Infrared thermal imaging of rat somatosensory cortex with whisker stimulation

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Suzuki T, Ooi Y, Seki J. Infrared thermal imaging of rat somatosensory cortex with whisker stimulation. J Appl Physiol 112: 1215–1222, 2012. First published January 26, 2012; doi:10.1152/japplphysiol.00867.2011.—The present study aims to validate the applicability of infrared (IR) thermal imaging for the study of brain function through experiments on the rat barrel cortex. Regional changes in neural activity within the brain produce alterations in local thermal equilibrium via increases in metabolic activity and blood flow. We studied the relationship between temperature change and neural activity in anesthetized rats using IR imaging to visualize stimulus-induced changes in the somatosensory cortex of the brain. Sensory stimulation of the vibrissae (whiskers) was given for 10 s using an oscillating whisker vibrator (5-mm deflection at 10, 5, and 1 Hz). The brain temperature in the observational region continued to increase significantly with whisker stimulation. The mean peak recorded temperature changes were 0.048 ± 0.028, 0.054 ± 0.036, and 0.097 ± 0.015°C at 10, 5, and 1 Hz, respectively. We also observed that the temperature increase occurred in a focal spot, radiating to encompass a larger region within the contralateral barrel cortex region during single-whisker stimulation. Whisker stimulation also produced ipsilateral cortex temperature increases, which were localized in the same region as the pial arterioles. Temperature increase in the barrel cortex was also observed in rats treated with a calcium channel blocker (nimodipine), which acts to suppress the hemodynamic response to neural activity. Thus the location and area of temperature increase were found to change in accordance with the region of neural activation. These results indicate that IR thermal imaging is viable as a functional quantitative neuroimaging technique.

infrared imaging; neural activity; brain temperature; whisker barrel; microcirculation

INCREASES IN CEREBRAL NEURAL activity are accompanied by changes in local brain metabolism, cerebral blood flow, and cerebral blood oxidation (28). The relationship between local neural activity changes and regional cerebral blood flow change is well known as neurovascular coupling. There are several types of functional neuroimaging techniques in use, which assume this tight coupling. Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) is one such technique that is widely accepted for functional brain imaging. Functional neuroimaging techniques detect changes in cerebral blood flow or cerebral blood oxidation in place of actual neural activity to provide an indirect measure of neural activity. It has been demonstrated that neural activations showing no change or a negative change in BOLD fMRI signals occur in response to sensory stimulation (23). An improved technique would obtain functional maps more closely relating to the actual neural activity. The purpose of this study is to validate that infrared (IR) thermal imaging offers a better measure of cortical neuronal activity than present neuroimaging techniques.

In the course of neural activation, cellular metabolic activity increases in advance of the vasodilation occurring within the brain. Astrocytes in layer IV are the primary site of glucose uptake during neuronal activity in the neocortex (31). At the same time, synaptically released glutamate boosts metabolism in the astrocytes. Weber et al. (32) indicated that the increase in cerebral oxidative metabolism in response to sensory stimulation is considerably faster and more localized than the cerebral blood flow response. This metabolic activity generates heat and increases local temperature within the brain (16). The subsequent changes in local cerebral blood flow alter the local heat transport. Therefore, the local temperature of the brain is affected bimodally due to increased metabolism and increases in blood flow and should be dependent on the level of local neural activity. The brain temperature is possibly a somewhat more direct indicator of brain function than the regional blood flow, although it is also influenced by the blood flow.

Localized thermal changes have been reported in the brain with visual and auditory stimulation (18). Visual stimulation raised the temperature in the lateral geniculate nucleus by 0.001–0.1°C, and auditory stimulation raised the temperature in the inferior colliculus by 0.001–0.015°C. Melzack and Casey (19) recorded a temperature increase of <0.005°C in primary and secondary somatosensory areas with somatosensory stimulus in the cat brain. Also, local brain temperature increased during cocaine self-administration in the rat with increases in neural activity that drives cocaine seeking (15).

Thermal imaging by an IR camera can noninvasively produce a temperature map with high spatial and temporal resolution, as well as with a high-temperature resolution. Brugge et al. (1) applied IR thermal imaging to successfully detect the thermal response to tactile stimulation of the face over the highly elaborated somatic sensory cortex in two species of rodent (the rat and gerbil). This technique was also used to measure cortical surface temperature distribution as a means of functional mapping after application of various stimuli in various species. For example, when visual, auditory, or sensory stimulation was given, a rapid change in IR radiation from the cranial top was observed in rats, rabbits, cats, monkeys, and humans (26). It has also been applied to intraoperative observation of human brains suffering from tumors, seizures, and vascular malformations (7, 13, 14). Gorbach et al. (12) reported an intraoperative application of IR imaging to measure func-
tional temperature change in the human brain. However, recent reports have been limited regarding the application of IR imaging as functional neuroimaging for both animals and humans. This may be due to several technical challenges. The expected temperature change within the brain induced by a short-term stimulus is quite small (0.001–0.01°C). It is difficult for thermal imaging to separate the effect of cerebral blood flow from neural and metabolic activities. In addition, the instruments used for the animal experiments did not have sufficient spatial resolution. More information is needed to greater establish the quantitative relationship between brain temperature and neural activity. Stimulus-response studies are needed using animals where localization and intensity of the induced neural activity are well documented.

The whisker barrel cortex in rodents is a standard model system for the study of cortical structure, function, and development (9). In the rat whisker barrel sensory system, sensory inputs from whiskers on the side of the rat’s face project to a localized group of cells within barrels in the contralateral somatosensory cortex, such that moving the whiskers increases electrical activity and blood flow in the contralateral barrel field (33). In the posteromedial barrel subfield of the primary somatosensory cortex, neurons above, below, and within a barrel are postulated to form a columnar functional module for processing of information, essentially from the corresponding single vibrissa or whisker (6, 27). Therefore, this sensory system should allow the study of detailed cerebral responses to the movement of vibrissae, including neuronal activity, metabolism, blood flow, and temperature.

The purpose of this study is to measure the regional temperature changes of the rat somatosensory cortex in response to whisker stimulation using IR imaging to analyze the spatiotemporal relationship between the local temperature change and neural activity.

MATERIALS AND METHODS

Animal preparation. The animal experiments were performed in accordance with Guidelines for Animal Experimentation, National Cerebral & Cardiovascular Center Research Institute. Experimental protocols were approved by the Animal Care Committee of the National Cerebral & Cardiovascular Center Research Institute. In total, 11 male Wistar rats (7–10 wk old) (Japan SLC) weighing 150–250 g were used. Five rats were used for whole-whisker stimulation, two were used for ipsilateral stimulation, one was used for single-whisker stimulation, and three were used for nimodipine injection. The rats were anesthetized with intraperitoneally injected urethane (1.25 g/kg). The right femoral artery was cannulated with a thermocouple. Femoral arterial pressure and infrared (IR) thermal images were recorded on a personal computer. Whiskers were deflected by a solenoid [branches of middle cerebral artery (MCA)] was imaged using a color charge-coupled device camera (DXC-C1, Sony) and a stereoscopic microscope (SMZ645, Nikon).

IR imaging. Temperature distribution of the cortex was measured using an IR camera (Merlin Mid, FLIR Systems). This camera contained an indium antimonide IR sensor capable of distinguishing temperature differences as small as 0.018°C. The IR sensor was an array of 320 × 256 pixels covering 3.84 × 3.07 mm of the object with ×2.5 objective. The maximum frame rate of the IR camera was 60 frames/s. IR images were captured synchronously with the stimulation using a personal computer. The power of the thermal radiation emitted from an object increases nonlinearly with increasing temperature of the object (4). The IR camera was calibrated between 24 and 40°C using a thermal radiation source.

Whisker stimulation. The whisker stimulator consisted of a power supply (LX035–1B, Takasago), a function generator (FG-281, Kenwood), a miniature solenoid (DC Open Frame Solenoid type GFCX03X00E13, Magnet-Schultz), and a nylon mesh screen (grid size: 1 mm × 1 mm) as shown in Fig. 1. These devices were controlled by a personal computer installed with custom-made software written with LabWindows/CVI (National Instruments). The left vibrissae were cut to a length of ~2 cm from the face and fitted through the mesh screen connected to a solenoid shaft via mechanical leverage. The mesh screen was set ~1 cm from the rat’s face. Whisker stimulation was applied for 10 s with a 5-mm deflection at 10, 5, and 1 Hz. We chose the amplitude, frequency, and duration parameters of the stimulator based on a previous study (10), reporting maximum blood flow change with these settings.

Suppression of cerebral vasodilation. To suppress an increase of the cerebral blood flow accompanying the neural activity during whisker stimulation, we used nimodipine, which is a centrally active L-type calcium channel blocker (11). Among voltage-gated calcium channels, the L-type is the major calcium channel that mediates vascular smooth muscle contraction. N-type and P/Q-type calcium channels, which are expressed primarily in neurons, are relatively unaffected by the L-type channel blocker (3, 22). Therefore, nimodipine is expected to suppress vasodilation with few effects on neuronal activity. Nimodipine was dissolved at a concentration of 2.5 g/l in a vehicle consisting of polyethylene glycol, physiological saline (0.9% NaCl), and ethanol in 2:2:1 ratio under dim light, as standardized previously (17). We intraperitoneally injected three rats with the solution of nimodipine at a dosage of 0.2 mg/kg rat body wt 30 min before the start of the stimulus response experiment.
Data collection and analysis. IR images were captured via an image capture board (Road Runner, BitFlow) and saved on a personal computer using image processing software (Image Pro-Plus, Nippon Roper K.K.). Arterial blood pressure was amplified with a strain amplifier (6M92, San-ei) and was collected at a rate of 1,000 samples/s via a data acquisition board (PCI-6280, National Instruments) on a personal computer. The obtained IR images were processed and analyzed using Image J (National Institutes of Health) and MATLAB (MathWorks). To amplify the temperature resolution of IR measurements, as well as to reduce noise, the images were averaged spatially (3 × 3 neighboring pixels) and temporally (3 consecutive images at intervals of 0.3 s). Moreover, we applied an ensemble average to multiple images obtained for the same subject under the same experimental conditions.

All experimental data are expressed as means ± SD. Statistical significance of the changes was assessed by an unpaired Student’s t-test for stimulus frequency dependence and one-way repeated-measures ANOVA for temporal changes in temperature. Statistical significance was established at 95% confidence level (P < 0.05).

RESULTS

Figure 2A shows an example of the acquired IR thermal images during the 10-s-duration whisker stimulus, depicting brain surface temperature increases within the barrel field. The temperature appeared to first increase in the area surrounding the MCA branch perfusing the barrel area. See video image (Fig. 2B). Subsequently, the heat appeared to diffuse from the MCA to the whole region of the image. The spatial distribution of peak temperature was irrespective of the vascular arrangement, as observed in Fig. 2A. The region of temperature increase corresponded to the barrel cortex on the sensory map of rats (5). In contrast, the region on the sensory map corresponding to the unresponsive zone (UZ) in the rat brain did not show a clear temperature increase. The UZ was used as a control region, since it has been identified using electrophysiological techniques as an area that is unresponsive to stimulus in anesthetized rats (5).

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**Fig. 2.** A: averaged images of temperature change in the barrel cortex in response to the whisker stimulus with 5-mm deflection for 10 s at 10 Hz for one animal (11 trials). The figures on each image indicate elapsed time from the beginning of stimulus. Red color in the image shows the maximum change of temperature (0.05°C). B: video image of the cranial window. The rectangle in the video image indicates the IR image size that corresponds to 3.84 × 3.07 mm. UZ, MC, and BF in A (10 s) and B indicate the position where the local temperature change was measured. UZ is on the zone unresponsive in anesthetized recording. MC is on a branch of middle cerebral artery (MCA). BF is in the whisker barrel field outside the branch. C: temporal change of temperature averaged over the entire image region from 4 animals (33 trials). Values are means ± SD. The horizontal line denotes the stimulus duration (10 s). D: temporal change of mean arterial blood pressure from 4 animals (40 trials). E: temporal change of temperature in the regions UZ, MC, and BF from 1 animal (11 trials). The control values were evaluated at 5 s before stimulus. The data points are shown at 0.33-s intervals, and the error bars are shown at 1-s intervals.
The average temperature over the entire brain image region increased significantly ($P < 0.05$) over time from the initial control value following the whisker stimulation, as shown in Fig. 2C, while the mean arterial blood pressure remained almost constant (Fig. 2D). The statistical significance of cortical temperature changes was analyzed by one-way repeated-measures ANOVA and the pairwise comparisons among means (Tukey’s test). These tests revealed that the mean temperatures at times between 9 and 13.67 s were significantly larger than the mean temperature at time 0.67 s with $P < 0.0001$. The peak temperature change was $0.04^\circ C$ and occurred $11$ s after the start of stimulation. More locally, the temperature was averaged over the smaller area of $12 \times 12$ pixels, corresponding to $0.02 \text{mm}^2$, for regions in the UZ, on a branch of the MCA passing through the barrel field (MC), and in the barrel field outside the MCA branch (BF), as shown in Fig. 2B. Figure 2E shows an example of local temperature changes in UZ, MC, and BF for one of the five animals (11 trials). Temperature in MC and BF significantly ($P < 0.05$) increased during stimulation. The peak values of MC and BF were higher than that of UZ, and the temperature change in UZ was not statistically significant. There were no significant differences in the peak values between MC and BF.

The effect of the frequency of whisker stimulation on the temperature change is shown in Fig. 4. We adopted three different frequencies (1, 5, and 10 Hz). There was a significant ($P < 0.001$) difference between the peak temperature increase for 1-Hz stimulation ($0.097 \pm 0.015^\circ C$, 15 trials) and that for 10-Hz stimulation ($0.048 \pm 0.028^\circ C$, 38 trials). There was also a significant ($P = 0.026$) difference between the temperature increase for 1-Hz stimulation and that for 5-Hz stimulation ($0.054 \pm 0.036^\circ C$, 16 trials). The temperature increase for 5 Hz tended ($P = 0.063$) to be higher than that for 10 Hz. Thus the peak temperature increase tended to decrease as the stimulus frequency increased.

Considering that sensory stimulus to vibrissae is projected to the whisker barrel cortex of the contralateral hemisphere, the
results shown thus far concern the contralateral side. The temperature change of the ipsilateral barrel cortex was also measured for comparison. We observed that the average temperature over the entire ipsilateral imaged region also significantly \((P < 0.05)\) increased from the initial control value with the whisker stimulation in a manner comparable to the contralateral barrel cortex. As for the spatial distribution, the temperature of the ipsilateral cortex increased exclusively around the branch of the MCA perfusing the barrel area during stimulation. This is shown in Fig. 5A, compared with the surface pial arterioles seen on the video image (Fig. 5B). The extent of cortical area with temperature increase due to whisker stimulation was more widely distributed on the contralateral cortex than the ipsilateral cortex, as can be seen in Figs. 2A and 5A.

To provide additional evidence for the cerebral temperature increase accompanied by the neural activity, cerebral hemodynamic responses were suppressed by applying a calcium channel blocker, nimodipine, to the rats in advance of the experiments. In the nimodipine-injected rats, the cortical surface temperature did not change along the branches of the MCA, but the cortical temperature of the barrel field increased during whisker stimulation, as shown in Fig. 6.

**DISCUSSION**

The main finding of the present study is that the local brain temperature increases in the barrel cortex during whisker stimulation due to heat sources other than the surface blood flow. However, it is controversial in literature whether local brain temperature is an appropriate indicator of neural activity. There are reports of brain temperature increases following functional brain activity (12, 18, 19), and there are also reports of temperature decreases following functional brain activity (35). These differences may be related to variable temperature measurement sites, i.e., surface or inner part of brain (29). The increase in regional blood flow induced by neural activity is considered to produce a heating or a cooling effect, depending on the relative temperature difference between arterial blood and brain tissue (21). However, the inner part of the cat brain, such as the ventral posteromedial nucleus (19) and lateral geniculate nucleus (18), showed an increase in temperature evoked by contralateral somatic stimuli and visual stimuli, respectively. Melzack and Casey (19) further described that the direction of thermal responses is not simply a function of blood-brain temperature differences. The temperature of the somatosensory cortex has been reported to be increased fol-
lowing ipsilateral somatic stimuli, approximating that of the contralateral stimuli (19, 24). In the latter reference, electrical stimuli (rectangular pulses with 3-mA amplitude at 5 Hz for 4 s) were applied on the rat hindpaw, and the mean systemic blood pressure was found to increase by 9.4 ± 1.7 mmHg, which was thought to produce similar increases in cortical temperature of both hemispheres.

In this study, we adopted whisker vibration as a sensory stimulus, since it was not expected to affect the systemic blood pressure even for stimulus of a long duration. We did not detect any significant changes in the mean blood pressure throughout the experiment. Furthermore, we expected that the whisker barrel field would indicate a large temperature change evoked by whisker vibration. The barrel field is an important area of the rat brain, collecting information about the surrounding environment. In the present study, the average temperature over the entire imaged region significantly increased ~0.04°C from the initial control value with whisker stimulation of 10-s duration at 10 Hz (Fig. 2C). Our temperature change was certainly larger than that reported by Seki et al. (24), who noted a <0.01°C temperature change in the S1 region of the cerebral cortex evoked by an electrical stimulus of a single pulse with a 3-mA amplitude and a 0.5-ms duration in the rat hindpaw. It appears the temperature increase with rat hindpaw stimulation occurred only in the peripheral region along the pial arterioles. Whisker stimulation, however, resulted in an observed temperature increase, not only along the pial arterioles, but also throughout the entire whisker barrel field region (Fig. 2A).

Our study indicates that IR thermal imaging is capable of localizing neural activity. When all whiskers were stimulated, the entire barrel field region exhibited an increase in temperature, while the area corresponding to the UZ (5) did not show a clear temperature increase (Fig. 2, A and E). Experimental stimulation of a single D4 whisker indicated a narrower or more focal regional temperature increase within the barrel field. This focal area’s epicenter coincided with a single barrel corresponding to the D4 whisker, as shown in Fig. 3A. In this example, a branch of the MCA passed through the D4 column. The temperature increased first along the MCA branch at 2.0 s, then radiated peripherally from this central area corresponding to the D4 column between 3 and 6 s. This study demonstrated the ability of IR thermal imaging to provide spatial localization of neural activity. However, the widespread use of this technique for functional neuroimaging will be limited due to the prerequisite of exposing the cortical surface.

Measurement of absolute change of the local brain temperature may allow quantification of neural activity in contrast to conventional neuroimaging techniques such as fMRI. We analyzed thermal responses to three frequencies of whisker stimulation (1, 5, and 10 Hz). See Fig. 4. Greater increases in temperature change were evident with a lower stimulus frequency. This is consistent with findings that the excitatory neurons in layer IV of whisker barrels exhibit low-pass characteristics with fewer spikes when whisker stimulation frequencies are >2 Hz (2). It has been reported that the area of brain activation is more diffuse when stimulated with frequencies at <1 Hz compared with stimuli at 5 and 10 Hz (25). In contrast, there is also a report indicating that the regional cerebral blood flow in the rat somatosensory cortex obtained by Laser-Doppler flowmetry increases linearly with increased whisker movement frequency (10).

Cortical temperature is inevitably influenced by the change of cerebral blood flow accompanying neural activity. The change of blood flow is divided into systemic and regional changes. In the present study, we collected data under two conditions to evaluate temperature, as well as blood flow. The first was to compare thermal responses of the barrel cortex between contralateral and ipsilateral whisker stimulations to discriminate the change of regional blood flow from the change of systemic blood flow. In the rat whisker barrel sensory system, whisker sensory nerves terminate in localized groups of cells within barrels in the contralateral somatosensory cortex (33). The cortical temperature was also found to increase for the ipsilateral whisker stimulus; however, the region of temperature increase was localized in the same region as the pial arterioles branching from the MCA as seen in Fig. 5. The contralateral stimulus, in contrast, produced a temperature increase in the wider region of the barrel field, including the pial arterioles. Furthermore, the spatial distribution of peak temperature for contralateral stimulation was irrespective of the vascular arrangement, as shown in Fig. 2A. The difference in thermal responses between ipsi- and contralateral stimuli indicates differing heat sources between them. In the contralateral whisker barrel field, there should be heat sources other than the blood flow of surface pial arterioles. The heat sources include the cell metabolic heat accompanying the neural activity and the blood flow in deep capillaries that were invisible from the surface.

The second part of this study involved suppression of the regional cerebral blood flow response to neural activity by the use of a calcium channel blocker. We applied nimodipine (4-dihydropyridine-derivative Ca²⁺ channel blocker) intraperitoneally in advance of the experiment. It is reported that Ca²⁺ signals in astrocytes play a central role in the neurovascular coupling (8, 20, 30). Nimodipine is so lipophilic that it passes through the blood-brain barrier, reaching brain and cerebrospinal fluid, and blocking calcium channels of the smooth muscle cells, which set the basal tone of blood vessels. Thus nimodipine was expected to suppress stimulus-induced vasodilation, although it may also affect blood pressure. Since the dosage of nimodipine adopted in this study, 0.2 mg/kg, was not high (11), the lowering effect of nimodipine on blood pressure was expected to be small. Figure 6 illustrates this finding. Specifically, the area of temperature increase in the barrel field of nimodipine-applied rats did not delineate or originate along the branching arterioles of MCA. Furthermore, the amount of peak temperature change following whisker stimulation in nimodipine-applied rats appeared somewhat larger than that in the rats without nimodipine (see Figs. 2A and 6A). This might be due to the lack of stimulus-induced vasodilation that functions as an effective heat transfer between tissue and blood.

Comparison of cortical temperature changes between ipsi- and contralateral barrel fields in response to the whisker stimulus suggested the presence of heat sources other than the surface blood flow in the contralateral cortex. This is also supported by the result that the local blood flow reached its peak value earlier than local brain temperature in response to whisker stimulation. Furthermore, the amount of heat brought about by the surface blood flow is supposed to be smaller than that by other sources, if we consider that the cortical temperature increase in the nimodipine-injected rats was somewhat larger than in normal rats.
It is noted that the barrel cortex in layer IV of the primary somatosensory cortex, which can be considered as the location of the initial heat source, is located 4–500 μm below the pia (34). However, the site of temperature measurement by the present technique is the dural surface. Time lag and spatial broadening are anticipated due to this effect; however, they are estimated to be minimal since the depth is small. When the heat is generated as a sharp spike at the depth of 500 μm, the time of peak temperature at the surface is estimated to be 0.28 s after the spike. The half width at half maximum of the temperature distribution is a measure of spatial broadening and is estimated to be 0.34 mm on the surface at that time. These values are obtained by solving the thermal conduction equation for a semi-infinite plane under the assumption that the thermal conductivity and the heat content of cortical tissue are the same as those of water (29). The depth of 500 μm is negligibly small compared with the size of the rat brain, and the radiation boundary condition is satisfied at the brain surface. Furthermore, the peak temperature increase measured on the surface should be lowered by the effects of spatial broadening compared with the temperature increase in layer IV.

The present study showed that the brain temperature obtained by IR thermal imaging increased in a focal region of the barrel cortex during whisker stimulation by an amount obtained by IR thermal imaging: a review of the literature and case report. *Neuroimage* 47, Suppl 2: T154–T162, 2009.


Woolsey TA, Van der Loos H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of

