Hyperthermia-induced Changes in EEG of Mice

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Abstract

Currently, the role of hypothermia in electroencephalography (EEG) is well established. However, few studies have investigated the effect of hyperthermia on EEG, an important physiological parameter governing brain function. The aim of this work was to determine how neuronal activity in mice is affected when the temperature rise above the physiological threshold mandatory to maintain the normal body functions. In this study, a temperature-elevation protocol, from 37 ºC to 42 ºC, was applied to four female mice of 2-3 months old while EEG was recorded simultaneously. We found that hyperthermia reduces EEG amplitude by 4.36% when rising from 37 to 38 degrees and by 24.33% when it is increased to 42 degrees. Likewise, increasing the body temperature produces a very large impact on the EEG spectral parameters, reducing the frequency power at the theta, alpha, and beta bands. Our results show that hyperthermia has a global effect on the electrical activity of the brain, with an impact on physiological responses under fever but also needs to be taken into account in those situations where the temperature triggers a pathological condition (e.g., a mouse model of seizures where temperature is the trigger parameter).

Introduction

Mammals keep their body temperature within certain boundaries, as it is the base of most biological processes. Considering the electrophysiological responses in the nervous system, changes during profound hypothermia have been described, consisting of a flat line or an isoelectric electroencephalography (EEG), with a total recovery once the temperature returns to normal [1,2]. While loss of EEG activity during hypothermia is widely accepted, data about the effect of hyperthermia on nervous system activity are less clear, as different brain areas have been studied, various exposure times and heating techniques have been used, and diverse animal species have been explored [3]. Using rats and a thermostatically controlled chamber, Gold *et al*. [4] found no significant correlation between changes in body temperature and EEG. Lifshitz *et al*. [5], in patients with fever and without neurologic disease, found that six of the fourteen subjects had completely normal EEG activity. Additionally, in humans, but with a protocol of deliberate hyperthermia, Dubois *et al*. [6,7] found a diffuse decrease in cortical activity that varied with the extent and duration of hyperthermia and was reversible after a few hours of cooling. With a similar protocol, Reilly *et al*. [8] demonstrated a heat-induced decline in both the frequency and amplitude of the EEG.

In recent years, there has been renewed interest in EEG recordings, which is largely due to both increased real-time EEG monitoring of brain function in critical patients such as coma or epileptic patients [9,10,11] and because neuroimaging techniques are not always available because of their elevated cost. In this scenario, and for many researchers, EEG is the preferred technique for data acquisition of cerebral activity [12], in humans and in experimental models.

In this sense, the mouse is becoming the reference animal in brain research; mice are affordable, require limited facilities, and transgenic methodology can be used to create mouse models of human diseases.

As genetic manipulations became more sophisticated, neuroscientists increased the availability of mouse models to work with. Particularly in epilepsy, there are several models where temperature is a critical factor for triggering the crisis [13,14,15]; however, to the best of our knowledge, there are no studies examining the changes in mouse EEG during hyperthermia. Because of this factor, and because elevated temperature may induce severe damage and complications in the nervous system, the aim of this investigation was to study the effect of whole-body hyperthermia on the brain activity of mice.

Results

Wild-type mouse cortical activity was continuously monitored by chronically implanted intracranial electrodes in the somatosensory (active) and visual cortices (reference). Animals were maintained in a state of light/medium anesthesia with sevoflurane. At 37 ºC, the EEG signal was dominated by low-frequency high-amplitude signals (Figs. 1 and 2). This kind of recording was maintained stable for long periods of time and was easily reproducible on different days in the same animal and even in different animals (Fig. 2), hence representing a solid baseline for establishing comparisons. Fig. 1 shows a representative 60-second EEG trace (A) and its spectrogram (C), where the dominance of lower frequencies is clearly visible. This figure also shows an expanded 1-second segment (B) and its spectrogram (D).

Increasing animal temperature had a dramatic effect on the recording amplitude, producing a reduction of 4.36% (±14.45) from 37 to 38 ºC and of 24.33% (±19.05) from 37 to 42 ºC. Fig. 2 shows a representative example of a complete session in 2 different animals (panels A and C) and an expanded sample at 37 and 42 degrees, where those changes in amplitude are visible (panels B and D).

Fig. 3 shows how the EEG amplitude changes with temperature. In panel A, data from a single session illustrate a linear regression of averaged recorded amplitude ($y = -0.037x + 1.842$; $R^2 = 0.84$). In panel C, the same data were averaged across all sessions ($n = 19$; $y = -0.025x + 1.4$; $R^2 = 0.9$). Panels B and D show the comparison between the amplitudes at 37 and 42 ºC for one session and averaged across sessions, respectively. For the example session, the mean ± SD normalized amplitudes were 0.46 ± 0.08 and 0.29 ± 0.05 at 37 and 42 ºC, respectively; the mean ± SD amplitudes across sessions were 0.46 ± 0.11 and 0.34 ± 0.09, respectively. In both cases, the difference was significant ($p<0.0001$ and $p<0.001$, respectively; permutation tests).

In addition to the comparison of the extremes, we ran binary comparisons for the amplitude at other temperatures against that at 37 ºC and found significant differences from 40 ºC on (Table 1).

Frequency analysis:

The EEG recorded was characterized mainly by the different oscillations included in the signal; thus, we analyzed how body temperature modified those frequencies. In anesthetized animals, EEG is dominated by lower frequencies, but different components up to the beta range (12 to 30 Hz) are detectable (Fig. 1B).

A temperature increase resulted in changes in the theta, alpha and beta bands while for the delta band there was no effect, as presented in Table 2 and Fig. 4. For each frequency band, we calculated the mean normalized power for each session and temperature and compared 37 with 42 ºC. The averaged power for the different frequency bands is shown in panel B.

Discussion

For many years, the effect of a temperature drop on EEG has been studied, mainly because of its clinical use, as it has protective effects on neurons in different conditions, such as acute ischemic stroke, traumatic brain injury, or inflammation of brain tissue [16]. Conversely, hyperthermia is a more common condition in nature, but it has received less attention, at least taking into consideration its relationship with brain activity in healthy subjects. With this investigation, we aim to study the impact of hyperthermia on neural activity, a condition in which core temperature rises above the physiological threshold necessary to maintain normal body functions. Our results demonstrate that hyperthermia has an important impact on neural activity measured by EEG. Hyperthermia decreased not only the amplitude of the recorded signal but also induced a reduction in the theta, alpha, and beta frequency bands when the temperature reached 42 ºC.

EEG signals represent a reliable tool for evaluating cortical function, a dynamic process that changes continuously and is affected by pathological conditions (e.g., infections) and physiological homeostasis. Regarding physiology, it is known that body temperature is an important factor driving neuronal activity, as the metabolism and integrity of brain cells depend on it [17].

EEG during febrile status has been intensively studied and analyzed and is usually related to other pathological processes, such as seizures, infections, cancer treatment [18,8] or syphilis [19]. However, the effect of hyperthermia on EEG in healthy subjects has not been deeply studied, even when fever is a common process that all of us suffer from at some point in life.

In the situations mentioned above, when EEG alterations are detected, it is often very difficult to disentangle which part can be attributed to the underlying pathological process and which to the temperature itself. Furthermore, the available results are not clear. Lifshitz *et al*. [20] concluded after studying the EEG of patients without neurological pathologies during episodic fever that *“fever in adults seems not to provoke rapidly reversible EEG changes.”* This conclusion is completely different from the profound depression of recorded activity reported by Cabral *et al*. [21]. In both cases, the increase in temperature was a consequence of a previous condition that could affect the results. On the other hand, Reilly *et al*. [8], studying cancer patients on hyperthermia treatment, found reversible changes in EEG signals related to temperatures above 41.5 ºC, mainly a decrease in the voltage signal and a shift to lower EEG frequencies.

The present study demonstrates a gradual reduction in signal intensity as the temperature increases, but in no case was there a suppression [21] or an increase [19] of EEG signal as previously reported, indicating that those extreme results could be the consequence of the specific pathology. On the other

hand, a gradual increase from 39 to 41 ºC followed by an abrupt decrease in EEG amplitude has also been reported in curarized rats [22]. Although it is a different animal model, it fits well with our data.

Our results also showed that in control conditions, the EEG was dominated by low frequencies; as a consequence of anesthesia, delta frequencies rule the spectrum [23], and this predominance was maintained without changes even during the high temperature peaks. However, significant changes were detected at theta, alpha, and beta frequencies, suggesting that these frequency bands are more sensitive to high temperatures.

We must keep in mind that our control situation is anesthetized mice; hence, anesthesia influences both the initial EEG and the EEG obtained during hyperthermia. There are a number of studies describing the effect of different anesthetics on EEG signals [24,25,26] and showing that anesthesia tends to produce a common pattern on EEG, reducing the variability that characterizes the awake resting state. This pattern is characterized by a prominent presence of delta frequencies, usually with no differences between anesthetic agents. Delta frequencies have been proposed to be the default activity pattern of cortical networks since they have been detected in disconnected preparations [27,28]. Theta, alpha and low beta frequencies are also present in anaesthetized animals, but the intensity and particular characteristics can vary with the anesthesia level and the anesthetic agent [25]. We are aware that our control situation cannot be defined as a normal physiological state, but on the other hand, it allows us to obtain a more stable standard initial point, decreasing the variability between animals. Additionally, anesthesia guarantees that changes are generalized, and two electrodes at distant places are sufficient to obtain a representative measurement of brain activity [29]. We observed a reduction in alpha, theta and beta frequencies, compatible with the described disruption of functional connectivity in the brain network under hyperthermia [30]. Only delta frequencies, which can be present even in slice preparations [27,28], maintained stable power.

These results show that hyperthermia is able to change cortical circuitry activity, affecting brain oscillations in anesthetized mice. Since alpha, theta and beta frequencies have been associated with different levels of awareness and different cognitive tasks [31,32,33] and hyperthermia has been negatively correlated with cognitive functions [34,35,36], it is tentative to speculate that those changes in cortico-cortical synchronization could be behind some of the cognitive effects. Additionally, such effects open the door to the possibility that small temperature changes in awake animals may have a strong effect on cortical oscillations, affecting gamma rhythm (not detectable in our anesthetized animals), which is related to sensory stimulation, attention and cognitive tasks [37,38,39]. Another open question worth considering in the future is whether longer periods of hyperthermia could produce stronger changes lasting longer or perhaps trigger some compensatory plastic phenomena.

In summary, acute elevation of body temperature is an important factor that is able to modify the electrical activity of the brain, probably impacting its metabolic activity. Our results showing the effect of hyperthermia on the amplitude and the synchronization of EEG signals pave the way for future research and represent a reference point for experiments involving a specific pathological condition in which

temperature is a trigger (e.g., a mouse model of seizures where temperature is the trigger parameter for seizures).

Methods

All the methods were carried out in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This study was conducted according to the rules of Physiological Spanish Society, International Council for Laboratory Animal Science, and the European Union guidelines (No. 2010/63/EU) for the protection of laboratory animals used for scientific purposes. The experimental protocol was approved by the ethics committee for animal research of the University Hospital of A Coruña.

Four female mice, 2- to 3-month-old C57-BalbC mice, were prepared and used for the experiments. Mice were maintained under standard laboratory conditions on a 12/12-hour light/dark cycle with free access to food and water.

Surgery

For surgery, animals were anesthetized with sevoflurane (3.5% for induction and 2-2.5% for maintenance) and positioned on a heating pad, with a rectal probe inserted, to maintain their core temperature at 37 °C (Thermostatic blanket low noise, model RTC-1, Cibertec, Madrid, Spain). Once the animal was stabilized, it was placed in a custom-made frame, the skin was open, and the connective tissue was removed until the lambda and bregma landmarks were visible. Using a high-speed dental drill, two small craniotomies were made in each mouse over the right somatosensory (AP -1.5 mm from the bregma, L 2.5 mm from the midline) and left primary visual (AP -2.5 mm, L 1.5 mm) cortices. At these locations, two copper wire electrodes were implanted on the dura for EEG recordings. Electrodes were fixed with a drop of fast drying superglue and a thin layer of dental acrylic. The electrode located on the somatosensory cortex was the active electrode, and the other electrode was the reference electrode.

Data acquisition

Recordings started one day after the surgery. The EEG signal was bandpass filtered (gain 1000, range 1 Hz-500 Hz), amplified using an A-M system differential amplifier (Model 1700 A-M System, LLC, Calsborg, WA, USA), digitized at 20 kHz (1401 CED A/D convertor card; Cambridge Electronic Design, UK) and stored (Spike 2 software; Cambridge Electronic Design, UK) in a PC for online checking and posterior analysis.

Study design

The experimental setup and the temperature-elevation protocol used in this study are illustrated in Figure 5. The measurements were conducted for up to six days in each animal for a total of 19 sessions. Each day the animal was anesthetized as described above, two alligator clips were clamped to the implanted electrodes, and the rectal temperature probe was placed and connected to the mouse temperature

controller. The body temperature was initially maintained for 5 minutes at 37 °C by a heat lamp positioned near the mouse (baseline). After this period, body temperature was continuously increased (0.5 °C/min) from 37 to 42 °C, where it was maintained for 5 minutes. Following this step, the mouse was cooled until the core temperature reached 37 °C. A standard session lasted for 25 minutes.

Data analyses

First, we bandpass filtered (1 – 50 Hz) and normalized (between 0 and 1) the raw EEG from each recording session. Then, the signal was binned using 5-sec epochs, and the maximum peak-to-peak amplitude within each epoch was measured. The temperature was averaged within each time epoch and round to the nearest whole number (epochs with temperatures below 37 °C or above 42 °C were discarded). For each temperature in the analyzed range (37 °C to 42 °C), we calculated the mean and SD of the maximum amplitude across epochs. Finally, we calculated the mean and SD of the maximum amplitude across recording sessions (n=19). For statistical analyses, we ran binary comparisons with the nonparametric permutation test, with 20000 iterations and an alpha level set at 0.05, using the MATLAB function permutationTest.m [40]. This procedure allows testing the null hypothesis that two different groups come from the same distribution without requiring any assumption about the sampling distribution.

Frequency domain

We divided each recording session in 5-sec epochs, and for each epoch, we analyzed the power spectrum (estimated with the MATLAB function *pspectrum*) in four frequency bands separately: delta (0.1 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 12 Hz) and beta (12 to 30 Hz). Then, we normalized the power spectra between 0 and 1 and calculated the average of the normalized power spectrum across epochs with the same temperature. To compare the results obtained at 37 and 42 °C, we used the permutation test (20000 iterations).

Declarations

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Author contributions

C.L., C.R. and J.C. designed the experiments, C.L., and C.R. performed the experiments, J.L.P-V. performed the analysis, and C.L., C.R. and J.C. wrote the manuscript. All authors contributed to the discussion and approved the final version of the manuscript.

Conflict of Interest/Disclosure Statement

The authors have no conflict of interest to report

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Tables

Table 1. EEG amplitude averaged across recording sessions as a function of the temperature and p-values obtained by comparing the amplitude at each temperature against that at 37 °C.

Temperature	*Mean*	*SD*	*p* (vs. 37°C)
37°C	0.461	0.109	-
38 °C	0.436	0.122	0.504
39 °C	0.429	0.118	0.385
40 °C	0.357	0.131	**<0.05**
41 °C	0.365	0.140	**<0.05**
42 °C	0.341	0.091	**<0.001**

Table 2. Average of the normalized power for different frequency bands as a function of the temperature and p-values obtained by comparing 37 °C with 42 °C with a permutation test (n=20000 iterations).

	37 °C		42 °C		
	Mean	*SD*	*Mean*	*SD*	*p*
Delta	0.059	0.028	0.051	0.023	0.3314
Theta	0.509	0.132	0.259	0.172	**<0.0001**
Alpha	0.495	0.147	0.230	0.162	**<0.0001**
Beta	0.319	0.075	0.222	0.098	**<0.005**

Figures

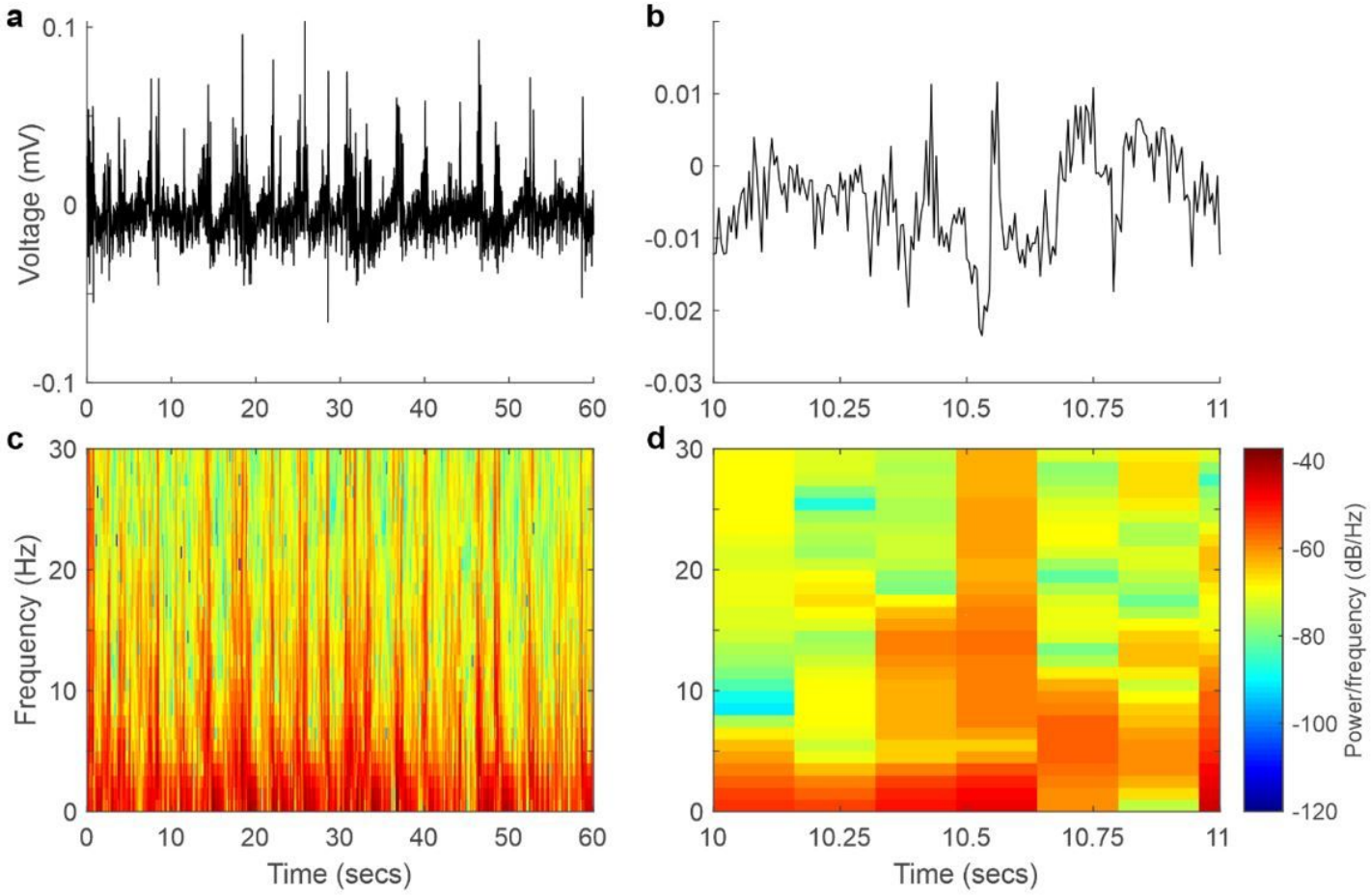

Figure 1

Example EEG signal and spectrogram at 37 °C. A. Sixty-second segment of the EEG signal. B. One-second recording expanded from A. C and D. Spectrograms of the EEG signals in A and B, respectively.

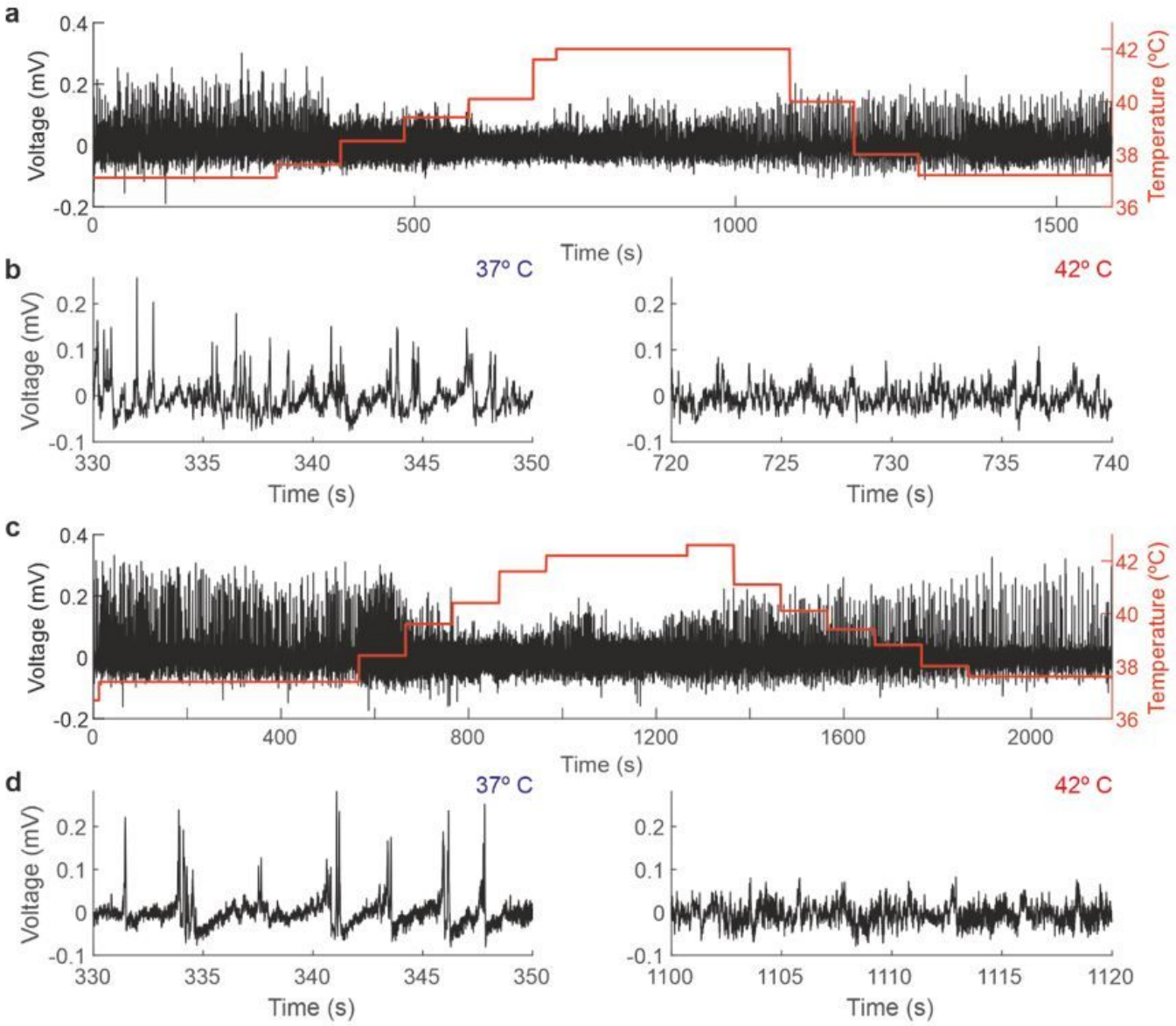

Figure 2

Changes in the EEG as a function of the core temperature recorded from two different mice. A and C. Raw EEG data (black trace, left y-axis) and core temperature (red trace, right y-axis). B and D. Details from the recordings depicted in A and C, respectively, showing 20 seconds during which the temperature of the mouse was 37 °C (left panels) and 42 °C (right panels).

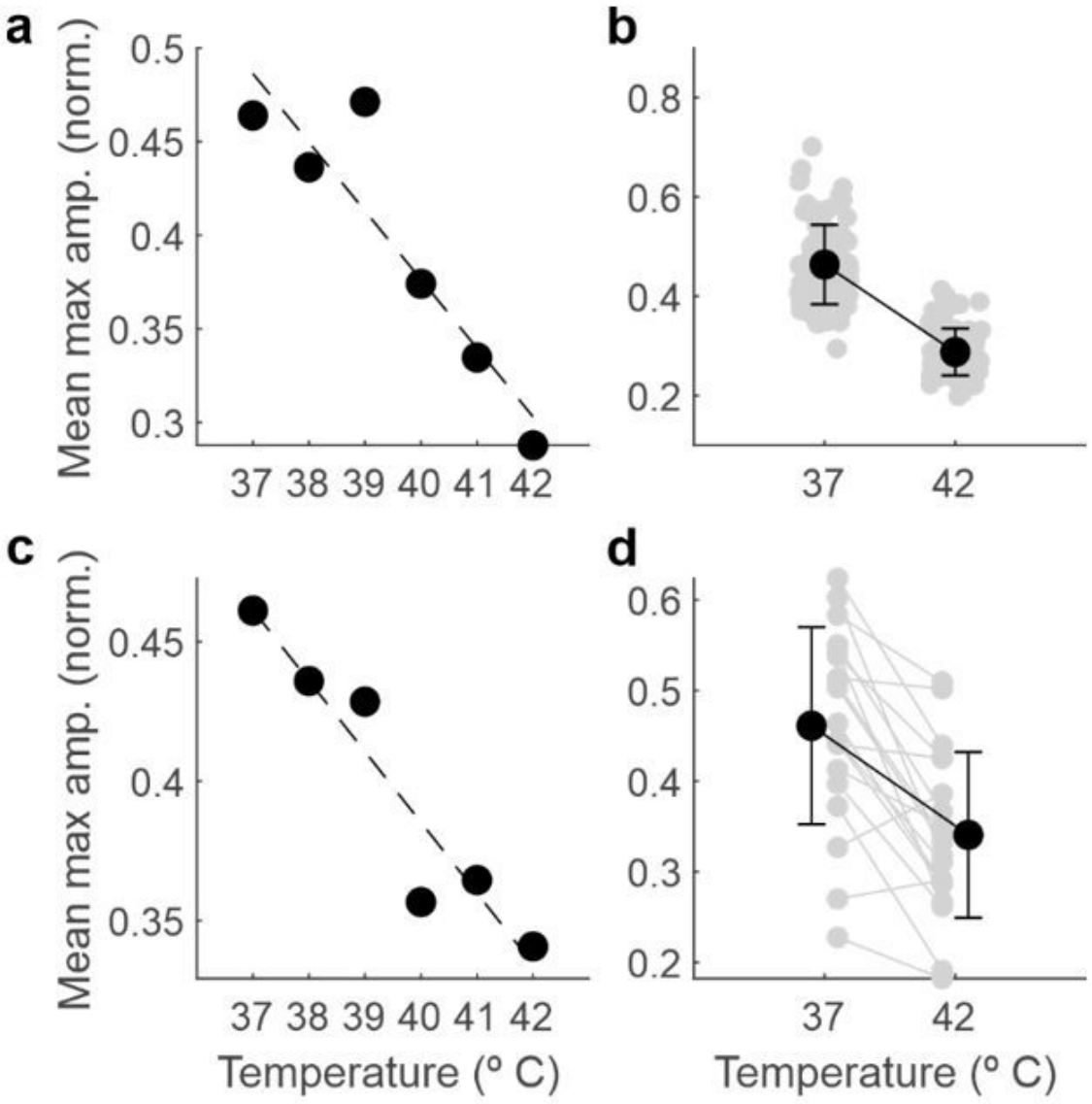

Figure 3

The amplitude of the EEG decreases as the temperature increases. A. Maximum amplitude of the normalized EEG signal within each 5-second epoch (averaged across epochs) as a function of core temperature, for one example recording session. B. Mean and SD (black dots) and individual values for each 5-second epoch (gray dots) comparing the maximum amplitude between 37 °C and 42 °C for the same session. C. The same as in A, but averaged across sessions. D. The same as in B but averaged across recording sessions (gray dots for individual sessions, n=19).

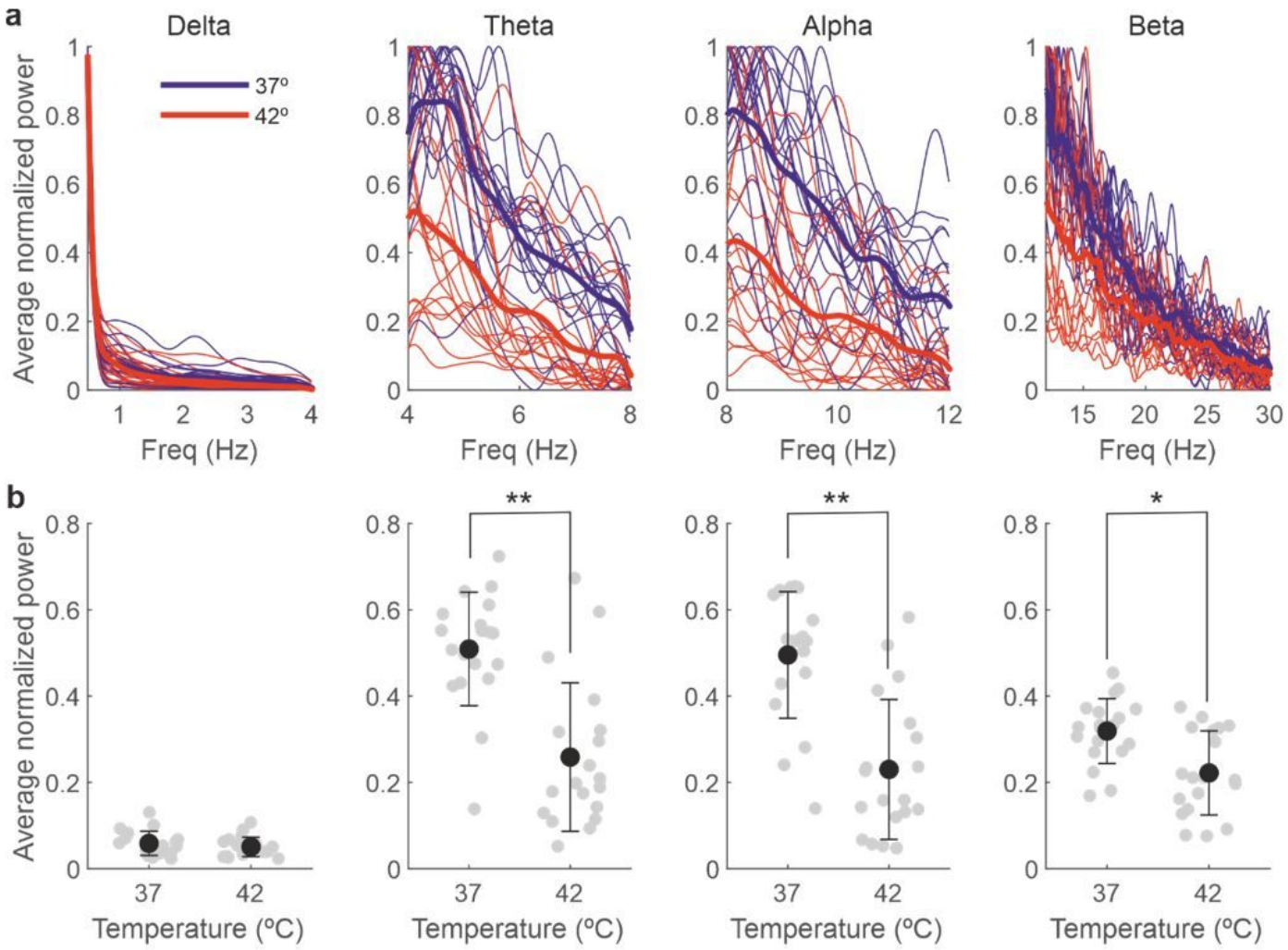

Figure 4

The effect of the temperature on the EEG is mostly due to changes in the theta and alpha bands. A. Power spectra for different frequency bands for each individual recording session, normalized between 0 and 1 and averaged across 5-second bins (thin lines) and averaged across sessions (thick lines). B. Power averaged across frequencies of the spectra in A. Gray dots represent individual sessions, and black dots represent the average across sessions (mean ± SD). * $p<0.005$; ** $p<0.0001$

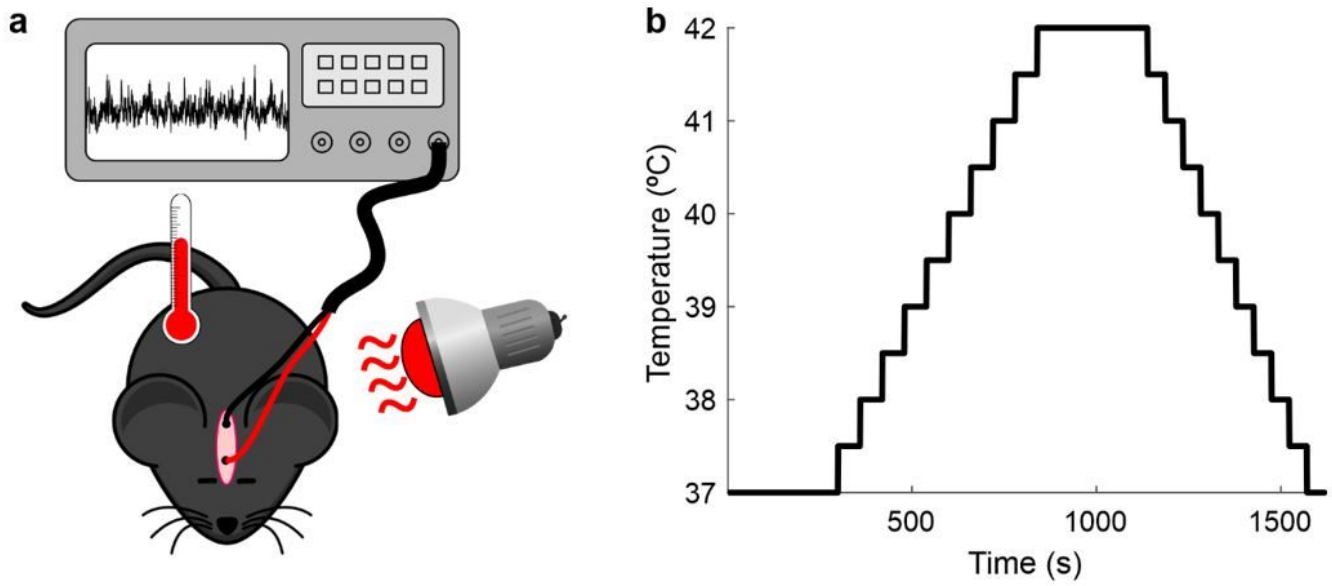

Figure 5

Recording setup. A. Experimental preparation. Anesthetized mice were placed under a heat lamp while the core temperature was monitored and the EEG recorded. B. Protocol for increasing the mice's temperature.