

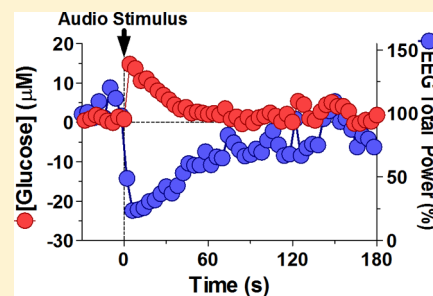
# Parsing Glucose Entry into the Brain: Novel Findings Obtained with Enzyme-Based Glucose Biosensors

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**ABSTRACT:** Extracellular levels of glucose in brain tissue reflect dynamic balance between its gradient-dependent entry from arterial blood and its use for cellular metabolism. In this work, we present several sets of previously published and unpublished data obtained by using enzyme-based glucose biosensors coupled with constant-potential high-speed amperometry in freely moving rats. First, we consider basic methodological issues related to the reliability of electrochemical measurements of extracellular glucose levels in rats under physiologically relevant conditions. Second, we present data on glucose responses induced in the nucleus accumbens (NAc) by salient environmental stimuli and discuss the relationships between local neuronal activation and rapid glucose entry into brain tissue. Third, by presenting data on changes in NAc glucose induced by intravenous and intragastric glucose delivery, we discuss other mechanisms of glucose entry into the extracellular domain following changes in glucose blood concentrations. Lastly, by showing the pattern of NAc glucose fluctuations during glucose-drinking behavior, we discuss the relationships between “active” and “passive” glucose entry to the brain, its connection to behavior-related metabolic activation, and the possible functional significance of these changes in behavioral regulation. These data provide solid experimental support for the “neuronal” hypothesis of neurovascular coupling, which postulates the critical role of neuronal activity in rapid regulation of vascular tone, local blood flow, and entry of glucose and oxygen to brain tissue to maintain active cellular metabolism.

**KEYWORDS:** Brain metabolism, amperometry, enzyme-based glucose sensors, neuronal activity, metabolic brain activation, cerebral blood flow



Brain cells continuously use glucose to fuel metabolic processes and glucose consumption is increased during metabolic brain activation.<sup>1,2</sup> The majority of glucose consumed by neuronal cells arrives from the peripheral circulation as glucose concentration in blood is always much higher than in the extracellular space.<sup>3–7</sup> This gradient-dependent transport of glucose is governed by a specialized type of GLUT-1 transporter densely expressed on the endothelial cells of the blood-brain barrier (BBB).<sup>8</sup> These transporters are capable of delivering two to three times more glucose than is normally metabolized by the brain.<sup>9</sup> This suggests that these transporters are more than capable of providing an adequate energy supply to neural cells under various physiological conditions.

As glucose transport capacity greatly exceeds the demands of neural activity, it is generally assumed that under physiological conditions more glucose enters neural tissue than is utilized for brain cell metabolism. Yet, our knowledge on this dynamic process is very limited due to an inability to measure extracellular glucose levels reliably, with sufficient temporal resolution, and under physiologically relevant conditions. In vivo microdialysis, a valuable approach for the direct measurement of brain chemicals, has insufficient time resolution (5–20 min), hampering the study of real-time glucose fluctuations. Moreover, microdialysis was rarely used for assessing physiological fluctuations in extracellular glucose levels.<sup>7,10–14</sup> The development of enzyme-based electrochemical sen-

sors<sup>15–18</sup> has made it possible to monitor fluctuations in brain glucose levels with second-scale resolution. This approach, however, has had a limited use due to technical complexities and, possibly, skepticism concerning the reliability of brain electrochemical measurements.

In this short Review, we present several sets of previously published and unpublished data utilizing enzyme-based glucose biosensors coupled with fixed-potential high-speed amperometry in freely moving rats. First, we consider basic methodological issues related to the reliability of electrochemical measurements of extracellular glucose levels in rats under physiologically relevant conditions. Second, we present data on glucose responses induced in the nucleus accumbens (NAc) by salient environmental challenges and discuss the relationships between local neuronal activation and rapid glucose entry into brain tissue. These data provide solid experimental support for the “neuronal” hypothesis of neurovascular coupling,<sup>19</sup> which postulates the critical role of neuronal activity in rapid regulation of vascular tone, local blood flow, and entry of glucose and oxygen to brain tissue. Third, by presenting recent data on changes in NAc glucose

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induced by intravenous (iv) and intragastric glucose delivery, we discuss other mechanisms of glucose entry into the extracellular domain following changes in glucose blood concentrations. Lastly, by showing the pattern of NAc glucose fluctuations during glucose drinking, we discuss the relationships between “active” and “passive” glucose entry to the brain, its connection to behavior-related metabolic activation, and the possible functional significance of these changes in behavioral regulation.

Most of our glucose measurements were obtained in the NAc shell, a critical integrative brain structure of the motivational-reinforcement circuit.<sup>20–22</sup> Since each brain structure has a specific pattern of neural and metabolic activity, often showing opposite responses to physiological stimuli and drugs, our discussion is focused on glucose dynamics in this representative brain structure. Literature data obtained in other brain structures (i.e., hippocampus, dorsal striatum, cortex, hypothalamus) as well as our own data obtained in the substantia nigra, pars reticulata suggest that glucose dynamics could have significant between-structure differences.

## 1. METHODOLOGICAL ISSUES RELATED TO RELIABLE EVALUATION OF LOCAL EXTRACELLULAR GLUCOSE LEVELS IN FREELY MOVING RATS

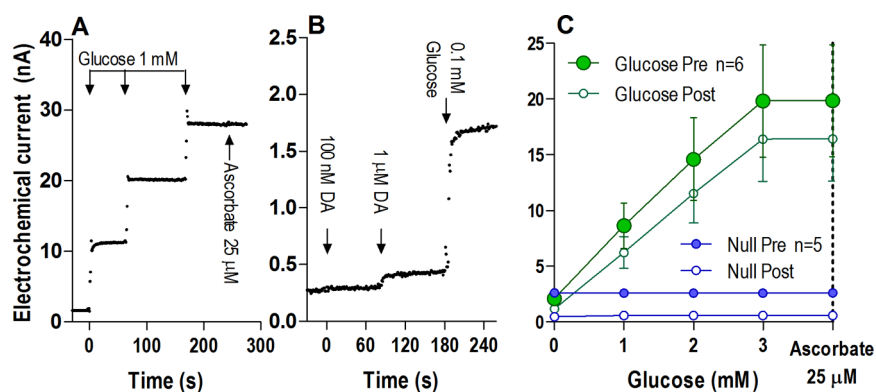
The quality of the sensing electrode is traditionally viewed as the most important factor in determining a high-quality electrophysiological recording and the same is true for electrochemical recordings. The substrate-sensitive sensor should not only be highly sensitive to the substance of interest, but also nonsensitive to all other chemical and physical interferents.

While the sensitivity of an enzyme-based sensor depends upon the amount of specific enzyme localized on the electrode's sensing area, the selectivity or insensitivity to other interferents is always relative and is usually represented as a selectivity ratio for major cationic or anionic interferents present in the extracellular space. While these selectivity ratios determined during prerecording or postrecording in vitro calibrations are important for verifying sensor's quality, chemical selectivity could be better represented with respect to basal levels of chemical interferents and the range of their physiological fluctuations. For example, microdialysis estimates of basal extracellular levels of dopamine, another electroactive interferent, are between 5 and 25 nM.<sup>23,24</sup> Conversely, transient physiological increases of dopamine detected in electrochemical studies and tonic dopamine changes detected by microdialysis typically lie between 30 and 100 nM.<sup>23,25–28</sup> Therefore, when considering dopamine as a possible nonspecific contributor, its influence appears to be minimal for enzyme-based glutamate and virtually absent for glucose sensors because basal concentrations of glutamate and glucose in the striatum are within 0.5–1.0  $\mu\text{M}$ <sup>29</sup> and  $\sim 0.5$  mM (0.47 mM,<sup>30</sup> 0.39 mM<sup>16</sup>), respectively. Although both glutamate and glucose sensors are similarly sensitive to dopamine in vitro (0.24 nA/ $1 \mu\text{M}$ ),<sup>31,32</sup> the current rise produced by a 100 nM increase in dopamine concentration (0.024 nA) is equivalent to 43 and 2  $\mu\text{M}$  concentration change induced by glutamate and glucose. With regard to glutamate sensors, this value corresponds to a  $\sim 5$ –10% concentration change (suggesting a possible contribution). Basal levels of glucose in the extracellular space, however, are  $\sim 50\,000$ -fold higher than that of dopamine and, as such, only produce a 0.2% concentration change.

There are, however, nonchemical interferents to electrochemical currents which are difficult, if not impossible, to exclude. For example, background electrochemical currents generated by enzyme-based sensors show a slow downward drift during a single-session, 8–9 h recording both in vitro and in vivo. Although the baseline currents are relatively stabilized 2–3 h after the recording start, baseline drift persists throughout the session and should be considered as a contributor to the slow current changes obtained during long-term recordings. This nonspecific influence, however, could be greatly reduced and possibly fully eliminated by preconditioning of sensors<sup>33</sup> or when the recordings are conducted with chronically implanted sensors. However, chronically implanted sensors present additional challenges associated with changes in sensor sensitivity and complexities of postrecording sensor calibrations.

The other well-known, but usually ignored, physical interferent is temperature. Although it is erroneously believed that brain temperature is stable, direct measurements reveal relatively large brain temperature fluctuations (1–3 °C) induced in rats by arousing stimuli and occurring during motivated behaviors (see ref 34 for review). Since electrochemical sensors are temperature-sensitive, naturally occurring changes in brain temperature are a critical factor affecting currents detected by substrate-sensitive sensors. As shown previously during in vitro tests of glutamate and glucose sensors at different ambient temperatures,<sup>31</sup> a 1 °C temperature rise produces a  $\sim 0.14$  nA increase in background electrochemical current; this is equivalent to a 26% presumed change in glutamate concentration, yet only a  $\sim 1\%$  change in glucose concentration. However, physiological brain temperature changes are relatively slow.<sup>35</sup> As such, the influence of temperature could be much more profound for longer analysis intervals (minutes to tens of minutes) than for short-duration analysis intervals (seconds to tens of seconds). Clearly the contribution of this physical factor should be carefully controlled in electrochemical evaluations, particularly when detecting glutamate.

As shown in our previous studies,<sup>31,32,36–39</sup> the use of enzyme-free null sensor is the best possible way to minimize all nonspecific influences from other electroactive chemical substances, changes in pH, oxygen, temperature, and basal current trends. The null sensor shares the same construction as the active sensor, but lacks the specific enzyme that reacts with the target molecule to yield a detectable electroactive compound. Although active and null sensors have minor differences in their sensitivity to major chemical interferents, they are equally temperature-sensitive and show similar performance both in vitro and in vivo. Therefore, identifying the difference between currents generated by active and null sensors under identical conditions greatly minimizes the influence of nonspecific interferents, improving reliability of neurochemical measurements. This approach appears to be very effective. For example, a total current response detected by a glutamate sensor in the NAc core during 3 min tail-pinch was mimicked 95% by a null sensor. This suggests that only 5% of the total current change resulted from changes in glutamate.<sup>31,39</sup> In addition, the difference in background currents detected by active and null sensors in vivo under identical conditions may also allow an estimate of basal levels of neurochemicals, a measure rarely achievable in most electrochemical studies.



**Figure 1.** Results of in vitro calibrations of enzyme-based Pinnacle glucose biosensors. (A,B) Changes in electrochemical currents induced by glucose, dopamine, and ascorbate in an example sensor. (C) Mean changes in electrochemical currents induced by glucose and ascorbate in enzyme-based (glucose) and enzyme-free (null) sensors during pre- and postrecording calibrations. See text for details.

In our studies, we used commercially prepared glucose sensors produced by Pinnacle Technologies, Inc. These sensors are prepared from Pt–Ir wire of 180  $\mu\text{m}$  diameter, with a sensing cavity of  $\sim 1$  mm length on its tip ( $\sim 0.56$  mm<sup>2</sup> area). The active electrode is incorporated with an integrated Ag/AgCl reference electrode. On the active surface, glucose oxidase converts glucose to glucono-1,5-lactone and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which is detected as an amperometric oxidation current generated by a +0.6 V applied potential.<sup>17</sup> The potential contribution of ascorbic acid to the measured current is competitively reduced by colocalizing ascorbic acid oxidase enzymes on the active surface of the sensor. This enzyme converts ascorbic acid to nonelectroactive dehydroascorbate and water. In addition, a negatively charged Nafion polymer layer under the enzyme layer serves to exclude endogenous anionic compounds.<sup>17</sup> Null sensors are prepared identically to glucose sensors except for the absence of glucose oxidase. While there are different designs of enzymatic and non-enzymatic glucose sensors that show excellent performance in vitro,<sup>40–43</sup> Pinnacle sensors are sufficiently sensitive and selective for the reliable evaluation of fluctuations in extracellular glucose in freely moving rats. These sensors are smaller than typical microdialysis probes, but larger than carbon fiber sensors. Although smaller-size sensors produce less structural damage and focus on a more precise detecting area, they are much more fragile and produce less current, thus making them more susceptible to electrical and mechanical artifacts during a behavioral experiment. All our experiments were repeated with glucose-null sensors (see below). The comparison of these data and quantitative analysis revealed that most current changes detected by glucose sensors in vivo result from oxidation of glucose and not from other nonspecific chemical or physical (i.e., temperature) influences.

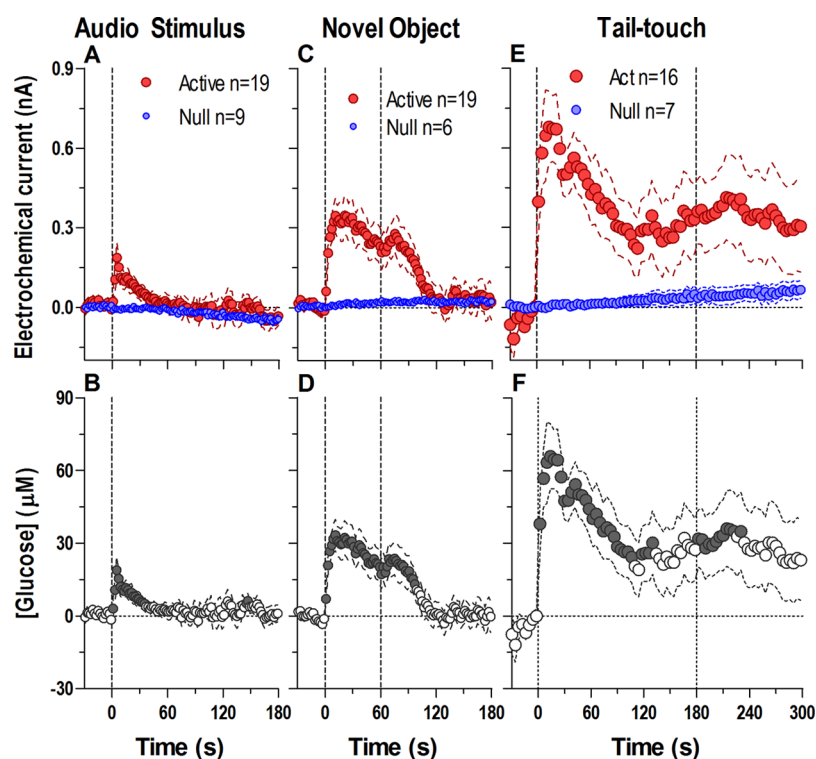
Figure 1 shows original examples and mean sensitivity/selectivity values of Pinnacle glucose sensors in vitro. As can be seen in panel (A), these sensors produced relatively large current increases following 1 mM additions of glucose [mean in two independent studies:  $7.11 \pm 0.84$  nA ( $n = 22$ )<sup>37</sup> and  $6.13 \pm 1.18$  nA ( $n = 6$ )<sup>32</sup> at 23 °C, and  $\sim 14.8$  nA and  $\sim 12.0$  nA, respectively, at 37 °C] and virtually no detectable changes with the addition of 25  $\mu\text{M}$  of ascorbate ( $0.06 \pm 0.02$  nA/25  $\mu\text{M}$ <sup>32</sup> at 37 °C  $0.56 \pm 0.10$  nA/250  $\mu\text{M}$ ,<sup>37</sup> respectively). These sensors generated oxidation currents with the addition of dopamine, but the response to 100 nM was virtually undetectable and much smaller than the response to 0.1 mM glucose (B). The

sensitivity and selectivity of these sensors remained virtually unchanged after an 8 h recording session in the brain as shown by pre- and postrecording calibrations (C). Null sensors had similar basal currents, were fully insensitive to glucose, and showed equally low responses to ascorbate. These sensors were also sensitive to dopamine, but their sensitivity was somewhat less than the dopamine sensitivity of glucose sensors (data are not shown here).

## 2. PHYSIOLOGICAL FLUCTUATIONS IN NAC EXTRACELLULAR GLUCOSE INDUCED BY SALIENT ENVIRONMENTAL STIMULI

While local extracellular glucose is a functionally important parameter that reveals the availability of glucose in brain tissue, its level depends upon two opposing variables: glucose entry from cerebral circulation and glucose consumption due to metabolic activity of brain cells. Although classic deoxyglucose data and recent attempts to image cellular glucose consumption<sup>44–48</sup> suggest that different forms of neural activation are associated with increased glucose use, the dynamics of this process are generally unknown. This lack of direct data on cellular glucose consumption complicates the interpretation of observed fluctuations in extracellular glucose, which occur under different experimental conditions. Brain structures that show high levels of glucose metabolism also tend to have a high density of GLUT-1 transporters.<sup>49</sup> Thus, glucose entry in these structures could be also higher than in structures with lower metabolic activity and less density of GLUT-1 transporters. Moreover, cellular glucose consumption and its inflow from arterial blood could differ between brain structures depending upon structure-specific neuronal activity and responsiveness.

It is known that basal glucose levels in peripheral blood fluctuate within 80–120 mg/dL (or 4.4–6.6 mM), but its levels in brain extracellular space determined by microdialysis in different brain structures of freely moving rats varied within 0.4–0.8 mM or  $\sim 15$ –20% of blood levels.<sup>3–7</sup> Our estimates of NAc levels made by comparison of currents detected by glucose and null sensors in rats under quiet resting conditions in two independent studies were:  $664 \pm 170$   $\mu\text{M}$ <sup>37</sup> and  $878 \pm 55$   $\mu\text{M}$ .<sup>32</sup> These values in good agreement with microdialysis estimates, suggesting glucose is indeed the major contributor to the difference in electrochemical currents detected by glucose and null sensors under physiologically relevant conditions. These values are also close to electrochemical estimates of basal



**Figure 2.** Mean ( $\pm$ SEM) changes in electrochemical currents and resulting changes in glucose concentration induced by different sensory stimuli in freely moving rats. Top graphs (A, C, E) show changes detected by glucose (red) and null (blue) sensors, and bottom graphs (B, D, F) show the resulting changes in glucose concentration calculated as a difference between active and null currents. Data for audio stimulus and novel object are shown with 2 s time resolution, and tail-touch shown with 4 s time resolution. In each case, two-way ANOVA revealed a significant current  $\times$  time interaction ( $F_{65,1690} = 1.95$ ;  $F_{90,2070} = 4.34$  and  $F_{180,2060} = 2.33$ ; each  $p < 0.05$ ) and the increases in glucose concentration were significant for each type of sensory stimuli (one-way ANOVA with repeated measures: sound,  $F_{18,1680} = 2.41$ ; novel object,  $F_{18,1620} = 14.90$ ; and tail-touch  $F_{99,396} = 2.50$ ; all  $p < 0.05$ ). Partially these data were reported in ref 32.

levels of extracellular glucose in the neighboring striatum ( $\sim 350 \mu\text{M}$ <sup>33</sup>).

Figure 2 shows mean changes in electrochemical currents detected by glucose and null sensors and resulting changes in NAc glucose concentration induced by several sensory stimuli of different duration and complexity. As can be seen in panel (A), a brief auditory signal (75 dB, 0.25 s) induced a rapid rise in currents detected by glucose sensors with no changes detected by null sensor. By transforming the currents into concentration, we found that glucose phasically increased relative to baseline within seconds after the stimulus onset, peaked at 6–8 s ( $\sim 20 \mu\text{M}$ ), and then rapidly fell to the prestimulus baseline (B). A larger and more pronounced glucose response occurred during a 1-min presentation of a novel object, another presumably stronger arousing stimulus (C, D). Similar to an auditory stimulus, glucose levels rapidly increased after the novel object was presented in the case, then slowly decreased, and slightly increased again after the novel object was removed from the cage. In this case, glucose increase was larger in magnitude ( $\sim 30 \mu\text{M}$ ) and more prolonged in duration. In contrast to glucose current, null current did not show evident changes except for a slow increase in baseline. While direct temperature recordings were not conducted with this stimulus, this current change could represent an initial component of brain temperature increase, which in the NAc is usually occurs with a  $\sim 20$ -s latency.<sup>35</sup>

The largest changes in NAc glucose were found with a 3-min tail-touch, a stronger arousing stimulus that induces motor activation (E, F). In this case, glucose currents rapidly rose

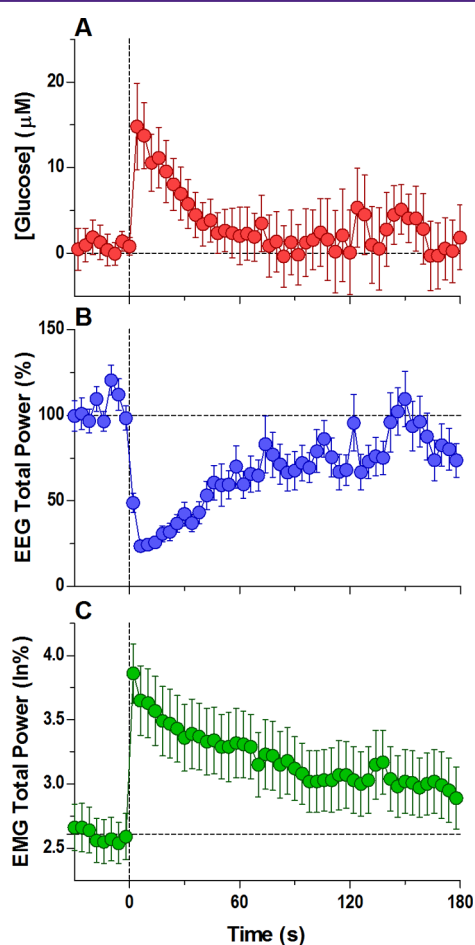
within the seconds after stimulus presentation, peaked at  $\sim 20$ – $30$  s ( $\sim 60 \mu\text{M}$ ), and slowly decreased toward baseline despite the continuation of stimulation. In contrast, null currents showed a slow, gradual increase, which, as shown previously, correlated with a slower temperature elevation induced in the NAc by this stimulus.<sup>35</sup> Increases in extracellular glucose induced by tail-pinch have been previously shown in the dorso-lateral striatum, a related but functionally distinct brain structure, by using high-resolution microdialysis<sup>3</sup> and enzyme-based sensors.<sup>50,51</sup> While glucose in dialysate rapidly increased during tail-pinch, electrochemically detected glucose began to increase later, after the initial slight fall. While technical differences could explain the discrepancy between these studies as well as between our and previous electrochemical studies, in light of our previous work suggesting robust between-structure differences in glucose responses,<sup>37</sup> the sensor location appears to be the most important factor.

Therefore, these results indicate that in the NAc glucose rapidly enters the extracellular space following sensory stimulation of different modalities. This rise in glucose concentration occurs with second-scale latencies suggesting a link with local neuronal activation. These data agree with the neural hypothesis of neurovascular coupling<sup>19</sup> which postulates that local neuronal activation is the primary force inducing rapid local vasodilation that increases entry of oxygen and glucose to an area in anticipation of its actual demands. Based on our brain thermorecording data, these changes precede increases in intrabrain heat production, a reliable index of metabolic brain activation, that occur slower and peak at much

later times.<sup>34,35</sup> Therefore, the brain is able to anticipate future metabolic needs for glucose by adjusting its delivery via rapid increases in local cerebral blood flow.

### 3. RELATIONSHIPS BETWEEN NEURONAL ACTIVITY AND GLUCOSE ENTRY TO THE BRAIN: DIRECT VERIFICATION

While the rapid time course of NAc glucose spikes suggests its triggering via local neural activation, we further tested this relationship by comparing mean changes in NAc glucose concentration with cortical EEG desynchronization, a direct measure of neural activation, and EMG activation that characterizes changes in tonic and phasic motor output (Figure 3). While under quiet conditions during sleep, EEG activity is

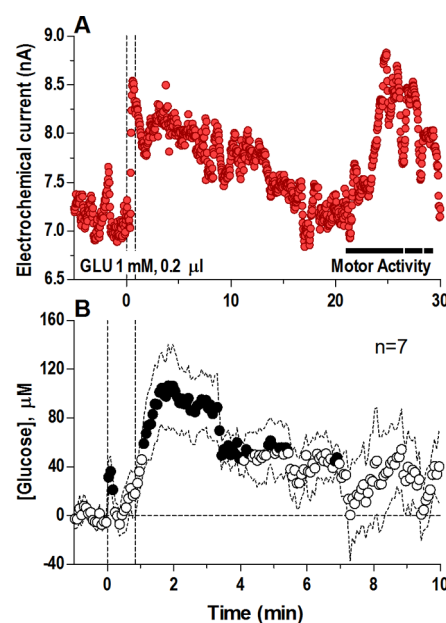


**Figure 3.** Mean ( $\pm$ SEM) changes in NAc glucose concentration (A), cortical EEG total power (B), and neck EMG total power (C) induced by a brief auditory stimulus. Data are shown with the same 4 s time resolution. Electrophysiological data were originally published in ref 60.

synchronized showing high-magnitude, low-frequency fluctuations, its amplitude decreases and frequency increases during sensory stimulation, resulting in rapid fall in EEG total power.<sup>52,53</sup> As shown in Figure 3, in response to the same brief auditory stimulus, changes in all three measures tightly correlate in time, with the largest changes in all parameters occurring rapidly within the first 4–8 s.

We also used another strategy to establish a link between local neural activation and rapid glucose entry in brain tissue. In

this case, a cannula used to insert the glucose sensor was combined with a microinjection cannula that allowed the delivery of drugs (glutamate and procaine) in close proximity to the glucose-recording site. Since glutamate universally excites all accumbal neurons,<sup>34</sup> we tested how low-dose microinjection of glutamate (1 mM, 0.2–0.5  $\mu\text{L}$ ) would affect glucose currents recorded during quiet resting conditions in freely moving rats. Figure 4 shows one original example obtained in these experiments (A) and the mean changes in NAc glucose concentration following low-dose intra-NAc glutamate microinjections (B).

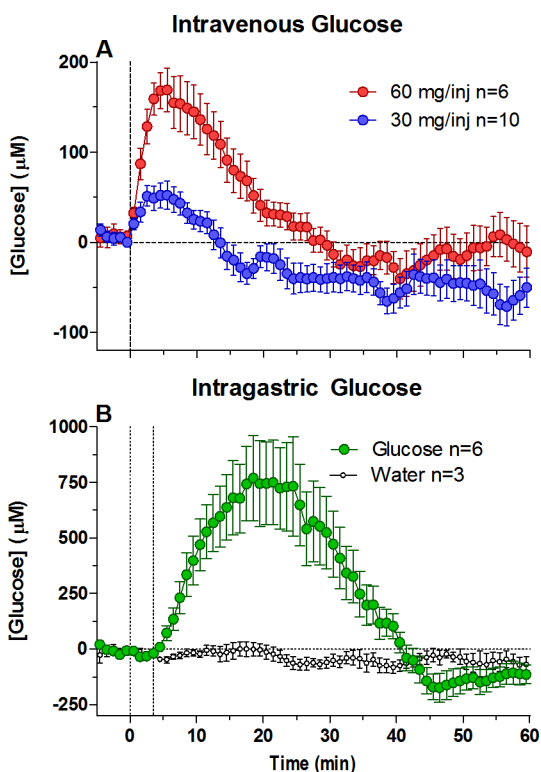


**Figure 4.** Original record (A) and mean changes (B) in electrochemical currents detected in the NAc by glucose biosensor during local NAc microinjections of glutamate nearby the recording site in freely moving rats. Data were originally reported in ref 37.

Glucose rapidly and strongly increased during the glutamate injection and then slowly fell toward baseline within next 20 min (A). Interestingly, a similarly strong increase in glucose concentration occurred during spontaneous behavioral activation (bold horizontal lines in A). Shown as mean changes (B), glucose also consistently increased following intra-NAc glutamate microinjection, peaking at  $\sim$ 120 s after the start of 50s microinjection. Interestingly, averaging multiple trials revealed another very short glucose rise at the start of microinjection. This increase was related the sound produced by microinjection pump and was virtually identical to the glucose response elicited by a brief auditory signal (see Figure 2A above).

### 4. OTHER MECHANISMS OF GLUCOSE ENTRY INTO EXTRACELLULAR SPACE: FINDINGS WITH IV AND INTRAGASTRIC GLUCOSE DELIVERY

While changes in local neuronal activity elicited by salient environmental stimuli could trigger rapid glucose entry to the brain tissue via an increase in local blood flow, glucose levels in the brain could be affected “passively” by changes in its concentration in peripheral blood (“gradient drive”). This possible mechanism was tested by direct measurements of NAc glucose following its iv and intragastric delivery (Figure 5).



**Figure 5.** Mean ( $\pm$ SEM) changes in NAc glucose concentration induced by intravenous (A) and intragastric (B) glucose delivery to freely moving rats. Glucose was intravenously injected at 30 and 60 mg doses (0.3 and 0.6 mL) and delivered intragastrically at the dose 500 mg in 5 mL solution. Panel (B) also shows changes in NAc glucose induced by intragastric delivery of water. In each case, one-way ANOVA revealed significant increase in glucose levels (30 mg,  $F_{9,540} = 12.48$ ; 60 mg,  $F_{5,300} = 19.94$ ; intragastric,  $F_{5,300} = 13.69$ ;  $p < 0.001$ ). Data shown in (B) were originally reported in 32.

While a similar approach, direct glucose measurements in different brain structures following systemic (ip or iv) or local glucose administration (via a microdialysis probe or micro-injection), has been previously used,<sup>33,43,55</sup> these tests were primarily methodological to demonstrate the substrate sensitivity of glucose sensor. Moreover, these tests were conducted in functionally different areas of the striatum or in anesthetized animal preparations. In our first test, freely moving rats were passively iv injected with solution containing either 30 or 60 mg of glucose. Since blood glucose levels are  $\sim 5$  mM, the blood volume in adult rat is  $\sim 30$  mL and the injection is relatively quick, the addition of 30 or 60 mg of glucose into bloodstream could theoretically transiently double or triple glucose levels in the peripheral blood. While not directly measured, these increases in peripheral blood should be smaller due to rapid glucose uptake and its removal from the bloodstream.

Passive injections of glucose increased its levels in the NAc, but the increase was relatively weak:  $\sim 50$   $\mu\text{M}$  for 30 mg and  $\sim 170$   $\mu\text{M}$  for 60 mg injections (Figure 5A). In contrast to the rapid glucose rise induced by sensory stimuli, these increases were relatively slow, peaking  $\sim 10$  min after the iv injections. These increases persisted up to 10 and 30 min longer after low- and high-dose injections, respectively. Finally, in both cases after glucose increases, its levels fell below baseline and only rebounded appreciably in the 60 mg injection group. While this decrease in glucose currents was weak, it could represent a

slight compensatory fall in glucose levels that occurs after its artificial increase due to passive injections. Therefore, passive rise in blood glucose increases NAc glucose levels, but only to a minimal extent. These “passive” increases, moreover, are relatively slow.

In the second test (Figure 5B), glucose was directly delivered (5 mL of 10% solution in water) into the stomach via a chronically implanted intragastric catheter. As shown in our behavioral experiments, rats easily consume this or larger volumes of a 10% glucose solution. While glucose delivered into the stomach slowly diffuses into the vessels, increasing its blood levels, the time-course of changes in blood glucose and subsequent changes in brain glucose levels were never measured before. NAc glucose concentration in this case increased more slowly ( $\sim 250$ -s latency from the start of 120 s intragastric infusion) than after an iv injection, but the increase was much larger ( $\sim 750$   $\mu\text{M}$ , or doubling of its levels) and with later peak (20–30 min). Although rats in this case received a relatively large amount of glucose (500 mg), only a very small amount reached the NAc.

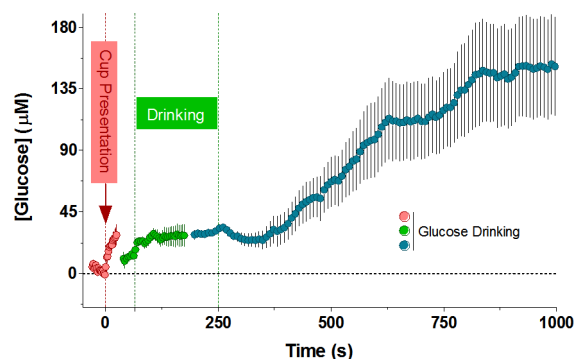
Therefore, although brain glucose levels could be affected by the changes in blood glucose levels, the brain appears to be reliably protected from peripheral hyperglycemia. These experiments also confirm previous data suggesting that the change in concentration gradient (and passive driving force) plays a minor role in glucose entry to the brain under conditions of functional brain activation.<sup>9</sup> However, the brain glucose response could be very different when blood glucose levels are significantly decreased. While this situation should not occur under normal physiological conditions because of large reserves for peripheral glucose production, it could be induced by an overdose of insulin. In such situations, blood glucose levels fall dramatically below the physiological range, resulting in life-threatening health complications and possible lethality. While the insulin-induced fall in glucose has been shown previously levels in the dorso-lateral striatum in freely moving rats,<sup>33,55</sup> it will be of interest to return to this issue and determine at which accumbal brain levels functional and behavioral deficit will occur.

## 5. GLUCOSE FLUCTUATIONS ASSOCIATED WITH GLUCOSE-DRINKING BEHAVIOR

Recently, we examined the pattern of NAc glucose changes during glucose-drinking behavior.<sup>32</sup> After minimal pretraining, rats were presented with a cup containing 5 mL of 10% glucose solution. After a variable latency, rats began to drink until the entire volume was consumed. Figure 6 shows the major results of this experiment taking into account both phasic and tonic changes in glucose concentration and essential behavioral variability.

Presentation of a glucose-containing cup results in rapid rise in NAc glucose concentration, which is maintained during the entire period of drinking (Figure 6). After the end of drinking, glucose levels began to slightly decrease before increasing again  $\sim 100$  s after the end of drinking. The second tonic increase was much larger in magnitude and it peaked at  $\sim 15$ – $20$  min after the end of glucose drinking.

While both the phasic increase in NAc glucose seen after presentation of a glucose-containing cup and its tonic elevation during drinking could reflect active glucose entry due to the activation of accumbal neurons, our data with iv and intragastric glucose delivery (see Figure 5 above) suggest that the second, larger elevation results from the rise in glucose in peripheral



**Figure 6.** Mean ( $\pm$ SEM) changes in NAc glucose concentration during glucose-drinking behavior. Because of between-test variability, we conducted three analyses of electrochemical currents focused on three critical behavioral events (cup presentation, initiation of drinking, end of drinking). Therefore, changes in glucose concentration are shown in three colors with respect to the initial pretest baseline (=0) and combined into one common time scale. The dashed vertical lines show the moment of cup presentation (0 s), median latency of the start of drinking (65 s), and a median end of drinking (250 s), respectively. Statistical analyses of these data were conducted for each of three individual events. Original data were presented in ref 32, where all methodological details can be found.

blood after its delivery into the stomach. Unexpectedly, the increase in NAc glucose levels after behavior-induced glucose consumption was significantly weaker than that seen after its passive, intragastric delivery. This seemingly paradoxical finding could be explained taking into account the second, usually hidden, force affecting extracellular glucose levels, its decrease due to cellular metabolism. Since in both cases the rats received the same amount of glucose, the weaker postingestion rise in NAc extracellular glucose after drinking could be attributed to behavior-associated consumption of glucose for cellular metabolism. This difference was surprisingly large, suggesting intense glucose use. As shown by using autoradiography, the ventral striatum is among brain structures with the largest increases in glucose utilization during drinking behavior.<sup>56</sup> Despite glucose consumption that tends to decrease its levels in the extracellular space, this process is opposed by intense gradient-dependent glucose entry from the peripheral blood (where its concentration is 5–8-fold higher) maintaining a positive balance (i.e., ready glucose supply) within the entire behavioral cycle. This finding underscores the importance of rapid, neural activity-regulated glucose entry in the brain, which is essential to anticipate its future use for cellular metabolism.

## 6. CONCLUSIONS AND FUNCTIONAL IMPLICATIONS

By using enzyme-based glucose biosensors coupled with fixed-potential amperometry in freely moving rats, we demonstrate that the extracellular glucose levels in the NAc are not stable and, in fact, show phasic increases following exposure to different salient environmental stimuli. The high-speed time resolution of this technique reveals that these increases occur with second-scale latencies, thus suggesting changes in local neural activity as their trigger. While establishing a direct correlation between NAc neuronal activity and fluctuations in glucose concentration is technically complex, a tight association between these parameters has been confirmed by independent monitoring of cortical EEG, neck EMG, and local activation of accumbal neurons by glutamate microinjections. Therefore, glucose is able to rapidly enter the extracellular space during

functional neural activation and prior to metabolic neural activation as detected by intrabrain heat production, a general measure of brain metabolism. In contrast to the rapid, transient glucose rise, brain heat production increases slowly, peaking at the stimulus offset and slowly returning to baseline within 20–30 min.

While our studies show the availability of glucose in the extracellular space outside brain cells, based on these data it is challenging to speculate on the dynamics of glucose consumption. However, our findings that NAc glucose concentration increased much stronger during passive, intragastric glucose delivery compared to behavior-induced glucose consumption could be explained by considering activity-related cellular metabolism, a factor not easily detected with an extracellular measure because intense glucose consumption that tends to decrease its levels in the extracellular space is continuously offset by greater glucose entry from the peripheral blood.

It should be noted, however, that the pattern of extracellular glucose fluctuations examined in the NAc may significantly differ in other brain structures. For example, glucose levels tonically decrease after sensory stimuli in the substantia nigra, pars reticulata,<sup>37</sup> where most neural cells have a high rate of basal activity and show inhibitions during sensory stimulation and motor activity.<sup>57–59</sup> In this structure, local microinjections of procaine, which inhibits neuronal activity via blockade of  $\text{Na}^+$  channels, decrease glucose levels.<sup>37</sup> Therefore, glucose dynamics in different brain structures could reflect structure-specific pattern of neuronal activity.

Nonetheless, it is clear that, in the nucleus accumbens shell, a critical component of the brain reinforcement and reward circuit, a positive balance (i.e., ready glucose supply) is maintained in response to salient stimuli and during the entire behavioral cycle. This finding underscores the importance of rapid, neural activity regulated glucose entry in the brain, which can be differentiated from slower changes in extracellular concentration that depend on its levels in arterial blood. This may indicate a complex interaction of multiple sources of glucose all essential for the maintenance of active brain metabolism.

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