models (Fig. 2, B and C) show that increases in cortical temperature slightly precede locomotion onset, indicating that increased neural activity likely drives increases in cortical temperature. The linear model was very effective at capturing the measured changes in cortical temperature, evidence that cortical temperature was tightly controlled. The biphasic response of temperature to locomotion specified by the impulse response is reminiscent of a negative feedback system, where the heat generated by neural activity is dissipated by the increased blood flow to the region (Zhu et al. 2006 2009; Yablonskiy et al. 2000), as is known to happen in sensorimotor cortex during voluntary locomotion (Huo et al. 2014, 2015). Specifically, local increases in neural activity during locomotion cause cerebral blood vessels to dilate (see Fig. 10A; Huo et al. 2015), and the increased blood flow could help dissipate neurally generated heat. The small amplitude of locomotion-induced temperature fluctuations predicted by the model and seen here is unlikely to have any significant effect on neural excitability.

Long-duration cooling associated with brief isoflurane anesthesia. Although temperature changes in response to locomotion were small, we found that brief exposure to isoflurane caused a long-duration depression in cortical and subcortical temperature. Previous studies have shown that pentobarbital sodium dramatically decreases brain and body temperature in rats by 3.5–4.5°C, with temperature returning to baseline 1 h after regaining consciousness (Kiyatkin and Brown 2005). We found that even brief exposure to isoflurane anesthesia until loss of responsiveness caused a significant decrease (~2°C) in mouse brain temperature for up to 30 min. These temperature decreases continued long after locomotion and neural activity had returned to normal. This result indicates that the decrease in cortical temperature we observed due to brief isoflurane anesthesia is likely not due to a decrease in heat production from metabolic processes associated with neural activity (Maekawa et al. 1986; Alkire et al. 1997). It is likely that brief exposure to isoflurane decreases the body temperature for the same time period, thus causing the cortical temperature to drop, likely due to a “toxic insult” response to isoflurane (Gordon et al. 1988).

Our experiments maintaining body temperature with a homeostatic warming blanket during prolonged anesthesia also showed a significant decrease in cortical and subcortical temperature caused by isoflurane (Fig. 4), suggesting that other mechanisms beyond core temperature changes may be responsible in this preparation. One mechanism that could be responsible for the decrease in brain temperature in these prolonged anesthetic exposures was the decrease in metabolic activity accompanying the decreased neural activity (Maekawa et al. 1986; Alkire et al. 1997). In addition to suppressing homeothermic regulation and lowering neuronal activity, isoflurane is a potent vasodilator (Iida et al. 1998; Masamoto and Kanno 2012; Mrozek et al. 2012). Another possible explanation for the dramatic decreases in cortical temperature we observed is excessive heat loss through the nasal breathing, which has been shown to effectively decrease brain temperature in other species, including humans (Einer-Jensen et al. 2000, 2002). Heat loss through nasal breathing could be augmented by the increased surface area of blood vessels both centrally and peripherally in the nasal mucosa, caused by the vasodilatory effects of isoflurane (Hayward and Baker 1968; Baker et al. 1973; Cabanac and Caputa 1979; Caputa et al. 1983; Blumberg and Moltz 1988). More recently, investigators have implicated the extensive surface area interaction of the cerebrospinal fluid with the cortical surface as a potential thermal mediator to explain the much reduced temperature of the cortex compared with deeper brain structures at rest (Wang et al. 2014). However, this explanation is speculative.

In summary, the mechanisms causing cortical temperature decrease in each of these experiments are likely different. In the brief anesthesia experiments, the toxic insult of isoflurane likely drives down core temperature and thus brain temperature despite an almost immediate return of neural generated heat and the return of vasodilation back to baseline. In the prolonged anesthesia experiments where body heat is maintained with a warming blanket, vasodilation (in the brain, nasal passages, and skin) and decreased neural activity are likely the key players cooling the brain.

Decoupling of brain temperature and neural activity. Brain temperature has traditionally been viewed to play a role in fundamental biochemical processes underlying neural activity; however, our results demonstrate decoupling of electrophysiological measurements and brain temperature in the postanesthetic state. The temperature decreases following brief isoflurane that we observed have been shown in other preparations to alter excitability (Andersen and Moser 1995), and comparable to temperature changes used to slow motor behaviors (Long and Fee 2008). Multiple studies show that large temperature changes of ~10°C cause significant changes in spiking pattern and transmitter release probability in cortical slices (Volgushev et al. 2004; Hedrick and Waters 2012). Other studies have shown that decreases in temperature of just a few degrees Celsius affect spiking activity, LFP fluctuations in up-down states, and neural excitability (Volgushev et al. 2000a; Reig et al. 2010). Surprisingly, we found that a prolonged 2°C decrease in cortical temperature did not significantly affect γ-band power, MUA firing rate, or the amount of locomotion. While other neural properties may have been altered, the unaffected γ-band power and MUA suggest that much larger temperature deviations from baseline are needed to significantly affect ECoG, LFP, and MUA measurements in mice. The resilience of neuronal activity to temperature fluctuations has been seen in both the crab pyloric central pattern generator, which can maintain rhythmicity of firing despite wide changes in temperature (Rinberg et al. 2013; Soofi et al. 2014), and in the lack of learning impairments in hypothalamic rodents (Moser et al. 1993; Moser and Andersen 1994; Andersen and Moser 1995). Thus, our data suggest that electrical activity can be robust despite alterations in underlying metabolic and biochemical processes. In summary, while our measures of neural activity were not significantly altered by the decrease in cortical temperature, other significant biochemical
changes may be present and suggest that experimenters be cautious in interpreting data from within 30 min after exposure to isoflurane. Furthermore, in light of the inability for body warming to warm cortex to its normal, awake temperature, investigators should use caution when interpreting relationships between neural activity and temperature in anesthetized data. Finally, these data have bearing on what the correct, physiologically relevant temperature should be for in vitro physiological recordings of cortical neurons to mimic the awake, in vivo animal.

Technical considerations. Although great lengths were taken to insulate the craniootomy and thermocouple with dental acrylic (an excellent thermal insulator), the possibility still remains that our cortical measurements were artificially lower than in an unperturbed cortex due to heat shunting from the thermocouple. This shift, however, would not affect the relative temperature changes measured in response to locomotion or anesthesia. The amount of heat shunting in our preparation was likely minimal, supported by the fact that our recorded data. Finally, these data have bearing on what the correct, relationships between neural activity and temperature in anesthetized cortex during voluntary locomotion and anesthesia. Core temperature relationships with spontaneous behavior in the rat. Physiol Behav 25: 69–75, 1980.


Wanner SP, Costa KA, Soares ADN, Cardoso VN, Coimbra CC. Physical exercise-induced changes in the core body temperature of mice depend more on ambient temperature than on exercise protocol or intensity. Int J Biometeorol 58: 1077–1085, 2013.


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