Application of a digital deconvolution technique to brain temperature measurement and its correlation with other physiological parameters.

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Abstract

The underlying reason for the local hyperthermia changes produced after a stimulus is not very well known and the relationship between local temperature changes and other physiological parameters has never been established. Current local temperature measurements are not completely accurate over time due to the physical constraints of the sensor, such as heat accumulation and dissipation. To clarify this tissue, simultaneous in vivo measurements of local temperature, local blood-flow by laser doppler flowmetry and neurotransmitter extracellular release using in vivo amperometry were performed with the aim of establishing their interrelationship. Local brain temperature measurements are usually obtained using thermocouples and thermistors, generally because of their small size and high level of accuracy. However, due to heat accumulation and dissipation effects on the sensor, the transient temperature measurement is not as accurate. In this paper, a simple method to obtain actual temperature fluctuations from measured values is proposed using classical digital signal processing techniques; the sensor was modeled via its transfer function. Deconvolution provides a method for obtaining actual temperature changes, enabling further comparative kinetic studies of all those physiological parameters, and helps to clarify the probable mechanism that underlies neurovascular coupling.

1. Introduction

The brain consists of neurons and glial cells that mutually influence each other and collaborate to keep the brain functional and to perform a spectrum of very complex tasks. Blood vessels are linked to neuronal functioning by the process of neurovascular coupling, i.e. the coupling of vessel diameter and thus blood flow, to neuronal activity [3]. Neuronal activity is determined by electric and synaptic activity, i.e. the action potential firing, the release of neurotransmitter vesicles from the presynaptic endings, and the subsequent postsynaptic chemical responses. Most of the energy needs of neuronal tissue derive from the restoration of transmembrane ion gradients that are temporarily disturbed by action potentials and postsynaptic currents [1]. A significant amount of heat in the brain probably comes from exothermic metabolic reactions during the above mentioned neuronal activity.
Following the above assumption, most of the generated heat comes from neural metabolism such as the restoration of membrane potentials after electrical discharges [7], suggesting a basic relationship between the electrical activity of neurons and metabolic neural activity. However, these are two different parameters. Metabolic activity involves both neurons and glial cells, a significant amount of energy is consumed by neural functions not directly related to spike generation (i.e. synthesis of macromolecules, transport of protons across mitochondrial membranes counteracting the proton leak in the opposite direction [12]), and it is still unclear whether neuronal inhibition, not only excitation, is energy-consuming and accompanied by heat production. Although not as specific as other measures of neuronal activity (i.e., single- and multi-unit electrical discharges, EEG – ElectroEncephaloGram) or brain metabolism (oxygen or glucose consumption, associated changes in blood flow), brain temperature monitoring provides a dynamic measure of both generalized and structure-specific changes in metabolic neural activity. In contrast to electrophysiological and other metabolic measurements, which often require anaesthesia and have a limited time window for experiments, temperatures may be recorded simultaneously from several brain and body areas in behaving animals during chronic experiments. This technical advantage was crucial for multiple experimental designs.

Neurovascular coupling matches the supply of blood and nutrients to the local needs of the brain cells and brings about an increased blood flow during or after local neuronal activation. For this reason several authors argue that hyperthermia produced after a stimulus is due to blood flow increase [15].

Local brain temperature measurements are usually obtained by using thermocouples [4, 5] and thermistors [8, 6], generally because of their small size and high accuracy. However, current temperature measurements are not completely accurate over time due to the
physical constraints of the sensor, such as heat accumulation and dissipation. Using classical digital signal processing techniques [9, 11, 14], we were able to reproduce the square pulse applied to the sensor using its output.

2. Material and methods

2.1. Temperature probes and recording instruments

The miniature probe consists of a Negative Temperature Coefficient (NTC) Thermistors (Betatherm NTC thermistor, Medical Catheter micro-Betachip Probe G22K7 MCD8). The major advantages of NTC thermistors are their large temperature coefficient of resistance, compared with RTD or thermo couples, simple electrical requirements, potentially good stability over time and small size (small size is correlated with fast response to temperature fluctuations). Thermistors major disadvantages: highly non linear temperature vs. resistance relationship and the need for calibration to obtain high accuracy, are not so important in our experiments where the non linearity is relatively small because the limited temperature range we used (36 to 38 \(^\circ\)C). A margin of error in this linear assumption in this range is below \(1 \times 10^{-4}\)\(^\circ\)C and the need for calibration is unnecessary because we worked with incremental or relative measurements. The hermetically sealed glass-encapsulated sensor (457 \(\mu\)m \(\phi\) and 3,18 mm long). The thermal time constant in liquids is 200 ms and the resolution achieved with it is 10-4\(^\circ\)C.

2.2. Animals, amperometric sensor, blood flow sensor and recording instruments

All experiments were performed in vivo on Sprague-Dawley albino male rats (300 to 400 gr). All protocols were carried out in compliance with the Local Animal and Use Committee.

The electrochemical microelectrode for dopamine (DA) measurement was prepared as described elsewhere [2]. This technique has been previously validated in our laboratory to measure brain extracellular DA levels [2].

The amperometric method is an electrochemical technique that quantifies neurotransmitter release in extracellular space. The current originated by the oxide reduction reaction on the carbon electrode surface is proportional to the in vivo neurotransmitter concentration. For voltammetry recording we used BECA (BioElectroChemical Analyzer, Tenerife, Spain). Electrical stimulation was done with “Stimulator S48” of GRASS and monitored with an oscilloscope (TDS 210, Tektronix, USA).

The microvascular blood perfusion (relative red blood cell flux) was recorded using laser-Doppler measurements. Optical probe consisted of two optical fibers, 100 \(\mu\)m diameter, which were used for continuous Laser (830 nm wavelength) Doppler flowmetry (ML191 Blood FlowMeter, ADInstruments, Australia). The probes were placed as close as possible to the temperature microthermistor and amperometric microelectrode.

2.3. Sensor model

The NTC thermistor probe temperature vs. time evolution follows a first order differential equation like \(dT/dt = -\tau(T - Ta)\) or \(T = Ta + (Ti - Ta)exp(-t/\tau)\), where \(T\) is the sensor temperature, \(\tau\) is the thermal time constant, \(Ta\) is the ambient or surrounding area temperature and \(Ti\) the initial sensor temperature. (\(t = \text{time.}\)). Because our sensor has a
small thermal mass, and is encapsulated within thin glass exposed to the medium, equation (1) gives accurate results. This is not the case for probes enclosed in larger metal cans or other materials. Equation 1 corresponds to a low pass filter equation where the 3 dB cut off frequency is $f = \frac{2\pi}{\tau}$. As already mentioned the thermistor thermal time constant $\tau$ is 200 milliseconds, so the 3 dB cut off frequency is 31.42 Hz. Because of the mathematical deconvolution we used to reconstruct our signal, see below, we can extend our bandwidth and use a cut off frequency of 20 db that corresponds with a cut off frequency of 314.2 Hz which corresponds with an attenuation of 10 in the temperature signal. This new cut off frequency allows us to be sure that the system follows almost all the thermal physiological transitions on the sampled area.

As stated before, the sensor is assumed to be linear and time invariant. This permits us model its behaviour via the systems impulse response. To characterize the sensor, a heat pulse was applied to it and the step response was obtained. As environmental conditions affect sensors transient response, several in vitro and in vivo experimental setups were studied.

The systems impulse response function was obtained via digital differentiation of the step response. Two different digital differentiators were studied, the first being the simple one-step backward method and the second being a more elaborated local five-point cubic polynomial fit. Finally, the transfer function is obtained by application of the Fourier Transform to the impulse response function obtained.

Once the transfer function is available, the steps involved with every measurement are simple. Firstly, the measurement is transformed to the frequency domain using the Fourier Transform. As the convolution operation between two functions is equivalent to a point-wise multiplication in the frequency domain, the deconvolution operation is reduced to the point-wise division of the measured data in the frequency domain and the transfer function. Finally, the Inverse Fourier Transform is applied to the obtained result.

3. Results and discussion

Neural metabolism requires intense energy consumption and is accompanied by heat production [7]. While metabolism-related heat production appears to be a primary fac-
tor determining brain temperature increases occurring under behavioural conditions [4], metabolic heat is continuously redistributed within brain tissue and removed from the brain by cerebral circulation to the lungs and then to the external environment. Changes in cerebral blood flow, therefore, may be another important contributor to brain temperature fluctuations occurring under behavioural conditions.

Figure 1 shows the step response of the sensor in four different environments. As can be seen, the step response function varies significantly, denoting very different heat dissipation speeds. Thus, a transfer function must be obtained for each different environment in which the sensor is used.

Simultaneous in vivo measurements of local temperature, blood flow and DA release were performed. Figure 2 shows the temperature fluctuation due to a biphasic electrical stimulation in MFB, 2000 ms at 40 Hz duration and frequency train respectively and its digital deconvolution. The square pulse of heat produced by the electrical stimulus is correctly estimated in its length and shape.

Figure 3 shows the time response curves of evoked neural activity in striatum by MFB electrical stimulation (40 Hz, 500ms, 150 μA). Note that the response of evoked DA release, deconvoluted temperature fluctuation and local blood flow are very similar. In contrast, time course of the blood flow of contralateral cerebral hemisphere was the opposite way. After stimulus onset, the signal increased very fast and was delayed by 0.5-1 s, followed by another ramp and a short plateau. The signal returned to the baseline with a similar decreasing ramp, although in our data, often with a prolonged post-stimulus undershoot. Our data show that this undershoot is often preceded by a marked inhibition in the neural response, so it may be related, at least in part, to changes in neural activity. The double increasing ramps could be explained by the heat generated by action potential propagation and neurotransmitter release. The second increase could be due to glial resting potential, neurotransmitter recycling and postsynaptic ion fluxes.

Figure 4 shows the changes observed during electrical stimulation of the MFB using different frequencies ranging from 10 to 60 Hz. The correlation between electrically evoked overflow of DA from the dopaminergic terminals and temperature increase (\(\Delta^\circ C\)) recorded very close to the amperometric electrode was very high \((r^2 = 0.99, p < 0.001)\). However, the correlation obtained from \(\Delta^\circ C\) and blood flow also recorded in a very small area close to the electrochemical probe was low \((r^2 = 0.79, p < 0.05)\). This suggests that brain temperature increases linearly induced by progressive increases of frequencies to the dopaminergic stimulation, moreover, have consistently shorter latencies and are stronger than those occurring in heart rate or local blood flow [4], suggesting brain metabolic activity as the primary heat source and a force behind more delayed body hyperthermia. Although metabolic brain activation is also accompanied by changes in cerebral blood flow [2], which is another indirect measure of metabolic neural activity [13], this factor seems unlikely to contribute significantly to electrically evoked or behaviour-associated local brain temperature increases, since brain tissue in intact organisms is always warmer than the arterial blood arriving to the brain [4]; thus, heat cannot be delivered to the brain from the periphery. This positive brain-arterial blood temperature gradient remains during both robust behavioural activation induced by various environmental challenges in animals [4] and intense physical exercise under conditions restricting body heat loss in humans [10]. Therefore, although temperature is usually omitted from equations relating brain metabolism and blood flow alterations [13], metabolism-related heat production and subsequent hyperthermia may be a significant contributor to adaptive increases of cerebral blood flow, which provides more
oxygen and nutrients to areas of high metabolic demand and (of no less importance) removes potentially harmful heat from brain tissue.

Future research will focus on studying the relationship between the transfer functions in different environments. Further biological studies will also focus on establishing the relationship between local temperature fluctuation and other relevant physiological parameters such as cerebral blood flow, neurotransmitter release, BOLD (blood oxygenation level dependent) signal etc., The role of heat control in the central nervous system by cerebral vasculature is probably more important and complex than has been believed to date. Moreover, the time course of haemodynamic response is more than a roughly low-pass-filtered expression of the total neural activity, and this suggests that vascular over response compared to the low tissue consumption could be explained by a new role which is more closely related to heat control (decrease of temperature in the activated area) than nutritional supply to the energetic demands of neuronal-glial activity.

References


