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Review

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In-Vivo Electrochemistry: What Can We Learn about Living Systems?

George S. Wilson* and Michael A. Johnson

Departments of Chemistry and Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045

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1. Introduction

Present in vivo electrochemistry has evolved through three distinct threads: the enzyme electrode/biosensor, direct electrochemistry of endogenous electroactive species, and potentiometric applications of ion selective electrodes. For the purposes of this review, we will confine discussion to the use of electrochemically based devices to single cells, cell cultures, tissue slices, and in vivo measurements. The latter thread, developed by Frant, Ross, Simon, Bakker, Meyerhoff, and others,[1] has provided important information on dynamic concentration changes in ions such as Ca^{2+}, Na^{+}, K^{+}, and, of course, the most important ion, H^{+}, coupled to various stimuli and will be mentioned subsequently. Discussion of the fundamentals and sensor design in this important area is outside the scope of this review. For enzyme-based sensors, the development of the so-called enzyme electrode, first described by Clark and Lyons in 1962,[2] has triggered significant interest in biosensor development due in large measure to its role in the diagnosis and treatment of diabetes. A major virtue of this device, based on the concentration-dependent enzyme-catalyzed oxidation of glucose, is that it can make continuous measurements of the analyte without the need of adding reagents. Although many devices have been described that are based on antibody−antigen or oligonucleotide interactions, most are not useful for continuous measurements because they require regeneration after the selective binding reaction has occurred. A biosensor is defined as "a self-contained device capable of providing specific quantitative or semiquantitative information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with a...transduction element".[3] Thus an ion selective electrode would not ordinarily be a biosensor even though it measures a biologically relevant species, although there are "designer" proteins now available for such purposes.[4] In the 1970s, Adams and co-workers demonstrated the utility of direct electrochemistry for the measurement of catecholamines in the central nervous system (CNS).[5,6] These analytes are very similar in structure and therefore have very similar electrochemistry, an issue that has been partly resolved. The deficiencies in selectivity have to be balanced against significantly faster response time compared to multilayered enzyme devices.

The application of both enzyme-based devices and direct electrochemistry is limited to those analytes that serve as enzyme−substrates or possess intrinsic electrochemical properties that can be exploited. Thus, increasing the number of accessible analytes requires removal of the sample from the site in question so that it can be analyzed using a more versatile range of bioanalytical tools. Two sampling techniques are used for this purpose: microdialysis and direct removal of small volumes of biological fluid. The former technique is most widely used, and target molecules and their metabolites can be separated by HPLC or capillary electrophoresis and detected electrochemically or by formation of a fluorescent derivative. Microdialysis tubing is limited to about 200 μm outside diameter, and temporal and spatial resolution as well as tissue damage resulting from implantation become significant limitations. Microfluidic approaches show more promise in addressing these key issues.[7]

The last 15 years has been characterized by a maturation in in vivo electrochemistry. Focus has passed from proof of

* To whom correspondence should be addressed: telephone, +1-785-864-3475; fax, +1-785-864-5272; e-mail, gwilson@ku.edu.

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George S. Wilson received his A.B. degree in Chemistry at Princeton University and his Ph.D. at the University of Illinois. Following a two-year postdoctoral stay in the biochemistry laboratory of Professor Lowell P. Hager, he joined the Chemistry Department at the University of Arizona. In 1987 he was appointed Higuchi Professor of Chemistry and Pharmaceutical Chemistry at the University of Kansas, a position he now holds. In addition, he has served as Associate Vice Provost for Research and Graduate Studies since 2004. He served as the Chairman of the Electrochemistry Commission and President of the Physical Chemistry Division of IUPAC. He is a Fellow of the AAAS and a recent winner of the C. N. Reilley Award in Electroanalytical Chemistry. His research interests are in the area of real-time in vivo monitoring using biosensors, analytical applications of biological recognition, flow injection analysis, and the redox chemistry of biologically important molecules.

Michael Johnson received his B.S. degree in Chemistry from the United States Air Force Academy in Colorado Springs, CO. After graduation, he embarked on an Air Force flying career in which he attained the rank of Captain and Senior Pilot. Following his military career, he attended graduate school at the University of Virginia, where he received his Ph.D. in Chemistry in 2002, working in the field of drug metabolism and oxidative stress. He then joined the laboratory of R. Mark Wightman as a postdoctoral researcher at The University of North Carolina, Chapel Hill, where he used fast-scan cyclic voltammetry to study dopamine release and uptake mechanisms in mammalian systems. In 2005, he joined the faculty at the Department of Chemistry, University of Kansas, where he is currently an assistant professor. Prof. Johnson's research group is focused on developing new methods to measure dynamic neurotransmitter concentration changes in living systems as well as understanding how neurotransmitter release and uptake is altered in selected neurological disease states.

principle to the application of in vivo electrochemistry to provide answers to questions where the answer is not already known. This has promoted added attention to understanding processes that are linked dynamically, such as coupling of transmembrane fluxes with neurotransmission or cell signaling.[8–10] Electrochemically based biosensors are necessarily invasive, thus added attention has been paid to studies of the electrode/tissue interface, since the sensor response is defined by the species present at the interface.

2. Challenges in Sensor Performance

The vast majority of reports relating to sensor performance literature fail to provide sufficient evidence that they could function effectively and reliably in a biological medium. Stability, selectivity, sensitivity, and spatial and temporal resolution are all important considerations. Such issues must be resolved in the context of what is being measured and under what conditions. Stability of enzyme-based sensors is generally measured in several ways: (a) measurement in substrate-containing buffer solution periodically over time; (b) continuous measurement in buffer at 37 °C; (c) measurement of shelf life when stored in a refrigerator. The influence of the biological medium can be assessed by testing the sensor in blood serum or serum containing polymorphonuclear granulocytes (PMN).[11] This is justified even if the sensor is not placed in the vascular bed. While in vitro testing of sensors does not necessarily predict their in vivo behavior, failure in vitro virtually guarantees failure in vivo, some but not all of the issues that limit the shelf life of enzyme-based sensors impact carbon fiber microelectrodes, given the absence of the biological recognition element. The carbon fiber sensor is typically evaluated/calibrated using a flow cell in which the species of interest is introduced to the flow, normally by a valve.

2.1. Sensor Performance Characteristics

2.1.1. Design of Oxidase Biosensors

In reporting sensor characteristics, there is frequently confusion between sensitivity and detection limit (LOD). Sensitivity is the slope of the dose−response curve and cannot necessarily be extrapolated to zero concentration because of the presence of a background signal. While it is not absolutely essential that this curve be linear, nonlinear response complicates calibration. To understand sensitivity, it is necessary to discuss the convolution of the enzyme reaction with the mass transfer of the substrate to the enzyme layer. Using oxidases as an example, the enzyme is known to follow what is called the "ping-pong" mechanism, as shown in the following reactions,

$$S_R + E_O \rightarrow S_O + E_R \quad (1)$$

$$E_R + M_O \rightarrow E_O + M_R \quad (2)$$

where S_R and S_O and M_R and M_O are the reduced and oxidized forms of the substrate and mediator, respectively. The mediator could be endogenous oxygen or an added electron acceptor for the enzyme such as ferrocene or Os(III).[12,13]

The rate of the overall reaction sequence is determined by measuring the rate of oxidation of the mediator.

$$M_R \rightarrow M_O + ne^- \quad (3)$$

The objective is to make reaction 1 the rate determining step, with the rate being proportional to the substrate concentration. At high concentrations, the enzyme can become fully reduced and the rate no longer increases with concentration. This is described by Michaelis−Menten kinetics, and the substrate concentration yielding a rate corresponding to half the maximum rate is the so-called Michaelis constant, K_M.

Taking glucose oxidase as an example, it is desired to monitor physiological glucose concentrations in the range 2−25 mM. The K_M for glucose is oxygen dependent ($M_O = O_2$) and is in the range 5−8 mM in air-saturated glucose solution. A sensor operating under such conditions will be linear to about 8−11 mM, after which deviations from linearity can be observed. This means that a linear response is not possible over the operating range unless the effective K_M is increased. There are two ways to do this. First, the rate of reaction 2 can be increased. Since there is little control over oxygen concentration in the biological medium, this strategy is only appropriate if an exogenous mediator is employed. The second method is to reduce the effective substrate concentration in the enzyme layer. This is accomplished by providing a diffusional barrier between the enzyme layer and the biological medium. Provided that the enzyme activity is sufficiently high, the rate of mass transfer into the enzyme layer is defined by a concentration gradient between the test solution and the reaction layer. This means that the sensor response does not depend on stirring of the test medium nor does it depend on enzyme activity as long as the substrate immediately reacts on arrival in the enzyme layer, a situation analogous to complete concentration polarization at an electrode. There is a second benefit of this approach. Since the sensor response depends on mass transfer rather than enzyme kinetics, the temperature dependence, 2.5%/°C vs ∼10%/°C, is much lower. The disadvantage is that the presence of a diffusional barrier, typically a membrane such as polyurethane, increases the sensor response time and decreases the sensitivity. Csöregi and Heller[14] have managed to increase the effective K_M to about 45 mM, more than sufficient to provide the necessary linear range. This is accomplished by making reactions 1 and 2 as rapid as possible and then coupling substrate transport to a diffusional barrier. This strategy may prove problematic when substrate concentrations are in the micromolar range because the signal is already small. While the K_M is independent of enzyme activity, the total response is not. It is therefore important, especially in critical applications involving variously low substrate concentrations and/or sensors with low surface area, to make the enzyme activity as high as possible. This can be done by increasing the microscopic surface area using Pt black or nanoparticles/nanotubes.[15,16] Such surfaces tend to be surface active and are subject to adsorption of species from the medium that can reduce the sensor sensitivity rapidly and significantly. We have reported on a procedure for electrodeposition of enzyme, which is very useful when dealing with electrode geometries where dip-coating of enzyme solutions is not satisfactory. The technique also has the advantage that controlled amounts of enzyme can be deposited.[17,18]

As noted, the response of an oxidase-based sensor can depend on ambient oxygen. One approach is to employ a diffusional barrier which is highly permselective for oxygen while reducing significantly the flux of the substrate, glucose. Such an adjustment is necessary because the concentration of oxygen in the tissue is about an order of magnitude lower than that of glucose. As a result, the oxygen in the reaction layer is in excess and a considerable variation in tissue oxygen can be tolerated.[19] An alternative approach is to measure the amount of oxygen consumed in reaction 2; however, to do so requires a difference measurement. One sensor measures ambient oxygen (no enzyme present); the other measures it in the presence of enzyme.[20] The difference corresponds to oxygen consumed. This approach has the disadvantage of greater complexity but is also used to correct for background current.[21] An obvious answer is to employ an alternative electron acceptor, M_O, an approach providing potentially higher response but which is not without complications. The mediator should be chosen to have a formal potential low enough that it would not be reduced by endogenous species such as ascorbate, and an appropriate potential would be applied to follow M_R oxidation. The mediator, which must be immobilized, must compete effectively with oxygen in accepting electrons from E_R because the applied potential for mediator monitoring will be much lower than that for peroxide oxidation. Thus, the resulting current will not detect peroxide formation and the involvement of oxygen has a parasitic effect on the detection of mediator oxidation. This is illustrated in the work of Mano et al.[22] At low glucose concentrations (2 mM), the response was 76% lower under oxygen and 35% lower under air than under argon. The differences are predictably less severe at higher glucose concentrations. The rather high potential required for the oxidation of peroxide is sufficient to oxidize endogenous species such as urate and ascorbate. These species can be excluded from the electrode using selective membranes, but with the attendant increase in response time.

2.1.2. Stability of Biosensors

The stability of a biosensor will depend on the time-dependent activity of the enzyme. In the case of flavoenzymes, such as glucose oxidase (GOx), activity loss appears to be strongly associated with the loss of the isoalloxazine (flavin) moiety. This is promoted by elevated temperature, physiological vs room temperature, or by excessive cross-linking during the immobilization step. Immobilization of enzyme using antibodies has proven quite successful without the loss of activity.[23,24] The most common immobilization procedure is to apply a solution of the enzyme and then bring the resulting surface in contact with glutaraldehyde solution or vapor. A second approach is to form an electropolymerized cross-linked enzyme layer using pyrrole or other monomers.[25,26] In our experience, this approach does not seem to yield stable enzyme layers lasting over several weeks in in vitro testing. There have been a number of studies based on the attachment of enzyme to nanostructures such as nanotubes and nanoparticles,[27,28] but little is known about how these perform in in vivo applications. A third approach involves holding the enzyme on the surface by electrostatic interactions generated by polyelectrolytes.[29,30] The deposition is accomplished using alternate layers of enzyme and polyelectrolyte of opposite charge, and this latter layer may also contain mediator centers to facilitate the "wiring" of the enzyme to the electrode.[31] A similar strategy involves the creation of alternating layers using an antibody or avidin/biotin linkage.[32,33] Finally, the previously mentioned approach based on the electrodeposition of enzyme on a Pt surface and driven primarily by the localized lowering of the pH due to the oxidation of water, yields stable enzyme.[17]

Once immobilized, even relatively stable enzymes such as GOx will start to lose activity. Thus, whether short-term (hours to days) or long-term (weeks to months) experiments are envisioned, the stability will, in the end, be defined by having sufficient enzyme activity. When a mass transfer (*vide supra*) membrane is employed, the sensitivity will appear

to remain constant for a considerable period of time, followed then by a significant decrease. This latter point corresponds to the situation where the biosensor response is now again defined by the enzyme kinetics and therefore enzyme activity. Since the permeability of the mass transfer limiting membrane will define the sensitivity, it is important to control permeability, thus placing strong emphasis on the design and manufacture of this membrane so that the characteristics are maintained during use. For a glucose biosensor, it is advantageous to have a multipolymer membrane with segments of controlled length to confer high permeability for oxygen (polydimethylsiloxane) and low permeability to glucose (a 2−10% hydrophilic soft segment, polyethylene oxide).[34]

2.1.3. Dehydrogenase-Based Biosensors

The overwhelming choice for enzymes is oxidases because the coreactant, oxygen, is presumed to be in sufficient quantity to support the oxidation of the substrate. It is unfortunate that dehydrogenases have not proven to be useful, especially considering that there are more than 450 enzymes of this type. The cofactor, NAD or NADP, has to be added, and immobilization and electrochemical recycling have not proven feasible. A subclass of this group, the so-called PQQ dehydrogenases, which contain pyrroloquinoline quinone, avoid the need for the cofactor, but they have so far not proven to be especially stable or selective and therefore not useful as a practical matter.[35,36]

2.1.4. Detection Limits

For substrates such as glucose and lactate, which are present at millimolar physiological concentrations, the limit of detection will be below the desired range and therefore will not pose a problem. However, for species such as glutamate in the brain, present at micromolar concentrations, background current can be a significant problem.

2.1.5. Fast Scan Cyclic Voltammetry (FSCV)

Several biologically active molecules, such as dopamine, serotonin, and norepinephrine, possess electroactive ring systems that are oxidized at potentials of about +0.6 V (versus Ag/AgCl reference electrode). Currently, one of the most popular methods employed to measure electroactive neurotransmitters in the brain is fast scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes. The development of FSCV for use in living systems was developed largely through the work of Wightman[37,38] and Millar.[39] For the measurement of catecholamines, the potential at the working electrode is typically scanned between −0.4 V and +1.0 V (versus Ag/AgCl) at scan rates on the order of hundreds of volts per second. The high scan rates employed allow for a large number of cyclic voltammograms to be collected per second, thereby providing sufficient temporal resolution to resolve the processes of vesicular neurotransmitter release and uptake. The above parameters may be adjusted depending on the particular application. The high scan rates are possible because the small dimensions of the carbon fiber electrode (5−50 μm diameter) allow for efficient diffusion of analytes to and from the electrode surface. Because a large capacitive current is produced, a background cyclic voltammogram (CV) must be subtracted from CVs that potentially contain currents arising from analyte oxidation or reduction. FSCV provides limits of detection ranging from 200 to 50 nM, depending on what potentials are applied to the working electrode.[40]

Due to electrode drift, FSCV is not well-suited for the continuous monitoring of analyte levels over extended periods of time. Nevertheless, this technique has sufficient temporal resolution to measure dopamine release and uptake as separate processes. Thus, dopamine release curves obtained from striatal brain slices can be modeled to calculate the Michaelis−Menten kinetic parameters that describe the efficiency of the dopamine transporter (DAT) uptake of dopamine.[41,42] FSCV has also found a niche in monitoring neurochemical events that occur on fast time scales and is, therefore, useful for correlating neurochemistry with specific behavioral episodes in real time. In particular, dopamine release transients have been measured with relevance to cocaine addiction,[43–47] alcohol addiction,[48] eating behaviors,[49] and sexual behaviors.[50,51] It is clear that this high resolution technique can find application to the study of a broad range of conditions in which the release of dopamine plays a role. Our laboratory is currently investigating the role of transient dopamine release events in transgenic rats that model the motor phenotype of Huntington's disease,[52] a neurodegenerative movement disorder.[53] As more transgenic rat models are developed, FSCV promises to have an even greater impact on the study of various genetic disease states. Constant potential amperometry is an electroanalytical method complementary to FSCV, which, because of its millisecond temporal resolution, is often used to directly measure the exocytosis of single vesicles of electroactive neurotransmitters.[54] Using this method, Hochstetler et al. demonstrated the detection of zeptomole quantities of dopamine from fluorescently labeled retinal neurons.[55] A number of excellent reviews on the various biological applications of these techniques have been published over the course of the past two decades and illustrate how extensively the field of electrochemical neurotransmitter measurement has matured.[56–65] In contrast with enzyme-mediated biosensors that measure nonelectroactive molecules, such as glutamate and glucose, the carbon-fiber microelectrode, as used in voltammetric and amperometric applications, is not considered a biosensor due to the lack of a biological recognition element at the electrode surface.

2.1.6. Interferences in Enzyme-Based Systems

For enzyme-based systems, interferences take on two different forms. The first are those which affect the enzymatic reaction itself and the second species that contribute to the current and therefore to the apparent substrate concentration. In the first case, two additional interactions have been suggested as causes for loss of enzyme activity: nonspecific oxidation of the enzyme structure by the peroxide formed or interference from metals such as Cu(II) that might form complexes with the isoalloxazine ring.[66,67] If a mediator other than oxygen is being monitored, then oxygen has to be considered an interference, as noted above. The second group would include species that are electroactive at the potential corresponding to the mediator oxidation. As noted above, lowering of the mediator potential to around 0.0 V vs AgCl/Ag reference means that few endogenous species are likely to interfere. Monitoring of hydrogen peroxide is typically carried out at 0.6 V vs AgCl/Ag reference, and in this region, a number of endogenous species such as ascorbate, urate, catecholamines, certain amino acids, and NO might interfere.

For glucose and lactate biosensors, this problem is easily solved using a permselective membrane placed between the enzyme layer and the electrode. These membranes have been constructed from cellulose acetate and Nafion. In the case of measurements of glutamate in the brain, there is a significant interference from ascorbate, present at concentrations 30−40 times higher. Moreover, the ascorbate concentration changes during neuronal stimulation, so a constant background cannot be assumed.[68,69] Passive membranes have proven applicable, but care has to be taken to ensure that the response time is not adversely affected. There have been a number of reports of electropolymerized films of 1,3-diaminobenzene, resorcinol, and pyrrole serving to confer permselectivity. Our experience has been that such films work well for a day or so but after that lose selectivity rapidly. Sol−gels have been used with some success.[70–73]

Alternatively, ascorbate oxidase can be employed. It catalyzes the oxidation of ascorbate, but peroxide is not formed and therefore does not contribute to the signal. The disadvantages are enzyme stability and the consumption of oxygen, which may be in short supply in cases where ischemia is being studied. In any case, it is extremely important to test the sensors in vitro to confirm selectivity, and this also has to be done in a time-dependent manner. A convenient mode is to measure the percent change in current output from the sensor resulting from addition of the physiological concentration of the interferent to the basal level of the substrate.[74] Since there is a good chance that the interferents can interact with each other, adding them sequentially to the test solution has some potential advantages.

2.1.7. Interferences in FSCV

Simple amperometric measurements of neurotransmitter release are not well-suited for in vivo applications without first confirming the identities of the electroactive molecules and their influence on the FSCV signal. Generally, cyclic voltammograms of electroactive species in the brain serve as "signatures" that are useful for compound identification. Thus, FSCV measurements can be used to sort out currents caused by the oxidation/reduction of different electroactive species and interferents. For example, when scanned at normal potentials, the catecholamines epinephrine and norepinephrine have similar voltammetric traces. However, these two species can be resolved by increasing the scan limit of the positive sweep to +1.5 V (versus Ag/AgCl). At this potential, an amine group on epinephrine is oxidized. Using this method, Wightman and co-workers found that 30% of bovine adrenal chromaffin cells release both epinephrine and norepinephrine.[75] Additionally, the use of FSCV allows for elimination of interfering electrochemical signals, which often are present in both the background and measurement scans.

Ascorbate and changes in pH are potentially troublesome interferents when collecting voltammetric and amperometric measurements in the brain. The application of Nafion coatings has again been used to decrease interference by ascorbate and other negatively charged species while obtaining measurements in brain tissue.[76–78] Such films have the ability to concentrate positively charged species such as dopamine. Changes in pH have been observed in connection with the release of dopamine in vivo[51] and in brain slice preparations.[79] Increases in local blood flow in vivo have been hypothesized to induce alkaline shifts in pH caused by the removal of carbon dioxide.[80] In many cases, these pH changes can interfere with the collection of voltammetric and amperometric data. One method used to mitigate the impact of ascorbate, pH changes, and other interferents is to apply a principal component regression (PCR) model in which each cyclic voltammogram is reduced to four or five principal components after obtaining a "training set."[81] Heien et al.[46] demonstrated the utility of this method in electrochemically resolving dopamine, 5-hydroxytryptamine, DOPAC (3,4-dihydroxyphenylacetic acid, a metabolite of dopamine), and ascorbate in a flow cell. Additionally, they applied this method toward mitigating pH related interferences in brain slices as well as resolving the release of norepinephrine and epinephrine in adrenal chromaffin cells. Recently, this method was also applied to resolving striatal dopamine release and changes in pH in ambulatory rats.[46]

2.1.8. Spatial and Temporal Resolution

As Wightman has pointed out,[82] chemical information in the form of a spatially resolved image has significant information content. In examining single cells or cell cultures, it is possible to study the dynamics of the cellular environment and, in particular, the time-dependent variations in the concentrations of key components. A spatially resolved concentration gradient up to a cell permits an estimation of flux of the analyte and therefore the rate of uptake or discharge. Such information facilitates understanding of the communication between cells and the control of release and uptake of species that may be controlled by membrane potentials or transmembrane flow of ions such as Ca^{2+}, H^+, or K^+. Fluorescent indicators as well as potentiometric measurements are often used for this purpose, and simultaneous measurements of different analytes are especially valuable. Amperometric measurements perturb the environment under study by consuming the analyte of interest and producing products that may become incorporated into the system under study. It must also be borne in mind that the electrode design and its positioning within the matrix under study will influence the observed response. As species move radially from the point of release by a cell under diffusion control, they become subject to dilution. Such effects must be deconvoluted in order to extract the correct rate information. In addition, the response time of the sensor must also be accounted for. In the case of enzyme-based sensors, there are typically several polymer layers through which the substrate(s) and products must move. In order to obtain a stable and reliable signal, a steady state condition must be established within the enzyme layer. This is a function of the thickness of the respective layers, so making the various layers as thin as possible (<1 μm) is an important design objective. The size of the sensor is also important: glucose sensors with diameters of 0.27 and 1.5 μm have been demonstrated to have response times of 92 and 190 ms, respectively.[83] This results in rather small signals, typically in the picoampere range. Response times are often measured in terms of the time required to transition from 10 to 90% of the maximum signal using a concentration step within the operating range of the sensor. It has proven useful to make such estimates in a flow injection experiment with a concentration increment plug preceded by an air bubble (Hu, Y. Unpublished results, 1994).

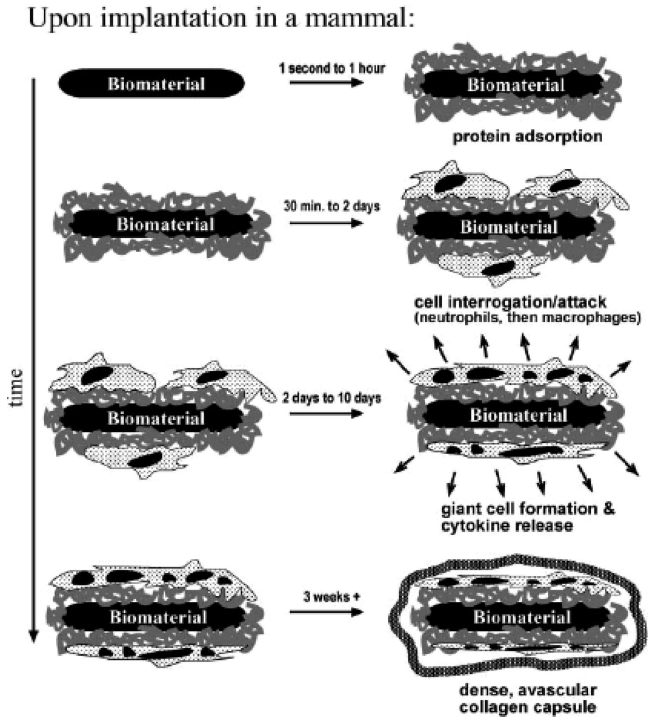

Figure 1. Time scale for the inflammatory response to an implant. Reprinted, with permission, from the *Annual Review of Biomedical Engineering*, Volume 6, Copyright 2004 by Annual Reviews www.annualreviews.org.

2.1.9. Biocompatibility

It was once assumed that, with proper design, implanted devices could be rendered "inert" so that there would be no tissue reaction to their presence. The current view is to consider a device biocompatible if the "host" does not adversely perturb the function of the device and the device does not adversely affect the function of the host.[84–86] It is not correct to talk about a "biocompatible" sensor using materials assumed to be biocompatible, since the size and morphology of the implant has an important and sometimes dominant effect on the tissue response. Since tissue interactions can have an important influence on sensor response, it is necessary to isolate and control such effects, as they may well determine the success of in vivo measurements.

2.1.9.1. The Acute Inflammatory Response. Figure 1 depicts the sequence of events that occurs when an implant is inserted into mammalian tissue, the so-called foreign body reaction. It should be emphasized that this is not the result of an infection but rather the response of the immune system in identifying and attempting to destroy the sensor implant. The first step in the process is the nonspecific adsorption of protein on the implant surface. Even if the implant is in subcutaneous tissue, there will be some destruction of capillaries and therefore activation of coagulative and thrombosis systems. Considerable attention has been focused on synthesis and surface modification of biomaterials with the objective of minimizing protein adsorption and cell adhesion.[87,88] Polyethylene glycol (PEG), also referred to as polyethylene oxide (PEO), is one such material. The specific requirements for surface modifiers have been characterized using self-assembled monolayers. Surfaces that resist the adsorption of proteins incorporate four molecular characteristics: (a) they are hydrophilic; (b) they include hydrogen bond acceptors; (c) their overall electrical charge is neutral; (d) they do not include hydrogen bond donors.[89] However, there are examples of protein resistant surfaces that do not possess the latter characteristic.[90] A common attribute of low interaction surfaces is their ability to interact strongly with water.[91] Materials created with pendant phospholipids showed reduced protein adsorption and reduced foreign body capsule formation. The strategy to minimize protein adsorption applies not only to the acute inflammatory response but also to processes that lead to attachment of cells to the implant. This process, generally called "biofouling", can lead to the accumulation of bacteria on the sensor and the resulting infection.[92–94]

Step two (Figure 1), which lasts over the first several days, is dominated by neutrophils, white blood cells that normally function to ingest foreign substances. After 24−48 h, the neutrophils gradually disappear and are replaced by monocytes and macrophages. These species produce superoxide and NO, which then react to form toxic peroxynitrite ($OONO^-$) and hydroxyl radicals. The macrophages attempt to ingest the implant, which is not possible because it is much larger, which leads to frustrated endocytosis and the formation of giant cells. This third step is controlled by the release of cytokines and other mediators of inflammation including leukotrienes, growth factors, and proteases.[95] Although giant cells and macrophages can exist for a considerable period of time, this marks the end of the acute inflammatory response. Since much of the use of biosensors is in this 1−5 day period, it will be the above reactions that will perturb the area around the implanted sensor. As there is also serious interest in chronically implanted sensors, the fourth stage is also important. The end result is the formation of a foreign body capsule, a fibrotic layer (50−200 μm thick) that surrounds the implant, attempting to isolate it from the rest of the tissue. Isolation, would, of course, be detrimental to sensor function. Here, the design of the surface morphology becomes important. It is known that if the surface of the implant is dense and relatively smooth, then the capsule will also be dense. If however the implant surface is porous, then this tends to lead to a less dense foreign body capsule which is also more extensively neovascularized (regeneration of capillaries). Another overlooked consequence of macrophage activity is the lowering of the local pH due to the extensive oxidative activity, which drives the surface pH to about 3.6.[96] A detailed description of the biological response to implants, which has been oversimplified here, has been reviewed.[95] Recent proteomic studies have focused on the role of cytokines and chemokines in activating inflammatory and wound healing processes.[97]

2.1.9.2. Approaches to Biocompatibility. There have been a variety of approaches, both biological and chemical, to modulate the tissue response. The first general approach is to modify the implant surface prior to implantation. This could involve the construction of self-assembled monolayers, which permits the formation of defined surface structures. These can then be tested for protein binding using surface plasmon resonance.[89] Alternatively, the surface might be modified using cell-adhesive peptides such as arginine-glycine-aspartic acid (RGD),[98] which is known to interact with matrix proteins such as fibronectin, laminin, collagen, and vitronectin.[99] The effect of this strategy is to promote selective cell adhesion, which may be compromised if the matrix protein is adsorbed in a modified, nonfunctional conformation. Hydrogels constitute another important class of surface materials. These are chemically cross-linked multivinyl monomers such as methacrylate and acrylate, as

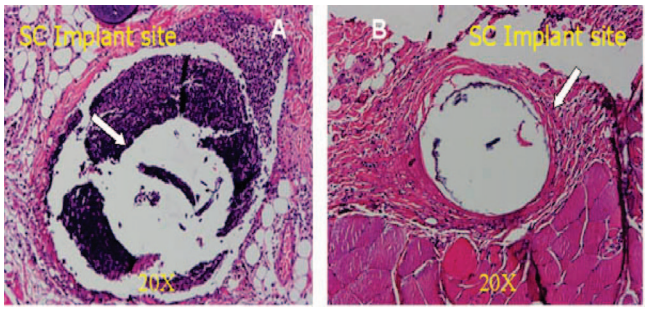

Figure 2. Histological cross section of a sensor implant site. (A) Control sensor (no NO); (B) NO evolving sensor. Reprinted with permission from ref 106. Copyright 2005 J. Wiley and Sons, Inc.

well as additional cross-linked components of poly(ethyleneglycol)-co-poly(lactic acid) or poly(vinyl alcohol) or natural polymers such as hyaluronic acid and chondroitin sulfate.

A second strategy is to release substances from the implant capable of modulating tissue response. Two general strategies have evolved. The first involves promoting angiogenesis, with a principal modulator being vascular endothelial growth factor (VEGF). Thus, VEGF is delivered from a coating on the sensor. This approach has been applied to the study of biosensors.[100] Release of an anti-inflammatory, dexamethasone, has been used to study modulation of its effect on foreign body response when combined with VEGF release.[101,102] VEGF appeared to increase inflammation, and dexamethasone appears to decrease neovascularization. In the latter case, the long-term effects were diminished because at the end of six weeks the drug-releasing hydrogel fibers were no longer functional and there was no difference between the treated sensors and the controls. This is a familiar theme: often short-term differences in materials are observed that may not be manifested at the level of the foreign body capsule. A third approach is to use sensors that release NO. Meyerhoff and co-workers have successfully used this approach for sensor measurements in blood, and this approach has also been applied to subcutaneously implanted glucose sensors.[103–106] Because the sensor is rather small, the capacity for NO-releasing compound is rather limited. Therefore, release occurs only over about the first 24 h. However, as a sample tissue section shows in Figure 2, there is a significant reduction in the extent of tissue reaction and improvement in sensor stability in the first several days of operation when the NO-evolving sensor and the control are compared. It is important to keep in mind that NO is electroactive at the potential corresponding to the oxidation of peroxide. Although several of the candidates mentioned produce beneficial effects on sensor performance, their intrinsic influence on the tissue will have to be examined, as they may be hazardous long-term.

2.1.9.3. Causes of Sensitivity Loss. One of the pervasive properties of in vivo sensors is their loss of sensitivity on implantation, ranging from 10 to 30%. This process occurs very rapidly and can lead to sensor instability over the first three days, after which the stability often improves. In the case of a glucose sensor, for example, the several different causes might be divided into two categories: (a) active and (b) passive. The former category would include loss of sensitivity due to a decrease in enzyme activity caused by some endogenous component. It could also be due to a decrease in the concentrations of either glucose or oxygen in the vicinity of the sensor caused by damage or trauma associated with the implant. The passive components could include blockage of the outer membrane by adsorbed protein, thus preventing passage of glucose into the enzyme layer or interference with electron transfer at the electrode due to adsorption of small molecules. Isolating the causes is not simple. We have shown[107] that if a sensor is implanted for a period of 24 h, followed by rapid removal from the tissue and placement in buffer solution containing glucose, there is essentially no difference between the in vivo sensitivity and the in vitro sensitivity after explantation. If the sensor remains in buffer for several hours, the sensitivity returns to its original in vitro value. This particular sequence of events suggests that whatever has modified the sensitivity of the sensor follows the sensor when it is explanted. Thus, loss of sensitivity cannot be primarily associated with lowered oxygen or glucose in the vicinity of the sensor. Because the recovery is reversible, this argues against irreversible modification of the enzyme. This leaves the possible interference in sensor operation due to passive blockage either at the level of the outer membrane or at the electrode surface. Addition of physiological concentrations of proteins such as IgG, serum albumin, or fibrinogen to a glucose solution produces little or no decrease in apparent sensitivity, when increases in viscosity are taken into account. On the other hand, small molecules and proteins (<15 kD) can have a large influence on sensor response.[108] Gerritsen and co-workers[11] have examined the response of glucose sensors in the presence of polymorphonuclear granulocytes and identify active components in serum that can contribute to protein degradation: myeloperoxidase and a variety of matrix metalloproteases. Some insight into this conundrum comes from analysis of the leachate when sensors are explanted and placed in buffer solution.[109] The leachate and the sensor membranes were analyzed in a proteomic study. Because of the very small amount of material taken up by the sensor, it was difficult to identify more than a few proteins. By analyzing the leachate as well as the entire sensor membrane assembly, it was clear that there were no intact proteins detected, suggesting that the effect of the acute inflammatory response was to convert much of the protein to smaller fragments that could then penetrate within the sensor. There are a large number of small nonprotein molecules that can also contribute to sensitivity loss, including interference with peroxide electron transfer.

Brain biocompatibility is less well understood, due to its greater structural density and complexity. The brain-based active response would involve the proliferation of astrocytes (glial fibrillary acidic protein (GFAP)) and microglia (CD11b/ED1).[110,111] Because the implant damages capillaries and compromises the blood−brain barrier, blood-borne inflammatory agents such as cytokines can also participate in the healing process. Ultrastructural analysis of the tissue surrounding a microdialysis probe suggests that the injury in the form of decreased neuronal density shows that tissue disruption can extend beyond 1 mm from the implantation site. It was suggested that this extensive damage may be the result of chemical signaling pathways rather than a direct effect.[112] Normal function can also be assessed by measuring local cerebral glucose metabolism and local cerebral blood flow. Time-dependent studies suggest that the initial tissue disruption subsides in about 24 h before the second stage (gliosis) becomes dominant in day 3−4.[113] Much of the early work was based on the use of a microdialysis probe in the

range of 250 μm o.d. Michael and co-workers[114] have examined the microstructure surrounding a 7 μm diameter carbon fiber, addressing specifically whether a smaller probe would cause less damage. It was observed that the spot of maximal damage had a radius of 2.5 μm, and about 6.5 μm from this annular region no significant damage was observed. However, since the interest is frequently in measuring functional neurons, this raises the question as to whether the region immediately surrounding the sensor has been seriously compromised.[115] Other workers[116] have measured phosphoethanolamine as an indicator of cellular membrane disruption and have found that the extent of disruption is clearly linked to the size of the implanted probe.

There have been a few efforts to develop chronically implanted glucose sensors where long-term interactions must be considered. The first effort,[117] directed toward sensors implanted in the vascular bed of dogs, demonstrated that meaningful results could still be obtained three months after implantation. The failure mode was not the loss of enzyme activity but the failure of the implanted electronics package. Similar results were obtained when the sensor was implanted in the subcutaneous tissue of humans.[118]

In the end, and as Reichert and co-workers have suggested,[119] issues of biocompatibility will be resolved through fundamental understanding of the interactions at the sensor/biological medium interface. The strategy may well be to elicit a particular and directed response rather than trying to render the implant inert. It is important to keep in mind that not only must the outer membrane present a favorable view to the tissue, it must also possess the requisite permselectivity characteristics. For devices that will be implanted for more than 30 days or which are implanted repeatedly, International Organization for Standardization (ISO), U.S. Pharmacopeia (USP), or American Society for Testing and Materials (ASTM) standards must be met. These include such measures as genotoxicity, carcinogenicity, cytoxicity, and various tests of irritation. If the device includes electronics, it also has to meet certain requirements. Major manufacturers of polymers used in implantable devices, have, for liability reasons, refused to permit their materials to be used in humans, so it cannot be assumed that presently available materials can be used for this purpose. The real challenge in this area is new materials which can be easily fabricated into devices with reproducible characteristics.

3. Applications

3.1. Analyte-Specific Sensors

For measurements of single cells, cell cultures, and tissue cultures and for in vivo measurements, electrochemical sensors have proven useful in several different areas. The majority of this work has revolved around neuroscience.[120,121] Ions such as K^+, Na^+, and Ca^{2+} [10,122,123] are monitored for their role in the control of membrane potentials, and H^+ is monitored for changes in pH that can be highly localized. Reactive oxygen species (ROS) such as NO,[124–128] O_2^-,[129–131] H_2O_2,[132] and $ONOO^-$ [128,133] have a transient existence and thus must be measured with sensors yielding low detection limits and rapid response times. There is a limited number of endogenous species that are intrinsically electroactive and tractable for monitoring: catecholamines and other species that influence neurotransmission, such as ascorbate.[68,69,134] The vast majority of species of interest are either electroinactive or have poorly defined electrochemistry: glucose,[121,135–137] lactate,[138–141] glutamate,[21,74,142–146] acetylcholine/choline,[147,148] ATP, and adenosine.[149–158] This does not include the multitude of peptides, proteins, and other species whose roles in cell function are becoming increasingly evident.

Two approaches have been taken to the analysis of ions, ion selective electrodes,[159] and fiber optic-based devices.[160] Measurements in the CNS have been especially important, as astrocytes have to maintain energy-dependent ion gradients for Ca^{2+}, Na^+, and K^+ as well as maintaining the extracellular pH. The role played by these ions in cell signaling and in regulating glutamate uptake has been reviewed.[122] Such investigations are most easily envisioned in cell cultures, where essentially a "closed" system can be employed where inputs and outputs from the culture can be readily identified. In studying metabolism-linked processes, it is often more important to know the gradient or *flux* of a particular species moving in or out of a cell, since this gives an indication of the rate of the process under study. This need has given rise to imaging techniques which permit the gradients to be mapped in a time-dependent fashion. It has proven possible, for example, to measure efflux from individual channels using a microelectrode, although it was noted that the potentiometric electrode response time limited the ability to measure the fastest events.[161] A two channel sensor has been used to simultaneously map Ca^{2+} and catecholamine exocytosis.[162]

3.1.1. Reactive Nitrogen Species

Over the last 15 years, NO has been implicated in a wide range of functions including regulating blood pressure and preventing coagulation. It can also serve as a cytostatic agent. NO is synthesized through the catalytic oxidation of L-arginine by three nitric oxide synthase (NOS) isoenzymes: neuronal (nNOS), endothelial (eNOS), and inducible NOS (iNOS). The most rapid scavenger of NO is superoxide, and the more stable peroxynitrite is formed with a rate constant of 6.7×10^9 M^{-1} s^{-1}.[163] The electrochemistry of NO is complicated because it can undergo both oxidation and reduction. On the oxidative side, NO is oxidized by one electron to yield NO^+, which then reacts with water to form NO_2^-. Further two-electron oxidation of this species results in the formation of NO_3^-. In the reducing direction, NO can be converted to hydroxylamine, ammonia, and eventually nitrogen.[164] Three methods have been employed for the electrochemical determination of NO, all involving its oxidation. The first is the "Clark Electrode" type, namely a Pt electrode protected by a gas-permeable membrane. The advantage of this device is its simplicity, with the disadvantages being a relatively slow response ($t_{1/2}$ for NO $\approx$ 3−5 s)[154] and the problem of formation of NO_2^-, which can undergo further oxidation. The applied potentials are only separated by 60−80 mV, so overlap of the two steps is significant. This problem is addressed in a second configuration where an electrode is coated with Nafion and/or cellulose acetate in combination with nonconducting polymer films using *o*-phenylenediamine,[127] which ideally will prevent the oxidation of NO^+. The third approach involves the use of transition metal porphyrins such as Ni(II) and metallophthalocyanines.[163,165] Amatore and co-workers[133] have argued that it is possible to distinguish reactive oxygen species (ROS) by judicious choice of applied potential, and for this purpose they have employed platinized carbon microelectrodes. For a series of ROS, peroxide is most easily oxidized, followed by $ONOO^-$, NO, and NO_2^- in PBS.

Using this system, they were able to observe reactive oxygen and nitrogen species at a single macrophage and to construct the fluxes associated with the production of O_2^- and NO. The release of NO from single neurons was studied.[127] This sensor yielded a detection limit of 2.8 nM and a sensitivity of 9.5 nA/μM and exhibited good elimination of uric acid, ascorbic acid, nitrite, and arginine; however, there was significant interference from catecholamines.

The similarity of ROS and RNS electrochemistry has already been noted, so selectivity, especially in in vivo applications, is a serious challenge. Peroxynitrite is an unstable species ($t_{1/2}$ < 1 s) with concentrations near the endothelial membrane less than micromolar. Depending on concentration, $ONOO^-$ can decompose into NO_3^- or it can cleave to form $OH^•$, $NO_2^•$, and NO_2^+.[128] This group has developed a triple nanosensor assembly that can simultaneously monitor NO, $ONOO^-$, and O_2^-. The two RNS are simultaneously monitored using Ni(II) tetrakis(3-methoxy-4-hydroxyphenyl)porphyrin and Mn(III)-[2,2]paracyclophenylporphyrin, respectively. Superoxide is monitored with an immobilized polypyrrole/horseradish peroxidase and SOD film. This system, using 200−250 μm diameter carbon fibers, was used to observe single endothelial cells and also the in vivo vasculature of a rat. The purpose of such experiments is to measure the [NO]/[$ONOO^-$] ratio, which is a measure of cardiovasculature disfunction during ischemia/reperfusion following myocardial infarction or stroke. Significant differences were observed between normotensive and hypertensive rats when a calcium ionophore (A23187) was used to stimulate NO, O_2^-, and $ONOO^-$ release.

3.1.2. Superoxide

The development and use of biosensors that employ selected proteins as the recognition element for the measurement of $^•O_2^-$ have received recent attention. $^•O_2^-$ is formed in living biological systems by the donation of an electron to molecular oxygen. The most important biological reaction responsible for $^•O_2^-$ formation is the oxidation of semiquinone-type radicals formed in the mitochondrial electron transport chain. Moreover, xenobiotics that contain quinone ring systems, such as the drugs adriamycin and mitomycin C, may also contribute to $^•O_2^-$ formation by the donation of electrons to molecular oxygen.[166] Thus, it is not surprising that well-oxygenated tissues, such as muscle and brain tissue, tend to produce especially high $^•O_2^-$ levels. A $^•O_2^-$ biosensor in which cytochrome *c* was incorporated as a sensing element has been developed and used to measure $^•O_2^-$ levels in vivo.[130] In a particularly interesting application of this biosensor, it was found that $^•O_2^-$ levels during the first 5 min of ischemia, induced in the muscle tissue of a rat, remain flat, suggesting that energy is not substantially depleted within this time period.[167] However, $^•O_2^-$ levels increased gradually at 10 min and robustly at 40 min until reaching a peak at 90−120 min. Thus, these experiments demonstrate that free radical production during ischemia/reperfusion injury gives rise to a period of oxidative stress that potentially lasts for hours. In a cell culture-based study, Manning et al. used a cytochrome *c* based biosensor to measure $^•O_2^-$ release from glioblastoma cells. These studies shed light on the potential relationship between production and release of nitric oxide and superoxide.[168]

Other $^•O_2^-$ biosensors have employed superoxide dismutase (SOD), an enzyme that scavenges superoxide according to following the net reaction at near-diffusion limited rates:[169]

$$2^•O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (4)$$

Recent biosensor designs have employed SOD immobilized on gold nanoparticles electrodeposited on carbon-fiber microelectrodes.[170] Another $^•O_2^-$ biosensor employed SOD immobilized on a Pt electrode surface with gelatin.[171] Using this design, superoxide levels were found to be elevated in homogenized cancerous brain tissue compared to normal human brain tissue. However, to our knowledge, the application of this class of $^•O_2^-$ biosensors for obtaining in vivo measurements has not yet been published.

3.1.3. Hydrogen Peroxide

H_2O_2 has long been known to be potentially toxic to biological systems because, under certain conditions, it can form highly reactive hydroxyl ($^•OH$) radicals, which have the potential to irreversibly alter DNA, lipid, and protein structure.[172] Key examples of $^•OH$ formation from H_2O_2 include nonenzymatic reactions with reactive metals, such as Fe(II), known as the Fenton reaction (reaction 5),[166] and reaction with superoxide anion ($^•O_2^-$), known as the Haber−Weiss reaction (reaction 6).[166]

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + {}^•OH + Fe^{3+} \quad (5)$$

$$H_2O_2 + {}^•O_2^- \rightarrow O_2 + OH^- + {}^•OH \quad (6)$$

Given the above reactions, it is easy to see why H_2O_2 is potentially harmful to living organisms: H_2O_2 passes through membranes either by diffusion or through channel proteins[172] with the potential of producing $^•OH$ at a one to one ratio. Consequently, free radical damage, mediated in part by H_2O_2, has generated a great deal of interest with regard to a number of neurological disease states, including Alzheimer's disease,[173] Huntington's disease,[174] Parkinson's disease,[175] and Lou Gehrig's disease.[176] In addition to its harmful effects, recent evidence has raised the possibility that H_2O_2 may also be an important mediator of glutamate dependent attenuation of dopamine release.[177–179] Thus, there is strong interest in the development of sensors capable of obtaining real-time measurements of H_2O_2 at high temporal resolution and low detection limits.

The oxidation of H_2O_2 is electrocatalyzed, which explains the poor response seen on gold and carbon electrodes. The reaction sequence for Pt, Pt/Ir, and Pd electrodes is given by[180,181]

$$H_2O_2 + Pt(OH)_2 \leftrightarrow Pt(OH)_2 \cdot H_2O_2 \quad (7)$$

$$Pt(OH)_2 \cdot H_2O_2 \leftrightarrow Pt + 2H_2O + O_2 \quad (8)$$

$$Pt + 2H_2O \leftrightarrow Pt(OH)_2 + 2H^+ + 2e^- \quad (9)$$

where the first step in the reaction is the formation of a complex between peroxide and Pt(II) sites on the electrode (reaction 7). The second step involves the surface transfer of electrons, followed by the restoration of Pt(II) sites on the electrode surface. There are several consequences of this situation. The first is to favor the rapid formation of the oxide layer, which requires an applied potential of about 0.6 V vs

AgCl/Ag reference. The oxide formation can be somewhat slow, such that it is generally not beneficial to change the electrode potential during the course of measurements (various forms of voltammetry). Endogenous species, such as chloride, can complex with Pt(II), dissolving the oxide[182] and thus interfering with peroxide oxidation. The polymeric coatings associated with biosensors appear to minimize this problem (Y. Zhang, unpublished results, 1993). In a recent study of reactive oxygen species, it was shown that peroxide could be detected at ~0.35 V. This is likely possible because peroxide concentrations are very low and therefore reaction 9 can proceed at a lower rate, which can be sustained at a lower applied potential.[133]

An alternative is to employ horseradish peroxidase (HRP), a heme protein of about 40 kD, which is oxidized by peroxide from a nominal Fe(III) to an Fe(V) state. The mechanism of this reaction has recently been studied in detail.[183] The reduction of the oxidized form of HRP can be carried out at an applied potential of 0.0 V vs AgCl/Ag reference, a region relatively free of endogenous electroactive interferences. The problem is that the Fe(V) form can react directly with endogenous electron donors. HRP can be coupled directly to carbon fiber electrodes[184] or via a mediator.[185] In such a study, a carbon fiber microelectrode was coated with conductive redox polymer containing horseradish peroxidase, which scavenged H_2O_2.[185] Using this design, the researchers found a biphasic increase in extracellular H_2O_2 levels in the brain striatum of anesthetized rats after the electrical stimulation of the dopaminergic pathway that innervates the striatal tissue.[132] In a study using an HRP-based biosensor, H_2O_2 release was induced from cultured rat cortical neurons upon administration of the endocrine disrupter tributyltin, administered at concentrations as low as 10 nM.[186] Thus, while current sensors that detect H_2O_2 and other biologically active molecules empower researchers with the ability to obtain important physiological data, studies in which these sensors have been applied to biological preparations appear to be lagging. Several other designs for H_2O_2-selective electrodes, in which other biological sensing elements are used, have been proposed. These designs include electrodes that incorporate hemoglobin,[187–189] myoglobin,[190] and catalase.[191] While in some cases the *in vitro* performance has been improved, the utility of these biosensor systems in collecting measurements in cell culture, organ tissues, and in vivo has not yet been fully demonstrated.

3.1.4. Ascorbate

Ascorbate constitutes both an important endogenous electrochemical interference and an important component in the CNS. It cannot be assumed, for example, that ascorbate levels remain constant during neuronal stimulation and indeed that ascorbate levels are linked to glutamatergic mechanisms.[68,69,134,192] Most in vivo applications have been aimed at eliminating the signal arising from ascorbate oxidation. For example, Nafion coatings have been commonly employed for electrochemical measurements to decrease the background current arising from ascorbate oxidation. It is now believed that ascorbate acts as a neuromodulator under some circumstances. For example, evidence suggests that modulation of extracellular ascorbate regulates corticostriatal glutamate transmission.[134] Therefore, it is useful not only to measure ascorbate but also to resolve the relationship between ascorbate levels and other neurotransmitters. In an important example in which this issue was addressed, Gonon and co-workers developed a method that used an electrochemically treated pyrolytic carbon fiber electrode to resolve the voltammetric peaks arising from catecholamines and ascorbate.[193,194] The electrode was prepared by applying a triangular wave potential (0−3 V, frequency 70 Hz) for 20 s with the electrode tips immersed in phosphate-buffered saline. Their measurements, collected using differential pulse voltammetry, reveal reproducible oxidation peaks that occur at about −50 mV and +100 mV (vs Ag/AgCl reference electrode). The peak occurring at −50 mV arises from ascorbate while the peak occurring at +100 mV arises from catecholamine oxidation. Importantly, this electrode was shown to resolve electrochemical peaks from catecholamines and ascorbate in the striatum of anesthetized rats.[193] This method was later used to resolve the effects of amphetamine administration on ascorbate and 3,4-dihydroxyphenylacetic acid (DOPAC; a metabolite of dopamine) levels in anesthetized rats. Substantial increases in ascorbate levels and a decrease in DOPAC levels were noted in the nucleus accumbens following amphetamine administration.[195] Additionally, Gonon and co-workers used electrochemically treated carbon fiber electrodes to obtain simultaneous measurements of catechols and ascorbate in ambulatory rats. Using this method, they estimated striatal ascorbate levels to be 306 μM and levels of DOPAC to be 17.7 μM.[196]

More recently, Gonon's method has been employed to test the hypothesis that ascorbate is released in vesicles with catecholamines from adrenal medullary cells. In this set of experiments, Cahill and Wightman used two carbon fiber microelectrodes to measure release simultaneously from each cell.[197] Both electrodes were beveled to form disks, each with a single electroactive surface. However, one electrode was electrochemically treated, similar to the treatment applied by Gonon et al.[194,196] while the other electrode was untreated. Using constant potential amperometry and square wave voltammetry, it was found that ascorbate is released upon treatment with digitonin, a detergent that permeabilizes the cell membrane, but is not released upon treatment with various secretagogues, which cause the cells to release catecholamines by vesicular exocytosis. Conversely, catecholamine release is readily evoked by application of secretagogues. Thus, it is apparent in this application that ascorbate is secreted from the cytosolic compartment, whereas catecholamines are released from vesicles by exocytosis.[197]

3.1.5. Enzyme-Based Sensors

3.1.5.1. Lactate. After glucose lactate sensors are probably the next most important biosensor. Normally associated with hypoxic (oxygen-deficient) conditions, biosensors have been envisioned for intensive care situations and also for sports medicine.[141] Lactate measurements can also serve as a measure of the extent of ischemia, triggered, for example, by myocardial infarction. Lactate is now regarded as a major source of energy in the brain, and this subject will be addressed in more detail subsequently. Studies of cortical lactate and glucose levels in rats during the sleep−awake cycles provide some insight into energy management within the brain, respectively.[140,198] Brain activity is assessed via electroencephalograms, and it is concluded that paradoxical sleep (PS) is a state highly dependent on available energy

and slow-wave sleep as energy saving. These observations are consistent with increased demand for lactate in the PS sleep state.

3.1.5.2. Glutamate. Glutamate is the most abundant excitatory neurotransmitter in the mammalian nervous system and for this reason has been a frequent target of neuroscientists. Glutamate at the synaptic level is implicated in both acute and chronic pathological states such as neuronal damage associated with brain trauma[199] and neurodegenerative diseases.[200] The glutamate biosensor presents a challenge because the basal level of glutamate is about 10 uM and response must be measured in the presence of ascorbate, which can be 100−300 μM. It cannot be assumed *vide supra* that the ascorbate level will remain constant during glutamate neuronal stimulation. It has been pointed out that stimulated release of lactate in cultured astrocytes[201] and in vivo[202] appears to be dependent on glutamate uptake. Glutamate has also been implicated in key roles in the ventromedial hypothalamus in mediating energy expenditure, insulin−glucose homeostasis, the sleep−awake cycle, and the neuroendocrine output of the pituitary gland.[203] Detailed understanding has been complicated by the fact that glutamate is a fast acting neurotransmitter and that it has a multiplicity of receptors. Glutamate has been implicated in the control of dopamine release in the striatum.[145] Other neuroscience applications of glutamate sensors include the effects of deep brain stimulation of the subthalamic nucleus on in vivo glutamate concentrations[204] and the influence of hypergravity on hypothalamic glutamate levels.[205] In the latter two cases, the glutamate sensor and a wireless head mount are commercially available from Pinnacle Technology, Lawrence, KS.[206] Other sources of commercially available glutamate sensors are Sarissa Biomedical Ltd., Coventry, U.K., Quanteon, LLC, Nicholasville, KY, and Sycopel Intl. Ltd., Jarrow, U.K. The latter vendor provides what is called a "microdialysis biosensor", meaning a biosensor placed inside a microdialysis probe. As a hybrid device, it has the disadvantages of microdialysis probes (large size, slow response) and the advantage of controlling the environment around the sensor because it is isolated from the tissue. Enzymes requiring a cofactor may be added, as may drugs to be diffused into the surrounding tissue. This device has been used to monitor postischemic glutamate uptake, a situation in which the usual oxidase-based sensor is a problem because of low oxygen levels.[143]

3.1.5.3. Acetylcholine. Acetylcholine (ACh) is an important neurotransmitter associated with a variety of normal and pathological conditions. The associated choline (Ch) is a neurotransmitter at certain nicotinic receptor sites.[148] The detection of choline is facilitated by the use of choline oxidase, which produces detectable peroxide.[147,207] The determination of Ach, on the other hand, requires the conversion to Ch, leading to peroxide in a two step process. The presence of endogenous choline (the Ch:Ach ratio in brain tissue is more than 10:1) means that, in determining ACh, the signal has to be corrected for the endogenous Ch. At present, this requires two sensors, one for total Ach + Ch and the other for Ch.

3.1.5.4. ATP/Adenosine. The purines ATP and ADP (P2 receptor active) and adenosine (P1 receptor active) are important signaling molecules that mediate diverse biological processes via cell surface receptors. ATP plays an important role in sensory transduction and is implicated in mediated glia signaling through the propagation of Ca^{2+} waves.[208]

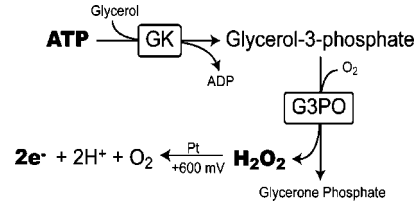

Figure 3. ATP sensor schematic. Reprinted in part with permission from ref 156. Copyright 2005 American Chemical Society.

There are two approaches to the ATP sensor. The first, originally suggested by Scheller,[149] based on a classical method for glucose analysis, employs two enzymes: glucose-6-phosphate, which requires ATP to effect glucose phosphorylation, and GOx, which measures the glucose remaining. GOx will not catalyze the oxidation of the phosphorylated derivative. The extent of phosphorylation is measured by determining the decrease in O_2 resulting from the glucose oxidase reaction. This requires a second measurement, the signal due to oxygen in the absence of the GOx reaction. This method was later enhanced by measuring peroxide instead, which simplifies the method. Two measurements are still required, but instead the glucose in the sample is measured in the presence and absence of the phosphorylation reaction.[150] A second approach has recently been proposed, and this is shown in Figure 3.[156] In this case, immobilized glycerol kinase (GK) uses the available ATP to form glycerol-3-phosphate, which is then oxidized by immobilized glycerol-3-phosphate oxidase to produce peroxide, detected in the usual manner. The enzymes were immobilized in a sol−gel built around Pt microelectrodes 25−100 μm in diameter. This approach depends on adding glycerol to the sample (up to ~2 mM), which appears not to interfere with normal biological processes. The risetime of the sensor was <10 s and yielded a linear response between 200 nM and 50 μM. Measurements were made on *Xenopus* embryo spinal cords. The free concentration of ATP measured at the sensor was estimated at 0.5−1.0 μM, and it is suggested that ATP does not accumulate but is rapidly broken down. The actual ATP concentration at the source of production could be as much as 10-fold higher, when diffusion is taken into account. Even considering the fast response of the sensor, it is clear that this response is insufficient to track the very rapid concentration changes in ATP resulting from stimuli, followed by degradation into adenosine. It is necessary to deal with interferences such as ascorbic acid, 5-HT, and urate. The maximum consumption of ATP by the sensor is about 420 fmol/s. Release of ATP due to hypoxia has been studied.[157] Adenosine is known to be released during cerebral hypoxia, which appears to trigger depression of excitatory synaptic neurotransmission, a neuroprotective step.[151] A sensor for adenosine (25−50 μm diameter) has been developed, but it is rather complicated, requiring three enzymes: adenosine deaminase (AD (EC 3.5.4.4)), nucleoside phosphorylase (NP EC 2.4.2.1), and xanthine oxidase (XO (EC 1.1.3.22)). AD converts adenosine into inosine, NP converts inosine into hypoxanthine, and the third enzyme (XO) converts hypoxanthine into xanthine and uric acid. Since inosine can be present in the biological sample, it is necessary to obtain the difference between two sensors, one containing all three enzymes, the second containing only NP and XO. These sensors were used to study adenosine release in hippocampal slices under hypoxic conditions.[151] A later work[152] simultaneously measured adenosine and ATP in hippocampal slices during in vitro ischemia and concluded

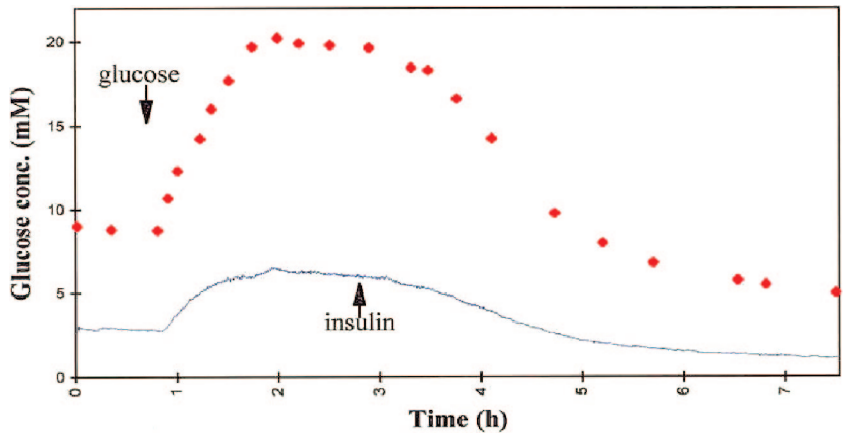

Figure 4. Relationship between plasma and hippocampus ECF glucose. Arrows indicate IP injection of 2 mL of 30% glucose and 14 U/kg of insulin, respectively. Reprinted with permission from ref 136. Copyright 1997 Blackwell Publishing.

that the two processes are mostly independent steps. These studies afforded the opportunity to apply various pharmacological agents to assess ATP and adenosine release. The ATP and adenosine sensors are commercially available from Sarissa Biomedical Ltd.

3.2. A Case for Measuring Fluxes

As biologists and chemists focus ever more sharply on monitoring of biological processes involving single cells, cell cultures, tissue slices, or intact organisms, the focus turns to understanding the dynamics of metabolism and cell signaling. Much of the previous effort has been devoted to the measurement of concentrations of key species at particular locations and possibly as a function of time. The resulting concentrations are a convolution of a burst from a cell involving femtomoles of analyte, which is rapidly diluted through diffusion, reaction with species in the milieu, or uptake by a receptor. The net result is an analyte concentration which, by itself, reveals little about what has actually transpired. By using microelectrodes with both high temporal and spatial resolution, Wightman and co-workers[53] have demonstrated the vesicular release of dopamine from single cells. Amatore and co-workers[125] generated oxidative bursts produced by macrophages. A key to resolving the burst is extracting the time-dependent flux data for the four species (H_2O_2, $ONOO^-$, NO_2^-, NO) that constitute the oxidative burst. To accomplish these measurements, a 10 μm diameter carbon fiber electrode is placed within 5 μm of the cell surface. Another approach, called the "self-referencing" method, involves the use of a microelectrode, typically 1−5 μm in diameter, that is moved slowly but repeatedly between two points.[209] If these two points are located within the concentration gradient of a cell, then the gradient can be estimated as $\Delta C/\Delta x$, where Δx is the distance between the two points, typically 10−20 μm. The sensor must be calibrated to determine C, and if the diffusion coefficient is known, the flux can be calculated. The method depends on the assumption that the gradient is not disturbed by the movement of the sensor (0.3 Hz) and that the response time of the sensor is sufficiently rapid to properly respond to changing concentrations. At each pole, data are collected for 70% of the cycle time or about 1 s. Ion selective electrodes (Ca^{2+}, K^+, and H^+), amperometric sensors (O_2, NO, H_2O_2, ascorbate) and biosensors (glucose) have been developed at the BioCurrents Research Center, Woods Hole, MA, for these studies.[210]

In the 1960s and 1970s, physiologists attempting particularly to measure tissue oxygen levels realized the importance of perturbation of tissue surrounding the measurement as a result of its implantation.[211–213] They also realized the importance of "flux balance", namely the balance between the amount of analyte consumed by the sensor (sensor flux) in relation to the flux of the analyte brought to the site by the organelles under study. This problem will be illustrated subsequently in the context of in vivo monitoring.

4. Understanding Biological Processes

4.1. Energy Utilization in the Brain

The human brain constitutes about 2% of the adult body weight but generates as much as 50% of resting glucose consumption.[214] This can amount to about 100 g/day. To enter the brain, glucose must cross the blood−brain barrier, and most glucose transport occurs via the facilitative GLUT 1 transporter, although some glucose may enter via simple diffusion. Once in the brain ECF, GLUT 3 serves as a facilitative transporter for glucose entrance into neurons. Despite the clear demand for energy, ECF glucose levels in the rat hippocampus remain at about 25% of the blood glucose levels, and this gradient is reflected in human studies as well.[214] This is illustrated in Figure 4. The ECF glucose levels[136] are consistent with those observed by another group[135] and significantly higher than the results previously reported (~0.5 mM).[215] However, it is important to note that the latter results were obtained on conscious, freely moving rats. Administration of anesthesia is known to elevate both the plasma and ECF glucose levels.

The principal energy consuming processes of the CNS are biosynthesis and transport of ions and neurotransmitters, especially glutamate. Glutamate is also a potent neurotoxin, and its clearance by conversion to glutamine is an important, energy consuming process. The availability of glucose for this purpose is a critical concern for diabetic patients, who often encounter low blood glucose and therefore ECF glucose levels, which can lead to loss of consciousness.

The CNS appears to have limited storage capacity for glucose (glycogen), and therefore, additional energy may be derived from other sources. A prime candidate is lactate, and the evidence for its direct utilization has been recently reviewed by Pellerin.[216,217] Studies have been carried out on brain slices and other in vitro preparations, indicating, for example, that lactate has about equal access to the TCA

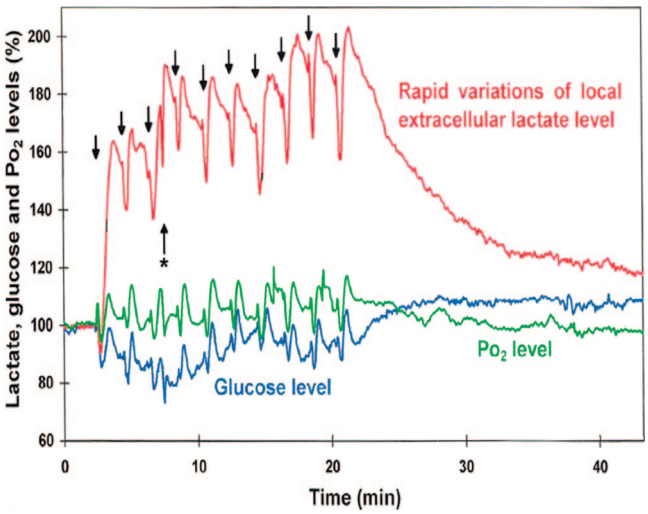

Figure 5. Stimulation of rat brain dentate gyrus glutamate neurons while monitoring lactate, glucose, and oxygen simultaneously with 5 s stimulations, with 2 min of rest between stimulations. Reprinted with permission from ref 139. Copyright 1997 Blackwell Publishing.

cycle, with the ultimate goal being the production of ATP. It is also suggested that "metabolic cooperation" between astrocytes and neurons occurs.[218,219]

To examine lactate utilization, three sensors were implanted in the dentate gyrus of hippocampus of an anesthetized rat.[139] Glutamate neurons were then stimulated through a perforant path. The simultaneous response of the three sensors is shown in Figure 5. Immediately on the first stimulation, the glucose and lactate levels decrease. Oxygen first increases and then seems to rise synchronously with rising lactate following stimulation. During the series of stimulations, the oxygen level remains at or above the basal level. Glucose rises synchronously with oxygen but, during the stimulation period remains 10−20% below the basal level. Although cerebral blood flow (CBF) was not measured, it is reasonable to assume that the increase in oxygen levels is due to such an increase. Aubert and co-workers[220] have modeled these results with the intent of providing additional evidence in support of lactate as an important energy source when neurons are activated. The flux balance for lactate is shown in Figure 6. LAC_e would correspond to the extracellular lactate that should be measured by the sensor. The net flux into the extracellular space would be

$$J_{tissue} = J_{mb} - J_{diff} \quad (10)$$

and this number is calculated as $\sim 5 \times 10^{-3}$ mM s^{-1}. One might add an additional flux, namely that due to the consumption of lactate by the sensor. The in vivo sensitivity is about 2.3 nA/mM, and assuming a lactate concentration of ~1 mM, this gives, by Faraday's law, 1×10^{-14} $mols^{-1}$. However, the sensor, depending on the exact geometry and multilayer thicknesses, collects only about 10% of the peroxide produced; therefore, the consumption of lactate is actually ten times higher. (This is a worse case estimate.) In a sample volume of 1 μL, this would correspond to a flux of $\sim 1 \times 10^{-4}$ mM s^{-1}. Based on a value of J_{tissue} of 5×10^{-3} mM s^{-1}, this would correspond to about 2% of the total flux. On the other hand, if a microdialysis probe had been employed, the lactate uptake by the probe would have been 10−100 times higher and would seriously perturb the concentrations of the extracellular space. A key component

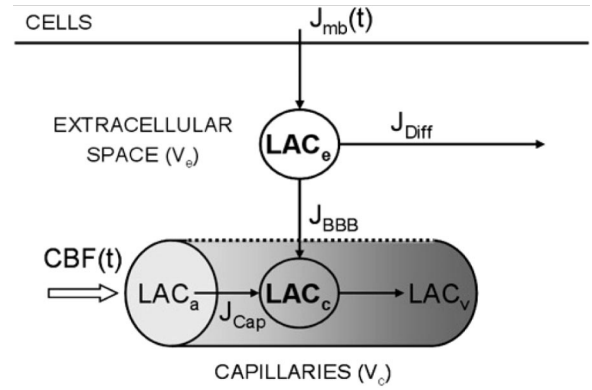

Figure 6. Model of lactate fluxes. LAC_e is the extracellular (interstitial) lactate concentration. $J_{mb}(t)$ is the difference between lactate release by some cells and lactate uptake by others, J_{diff} is the flux of diffusion of lactate through the extracellular space, J_{BBB} is the rate of lactate transport through the BBB, and J_{cap} is the blood flow [CBF(t)] contribution to the capillary lactate (LAC_c) variation. Reprinted with permission from: Aubert et al. Proc. Natl. Acad. Sci. 2005, 102, 16448.[220] Copyright 2005 National Academy of Sciences, U.S.A.

in this model is the communication between astrocytes and neurons, and this requires dealing with Na^+/K^+ pumps and the production of ATP. Measurement of the NADH/NAD^+ ratio helps to understand the intracellular activity.[217,221] Functional brain imaging has also provided valuable insight.[222]

4.2. Diabetes

The Ebers papyrus (1550 BC) seems to be the earliest and most comprehensive description of diabetes characterized by excessive urination, and the name diabetes is taken from the Greek "siphon". It was, however, in 1766, that Matthew Dobson, a British physician and chemist, suggested that the sweetness in both urine and blood was due to sugar.[223] In the mid-19th century, the eminent French physiologist, Claude Bernard, applied the method of Bouchardat, based on the Fehling's solution reduction of Cu(II) to Cu(I), to the analysis of blood samples. He was able to demonstrate the appearance of glucose in blood not attributable to carbohydrate ingestion (gluconeogenesis).[224] With the isolation of insulin in 1921, the importance of patient involvement in insulin administration became important, and in 1956, Bayer developed a dip and read test (Clinistix) based on the use of glucose oxidase and peroxide, leading to color development resulting from the oxidation of *o*-tolidine. This was then read against a printed color scale: negative, light, medium, or dark. As glucose appears in the urine only at high blood glucose concentrations, this device had limited use. In 1964, however, a test strip designed for blood glucose analysis was introduced (Dextrostix), and by the mid-1980s a home-use meter, based on reflectance, became available.

Leland Clark, recognized as the inventor of the electrochemically based glucose sensor, described a device[2] that evolved out of studies of oxygen in biological fluids. A miniaturized version of this sensor appeared in 1967,[225] and in 1974, the Yellow Springs Model 23 glucose analyzer, based on Clark's design, became available and is used to this day for clinical glucose measurements. In 1985 a test strip based on the ferrocene-mediated enzyme-catalyzed oxidation of glucose was reported,[226] and this device became the basis for the Medisense ExacTech system introduced in 1987. At present, electrochemistry is the dominant detection

mode, and more than 20 different companies make such "fingerstick" systems, which have improved significantly. It is still necessary to obtain a drop of blood, but this process now requires less material (now around 0.2 μL), less time for measurement, and less pain. Although these systems are simple to use, patients find the process boring and expensive (more than $0.50/strip).

In the mid-1980s, increased effort was devoted to the development of electrochemically based sensors that could continuously monitor blood glucose. The performance of a needle-type sensor was reported by Shichiri[227] for studies in a pancreatectomized dog. Additional subcutaneous implant studies on dogs were reported by Fischer and co-workers,[228] and in 1990, the performance of a sensor implanted in the vascular bed of a dog was reported.[117] In 1993 we reported on a wearable glucose monitoring system employed in humans.[229]

In 1993, the report of a ten-year study by the Diabetes Control and Complications Trial Study Group (DCCT) addressed the importance of tight control of blood glucose among patients (type 1) that depend on regular injections of insulin.[230] Intensive insulin therapy involving multiple insulin injections per day as well as 6−8 discrete glucose measurements resulted in a 30−70% reduction in the complications of diabetes (blindness, amputation of limbs, and kidney failure). Coupled with this was a 300% increase in the incidence of hypoglycemia (low blood sugar), which can lead to loss of consciousness, a condition that patients and their physicians specifically want to avoid. Recent studies have suggested that the characteristic wide excursions in blood glucose, which can vary over a factor of 4,[231] are also problematic.[232] Since most patients are unwilling to devote the time and effort required to monitor glucose at the required level, the appeal of a continuous monitoring system is significant.

In response to the need for continuous systems, several devices have been developed: Medtronic Minimed CGMS Gold, DexCom STS, Cygnus/Animas GlucoWatch G2 Biographer, and TheraSense/Abbott Free Style Navigator. The first three have FDA approval, and approval has recently been obtained for the latter system. Two systems, based on microdialysis, have been developed in Europe: Menarini Diagnostics GlucoDay,and Roche SCGM1. The electrochemical systems have so far proven superior to various spectroscopic approaches, some noninvasive, some minimally invasive. Because they exhibit instability over the approved three day period of implantation, patients are accordingly advised to make as many as four fingerstick measurements a day to verify performance and to only rely on the monitoring system to detect trends. There is only one published example of a chronically implanted subcutaneous sensor in humans.[118] Out of five sensors, one was functioning normally after six months. The principal mode of failure was the electronics packaging. The sensors needed to be calibrated every 1−4 weeks.

It must first be emphasized that the performance of a new sensing device is always evaluated against blood glucose. Since virtually all of the presently available devices sample interstitial fluid in the subcutaneous tissue, the temporal glucose distribution relationship between these two compartments must be understood. Experimentally, whether in laboratory animals or in humans, for rising glucose concentrations, induced through an intravenous glucose tolerance test (IVGTT) or by oral ingestion of glucose (OGTT),

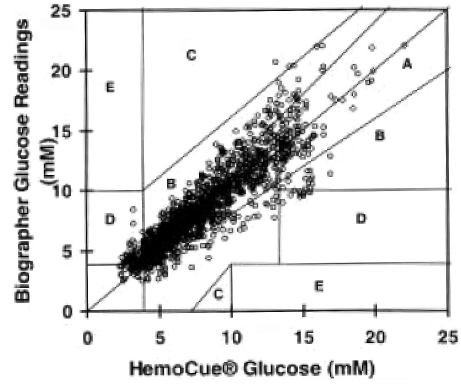

Figure 7. Error Grid Analysis. Clinical data for 32 type 1 subjects: A domain, result clinically accurate; B domain, result clinically acceptable. 96% of the results fall in the A and B zones. Copyright 1999 American Diabetes Association. From Diabetes Care Vol. 22, 1999, 1708−1714.[237] Reprinted with permission from *The American Diabetes Association*.

glucose concentrations in the tissue always lag those in the blood. There are three possible reasons for this: (a) the intrinsic response time of the sensor; (b) response delays created by signal filtering; and (c) physiological delays. For decreasing glucose concentrations, triggered by insulin injection, the tissue glucose may lead, lag, or track the blood values. This situation has proven to be complicated and dependent on the extent of peripheral insulin.[233,234] In addition, as Baker and Gough have pointed out, the sensor dynamic response is also dependent on the magnitude of the glucose change.[235] Because the calibration of the sensor is critical to the subsequent measurements, detailed understanding of the plasma/interstitial glucose levels is essential. This ratio will be unity when the glucose concentration is not changing and no insulin has recently been injected. Thus, calibration in the morning before breakfast is ideal. It is important to emphasize that the observed differences between the two compartments are not simply the result of a time lag, as the rise and fall of glucose values are controlled by different processes. It is necessary to distinguish the calibration issues from the effects of tissue interaction leading to variations in sensor response.

The performance of glucose sensors has frequently been characterized using Clarke Error Grid Analysis (EGA),[236] as shown in Figure 7.[237] The normal correlation plot would generate a 45° line, and perfect correlation would result in all points falling on the line. Surrounding the line are zones that designate the clinical significance of a decision based on the observed glucose value. The A zone denotes clinically accurate, B, clinically acceptable, etc., with the other letters suggesting decreasing levels of clinical significance. Sensors are generally considered acceptable if <98% of the values fall in the A and B zones. The D zone is of the greatest concern because it corresponds to a situation in which the patient believes blood glucose is in an acceptable range, whereas it may be dangerously low. The original EGA procedure was designed for evaluating fingerstick systems in which the various values represent a series of independent measurements. More recently this procedure has been modified to accommodate continuous measurements (CG-EGA).[238] This latter approach takes into account two aspects of sensor performance: Point Error Grid Analysis (P-EGA), which considers the accuracy of blood glucose measurements, and Rate Error Grid Analysis (R-EGA), which determines whether the sensor correctly captures the direction

and rate of blood glucose variations. Not surprisingly, sensors tend to perform better at normal and high glucose levels than at low ones. This can be a matter of concern if the objective is to detect hypoglycemia.

The value of the 3−4 day implants is primarily the insight gained by clinicians concerning the management of diabetes. Continuous monitoring has revealed the higher than suspected incidence of "nocturnal hypoglycemia", an occurrence most common at about 4−5 a.m.[239,240] This can be followed by a rebound, via the "counterregulatory mechanism" in which K_{ATP} channels in the ventromedial hypothalamus are suggested to function as a "glucose sensor". They are believed to be linked to glucose-sensitive neurons via glucokinase, the same apparatus used to detect glucose in the pancreatic β cells,[241] through a complicated cascade epinephrine is released in the brain, which triggers the release of glucagon by the pancreatic beta cells and subsequent conversion of glycogen to glucose in the liver (gluconeogenesis). Unless the nadir is detected when it occurs, there may be no indication of the event at say 7:00 a.m. because the glucose level will have returned to normal. There is great concern, particularly by parents of young children, that a hypoglycemic event may occur at night but the child never recovers. This is known as the "dead in bed" syndrome[242] and is a major reason why continuous monitoring/alarm systems have significant appeal. Complete 24 h results are also valuable in assisting patients in regulating their carbohydrate and insulin intake so as to maintain normoglycemic levels. It has also been recently observed that the survival rate of seriously ill patients, who are not diabetic, is significantly improved if glucose levels are monitored and maintained near normal.[243]

4.3. Interactions among Neuromodulators: Dopamine, Glutamate, GABA, and Hydrogen Peroxide

Important contributions have been made toward understanding how different neurotransmitter systems in the CNS influence one another. Much of this work has been accomplished in acutely dissociated brain slices, which may be regarded as living systems. Brain slices provide several advantages, summarized by Avshalumov et al., over the use of single cells and whole animals.[244] First, the local function of synaptic connections as well as interactions between neurons and glia are maintained in brain slice preparations. The preservation of these characteristics makes possible analyses of the functional interactions between the different cell types. Second, the three-dimensional architecture, including the normal intracellular and extracellular compartments, is preserved; thus, neuromodulating molecules, released by exocytosis and other mechanisms, diffuse in a manner similar to that which occurs in vivo. Third, the use of brain slice preparations facilitates precise electrode placement, allows for the application of imaging methods, and permits the rapid application of pharmacological agents at well-defined concentrations.

Using striatal brain slices, harvested acutely from guinea pigs and rats, Rice and co-workers have uncovered a role for hydrogen peroxide, a reactive oxygen species (ROS), as an important intermediate in the neuromodulation of dopamine by glutamate and γ-aminobutyric acid (GABA). Initial carbon fiber voltammetry experiments, in which trains of multiple stimulation pulses were applied locally to the sites of measurement, revealed that the exogenous application of 1.5 mM H_2O_2 to the brain slice decreases peak dopamine release ($[DA]_0$) by 30 to 40%.[177] Treatment of brain slices with mercaptosuccinate (MCS), which amplifies H_2O_2 levels by inhibiting the antioxidant enzyme glutathione peroxidase, also decreased $[DA]_0$ by 30 to 40%.[178] Thus, it is apparent that endogenous H_2O_2 might serve to mediate striatal dopamine release. It is important to note that single pulse experiments did not reveal the apparent differences in $[DA]_0$ that were present when multiple-pulse stimulation regimens were used following MCS treatment of brain slices.[178] This observation suggests that extracellular H_2O_2 levels may be dynamically enhanced upon the initial stimulus pulse and that interaction with other neurotransmitters, such as glutamate and GABA, may occur.[244]

To investigate a possible regulatory role for glutamate on dopamine neurotransmission, dopamine release was measured voltammetrically in striatal brain slices before and after selective pharmacological inhibition of glutamatergic AMPA receptors.[178,179] In the presence of the AMPA receptor antagonist, GYKI-52466, evoked $[DA]_0$ was enhanced. Furthermore, AMPA receptor antagonism blunted the inhibitory effects of enhanced H_2O_2 levels, brought about by MCS application, on stimulated dopamine release. Thus, Rice and co-workers have uncovered a modulatory role for glutamate on striatal dopamine release. Moreover, their efforts reveal compelling evidence that H_2O_2 is not only a reactive byproduct of mitochondrial respiration but that it also serves as an important signaling molecule that mediates the attenuating effects of glutamate on dopamine release. Recent evidence also suggests that H_2O_2 modulates the enhancing effects of GABA, acting through $GABA_A$ receptors, on striatal dopamine release.[245]

From these voltammetric studies conducted in brain slices, it has been shown that, in the striatum, neurotransmitters act in concert to influence neuronal function. Such studies reveal a need not only to measure dopamine oxidation at the electrode surface but also to simultaneously measure other important bioactive molecules, such as the neurotransmitters GABA and glutamate and the neuromodulators H_2O_2 and nitric oxide (NO), using electrochemical approaches. Application of this multifunction sensor approach is sure to yield an even more detailed account of how these neurochemicals interact to regulate neuronal function.

4.4. Neurological Disorders

Neurological disease states exact a tremendous toll at multiple levels, including individual, family, and governmental. For example, Parkinson's disease is estimated to afflict 1,000,000 in the U.S. alone and increases U.S. health care costs by $34 billion.[246] Thus, there is substantial motivation for the detailed investigation of the underlying mechanisms of human disorders using the most advanced biomedical technologies available. Electrochemical monitoring can make a meaningful contribution toward understanding the underlying pathology of selected disease states.

4.4.1. Huntington's Disease

Among the most relentless of neurological conditions is Huntington's disease (HD), a fatal, genetic, neurodegenerative movement disorder for which there is no cure or effective treatment.[53] In his original communication to *The Medical and Surgical Reporter* in 1872, George Huntington, then a

physician in Pomeroy, OH, gave an account of "a disease of the nervous system" given the name "chorea...on account of the dancing propensities of those who are affected by it."[247] HD is a hereditary disease in which individuals develop mental and motor deficits including chorea, defined more recently as "A state of excessive, spontaneous movements, irregularly timed, randomly distributed, and abrupt..."[248] There is currently no cure for HD; however, tetrabenazine (TBZ; Xenazine), which decreases the vesicular release of dopamine by blocking the vesicular monoamine transporter (VMAT), has emerged as a treatment to potentially suppress chorea. Thus, dopamine regulation in the central nervous system appears to play an important role in regulating movement in HD. It has been established previously that the mutation that causes HD is an expanded CAG repeat segment on the gene encoding a protein called huntingtin,[249] which is thought to associate with vesicles. In humans, a segment of greater than 40 CAG repeats makes highly probable the emergence of HD at some point in life. Additionally, longer repeat lengths are associated with the development of overt disease symptoms earlier in life. Only three years after the identification of the causative HD mutation was published, the development of the first line of transgenic mice, known as R6, was published.[250] These mice were shown to develop an overt phenotype that resembles human HD in many ways. Since this development, several other transgenic mouse models (and one rat model) that approximate the neurological phenotype of HD have been developed.

Initial in vivo electrochemical neurotransmitter measurements in chemically induced HD model animals were conducted in ambulatory Wistar rats following intrastriatal injection with the excitotoxic glutamate agonist kainic acid.[251] Kainic acid treatment produces lesions and induces the expression of motor symptoms resembling that of human HD.[252,253] In this study, Nakazato and Akiyama used an electrochemical technique in which slow scan voltammetry (scan rate of 10 V/s) was combined with differential pulse voltammetry at carbon fiber microelectrodes.[254] In this way, dopamine and 5-hydroxytryptamine were measured independently. Using this approach, a biphasic increase in dopamine levels was found to occur at ~1 h and ~4.5 h after injection and a gradual increase in 5-hydroxytryptamine levels was observed at ~20−60 min after injection. The authors hypothesized that this increase in neurotransmitter release was caused by decreased inhibition from GABAergic neurons in the striatum, which inhibit the dopaminergic neurons that project to the striatum.[255,256] Thus, kainic acid-induced lesioning of GABAergic neurons in the striatum would be expected to increase the firing rates of dopaminergic neurons and, therefore, increase striatal dopamine release.[257,258] HD is characterized by the selective degeneration of striatal GABAergic neurons;[53] thus, this study suggests that dopaminergic overactivation may also occur in transgenic HD model rodents and HD patients.

While chemically induced models of HD have been useful as "pure" models, i.e. a specific malfunction is induced in the brain, rodents that are genetically engineered to express the mutation associated with HD likely represent the most authentic model of HD. The most common transgenic mouse model of HD is the R6/2 mouse. We have previously found, from voltammetric measurements conducted in striatal brain slices, that dopamine release is decreased in R6/2 mice compared to WT mice,[259] likely due to impairments in VMAT function, which may arise from energy deficits.[260] The role that this decrease in dopamine release plays on the motor phenotype is unclear. Naturally occurring dopamine release transient measurements, obtained using FSCV, should be extremely helpful in resolving this issue because the measurements would be collected as the motor phenotype occurred. However, due to their small size, measurements of dopamine release transients in awake mice are difficult. For this reason, the use of transgenic HD model rats should provide valuable insight. Measurements of this type will establish whether the frequency of phasic dopamine release events in the striatum is elevated compared to controls.

Another case in which in vivo electrochemical measurements have proven to be beneficial in understanding the underlying neurochemical mechanisms of HD is the measurement of ascorbate. Rebec et al. used slow scan cyclic voltammetry to measure striatal ascorbate levels in R6/2 mice during anesthesia and behavioral recovery.[261] During anesthesia, ascorbate levels in R6/2 mice were similar to those measured in wild-type control mice. However, as the animals recovered, a 25−50% decrease in R6/2 ascorbate levels was found, suggestive of inadequate antioxidant protection. In fact, chronic ascorbate injections after overt phenotype onset were shown to attenuate the neurological motor signs of HD[262] and normalize the elevated firing rates of striatal GABAergic neurons in R6/2 mice.[263] Given that ascorbate has been shown to modulate glutamate levels in the brain, it is important to obtain accurate measurements of dynamic glutamate levels in R6/2 mice during the anesthesia and the recovery period. Obtaining these data using conventional microdialysis measurements would be difficult given the limited temporal resolution; conversely, this application would be well-suited for measurements obtained using an amperometric glutamate biosensor. Such measurements may shed more light on the role of glutamate on the HD-like motor phenotype expressed by R6/2 mice.

4.4.2. Parkinson's Disease

Parkinson's disease (PD), first described in scientific detail in 1817 by British physician James Parkinson, is a chronic, progressive neurodegenerative disorder in which the primary motor abnormalities include bradykinesia (slow movement), tremor, rigidity, and impaired balance. PD is characterized by degeneration of dopamine neurons in the substantia nigra, resulting in decreased nigrostriatal dopaminergic input to the striatum.[264] Proper function of the nigrostriatal dopamine system is important in the regulation of purposeful movement; thus, when it is disrupted, neurons fire abnormally in the brain, impairing movement.[265] Interestingly, studies have shown that PD patients have lost about 50% of their dopaminergic neurons in the substantia nigra and have an 80% loss of striatal dopamine by the time clinical symptoms are expressed, suggesting the presence of a compensatory effect.[266] Given that PD is characterized by a decrease in striatal dopaminergic function, it is not surprising that the measurement of dopamine has been a focus in recent work. Most of the work in vivo has focused on pulse voltammetric and chronoamperometric methods to study the effects of antiparkinsonian drugs on established models of PD[267,268] as well as to investigate new PD model animals.[269,270]

FSCV has also been used to study dopaminergic system function in PD. Garris et al. showed that normal extracellular dopamine levels can be generated by remote electrical stimulus pulses in the partially lesioned striatum of rats

treated with 6-hydroxydopamine (6-OHDA).[271] Moreover, both the concentration of dopamine released per pulse and the maximum rate of dopamine uptake (V_{max}) decreased in proportion to lesion severity. These findings imply that PD patients may not exhibit overt symptoms until a specific threshold of dopaminergic cell loss occurs. Additional work is aimed at understanding why the fundamental symptoms of experimental and clinical parkinsonism are ameliorated following the intrastriatal transplantation of fetal midbrain dopamine neurons.[272] FSCV revealed that the maximum rate of V_{max} is decreased in grafted brain tissue, thereby extending the time that released dopamine stays in the intracellular space. It is hypothesized that this decreased uptake extends the diffusion sphere of released dopamine, resulting in the normalization of ambient dopamine levels. These examples demonstrate the importance of measuring dopamine release and uptake as separate processes; thus, FSCV has made these studies of PD possible by allowing for high temporal resolution measurements.

Glutamate may also play an important role in PD and other neurological disorders reviewed previously.[273,274] Glutamate input from the motor cortex is thought to regulate dopaminergic input to GABAergic neurons in the striatum and thereby facilitate regulation of movement in the thalamocortical circuit.[265] Despite this modulatory role, published studies that use biosensors in vivo to measure striatal glutamate levels at high temporal resolutions are sparse. However, a recent study using a glutamate biosensor has shed light on glutamate levels during high frequency stimulation of the subthalamic nucleus (STN), a deep brain stimulation procedure that is effective in treating tremor in Parkinson's disease.[275–277] Because high frequency stimulation of the STN has similar effects as surgically lesioning the STN, it has long been assumed that the beneficial effects of high frequency stimulation arise from silencing of the neurons in the stimulated structure.[278] In contrast, it has been reported that neurons in the STN become excited following high frequency stimulation in vivo[279–281] and in thalamic brain slices.[282] Moreover, antagonism of glutamate receptors inhibited depolarizing effects in thalamic brain slices from rats.[280,282] These findings led Lee et al. to hypothesize that glutamate levels in the STN would increase following high frequency stimulation.[204] Using an enzyme-based glutamate electrode, they were able to confirm this hypothesis by stimulating the STN at different frequencies, durations, and current intensities, and measuring up to a 500 μM increase in glutamate levels. These measurements would not have been practical using conventional microdialysis procedures because of the high temporal resolution required.

Collectively, the studies mentioned in above section illustrate the importance of combining in vivo electrochemical measurements with other methods to understand disease pathologies. The wide array of biosensing methods currently available for the measurement of important biological molecules that play important roles in neurotransmission (e.g., glutamate), neuromodulation (H_2O_2), and oxidative stress (superoxide) are presently underutilized. The use of biosensing methods in this case may provide important insight into disease pathology.

5. Conclusion

As electrochemical methodologies become more reliable, scientists outside the field of electrochemistry have begun to make use of in vivo electrochemistry. The understanding of complicated problems will require insights from imaging based on spectroscopic studies and the application of molecular biology in altering metabolic and catabolic pathways. It seems likely that in vivo electrochemistry will find the most prominent application in neuroscience, although there are also advances in cardiology that might be noted.[283,284] The challenge for electrochemists will be to develop methodology that is carefully validated with respect to sensitivity, selectivity, and stability.

6. Acknowledgments

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7. References

(1) Buck, R. P.; Lindner, E. *Anal. Chem.* **2001**, *73*, 88A.
(2) Clark, L. C.; Lyons, C. *Ann. N. Y. Acad. Sci.* **1962**, *102*, 29.
(3) Thévenot, D. R.; Toth, K.; Durst, R. A.; Wilson, G. S. *Pure Appl. Chem.* **1999**, *71*, 2333.
(4) Rowe, L.; Ensor, M.; Scott, D.; Deo, S.; Daunert, S. *Proc. SPIE—Int. Soc. Opt. Eng.* **2006**, *6098*, 60980H/1.
(5) Adams, R. N. *Anal. Chem.* **1976**, *48*, 1126A.
(6) Kissinger, P. T.; Hart, J. B.; Adams, R. N. *Brain Res.* **1973**, *55*, 209.
(7) Shackman, J. G.; Dahlgren, G. M.; Peters, J. L.; Kennedy, R. T. *Lab Chip* **2005**, *5*, 56.
(8) Dahlgren, G. M.; Kauri, L. M.; Kennedy, R. T. *Biochim. Biophys. Acta* **2005**, *1724*, 23.
(9) Jung, S.-K.; Kauri, L. M.; Qian, W.-J.; Kennedy, R. T. *J. Biol. Chem.* **2000**, *275*, 6642.
(10) Kennedy, R. T.; Kauri, L. M.; Dahlgren, G. M.; Jung, S.-K. *Diabetes* **2002**, *51*, S152.
(11) Gerritsen, M.; Jansen, J. A.; Kros, A.; Vriezema, D. M.; Sommerdijk, N. A. J. M.; Nolte, R. J. M.; Lutterman, J. A.; Van Hovell, S. W. F. M.; Van der Gaag, A. *J. Biomed. Mater. Res.* **2000**, *54*, 69.
(12) Heller, A. *Annu. Rev. Biomed. Eng.* **1999**, *1*, 153.
(13) Campbell, C. N.; Heller, A.; Caruana, D. J.; Schmidtke, D. W. *Electroanal. Meth. Biol. Mater.* **2002**, 439.
(14) Csöregi, E.; Schmidtke, D. W.; Heller, A. *Anal. Chem.* **1995**, *67*, 1240.
(15) Cui, X.; Liu, G.; Lin, Y. *J. Biomed. Nanotechnol.* **2005**, *1*, 320.
(16) So, H.-M.; Won, K.; Kim, Y. H.; Kim, B.-K.; Ryu, B. H.; Na, P. S.; Kim, H.; Lee, J.-O. *J. Am. Chem. Soc.* **2005**, *127*, 11906.
(17) Matsumoto, N.; Chen, X.; Wilson, G. S. *Anal. Chem.* **2002**, *74*, 362.
(18) Chen, X.; Matsumoto, N.; Hu, Y.; Wilson, G. S. *Anal. Chem.* **2002**, *74*, 368.
(19) Bindra, D. S.; Zhang, Y.; Wilson, G. S.; Sternberg, R.; Thévenot, D. R.; Moatti, D.; Reach, G. *Anal. Chem.* **1991**, *63*, 1692.
(20) Gough, D. A.; Lucisano, J. Y.; Tse, P. H. S. *Anal. Chem.* **1985**, *57*, 2351.
(21) Burmeister, J. J.; Palmer, M.; Gerhardt, G. A. *Biosens. Bioelectron.* **2005**, *20*, 1772.
(22) Mano, N.; Mao, F.; Heller, A. *J. Electroanal. Chem.* **2005**, *574*, 347.
(23) De Alwis, W. U.; Hill, B. S.; Meiklejohn, B. I.; Wilson, G. S. *Anal. Chem.* **1987**, *59*, 2688.
(24) Demaille, C.; Moiroux, J.; Saveant, J. M.; Bourdillon, C. In *Protein Architecture: Interfacing Molecular Assemblies and Immobilization Biotechnology*; Lvov, Y., Möhwald, H., Eds.; Marcel Dekker: New York, 2000.
(25) Kranz, C.; Wohlschlaeger, H.; Schmidt, H.-L.; Schuhmann, W. *Electroanalysis* **1998**, *10*, 546.
(26) Cosnier, S. *Appl. Biochem. Biotechnol.* **2000**, *89*, 127.
(27) Yu, X.; Sotzing, G. A.; Papadimitrakopoulos, F.; Rusling, J. F. *Anal. Chem.* **2003**, *75*, 4565.
(28) Yu, C.; Irudayaraj, J. *Anal. Chem.* **2007**, *79*, 572.
(29) Calvo, E. J.; Battaglini, F.; Danilowicz, C.; Wolosiuk, A.; Otero, M. *Faraday Discuss.* **2000**, *116*, 47.
(30) Rusling, J. F. In *Protein Architecture*; Marcel Dekker: 2000.
(31) Calvo, E. J.; Wolosiuk, A. *ChemPhysChem* **2004**, *5*, 235.
(32) Anicet, N.; Bourdillon, C.; Moiroux, J.; Saveant, J.-M. *Langmuir* **1999**, *15*, 6527.
(33) Limoges, B.; Marchal, D.; Mavre, F.; Saveant, J.-M. *J. Am. Chem. Soc.* **2006**, *128*, 2084.
(34) Ward, R. S.; Tian, Y.; Ward, W. K.; Anderson, E.; House, J. 2006.
(35) Laurinavicius, V.; Razumiene, J.; Ramanavicius, A.; Ryabov, A. D. *Biosens. Bioelectron.* **2004**, *20*, 1217.
(36) Razumiene, J.; Vilkanauskyte, A.; Gureviciene, V.; Barkauskas, J.; Meskys, R.; Laurinavicius, V. *Electrochim. Acta* **2006**, *51*, 5150.

(37) Kuhr, W. G.; Ewing, A. G.; Caudill, W. L.; Wightman, R. M. *J. Neurochem.* **1984**, *43*, 560.
(38) Ewing, A. G.; Bigelow, J. C.; Wightman, R. M. *Science* **1983**, *221*, 169.
(39) Stamford, J. A.; Kruk, Z. L.; Millar, J.; Wightman, R. M. *Neurosci. Lett.* **1984**, *51*, 133.
(40) Hafizi, S.; Kruk, Z. L.; Stamford, J. A. *J. Neurosci. Meth.* **1990**, *33*, 41.
(41) Jones, S. R.; Garris, P. A.; Wightman, R. M. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 396.
(42) Jones, S. R.; Garris, P. A.; Kilts, C. D.; Wightman, R. M. *J. Neurochem.* **1995**, *64*, 2581.
(43) Carelli, R. M. *Neuropharmacology* **2004**, *47 Suppl 1*, 180.
(44) Phillips, P. E.; Robinson, D. L.; Stuber, G. D.; Carelli, R. M.; Wightman, R. M. *Methods Mol. Med.* **2003**, *79*, 443.
(45) Stuber, G. D.; Roitman, M. F.; Phillips, P. E.; Carelli, R. M.; Wightman, R. M. *Neuropsychopharm* **2005**, *30*, 853.
(46) Heien, M. L.; Khan, A. S.; Ariansen, J. L.; Cheer, J. F.; Phillips, P. E.; Wassum, K. M.; Wightman, R. M. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 10023.
(47) Phillips, P. E.; Stuber, G. D.; Heien, M. L.; Wightman, R. M.; Carelli, R. M. *Nature* **2003**, *422*, 614.
(48) Robinson, D. L.; Volz, T. J.; Schenk, J. O.; Wightman, R. M. *Alcohol.: Clin. Exp. Res.* **2005**, *29*, 746.
(49) Roitman, M. F.; Stuber, G. D.; Phillips, P. E.; Wightman, R. M.; Carelli, R. M. *J. Neurosci.* **2004**, *24*, 1265.
(50) Robinson, D. L.; Heien, M. L.; Wightman, R. M. *J. Neurosci.* **2002**, *22*, 10477.
(51) Robinson, D. L.; Phillips, P. E.; Budygin, E. A.; Trafton, B. J.; Garris, P. A.; Wightman, R. M. *Neuroreport* **2001**, *12*, 2549.
(52) von Hörsten, S.; Schmitt, I.; Nguyen, H. P.; Holzmann, C.; Schmidt, T.; Walther, T.; Bader, M.; Pabst, R.; Kobbe, P.; Krotova, J.; Stiller, D.; Kask, A.; Vaarmann, A.; Rathke-Hartlieb, S.; Schulz, J. B.; Grasshoff, U.; Bauer, I.; Vieira-Saecker, A. M.; Paul, M.; Jones, L.; Lindenberg, K. S.; Landwehrmeyer, B.; Bauer, A.; Li, X. J.; Riess, O. *Hum. Mol. Genet.* **2003**, *12*, 617.
(53) Bates, G. P.; Harper, P. S.; Jones, L. *Huntington's Disease*, 3rd ed.; Oxford University Press: Oxford, 2002.
(54) Wightman, R. M.; Jankowski, J. A.; Kennedy, R. T.; Kawagoe, K. T.; Schroeder, T. J.; Leszczyszyn, D. J.; Near, J. A.; Diliberto, E. J.; Viveros, O. H. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 10754.
(55) Hochstetler, S. E.; Puopolo, M.; Gustincich, S.; Raviola, E.; Wightman, R. M. *Anal. Chem.* **2000**, *72*, 489.
(56) Chen, G.; Ewing, A. G. *Crit. Rev. Neurobiol.* **1997**, *11*, 59.
(57) Garris, P. A.; Rebec, G. V. *Behav. Brain Res.* **2002**, *137*, 47.
(58) Greco, P. G.; Garris, P. A. *Eur. J. Pharmacol.* **2003**, *479*, 117.
(59) Robinson, D. L.; Venton, B. J.; Heien, M. L.; Wightman, R. M. *Clin. Chem.* **2003**, *49*, 1763.
(60) Stamford, J. A. *Brain Res.* **1985**, *357*, 119.
(61) Wightman, R. M. *Science* **2006**, *311*, 1570.
(62) Carelli, R. M.; Wightman, R. M. *Curr. Opin. Neurobiol.* **2004**, *14*, 763.
(63) Troyer, K. P.; Heien, M. L.; Venton, B. J.; Wightman, R. M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 696.
(64) Wightman, R. M.; Troyer, K. P.; Mundorf, M. L.; Catahan, R. *Ann. N.Y. Acad. Sci.* **2002**, *971*, 620.
(65) Troyer, K. P.; Mundorf, M. L.; Fries, H. E.; Wightman, R. M. *Ann. N.Y. Acad. Sci.* **2002**, *971*, 251.
(66) Dowling, M. G.; Clarke, M. J. *Inorg. Chim. Acta* **1983**, *78*, 153.
(67) Kaim, W.; Schwederski, B.; Heilmann, O.; Hornung, F. M. *Coord. Chem. Rev.* **1999**, *182*, 323.
(68) Cammack, J.; Ghasemzadeh, B.; Adams, R. N. *Brain Res.* **1991**, *565*, 17.
(69) Cammack, J.; Ghasemzadeh, B.; Adams, R. N. *Neurochem. Res.* **1992**, *17*, 23.
(70) Avnir, D.; Coradin, T.; Lev, O.; Livage, J. *J. Mater. Chem.* **2006**, *16*, 1013.
(71) Dunn, B.; Zink, J. I. *Acc. Chem. Res.* **2007**, *40*, 747.
(72) Liu, S.; Sun, Y. *Biosens. Bioelectron.* **2007**, *22*, 905.
(73) Xu, Z.; Chen, X.; Dong, S. *Trends Anal. Chem.* **2006**, *25*, 899.
(74) Hu, Y.; Mitchell, K. M.; Albahadily, F. N.; Michaelis, E. K.; Wilson, G. S. *Brain Res.* **1994**, *659*, 117.
(75) Pihel, K.; Schroeder, T. J.; Wightman, R. M. *Anal. Chem.* **1994**, *66*, 4532.
(76) Wiedemann, D. J.; Basse-Tomusk, A.; Wilson, R. L.; Rebec, G. V.; Wightman, R. M. *J. Neurosci. Methods* **1990**, *35*, 9.
(77) Gerhardt, G. A.; Oke, A. F.; Nagy, G.; Moghaddam, B.; Adams, R. N. *Brain Res.* **1984**, *290*, 390.
(78) Ohara, T. J.; Rajagopalan, R.; Heller, A. *Anal. Chem.* **1994**, *66*, 2451.
(79) Kraig, R. P.; Ferreira-Filho, C. R.; Nicholson, C. *J. Neurophysiol.* **1983**, *49*, 831.
(80) Urbanics, R.; Leniger-Follert, E.; Lübbers, D. W. *Pfluegers Arch.* **1978**, *378*, 47.
(81) Heien, M. L.; Johnson, M. A.; Wightman, R. M. *Anal. Chem.* **2004**, *76*, 5697.
(82) Wightman, R. M.; Runnels, P.; Troyer, K. *Anal. Chim. Acta* **1999**, *400*, 5.
(83) Meyerhoff, J. B.; Ewing, M. A.; Ewing, A. G. *Electroanalysis* **1999**, *11*, 308.
(84) Sharkawy, A. A.; Klitzman, B.; Truskey, G. A.; Reichert, W. M. *J. Biomed. Mater. Res.* **1997**, *37*, 401.
(85) Sharkawy, A. A.; Klitzman, B.; Truskey, G. A.; Reichert, W. M. *J. Biomed. Mater. Res.* **1998**, *40*, 598.
(86) Sharkawy, A. A.; Klitzman, B.; Truskey, G. A.; Reichert, W. M. *J. Biomed. Mater. Res.* **1998**, *40*, 586.
(87) Desai, N. P.; Hubbell, J. A. *Biomaterials* **1991**, *12*, 144.
(88) Tirelli, N.; Lutolf, M. P.; Napoli, A.; Hubbell, J. A. *Rev. Mol. Biotechnol.* **2002**, *90*, 3.
(89) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605.
(90) Luk, Y. Y.; Kato, M.; Mrksich, M. *Langmuir* **2000**, *16*, 9604.
(91) Goreish, H. H.; Lewis, A. L.; Rose, S.; Lloyd, A. W. *J. Biomed. Mater. Res., Part A* **2003**, *68A*, 1.
(92) Patel, J. D.; Ebert, M.; Ward, R.; Anderson, J. M. *J. Biomed. Mater. Res., Part A* **2007**, *80A*, 742.
(93) Hetrick, E. M.; Schoenfisch, M. H. *Chem. Soc. Rev.* **2006**, *35*, 780.
(94) Shin, J. H.; Schoenfisch, M. H. *Analyst* **2006**, *131*, 609.
(95) Anderson, J. M. *Annu. Rev. Mater. Res.* **2001**, *31*, 81.
(96) Silver, I. A.; Murrills, R. J.; Etherington, D. J. *Exp. Cell Res.* **1988**, *175*, 266.
(97) Jones, J. A.; Chang, D. T.; Meyerson, H.; Colton Il, E.; Kwon, K.; Matsuda, T.; Anderson, J. M. *J. Biomed. Mater. Res.* **2007**, *83A*, 585.
(98) Hern, D. L.; Hubbell, J. A. *J. Biomed. Mater. Res.* **1998**, *39*, 266.
(99) Ratner, B. D.; Bryant, S. J. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41.
(100) Ward, W. K.; Quinn, M. J.; Wood, M. D.; Tiekotter, K. L.; Pidikiti, S.; Gallagher, J. A. *Biosens. Bioelectron.* **2003**, *19*, 155.
(101) Hickey, T.; Kreutzer, D.; Burgess, D. J.; Moussy, F. *J. Biomed. Mater. Res.* **2002**, *61*, 180.
(102) Norton, L. W.; Tegnell, E.; Toporek, S. S.; Reichert, W. M. *Biomaterials* **2005**, *26*, 3285.
(103) Mowery, K. A.; Schoenfisch, M. H.; Baliga, N.; Wahr, J. A.; Meyerhoff, M. E. *Electroanalysis* **1999**, *11*, 681.
(104) Frost, M. C.; Reynolds, M. M.; Meyerhoff, M. E. *Biomaterials* **2005**, *26*, 1685.
(105) Frost, M. C.; Batchelor, M. M.; Lee, Y.; Zhang, H.; Kang, Y.; Oh, B.; Wilson, G. S.; Gifford, R.; Rudich, S. M.; Meyerhoff, M. E. *Microchem. J.* **2003**, *74*, 277.
(106) Gifford, R.; Batchelor, M. M.; Lee, Y.; Gokulrangan, G.; Meyerhoff, M. E.; Wilson, G. S. *J. Biomed. Mater. Res., Part A* **2005**, *75A*, 755.
(107) Thomé-Duret, V.; Gangnerau, M. N.; Zhang, Y.; Wilson, G. S.; Reach, G. *Diabetes Metab.* **1996**, *22*, 174.
(108) Elbicki, J. N.; Weber, S. G. *Biosensors* **1988**, *4*, 251.
(109) Gifford, R.; Kehoe, J. J.; Barnes, S. L.; Kornilayev, B. A.; Alterman, M. A.; Wilson, G. S. *Biomaterials* **2006**, *27*, 2587.
(110) Spataro, L.; Dilgen, J.; Retterer, S.; Spence, A. J.; Isaacson, M.; Turner, J. N.; Shain, W. *Exp. Neurol.* **2005**, *194*, 289.
(111) Szarowski, D. H.; Andersen, M. D.; Retterer, S.; Spence, A. J.; Isaacson, M.; Craighead, H. G.; Turner, J. N.; Shain, W. *Brain Res.* **2003**, *983*, 23.
(112) Clapp-Lilly, K. L.; Roberts, R. C.; Duffy, L. K.; Irons, K. P.; Hu, Y.; Drew, K. L. *J. Neurosci. Methods* **1999**, *90*, 129.
(113) Benveniste, H.; Diemer, N. H. *Acta Neuropathol. (Berlin)* **1987**, *74*, 234.
(114) Peters, J. L.; Miner, L. H.; Michael, A. C.; Sesack, S. R. *J. Neurosci. Methods* **2004**, *137*, 9.
(115) Borland, L. M.; Shi, G.; Yang, H.; Michael, A. C. *J. Neurosci. Methods* **2005**, *146*, 149.
(116) Kennedy, R. T.; Thompson, J. E.; Vickroy, T. W. *J. Neurosci. Methods* **2002**, *114*, 39.
(117) Armour, J. C.; Lucisano, J. Y.; McKean, B. D.; Gough, D. A. *Diabetes* **1990**, *39*, 1519.
(118) Gilligan, B. C.; Shults, M.; Rhodes, R. K.; Jacobs, P. G.; Brauker, J. H.; Pintar, T. J.; Updike, S. J. *Diabetes Technol. Ther.* **2004**, *6*, 378.
(119) Koschwanez, H. E.; Reichert, W. M. *Biomaterials* **2007**, *28*, 3687.
(120) O'Neill, R. D.; Lowry, J. P.; Mas, M. *Crit. Rev. Neurobiol.* **1998**, *12*, 69.
(121) O'Neill, R. D.; Lowry, J. P. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Jr., Ed.; John Wiley and Sons, Ltd.: 2000.
(122) Kahlert, S.; Reiser, G. *Cell Calcium* **2004**, *36*, 295.
(123) Smith, P. J. S.; Hammar, K.; Porterfield, D. M.; Sanger, R. H.; Trimarchi, J. R. *Microsci. Res. Technol.* **1999**, *46*, 398.
(124) Allen, B. W.; Liu, J.; Piantadosi, C. A. *Methods Enzymol.* **2005**, *396*, 68.

(125) Amatore, C.; Arbault, S.; Bouret, Y.; Cauli, B.; Guille, M.; Rancillac, A.; Rossier, J. *ChemPhysChem* **2006**, *7*, 181.
(126) Bedioui, F.; Villeneuve, N. *Electroanalysis* **2002**, *15*, 5.
(127) Patel, B. A.; Arundell, M.; Parker, K. H.; Yeoman, M. S.; O'Hare, D. *Anal. Chem.* **2006**, *78*, 7643.
(128) Kubant, R.; Malinski, C.; Burewicz, A.; Malinski, T. *Electroanalysis* **2006**, *18*, 410.
(129) Tammeveski, K.; Tenno, T. T.; Mashirin, A. A.; Hillhouse, E. W.; Manning, P.; McNeil, C. J. *Free Radical Biol. Med.* **1998**, *25*, 973.
(130) Scheller, W.; Ehrenreich-Foerster, E.; Ge, B.; Lisdat, F.; Buettemeier, R.; Wollenberger, U.; Scheller, F. W. *Electroanalysis* **1999**, *11*, 703.
(131) McNeil, C. J.; Manning, P. *Rev. Mol. Biotechnol.* **2002**, *82*, 443.
(132) Kulagina, N. V.; Michael, A. C. *Anal. Chem.* **2003**, *75*, 4875.
(133) Amatore, C.; Arbault, S.; Bouton, C.; Coffi, K.; Drapier, J.-C.; Ghandour, H.; Tong, Y. *ChemBioChem* **2006**, *7*, 653.
(134) Rebec, G. V.; Witowski, S. R.; Sandstrom, M. I.; Rostand, R. D.; Kennedy, R. T. *Neurosci. Lett.* **2005**, *378*, 166.
(135) Silver, I. A.; Erecinska, M. *J. Neurosci.* **1994**, *14*, 5068.
(136) Hu, Y.; Wilson, G. S. *J. Neurochem.* **1997**, *68*, 1745.
(137) Fillenz, M.; Lowry, J. P.; Boutelle, M. G.; Fray, A. E. *Acta Physiol. Scand.* **1999**, *167*, 275.
(138) Mo, J.-W.; Smart, W. *Frontiers Biosci.* **2004**, *9*, 3384.
(139) Hu, Y.; Wilson, G. S. *J. Neurochem.* **1997**, *69*, 1484.
(140) Shram, N.; Netchiporouk, L.; Cespuglio, R. *Eur. J. Neurosci.* **2002**, *16*, 461.
(141) Meyerhoff, C.; Bischof, F.; Mennel, F. J.; Sternberg, F.; Bican, J.; Pfeiffer, E. F. *Biosens. Bioelectron.* **1993**, *8*, 409.
(142) Lowry, J. P.; Ryan, M. R.; O'Neill, R. D. *Anal. Commun.* **1998**, *35*, 87.
(143) Asai, S.; Zhao, H.; Kohno, T.; Takahashi, Y.; Nagata, T.; Ishikawa, K. *Brain Res.* **2000**, *864*, 60.
(144) Porras, O. H.; Loaiza, A.; Barros, L. F. *J. Neurosci.* **2004**, *24*, 9669.
(145) Borland, L. M.; Michael, A. C. *J. Neurochem.* **2004**, *91*, 220.
(146) Chen, X.; Wang, L.; Zhou, Y.; Zheng, L.-H.; Zhou, Z. *J. Neurosci.* **2005**, *25*, 9236.
(147) Garguilo, M. G.; Michael, A. C. *TrAC, Trends Anal Chem* **1995**, *14*, 164.
(148) Mitchell, K. M. *Anal. Chem.* **2004**, *76*, 1098.
(149) Scheller, F. W.; Pfeiffer, D. *Anal. Chim. Acta* **1980**, *117*, 383.
(150) Compagnone, D.; Guilbault, G. G. *Anal. Chim. Acta* **1997**, *340*, 109.
(151) Frenguelli, B. G.; Llaudet, E.; Dale, N. *J. Neurochem.* **2003**, *86*, 1506.
(152) Frenguelli, B. G.; Wigmore, G.; Llaudet, E.; Dale, N. *J. Neurochem.* **2007**, *101*, 1400.
(153) Dale, N.; Hatz, S.; Tian, F.; Llaudet, E. *Trends Biotechnol.* **2005**, *23*, 420.
(154) Kueng, A.; Kranz, C.; Mizaikoff, B. *Biosens. Bioelectron.* **2004**, *19*, 1301.
(155) Llaudet, E.; Botting, N. P.; Crayston, J. A.; Dale, N. *Biosens. Bioelectron.* **2003**, *18*, 43.
(156) Llaudet, E.; Hatz, S.; Droniou, M.; Dale, N. *Anal. Chem.* **2005**, *77*, 3267.
(157) Gourine, A. V.; Llaudet, E.; Dale, N.; Spyer, K. M. *J. Neurosci.* **2005**, *25*, 1211.
(158) Gourine, A. V.; Llaudet, E.; Dale, N.; Spyer, K. M. *Nature* **2005**, *436*, 108.
(159) Bakker, E.; Yu, Q. *Anal. Chem.* **2006**, *78*, 3965.
(160) Wolfbeis, O. S. *Anal. Chem.* **2006**, *78*, 3859.
(161) Messerli, M. A.; Corson, E. D.; Smith, P. J. S. *Biophys. J.* **2007**, *92*, L52.
(162) Xin, Q.; Wightman, R. M. *Anal. Chem.* **1999**, *70*, 1677.
(163) Malinski, T. In *Encyclopedia of Electrochemisry*; Strattman, P., Bard, A. J., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 9.
(164) Inzelt, G.; Horányi, G. In *Encyclopedia of Electrochemistry*; Bard, A. J., Stratmann, M., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006; Vol. 7a.
(165) Griveau, S.; Dumezy, C.; Seguin, J.; Chabot, G. G.; Scherman, D.; Bedioui, F. *Anal. Chem.* **2007**, *79*, 1030.
(166) Eberhardt, M. K. *Reactive Oxygen Metabolites: Chemistry and Medical Consequences*; CRC Press: Boca Raton, FL, 2001.
(167) Buttemeyer, R.; Philipp, A. W.; Mall, J. W.; Ge, B.; Scheller, F. W.; Lisdat, F. *Microsurgery* **2002**, *22*, 108.
(168) Manning, P.; McNeil, C. J.; Cooper, J. M.; Hillhouse, E. W. *Free Radical Biol. Med.* **1998**, *24*, 1304.
(169) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049.
(170) Tian, Y.; Mao, L.; Okajima, T.; Ohsaka, T. *Biosens. Bioelectron.* **2005**, *21*, 557.
(171) Emregul, E. *Anal. Bioanal. Chem.* **2005**, *383*, 947.
(172) Bienert, G. P.; Schjoerring, J. K.; Jahn, T. P. *Biochim. Biophys. Acta* **2006**, *1758*, 994.
(173) Tuppo, E. E.; Forman, L. J. *J. Am. Osteopath. Assoc.* **2001**, *101*, S11.
(174) Butterfield, D. A.; Howard, B. J.; LaFontaine, M. A. *Curr. Med. Chem.* **2001**, *8*, 815.
(175) Jenner, P. *Ann. Neurol.* **2003**, *53 Suppl 3*, S26.
(176) Robberecht, W. *J. Neurol.* **2000**, *247 Suppl 1*, I1.
(177) Chen, B. T.; Avshalumov, M. V.; Rice, M. E. *J. Neurophysiol.* **2001**, *85*, 2468.
(178) Avshalumov, M. V.; Chen, B. T.; Marshall, S. P.; Pena, D. M.; Rice, M. E. *J. Neurosci.* **2003**, *23*, 2744.
(179) Avshalumov, M. V.; Rice, M. E. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11729.
(180) Zhang, Y.; Wilson, G. S. *J. Electroanal. Chem.* **1993**, *345*, 253.
(181) Hall, S. B.; Khudaish, E. A.; Hart, A. L. *Electrochim. Acta* **1997**, *43*, 579.
(182) Hall, S. B.; Khudaish, E. A.; Hart, A. L. *Electrochim. Acta* **2000**, *45*, 3573.
(183) Andreu, R.; Ferapontova, E. E.; Gorton, L.; Calvente, J. J. *J. Phys. Chem. B* **2007**, *111*, 469.
(184) Lindgren, A.; Ruzgas, T.; Gorton, L. *Curr. Top. Anal. Chem.* **2001**, *2*, 71.
(185) Garguilo, M. G.; Huynh, N.; Proctor, A.; Michael, A. C. *Anal. Chem.* **1993**, *65*, 523.
(186) Kurita, R.; Hayashi, K.; Torimitsu, K.; Niwa, O. *Anal. Sci.* **2003**, *19*, 1581.
(187) Zong, S.; Cao, Y.; Zhou, Y.; Ju, H. *Anal. Chim. Acta* **2007**, *582*, 361.
(188) Xu, Y.; Liang, J.; Hu, C.; Wang, F.; Hu, S.; He, Z. *J. Biol. Inorg. Chem.* **2007**, *12*, 421.
(189) Gao, L.; Gao, Q. *Biosens. Bioelectron.* **2007**, *22*, 1454.
(190) Zong, S.; Cao, Y.; Zhou, Y.; Ju, H. *Biosens. Bioelectron.* **2007**, *22*, 1776.
(191) O'Brien, K. B.; Killoran, S. J.; O'Neill, R. D.; Lowry, J. P. *Biosens. Bioelectron.* **2007**, *22*, 2994.
(192) Kulagina, N. V.; Shankar, L.; Michael, A. C. *Anal. Chem.* **1999**, *71*, 5093.
(193) Gonon, F.; Buda, M.; Cespuglio, R.; Jouvet, M.; Pujol, J. F. *Nature* **1980**, *286*, 902.
(194) Gonon, F.; Fombarlet, C. G.; Buda, M.; Pujol, J. F. *Anal. Chem.* **1981**, *53*, 1386.
(195) Louilot, A.; Gonon, F.; Buda, M.; Simon, H.; Le Moal, M.; Pujol, J. F. *Brain Res.* **1985**, *336*, 253.
(196) Gonon, F.; Buda, M.; Cespuglio, R.; Jouvet, M.; Pujol, J. F. *Brain Res.* **1981**, *223*, 69.
(197) Cahill, P. S.; Wightman, R. M. *Anal. Chem.* **1995**, *67*, 2599.
(198) Netchiporouk, L.; Sharm, N.; Salvert, D.; Cespuglio, R. *Eur. J. Neurosci.* **2001**, *13*, 1429.
(199) Faden, A. I.; Demediuk, P.; Panter, S. S.; Vink, R. *Science* **1989**, *244*, 798.
(200) Diemer, N. H.; Valente, E.; Bruhn, T. M. B.; Jorgensen, M. B.; Johansen, F. F. *Prog. Brain Res.* **1993**, *96*, 145.
(201) Pellerin, L.; Magistretti, P. J. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 10625.
(202) Fray, A. E.; Forsyth, R. J.; Boutelle, M. G.; Fillenz, M. *J. Physiol. (Oxford, U.K.)* **1996**, *496*, 49.
(203) Tong, Q.; Ye, C.; McCrimmon, R. J.; Dhillon, H.; Choi, B.; Kramer, M. D.; Yu, J.; Yang, Z.; Christiansen, L. M.; Lee, C. E.; Choi, C. S.; Zigman, J. M.; Shulman, G. I.; Sherwin, R. S.; Elmquist, J. K.; Lowell, B. B. *Cell Metab.* **2007**, *5*, 383.
(204) Lee, K. H.; Kristic, K.; van Hoff, R.; Hitti, F. L.; Blaha, C.; Harris, B.; Roberts, D. W.; Leiter, J. C. *Brain Res.* **2007**, *1162*, 121.
(205) Morita, H.; Abe, C.; Awazu, C.; Tanaka, K. *Neurosci. Lett.* **2007**, *412*, 201.
(206) Johnson, D. A.; Wilson, G. S. In *Electrochemical Methods for Neuroscience*; Michael, A. C., Borland, L. M., Eds.; CRC Press: Boca Raton, FL, 2007.
(207) Cui, J.; Kulagina, N. V.; Michael, A. C. *J. Neurosci. Methods* **2001**, *104*, 183.
(208) Cotrina, M. L.; Lin, J. H. C.; Lopez-Garcia, J. C.; Naus, C. C. G.; Nedergaard, M. *J. Neurosci.* **2000**, *20*, 2835.
(209) *Principles, Development and Applications of Self-Referencing Electrochemical Microelectrodes to the Determination of Fluxes at Cell Membranes*; Smith, P. J. S., Sanger, R. H., Messerli, M. A., Eds.; CRC Press: Boca Raton, FL, 2007.
(210) Porterfield, D. M. *Biosens. Bioelectron.* **2007**, *22*, 1186.
(211) Silver, I. A. In *A Symposium on Oxygen Measurements in Blood and Tissues and their Significance*,; Payne, J. P., Hill, D. W., Eds.; J. A. Churchill, Ltd.: London, 1966.
(212) Lübbers, D. W.; Baumgärtl, H.; Grunewald, W.; Leninger-Follert, E.; Wodick, R. *Bibliotheca Anat.* **1975**, *13*, 53.
(213) Leninger-Follert, E.; Lübbers, D. W.; Wrabetz, W. *Pfluegers Arch.* **1975**, *359*, 81.
(214) Evans, M. L.; Sherwin, R. S. *Diabetes Nutr. Metab.* **2002**, *15*, 294.
(215) Lowry, J. P.; O'Neill, R. D.; Boutelle, M. G.; Fillenz, M. *J. Neurochem.* **1998**, *70*, 391.
(216) Pellerin, L.; Bonvento, G.; Chatton, J. Y.; Pierre, K.; Magistretti, P. J. *Diabetes Nutr. Metab.* **2002**, *15*, 268.

(217) Pellerin, L. *Neurochem. Int.* **2003**, *43*, 331.
(218) Morgenthaler, F. D.; Kraftsik, R.; Satsicas, S.; Magistretti, P. J.; Chatton, J.-Y. *Neuroscience* **2006**, *141*, 157.
(219) Pellerin, L.; Magistretti, P. J. *Science* **2004**, *305*, 50.
(220) Aubert, A.; Costalat, R.; Magistretti, P. J.; Pellerin, L. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16448.
(221) Aubert, A.; Costalat, R. *J. Cereb. Blood Flow Metab.* **2005**, *25*, 1476.
(222) Raichle, M. E.; Mintum, M. A. *Annu. Rev. Neurosci.* **2006**, *29*, 449.
(223) Papaspyros, N. S. *The History of Diabetes Mellitus*, 2nd ed.; Georg Thieme Verlag: Stuttgart, 1964.
(224) Bernard, C. *Leçons sur Le Diabète et La Glycogenèse Animale*; J-B Balbière et Fils: Paris, 1877.
(225) Updike, S. J.; Hicks, G. P. *Nature* **1967**, *214*, 986.
(226) Cass, A. E. G.; Davis, G.; Francis, G. D.; Hill, H. A. O.; Aston, W. J.; Higgins, I. J.; Plotkin, E. V.; Scott, L. D. L.; Turner, A. P. F. *Anal. Chem.* **1984**, *56*, 667.
(227) Shichiri, M.; Kawamori, R.; Yamasaki, Y.; Hakui, N.; Abe, H. *Lancet* **1982**, *2*, 1129.
(228) Fischer, U.; Ertle, R.; Abel, P.; Rebrin, K.; Brunstein, E.; Hahn von Dorsche, H.; Freyse, E. J. *Diabetologia* **1987**, *30*, 940.
(229) Poitout, V.; Moatti-Sirat, D.; Reach, G.; Zhang, Y.; Wilson, G. S.; Lemonnier, F.; Klein, J. C. *Diabetologia* **1993**, *36*, 658.
(230) The Diabetes Control and Complications Trial Research Group (DCCT). N. Engl. J. Med. 1993, *329*, 977.
(231) Wilson, G. S.; Gifford, R. *Biosens. Bioelectron.* **2005**, *20*, 2388.
(232) Hirsch, I. B.; Brownlee, M. *J. Diabetes Complicat.* **2005**, *19*, 178.
(233) Aussedat, B.; Dupire-Angel, M.; Gifford, R.; Klein, J. C.; Wilson, G. S.; Reach, G. *Am. J. Physiol.* **2000**, *278*, E716.
(234) Steil, G. M.; Rebrin, K.; Hariri, F.; Jinagonda, S.; Tadros, S.; Darwin, C.; Saad, M. F. *Diabetologia* **2005**, *48*, 1833.
(235) Baker, D. A.; Gough, D. A. *Anal. Chem.* **1996**, *68*, 1292.
(236) Clarke, W. L.; Cox, D.; Gonder-Frederick, L. A.; Carter, W.; Pohl, S. L. *Diabetes Care* **1987**, *10*, 622.
(237) Garg, S. K.; Potts, R. O.; Ackerman, N. R.; Fermi, S. J.; Tamada, J. A.; Chase, H. P. *Diabetes Care* **1999**, *22*, 1708.
(238) Kovatchev, B. P.; Gonder-Frederick, L. A.; Cox, D. J.; Clarke, W. L. *Diabetes Care* **2004**, *27*, 1922.
(239) Buckingham, B.; Block, J.; Burdick, J.; Kalajian, A.; Kollman, C.; Choy, M.; Wilson Darrell, M.; Chase, P. *Diabetes Technol. Ther.* **2005**, *7*, 440.
(240) Tamborlane, W. V. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 815.
(241) Evans, M. L.; McCrimmon, R. J.; Flanagan, D. E.; Keshavarz, T.; Fan, X.; McNay, E. C.; Jacob, R. J.; Sherwin, R. S. *Diabetes* **2004**, *53*, 2542.
(242) Bell, D. S. *Diabetes, Obes., Metab.* **2006**, *8*, 261.
(243) Kondepati, V. R.; Heise, H. M. *Anal. Bioanal. Chem.* **2007**, *388*, 545.
(244) Avshalumov, M. V.; Bao, L.; Patel, J. C.; Rice, M. E. *Antioxid. Redox Signaling* **2007**, *9*, 219.
(245) Sidlo, Z.; Reggio, P. H.; Rice, M. E. *Neurochem. Int.* **2008**, *52*, 80.
(246) Noyes, K.; Liu, H.; Li, Y.; Holloway, R.; Dick, A. W. *Mov. Disord.* **2006**, *21*, 362.
(247) Huntington, G. *On Chorea* **1872**, *26*, 789.
(248) Barbeau, A.; Duvoisin, R. C.; Gerstenbrand, F.; Lakke, J. P.; Marsden, C. D.; Stern, G. *J. Neurol. Sci.* **1981**, *51*, 311.
(249) The Huntington's Disease Collaborative Research Group. *Cell* **1993**, *72*, 971.
(250) Mangiarini, L.; Sathasivam, K.; Seller, M.; Cozens, B.; Harper, A.; Hetherington, C.; Lawton, M.; Trottier, Y.; Lehrach, H.; Davies, S. W.; Bates, G. P. *Cell* **1996**, *87*, 493.
(251) Nakazato, T.; Akiyama, A. *J. Neurochem.* **1997**, *69*, 2039.
(252) Mason, S. T.; Sanberg, P. R.; Fibiger, H. C. *Science* **1978**, *201*, 352.
(253) Mason, S. T.; Sanberg, P. R.; Fibiger, H. C. *Life Sci.* **1978**, *22*, 451.
(254) Nakazato, T.; Akiyama, A. *Biog. Amines* **1988**, *5*, 339.
(255) Braszko, J. J.; Bannon, M. J.; Bunney, B. S.; Roth, R. H. *J. Pharmacol. Exp. Ther.* **1981**, *216*, 289.
(256) Reid, M. S.; Herrera-Marschitz, M.; Hokfelt, T.; Lindefors, N.; Persson, H.; Ungerstedt, U. *Exp. Brain Res.* **1990**, *82*, 293.
(257) Naudon, L.; Dourmap, N.; Leroux-Nicollet, I.; Costentin, J. *Brain Res.* **1992**, *572*, 247.
(258) Santiago, M.; Westerink, B. H. *Neurochem. Int.* **1992**, *20 Suppl*, 107S.
(259) Johnson, M. A.; Rajan, V.; Miller, C. E.; Wightman, R. M. *J. Neurochem.* **2006**, *97*, 737.
(260) Johnson, M. A.; Villanueva, M.; Haynes, C. L.; Seipel, A. T.; Buhler, L. A.; Wightman, R. M. *J. Neurochem.*, accepted.
(261) Rebec, G. V.; Barton, S. J.; Ennis, M. D. *J. Neurosci.* **2002**, *22*, RC202.
(262) Rebec, G. V.; Barton, S. J.; Marseilles, A. M.; Collins, K. *Neuroreport* **2003**, *14*, 1263.
(263) Rebec, G. V.; Conroy, S. K.; Barton, S. J. *Neuroscience* **2006**, *137*, 327.
(264) Moore, D. J.; West, A. B.; Dawson, V. L.; Dawson, T. M. *Annu. Rev. Neurosci.* **2005**, *28*, 57.
(265) Kandel, E. R.; Schwartz, J. H.; Jessell, T. M. *Principles of Neural Science*, 4th ed.; McGraw-Hill: New York, 2000.
(266) Hodaie, M.; Neimat, J. S.; Lozano, A. M. *Neurosurgery* **2007**, *60*, 17.
(267) Wesemann, W. *J. Neural Transm.* **1992**, *38*, 45.
(268) Gerlach, M.; van den Buuse, M.; Blaha, C.; Bremen, D.; Riederer, P. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2004**, *370*, 388.
(269) Wesemann, W.; Solbach, M.; Nafe, R.; Grote, C.; Sontag, K. H.; Riederer, P.; Jellinger, K.; Mennel, H. D.; Clement, H. W. *J. Neural. Transm. Suppl.* **1995**, *46*, 175.
(270) De Leonibus, E.; Pascucci, T.; Lopez, S.; Oliverio, A.; Amalric, M.; Mele, A. *Psychopharmacology (Berlin, Ger.)* **2007**, 517.
(271) Garris, P. A.; Walker, Q. D.; Wightman, R. M. *Brain Res.* **1997**, *753*, 225.
(272) Cragg, S. J.; Clarke, D. J.; Greenfield, S. A. *Exp. Neurol.* **2000**, *164*, 145.
(273) Lange, K. W.; Kornhuber, J.; Riederer, P. *Neurosci. Biobehav. Rev.* **1997**, *21*, 393.
(274) Blandini, F.; Porter, R. H.; Greenamyre, J. T. *Mol. Neurobiol.* **1996**, *12*, 73.
(275) Benabid, A. L.; Pollak, P.; Seigneuret, E.; Hoffmann, D.; Gay, E.; Perret, J. *Acta Neurochir. Suppl. (Wien.)* **1993**, *58*, 39.
(276) Benabid, A. L.; Koudsie, A.; Benazzouz, A.; Fraix, V.; Ashraf, A.; Le Bas, J. F.; Chabardes, S.; Pollak, P. *Arch. Med. Res.* **2000**, *31*, 282.
(277) Vercueil, L.; Benazzouz, A.; Deransart, C.; Bressand, K.; Marescaux, C.; Depaulis, A.; Benabid, A. L. *Epilepsy Res.* **1998**, *31*, 39.
(278) Benazzouz, A.; Piallat, B.; Pollak, P.; Benabid, A. L. *Neurosci. Lett.* **1995**, *189*, 77.
(279) Lee, K. H.; Roberts, D. W.; Kim, U. *Stereotact Funct. Neurosurg.* **2003**, *80*, 32.
(280) Lee, K. H.; Chang, S. Y.; Roberts, D. W.; Kim, U. *J. Neurosurg.* **2004**, *101*, 511.
(281) Garcia, L.; Audin, J.; D'Alessandro, G.; Bioulac, B.; Hammond, C. *J. Neurosci.* **2003**, *23*, 8743.
(282) Anderson, T.; Hu, B.; Pittman, Q.; Kiss, Z. H. *J. Physiol.* **2004**, *559*, 301.
(283) Dutta, A. K.; Sabirov, R. Z.; Uramoto, H.; Okada, Y. *J. Physiol. (Oxford, U.K.)* **2004**, *559*, 799.
(284) Marzouk, S. A. M.; Buck, R. P.; Dunlap, L. A.; Johnson, T. A.; Cascio, W. E. *Anal. Biochem.* **2002**, *308*, 52.

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