Temperature 1:1, 12–13; April/May/June 2014; © 2014 Landes Bioscience

# Brain temperature could affect neurochemical evaluations

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**This article demonstrates the importance of natural brain temperature fluctuations as a critical factor affecting electrochemical detection of extracellular glutamate in awake rats and proposes a viable strategy to exclude this inescapable influence, thereby increasing the reliability of electrochemical measurements of glutamate in behaving animals.**

The living brain is an exceptionally complex symphony of chemical reactions and changes occurring simultaneously at different speeds in different spatial locations. To understand the mechanisms underlying neural activity and brain functions, neuroscientists employ different tools to evaluate these reactions and changes. However, any specific measurement in the living brain is affected by multiple non-specific chemical and physical changes that affect measurement precision. In a recent paper,[1] the Authors present reliable evidence that naturally occurring changes in brain temperature are an important factor affecting electrochemical detection of extracellular glutamate (GLU) in awake, behaving rats. The Authors also propose a viable strategy to exclude this inescapable influence, thereby increasing the reliability and accuracy of electrochemical measurements with enzyme-based microsensors in behaving animals.

Comment on: Kiyatkin EA, Wakabayashi KT, Lenoir M. Physiological fluctuations in brain temperature as a factor affecting electrochemical evaluations of extracellular glutamate and glucose in behavioral experiments. ACS Chem Neurosci 2013; 4:652-65; PMID: 23448428 http://dx.doi.org/10.1021/cn300232m

**Keywords:** brain temperature, glutamate, glucose, enzymatic reactions, electrochemistry, enzyme-based biosensors

**Abbreviations:** GLU, extracellular glutamate; NAcc, nucleus accumbens

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Submitted: 12/24/2013

Accepted: 01/13/2014

Published Online: 01/24/2014

http://dx.doi.org/10.4161/temp.27666

Any chemical reaction depends upon temperature; this fundamental law is applicable in various degrees to any neurochemical parameter recorded from brain tissue. This law is also applicable to enzymatic chemical reactions (e.g., oxidation of GLU by glutamate oxidase at a specific voltage), which are used to measure fluctuations of GLU and other substances in the extracellular space of a living brain. Although the substrate selectivity of enzyme-based biosensors is usually and perhaps rightly viewed as the most important factor influencing the accuracy of electrochemical measurements,[2] a possible temperature influence on electrochemical measurements has been generally ignored due to the erroneous view that brain temperature is highly stable. However, multiple evidence obtained with direct brain thermorecording in awake animals, including our work on rats,[3,4] revealed relatively rapid (tens of seconds-minutes), large (2–4 °C) and prolonged (tens of minutes-hours) fluctuations in brain temperature that occur in response to different arousing stimuli, drugs and during various motivated behaviors (**Fig. 1**). Therefore, neurochemical evaluations under these conditions could be affected by concomitant changes in brain temperature.

In this featured paper, which extends the results of an earlier study,[5] it is demonstrated that rats exposed to various arousing stimuli show relatively long-term (20–40 min) increases in electrochemical currents detected by GLU-selective sensors; these increases are about the same in magnitude (~50–150 pA) in 2 compartments of the nucleus accumbens (NAcc), its shell and core. Based on sensor sensitivity detected in vitro and corrected for 37 °C ("mean brain temperature"), peak current increases induced by a 3 min tail-pinch are equivalent to a ~200 nM rise in extracellular GLU levels. However, when similar recordings were conducted with enzyme-free GLU-null sensors, non-specific electrochemical currents also increased within the same time scale; although these increases were slightly lower and more tonic. Since GLU and GLU-null sensors are identically constructed and have similar sensitivity to various chemical and physical factors, the current changes detected by GLU-null sensors reflect a total contribution of all these non-specific factors. When these non-specific changes were subtracted from those recorded by GLU sensors, the resulting differences revealed the GLU contribution, which was much smaller, more phasic, and different in each NAcc compartment. During a tail-pinch this difference was significant in the NAcc shell, suggesting a phasic GLU rise peaking at ~80 nM, but not significant (~20 nM rise) in the core, where both GLU and GLU-null currents were almost superimposable. While oxidation of multiple chemical substances other than GLU (i.e., ascorbate, catecholamines, its metabolites, urate, etc.) could also be responsible for the current increases detected by GLU-null sensors, the direct comparison of changes in NAcc temperatures and GLU-null currents during identical tail-pinch procedures revealed a near-perfect positive correlation, suggesting a tight interdependence between these 2 measures. Further calculations revealed that temperature influence accounts to 70–80% and > 90% of total current increases induced by natural arousing stimuli in the NAcc shell and core, respectively.

A similar approach was applied for evaluating physiological fluctuations in extracellular glucose levels in the NAcc shell.[1,6] In this case, stimulus-induced

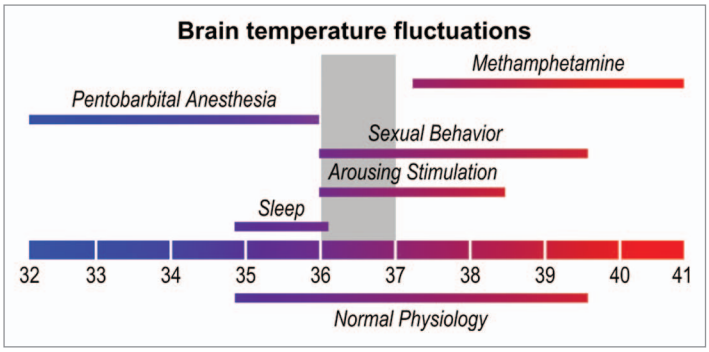


**Figure 1.** The range of brain temperature fluctuations in rats under different experimental conditions. While it is usually believed that normal basal brain temperature in deep ventrally located structures is within 36–37 °C (gray area), it could phasically reach higher levels during exposure to arousing stimuli (> 38 °C) and performance of motivated behavior (up to > 39.5 °C during copulatory behavior) and fall to lower levels (< 35.0 °C) during a slow-wave sleep. Brain temperature could also decrease well below its physiological range during pentobarbital-induced general anesthesia without body warming (to 32–30 °C) and rise to clearly pathological levels (> 40 °C) during acute intoxication with psychomotor stimulants such as methamphetamine.

increases in electrochemical currents detected by glucose sensors were much larger (~0.8–1.2 nA for tail-pinch) and, although glucose oxidase-free null sensors also showed significant changes, they were incomparably smaller (~0.05 nA) and very similar to those detected by GLU-null sensors under identical conditions. Therefore, the impact of temperature in electrochemical evaluations of glucose is insignificant due to its much larger basal levels in the extracellular space (~0.5–0.7 mM) and much larger range of its physiological fluctuations (60–80 μM). In marked contrast, this influence of temperature is robust for changes in NAcc GLU, which is maintained in the extracellular space at much lower basal levels (1 μM), showing physiological fluctuations within much lower range (50–200 nM).

While these studies clearly demonstrate that naturally occurring temperature fluctuations could profoundly influence the results of electrochemical measurements in awake, behaving animals, they also suggest that this influence varies depending upon the substance under study, the brain structure, where these measurements are conducted, and measurement duration. Since the contribution of this temperature factor could be large and greatly affect measurement results, this factor should be carefully controlled. The use of identical, enzyme-free sensors appears to be a valuable tool to exclude these and other non-specific influences and thus provide more reliable electrochemical measurements. These findings could also provoke multiple questions for further discussion. For example, our classic neurophysiological knowledge is primarily built on data obtained with cold in vitro or anesthetized preparations; to what extent could the data be affected if the factor of normal temperature (37 °C) and its physiological fluctuations (2–4 °C) are considered? Since trans-membrane diffusion is also temperature-dependent, to what extent do behavior-associated changes in brain temperature affect relatively slow and weak changes in neurotransmitter levels occurring under behavioral conditions? Since all processes governing neuronal activity, transmitter release and uptake are temperature-dependent, how does temperature affect stimulus- and drug-induced changes in these processes in awake animals? While these and many other related questions are complex, require thinking, and could initiate a "hot" discussion, it is evident that brain temperature is a unique physiological parameter that simultaneously depends upon neural activity, influences this neural activity and neural functions, and has profound, yet not clearly realized, effects on the various measurements conducted in the living brain.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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