The Effect of Neural Activity on Brain Temperature

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Abstract

The brain is a temperature-sensitive organ that needs to be within a narrow temperature range to function properly. The regulation of proper regulation of brain temperature is essential, but the mechanisms controlling brain temperature are not well understood. Cerebral blood flow (CBF) has been hypothesized to help in regulating local temperature by carrying heat away from an area. Neural activity generates heat and increases in CBF are coupled with neural activity. In this study, we aim to investigate the effect of independently manipulating CBF and neural activity on local brain temperature.

Brain temperature in awake mice will be measured with a K-type thermocouple implanted in the cortex. We can decrease neural activity and blood flow using local infusions of the GABA-A agonist muscimol or decrease blood flow without changing neural activity with the nitric oxide synthase inhibitor L-NAME. We will compare any temperature changes evoked by drug infusions to a vehicle control.

We expect this study to demonstrate one of two possibilities: either changing neural activity without changing blood flow alters brain temperature or the temperature changes caused by changes in neural activity are small. These experiments will help determine the role of blood flow in regulating brain temperature.

Introduction

The brain is a prominent organ that enables bodily function, and maintenance of its temperature is essential. Extreme deviation from a normal, baseline temperature leaves the brain vulnerable to incapacitation in the form of physiological deformation, increased susceptibility to toxins and toxicity, and death (Trübel et al., 2005; Wang et al., 2014). Brain temperature has been documented to fluctuate in response to environmental, physical, and behavioral stimuli **(**Aronov et al, 2012; Delgado et al., 1966; O'Herron et al., 2016).

However, despite the type of stimuli, multiple physiological mechanisms aid in maintaining a baseline temperature necessary for optimal brain function. Cerebral blood flow, for example, helps to carry away excess heat generated from metabolic activity occurring throughout the brain (Trübel et al. 2005). The shielding effect further stabilizes interior areas of the brain from drastic temperature changes (Zhu et al., 2006), while surface temperatures of the brain are more prone to higher degrees of temperature fluctuation (Kiyatkin et al., 2010)

Neural activity contributes to a significant amount of the heat generated in the brain, and thus contributes to fluctuations in regional brain temperature. Existing physiological mechanisms

like cerebral blood flow have been observed to couple with neural activity (Yablonskiy et al., 2001), but few studies have investigated how regional brain temperature is affected with neural activity and blood flow are independently modulated from one another.

Drugs such as muscimol (which hyperpolarizes all neurons) and L-NAME (which will block the synthesis of the vasodilator nitric oxide) have been used as tools in investigating brain-modulated behavioral changes. Muscimol is a GABA-A agonist that temporarily decreases metabolic processes in the injected site (Majchrzak and Di Scala, 2000). Both neural activity and blood flow are downregulated in response to muscimol, and the effects of the agonist are reversible in experimental models. L-NAME acts in a similar way to muscimol; rather than affecting neural activity, L-NAME will cause vasoconstriction of the blood vessels in the injection site by inhibiting nitric oxide synthase (NOS) activity. This agonist targets nitric oxide synthase (nNOS), an enzyme responsible for inducing vasodilation, and decreases its production, which in turn decreases blood flow through via vasoconstriction. Utilizing muscimol and L-NAME as tools to modulate neural activity and blood flow, we aim to investigate and understand how independent modulation of either factor affects brain temperature.

Methods

Construction and circuit design

Thermocouples were designed for prolonged use and long-term cranial implantation. K-type thermocouples (5TC-TT-K-40-36; Omega Engineering), and wire ends were fused together using colloidal silver. Polyimide tubing insulated exposed areas of the thermocouple wire, adding to more accuracy of thermal measurements.

Constructed, and subsequently implanted, thermocouples were connected to an amplifier. The amplifier's circuit design was adapted from Aronov et al. (2012) and subsequently attached to a power supply unit, the thermocouple, and a data acquisition system (LabView). Wires soldered to the cold junction compensator (LT1025 Linear Technology) and operational amplifier (LTC1050 Linear Technology) were coated and insulated with epoxy.

Animals

CBL567 mice (Jackson Laboratory) were used for experiments and data acquisition. Housing, specimen handling, and later described procedures followed the guidelines specified by the Institutional Animal Care and Use Committee (IACUC) of Penn State. Mice were housed individually in cages for the duration of the period of experiments and housed in an area that underwent 12-hour light/dark cycles. Mice were fed and watered ad libitum.

Surgical implantation

Male mice (n = 4) were between 3-8 months of age throughout the duration of experiments and weighed prior to surgery (26.9 ± 2.5). Anaesthetization included the use of isoflurane. Mice scalps were resected, and a thermocouple and custom-made titanium metal bar was implanted. Control mice (n = 2) were not implanted with a cannula. Cyanoacrylate glue (Vibra-Tite, 32402) was used to fix the metal bar to the skull along the midline and posterior to the lambda cranial suture. The skull was stabilized via self-tapping screws (#000,3/32", JI-Morris, Southbridge, MA) that were placed into the contralateral parietal and ipsilateral frontal bone. Thermocouples were implanted at a 30^{o} angle and depth of 700μm near the FL/HL representation of the somatosensory cortex. Mice implanted with a cannula along with the thermocouple (n = 2) had the cannula placed in the FL/HL representation of the somatosensory cortex. Metal bars were implanted and used to fix a mouse's head as it ran on a stationary ball. All implanted devices and exposed areas of the head were covered using black dental acrylic.

After surgery, mice were housed in separate cages in the housing unit and allowed to recover between 2 and 3 days prior to habituation. Weight was documented daily for 8 days and subsequently documented on a weekly basis for the duration of the period of experiments.

Habituation

Mice were habituated to the environment of future experiments. The implanted headbars were used to fix a mouse's position on a stationary ball. Throughout a 3-day period, a mouse was head-fixed to the stationary ball for a duration of 15 minutes, 1 hour, and 2 hours, respectively. Mice were enclosed in a near-room temperature (23.12 ± 0.025^{o}C) rig that limited light exposure and visual stimuli from the external environment. Habituation occurred between 1:00pm and 5:00pm.

Data acquisition

Mice were head-fixed to the stationary ball for the duration of each experimental trial. Trials lasted 3 hours, and velocity recordings were low-pass filtered at 1Hz (Butterworth). Velocity was then binarized to describe events as either "running" or "not running." Temperature data was also low-pass filtered at 1Hz (Butterworth).

Mice implanted with only a thermocouple were injected with a diluted dimethyl sulfoxide (DMSO)-water solution and diluted clozapine n-oxide (CNO)-water solution. A mouse was injected with 0.05mL of either chemical solution and allowed 48 hours to recover before another injection of the other chemical solution was performed. Injections were performed an average of 30 minutes after the start of the experimental trial.

Cannula-implanted mice were infused with 0.5μL of muscimol at a rate of 0.025μL/min via a Hamilton syringe. L-NAME and muscimol infusions lasted an average of 20 minutes, and infusion setup was initiated an average of 1 hour after the start of each experimental trial.

Experiments were conducted in a rig that limited the amount of light and visual stimuli to the mouse during data acquisition. Experiments were conducted between 1:00pm and 5:00pm.

Histology

Mice were anesthetized and perfused initially with heparinized saline and then 4% paraformaldehyde (PFA). Implanted devices such as the metal bar, screws, thermocouple, and cannula were removed prior to immersing the mouse head in 4% PFA. The head was allowed to saturate in the PFA solution for at least 24 hours before brain extraction and subsequent immersion in 4% PFA with 30% sucrose. The brain was removed from the solution after 24 hours and sagittally sectioned for Nissl staining. Each section was on average 90μm in thickness and was used to verify the location and depth of thermocouple implantation.

Statistical analysis

Paired two-tail *t*-tests were performed injection trials (n = 3) of either DMSO or CNO underwent. This test was performed for each both mice injected with DMSO and CNO. Two-way ANOVA was utilized to compare average brain temperature among pre- and post-DMSO and CNO injections. Statistical tests and analyses were performed in Matlab (Mathworks).

Results

Comparison of temperature change caused by CNO and DMSO injection. Mice (n = 2) implanted with only a thermocouple were subjected to CNO and DMSO injections in order to determine if either chemical solution affected brain temperature. Brain temperature prior to CNO or DMSO injection averaged 37.6 ± 0.012ºC and 37.7 ± 0.054ºC, respectively. Brain temperature was slightly higher prior to DMSO injection, but both average temperatures had high standard deviation values. After injection of either CNO or DMSO, brain temperature averaged to 37.2 ± 0.107 ºC and 37.1 ± 0.228ºC, respectively. Deviations were high due to the average temperatures of the experimented mice, both before CNO injection (S4: 36.9 ± 0.380ºC *vs* S11: 38.2 ± 0.333ºC) or DMSO injection (S4: 36.8 ± 0.654ºC *vs* S11: 38.7 ± 0.198ºC), and after CNO injection (S4: 36.5 ± 0.329 *vs* S11: 37.8 ± 0.367ºC) and DMSO injection (S4: 36.4 ± 0.458 *vs* S11: 37.9 ± 0.292ºC).

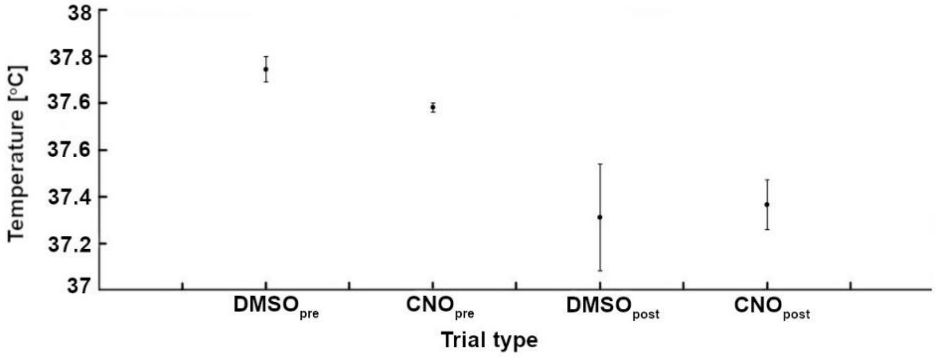

Figure 1. Average temperature pre- and post-DMSO and CNO injections. Pre-injection temperature of both CNO and DMSO were higher than their post-injection counterparts.c Individual pre- and post CNO injections of the mice in the experimental group did not significantly vary (S4: p = 0.208 *vs.* S11: 0.220). Temperature change in pre- and post-DMSO injection did not significantly vary in one mouse (S4: p = 0.143) while the other demonstrated significance in pre-/post-DMSO injections (S11: p = 0.005).

Experimental trials were conducted where both mice were not subjected to injection of either chemical solution. This control served as a control for both injections and served as the baseline when comparing how much brain temperature changed in response to DMSO or CNO injection, as shown in Figure 2. Prior to CNO injection, average temperature change among both mice were within 0.5ºC (S4: 0.021 ± 0.051ºC *vs* S11: -0.179 ± 0. 062oC.After injection of CNO, brain temperature remained lower than the

baseline as it had been prior to injection. Temperature change did not become higher than the baseline for CNO injection until more than 60 minutes had passed after injection. Temperature change of CNO injection decreased below the baseline, but eventually increased to above the baseline near the end of the trial. Overall, brain temperature change stayed within 0.25°C of the baseline pre- and post-CNO injection.

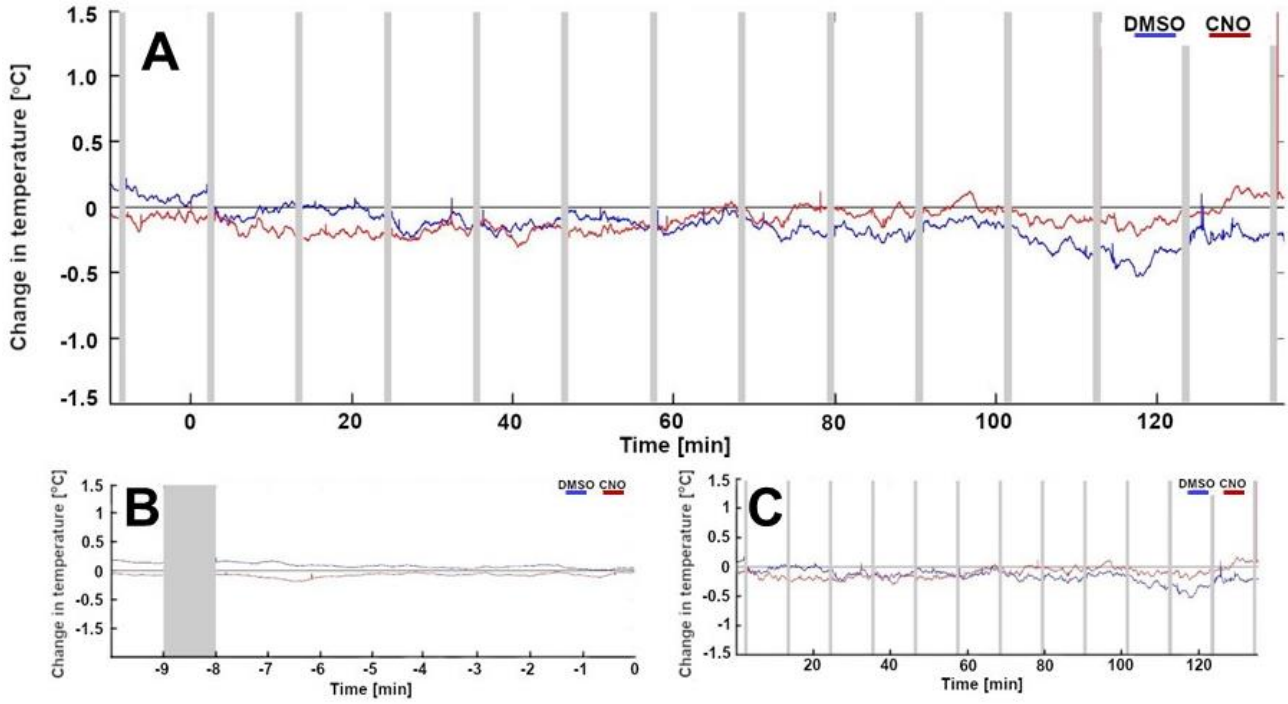

Figure 2. Temperature change in response to CNO or DMSO injection. Time of injection was designated as t = 0. Data was acquired in 10-minute intervals with 1-minute intervals in between each 10-minute bin.

Temperature change remained within 0.250°C (S4: 0.250 ± 0.046°C *vs* S11: 0.246 ± 0.090°C) above baseline temperature prior to DMSO injection. DMSO injection did not demonstrate an immediate effect in how much brain temperature changed relative to the baseline. Temperature change averaged within 0.250°C above or below (S4: 0.216 ± 0.115°C *vs* S11: -0.206 ± 0.209°C) the baseline post-DMSO injection. A slight decrease in temperature was demonstrated around 70 minutes after DMSO injection, but temperature increased toward the baseline near the end of the trial. Bouts where the average brain temperature difference between DMSO and the baseline was negative and positive fluctuated throughout the trial, but for a majority of the time temperature change was below the baseline for the DMSO injection trial. Brain temperature for most of the trial did not deviate outside of 0.5°C of the baseline.

Comparison of temperature change caused by aCSF, L-NAME, and muscimol infusion. Cannula-implanted mice (n = 2) underwent 3-hour experimental trials, as seen in Figure 2A. The cannula failed for one of the implanted mice, so data in Figure 2 represents the results of aCSF,L-NAME, and muscimol of one mouse. Separate infusions of aCSF, muscimol, and L-NAME were performed in 48-hour intervals. The average brain temperature before aCSF, L-NAME, or muscimol infusion was 37.0 ± 0.435°C. Figure 2B demonstrates that brain temperature remained steady during the pre-infusion portion of each experimental trial. Average pre-infused brain temperature of aCSF-infused mice was 37.1 ± 0.156°C. Pre-infusion temperature of L-NAMED-

infused and muscimol-infused mice was 36.6 ± 0.111ºC and 37.4 ± 0.140ºC, respectively. Post-infusion temperature was lower than pre-infusion, as brain temperature averaged 36.4 ± 0.268ºC when including all three infusion types (Figure 2C). Post-infusion brain temperature of the mice infused with L-NAME averaged a temperature of 36.1 ± 0.114ºC. Mice infused with muscimol demonstrated an average brain temperature of 36.4 ± 0.210ºC post-infusion. Both L-NAME and muscimol infusions resulted in a slight decrease in brain temperature when compared to the aCSF infusion. aCSF served as a control for the other infusions performed on the cannula-implanted mice, and brain temperature remained the least changed with the aCSF infusion (pre: 37.1 ± 0.156ºC *vs* post: 36.6 ± 0.123ºC) when compared to L-NAME and muscimol.

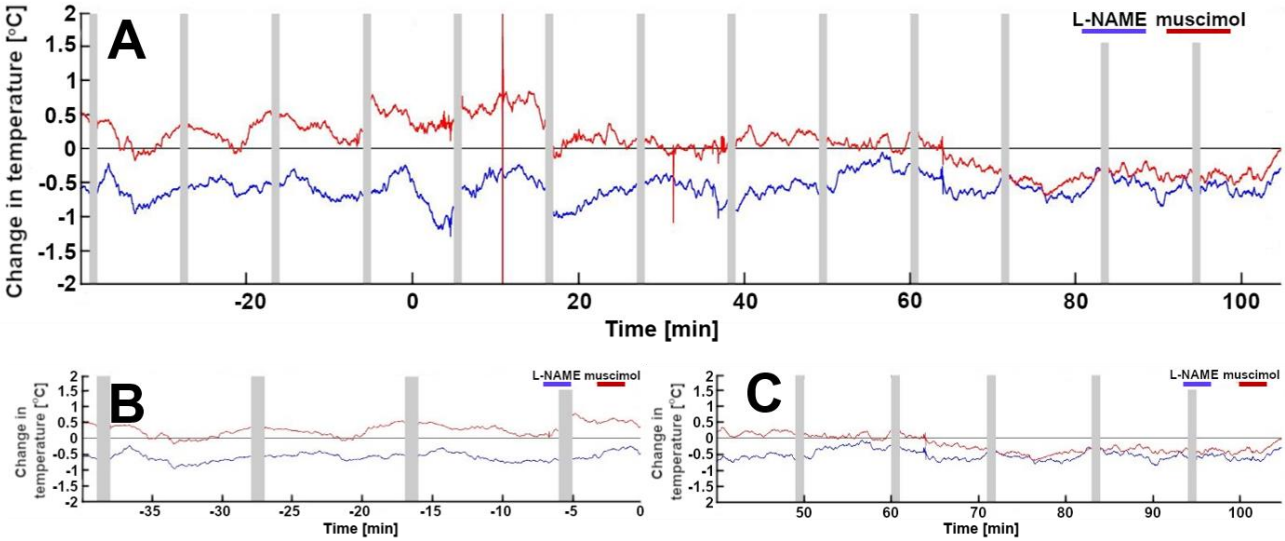

Figure 3. A: Infusion trials lasted 3 hours, and infusion setup started 60 minutes after the start of each trial. Data analysis accounted for 40 minutes prior to infusion setup and 40 minutes after infusion setup. The first and last 20 minutes of each experimental trials were not used in analysis. Infusions lasted an average of 20 minutes. t = 0 min indicates the time at which infusion setup started. B: Change in temperature between L-NAME and muscimol during the pre-infusion period remained within 1ºC of aCSF trials, the latter substance serving as the baseline for comparison. C: After infusion completion and onset of L-NAME and muscimol, brain temperature exhibited a decrease in temperature when compared to aCSF infusion. Brain temperature of both L-NAME and muscimol were within 1ºC less than the baseline (aCSF). Both infusions did not demonstrate a plateau in the rate of temperature decrease by the end of their respective experimental trial.

Discussion

DMSO and CNO injections provided no substantial change to brain temperature. The effect of DMSO and CNO injections was measured and compared to a running-only trial. With respect to the running trial as a baseline, brain temperature after either injection type did not demonstrate significant temperature deviation. This is supported by the functions of both drugs as described in previous studies; DMSO is a substance typically used as a vehicle control to other substances utilized in experiments. Its properties that attribute it to being used as a control supports the data acquired in the DMSO-CNO injections.

Similar to DMSO injections, CNO injections did not contribute to a drastic change in brain temperature. Pre- and post-CNO injections were within half a degree of change with respect to the baseline. The effect of CNO injection is to be expected because CNO is typically utilized congruently with designer receptors exclusively activated by designer drugs (DREADDs) (Manvich et al., 2018). No mice used throughout DMSO-CNO experiments contained DREADDs, so it is expected that the effects of CNO were not to be demonstrated in these experiments. Therefore, brain temperature was to not be drastically altered due to CNO injections.

Temperature changes were more likely influenced by frequency and duration of locomotion events than DMSO and CNO injections. Locomotion has been found to slightly increase cortical temperatures by 0.1^{o}C when short bouts of locomotion is performed (Shirey et al., 2015). However, continues periods of running have been demonstrated to contribute to larger increases in temperature as well as the rate in which this temperature increase occurs (Kunstetter et al., 2014). Pre-injection data contains the most prolonged periods of locomotive events, and in turn brain temperature in this experimental period was the highest. A constant pattern in which locomotion decreased as experiments, regardless of it being a CNO-DMSO injection or aCSF-L-NAME-muscimol infusion, progressed was prevalent. Decreases in temperature were apparent as well.

Brain temperature decreased slightly following muscimol and L-NAME infusions. Brain temperature decreased following infusions of muscimol and L-NAME. The degree of this change was not distinct between either drug, but it should be noted that a decrease in temperature was exhibited when blood flow, neural activity, or a both factors were manipulated.

Conclusions

Brain temperature is affected and regulated by multiple factors that happen internally and externally from the body. Current research has hypothesized a coupling relationship between neural activity and blood flow. In this study, we aimed to investigate whether independent modulation of either neural activity or blood flow affected brain temperature. We found agonists L-NAME and muscimol slightly decreased brain temperature upon infusion. Although the change was not drastic, modulation of neural activity and blood flow suggests the role both factors have in brain temperature. Our findings need to contain more muscimol, aCSF, and L-NAME infusion trials and a larger pool of animal subjects to validate and support our findings.

Future studies will utilize DREADDs to further modulate neural activity while not affecting blood flow. To conduct further studies using DREADDs, we investigated whether CNO, an activator for DREADDs, affected brain temperature. In this study, we compared how much temperature changed with respect to DMSO or CNO injections. We found that temperature change values were similar between the two substances, suggesting that CNO does not provide substantial change in brain temperature after injection. This allows us to utilize DREADDs as a tool for expanding this research in the field. DREADDs-containing mice will provide more insight in the effect of upregulating or downregulating neural activity while maintaining blood flow.

Understanding how brain temperature changes with respect to neural activity and blood flow is essential in a clinical setting. Further understanding is needed in what factors contribute to hypothermic and hyperthermic conditions that may lead to irreparable damage and decreased functionality of the brain.

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