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A New Trend on Biosensor for Neurotransmitter Choline/Acetylcholine—an Overview

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Abstract Technology always has been an indispensible part in the development of biosensors. The performance of biosensors is being tremendously improved using new materials as transducer as well as binding material in their construction. The use of new materials allowed innovation on transduction technology in biosensor preparations. Because of the submicron dimensions of these sensors, simple and rapid analyses in vitro as well as in vivo are now possible. Portable instruments capable of analysing multiple components are becoming available, too. Sensors that provide excellent temporal and spatial resolution for in vivo monitoring such as for measurement of neurotransmitters have become prominent. The interest to improve the stability, sensitivity and selectivity of the sensors is paramount. This study tries to give an overview of the present status of the material-based biosensor design and new generation of choline/acetylcholine neurotransmitter biosensors.

Keywords Neurotransmitter · Biosensor · Acetylcholine · Choline · Amperometry

Introduction

Biosensors have been under development for over 20 years, and research in this field has yet to slow down. Of all types of biosensors, the electrochemical version is the most studied, oldest and successful in the marketplace. An example of these is the glucose sensor, which uses glucose oxidase as the mediator, and the amount of analyte (glucose) is measured

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amperometrically. However, in perspective, biosensor research needs some rethinking especially in relation to public expectations, funding and marketability.

Neurotransmitters are chemicals that transmit signals from one neuron to the next across synapses. These chemicals are produced in pituitary and adrenal glands and are normally found at the axon endings of motor neurons in muscle fibres. Neurotransmitters are synthesised from simple precursors, such as amino acids, which are readily available from the diet and which require a small number of biosynthetic steps to convert. There are many types of neurotransmitters, and they can be classified as amino acids, peptides and monoamines. Over 50 neuroactive peptides have been found, and new ones are discovered regularly.

In recent years, neurochemical measurements have led to much improvement in our understanding of the relationship between chemistry in the central nervous system (CNS) and the behavioural and emotional state of an organism. Abnormal neurotransmission is linked to a wide range of conditions including depression [1], drug dependence [2], schizophrenia [3] and degenerative diseases [4]. In vivo dynamic measurements of neurotransmitters in the extracellular space of the CNS have been important in the study of these diseases. Making such measurements is fraught with difficulty because of the complex and delicate tissue, requirements for stable measurements with high selectivity, temporal and spatial resolutions, and difficult interpretations of data. Thus, these are the challenges that exist. While progress has been done in such measurements, the techniques used to measure these neural messengers are still limited in their ability to measure accurately and rapidly in heterogeneous changes that occur in the extracellular space of the CNS [5]. Neurotransmitter content and effect of different drugs on release are also studied in vitro through analysis of cells in culture and ex vivo tissue preparations such as brain slices.

The techniques used for measurement of neurotransmitters include microelectrodes, biosensors, liquid chromatography, capillary electrophoresis separations and mass spectrometry (MS). For measuring dynamics of neurotransmitters, microelectrodes and biosensors may be directly used.

A minority of neurotransmitters can be detected by direct redox activity at an electrode [6]. Measurement often complicates by interference from other electroactive neurotransmitters and high concentration of electroactive metabolites such as ascorbic acid and electrode fouling [7–10]. These electrochemical techniques may experience background drift and require long periods for the sample to accumulate on the sensor, both of which limit the length and frequency of their monitoring periods. There are only a few biosensors or microelectrodes that are capable of detecting more than one analyte at any given time. This review tries to address issues on ways to minimise the limitations of the common techniques for monitoring neurotransmitters. It covers the gathering of types, method developments and fabrication for in vivo and in vitro measurements. Recent novel developments on techniques used for tissue analysis are also cited.

Designs of Biosensors for the Brain

Biosensors are powerful analytical tools whose range of applications in medical diagnostics [11], food quality control [12] and environmental monitoring [13] is rapidly expanding. The design of an electrochemical biosensor started with the target analyte and then choice of appropriate biological element, e.g. L-glutamate detection has been achieved using glutamate receptor ion channels, glutamate oxidase [14] or glutamate dehydrogenase [15] and finally the subsequent electrochemical processes. The vast majority of enzyme-based amperometric

biosensors exploit the biocatalytic oxidation of analyte by oxidase enzymes containing the prosthetic group, flavin adenine dinucleotide (FAD). With this common foundation, a wide range of oxidase-based devices have been designed making use of different signal transduction mechanisms and diverse selectivity strategies [16–19].

Another key factor shaping the biosensor design is the nature of the intended application matrix. Devices for monitoring the dynamic analyte concentration in vivo must be biocompatible over the time course of the implantation, both in terms of tissue effects on sensor functionality and physiological reaction to the probe [20, 21]. In addition to possessing the appropriate level of biocompatibility, implantable oxidase-based biosensors must also fulfil the following minimum criteria for reliable analyte monitoring, viz. appropriate size and geometry [22, 23], good sensitivity to the enzyme substrate [24], effective rejection of electroactive interference [25, 26] and low sensitivity to changes in oxygen partial pressure (pO_2) over the range of substrate and oxygen concentrations relevant to the intended application [27]. Even different tissues present distinct challenges; for example, biosensors designed to monitor glucose in blood or subcutaneous fat in vivo need a significantly greater oxygen tolerance compared to one for monitoring brain extracellular fluid (ECF) [28]. This is because glucose levels are an order of magnitude higher in these peripheral tissues than in brain ECF [29].

Nevertheless, various types of biosensor designs have been developed and applied to monitor concentration dynamics of specific brain analytes in vitro and in vivo. Among the first to be reported were those based on organic metals tetrathiafulvalene tetracyanoquinodimethane (TCNQ) [30, 31]. Although initially thought to involve direct electron transfer between the FAD centre and this novel conducting surface, there is now evidence that signal generation is only mediated by TCNQ [32, 33]. It is interesting to note that one of these seminal studies applied the concept of a blank sensor (one containing all the design elements of the biosensor but omitting the enzyme) implanted beside the biosensor in order to minimise electroactive interference, using subtraction. This strategy was necessary because interference by ascorbic acid (AA), archetypal electroactive species that contaminates biosensor signals in vivo, can occur even at the low anodic potentials used for this class of biosensor [34]. The use of blank sensors coupled with biosensors in difference mode is now seen as a vital component of reliable brain monitoring [35–40], although its (blank sensor) use does not mitigate the problem of oxygen dependence of oxidase-based signals. A more common design of biosensor used for analysis of brain matrices (including brain slices in vitro and intact tissue in vivo) incorporates redox hydrogels, e.g. osmium poly(vinyl pyridine) redox polymer [41–43]. This biosensor design has also been implanted in peripheral tissues [44]. The osmium-gel-horseradish peroxidase (Os-gel-HRP) provides electron transfer mediated by the HRP, which reacts with the H_2O_2 generated in the electrode processes at mild cathodic applied potentials. Such devices may also incorporate ascorbic acid oxidase and Nafion to further reduce interference by AA. Neurochemical studies in vivo, using these probes, however, have been limited to acute implantation in anaesthetised preparations. It is still presently unclear at present whether this class of biosensor is stable enough to allow long-term brain monitoring in freely behaving animals.

Another important aspect of in vivo monitoring is the presence of anaesthetic, which may cause different and sometimes adverse effects on neurochemical dynamics. Thus, for example, it is unwise to test the glutamate/ascorbate heteroexchange hypothesis in chloral hydrate anaesthetised rats, using this class of biosensor, when it has been demonstrated explicitly that chloral hydrate blocks this exchange [45]. A ceramic-based biosensor has recently been implanted successfully for recording a chronic brain of unanaesthetised animals reveals that the anion-rejecting properties of Nafion are the main strategy for reducing electroactive interference [46], in addition to a blank or sentinel sensors to increase

further the selectivity of the detection system. The biosensor signal is generated by direct oxidation of H_2O_2 on the electrode surface at a relatively high applied anodic potential. Studies carried out over 7 days showed that there was little loss of the sensitivity of the implanted differential device, which indicates a promising future for this approach. However, an issue that needs to be addressed before widespread applications are undertaken is the oxygen dependence of these biosensors, which has yet to be reported.

A variation on this design is the incorporation of additional polymer layers deposited over a platinum substrate that has been applied for acute recording of brain glucose [47], lactate [48] and glutamate. The use of poly-*ortho*-phenylenediamine (PoPD) as a permselective membrane in the fabrication of implantable sensors and biosensors has been reported by a number of laboratories [49–54]. Indeed, PoPD has been used in the construction of biosensors for a range analytes in different target media [55–61]. The PoPD can be deposited electrochemically from *o*-phenylenediamine solutions at neutral pH to produce a thin, self-sealing, insulating polymer on the electrode surface. The estimations of film thickness for PoPD generated under these conditions are typically in the region of 10–30 nm [62–64]. In addition to possessing excellent interference rejection characteristics, PoPD also serves to immobilise enzymes and protects the electrode surface from fouling, making it ideal for biosensor applications in vivo. The signal in this design is also generated by the direct oxidation of H_2O_2 on the electrode surface at relatively high applied anodic potentials.

The main advantage of PoPD-based glucose biosensors is their stability, even during amperometric recording over days in freely behaving animals [61, 62]. Even for the pO_2 dependence glucose [65, 66] and glutamate sensors [67], the quantification has been shown to be adequate for brain monitoring. Each design of biosensor for brain monitoring has its own benefit, e.g. the small cross section of Os-gel-HRP-based carbon fibre electrodes [68], the self-referencing capability of the ceramic-based electrodes and the overall long-term stability and oxygen tolerance of PoPD-based biosensors, which make them attractive for further development [69, 70].

Acetylcholine

Acetylcholine (ACh) was the first neurotransmitter to be discovered. It was isolated in 1921 by a German biologist named Otto Loewi, who later won the Nobel Prize for his work. ACh has many functions. It is responsible for much of the stimulation of muscles, including the muscles of the gastrointestinal system. It is also found in sensory neurons and in the autonomic nervous system and has a part in scheduling rapid eye movement in sleep.

The curare and hemlock poison plants cause paralysis to humans due to the blocking of ACh receptor sites of muscle cells. Others like the well-known botulin also cause paralysis by preventing the vesicles in the axon ending from releasing ACh. Unfortunately, botox, a botulin derivative, is used, rampantly, by many to temporarily eliminate wrinkles. There is a link between ACh with Alzheimer's disease as about 90 % loss of ACh is found in the brains of people suffering from Alzheimer's disease.

Methods for the detection of neurotransmitters have been developed, such as biochemical analysis using radioactive labels [71, 72], HPLC [73–75] and microdialysis with electrochemical detection [76, 77]. The main drawbacks of these methods are their poor temporal and spatial resolutions and the complexity of the accompanying technical set-up. The use of implantable microbiosensors may overcome these disadvantages, with carbon fibre-based electrodes looking especially promising [78–84]. Design and schematic diagram for carbon fibre microelectrode are shown in Fig. 1.

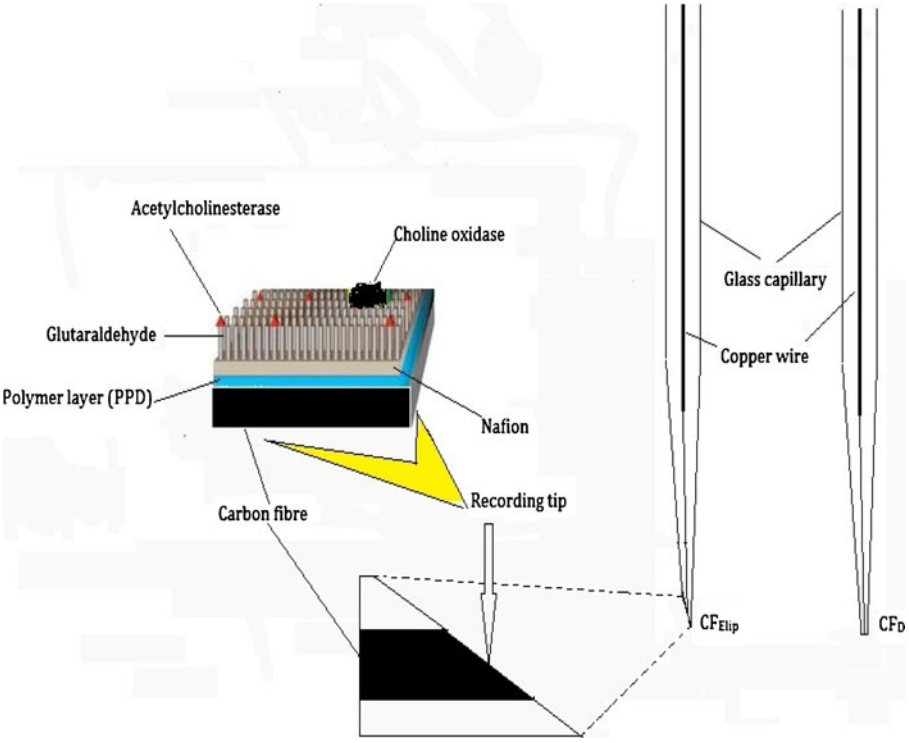

Fig. 1 Model of the carbon fibre microelectrode with immobilised biomolecules

The present-day ACh biosensor usually contains two enzymes, viz. acetylcholinesterase (AChE) and choline oxidase (ChOx) functioning through sequential biochemical reactions [79, 85, 86]. The use of AChE ensures the conversion of acetylcholine into choline (Ch) and acetate, while ChOx converts choline into betaine and H_2O_2. Hydrogen peroxide is an electrochemically active product that can be detected using electrodes constructed from carbon fibre, which offers biocompatibility and availability at micrometric dimensions (7–30 μm). However, the abovementioned biosensors would detect two substrates, i.e. ACh and Ch. Hence, the selective detection of ACh requires the use of two biosensors, one based on AChE and ChOx and the other on ChOx alone. Thus, the difference of signal between the two sensors relates to the ACh concentration.

Considerable interest has been devoted to the in vitro and in vivo determination of ACh and Ch, in connection with neuropsychological and neuropsychiatric disorders such as Alzheimer's disease and progressive dementia [87]. Unfortunately, ACh and Ch are neither oxidisable nor possess structural characteristics (e.g. chromophore groups) allowing a sensitive detection. Thus, the majority of the methods developed for their determination require a conversion into more easily detectable compounds, usually as its derivative. Several methods, including bio- or radiometric assay and gas chromatography (GC) and mass spectrometry (MS), have been developed for ACh and/or Ch determination in biological tissues and fluids [88, 89]. HPLC coupled to post-column enzymic reaction (PER) with AChE and ChOx is, by far, the most widely used method [90]. Hydrogen peroxide, released by the enzymatic reaction, is detected either by chemiluminescence or, more usually, by electrochemical detection (ECD) at a platinum electrode. Notwithstanding some improvements [91–93] achieved for example by

enzyme immobilisation in packed bed reactors, HPLC-PER-ECD methods still suffer from some drawbacks [94–96]. Amperometric biosensors, in which the enzymatic conversion and the electrochemical detection of hydrogen peroxide occur in the same physical device, could represent a valid alternative to PER-ECD. Several ACh/Ch amperometric biosensors with ChOx/AChE enzymes immobilised on discrete membranes [97–101], entrapped in photo-cross-linkable polymers [102], cross-linked redox polymers [103] and Nafion [104] have been described. Unfortunately, most of these sensors are useless in HPLC detection because they cannot satisfy certain requirements such as response time, sensitivity and adaptability to the cell geometry usually employed in HPLC detectors.

The development of methods for rapid determination of neurotransmitters and their precursors was a challenging goal for many years. This is so since they provide valuable information for the characteristic dynamic rather than static levels of neurotransmitters. ACh, the main neurotransmitter in both the peripheral and CNS, is found in mammalian brain tissues. Despite intensive research, the roles of neurotransmitters in the CNS are much less delineated although it is generally considered that ACh is involved in temperature and blood pressure regulation, motor coordination, learning, memory, etc. [105]. Furthermore, ACh and Ch levels in the brain are very important due to the significant role in the neuropsychiatric diseases [106–110]. Ch serves both as the precursor and metabolite of ACh.

Numerous chromatographic methods have been described for the simultaneous measurement of Ch and ACh [111]. This also includes hyphenated GC-MS technique with time-consuming clean-up and derivatisation procedures [112]. Although HPLC-MS or HPLC-MS-MS requires minimum sample preparation and with no derivatisation, it had relatively inadequate quantitation limits [113–116]. HPLC-ECD with immobilised AChE and ChOx reactors as column reactor [117, 118] or with co-immobilised AChE/ChOx bienzyme electrode [119] was suited for the sensitive determinations of Ch and ACh. The detection limits and samples throughout have been reported to be 1 and 3 nM for Ch and ACh, respectively, and 3/h. The slower flow rate is preferred to the conversion efficiencies for the analytes to H_2O_2 and to the sensitive detection of the H_2O_2, but the decreased sample throughout is undesirable to follow minutely the dynamics of ACh and Ch. For the rapid assays of Ch or ACh, flow injection analysis method is used coupled with immobilised ChOx or co-immobilised AChE/ChOx [96, 120–123]. A split stream system with chemiluminometric detection using three immobilised enzyme column reactors, such as an immobilised ChOx, an immobilised catalase and a co-immobilised AChE/ChOx and using ferricyanide-catalyzed luminol reaction, have also been developed for simultaneous determination of Ch and ACh [124]. In a split stream system with column reactors, generally, sample plugs from the reactors disperse into the carrier stream and are, moreover, diluted with the carrier solution from other streams; the resulting peak broadening causes lowering of sensitivity and sample throughput. Flow-through sensors based on the integration of conversion reaction and detection reaction in a flow cell have been designed to solve the problems [125–127]. Chemiluminometric biosensors for simultaneous determination of oxidase substrates on the basis of integration of oxidase reactions and peroxidase-catalyzed H_2O_2/luminol detection reactions, which involved no separation process, have been recently proposed [128–130]. This is possibly another challenge to get a rapid and sensitive sensor for the simultaneous determination of ACh and Ch without separation of the analytes. In this work, four kinds of immobilised enzymes were packed into a polyethylene tetrafluoroethylene or Teflon® tube. ChOx and peroxidase were co-immobilised on hydrophilic vinyl polymer beads to obtain co-immobilised enzymes. Types of biosensor and their detection limit are given in Table 1 [131–145].

Modern Technology, CNiFERs and Biosensor

Identifying the neural basis of behaviour is the main focus of neuroscience. One prominent methodology for this is monitoring the neurotransmitters that underlie communication between neurons. In this regards, Clark et al. [146] describe a microelectrode that retains the capability for sub-second dopamine measurements.

Nguyen et al. [147] used these recent advances in biosensing to develop electrode-free, non-invasive methodology for monitoring ACh. In their cell-based reporters, called as CNiFERs, that is a clever twist of both the moniker and the approach of the original 'sniffer pipette' detection is also based on cholinergic receptors, in this case, a metabotropic receptor. CNiFERs are an immortal cell line genetically engineered to express a fluorescent Ca^{2+} protein sensor and the M1 muscarinic receptor. Binding of acetylcholine initiates a biochemical cascade involving a G protein, phospholipase C and the second messenger inositol trisphosphate, ultimately leading to increased intracellular Ca^{2+} levels and altered fluorescence. When chronically implanted in the rat cortex and monitored by two-photon laser scanning microscopy, CNiFERs robustly respond to basal and electrically evoked levels of acetylcholine for up to 6 days [147]. Suggestive of a physiological role, measured signals temporally coincided with the electrophysiological response recorded locally. Most importantly, there was minimal tissue damage at the implantation site, as indicated by few reactive astrocytes, no cell proliferation and an intact vasculature. What does the future hold for these new neurotransmitter sensing technologies? As it is already coupled to an established monitoring technique, the new CFM developed by Clark et al. [146] appears poised for immediate applications. The small shaft size compared to the conventional CFM (80 μm vs ~1 mm) permits implantation of several probes in a single animal for concurrent assessment of regional differences in dopamine dynamics. Incorporating telemetry into the probe design would also remove the restriction of the cable tether linking animal and recording equipment. CNiFERs developed by Nguyen et al. [147] are at a more nascent stage. As the monitoring scheme based on G protein-coupled receptors is common to most neurotransmitters, this methodology holds great promise for broad applications in neurochemical measurement in vivo. Extending these cell-based measurements to deep brain structures and to awake, especially freely behaving animals, would be very attractive as well.

Biosensors, which use a 'biological recognition element' to interact with an analyte, have been developed for monitoring non-electroactive neurotransmitters that go undetected with voltammetry [148]. A most important design for such biosensors incorporates an oxidase enzyme generating the electroactive hydrogen peroxide.

One of the earliest biosensors was the sniffer pipette, constructed from an excised membrane patch containing ionotropic cholinergic receptors [149]. Binding of acetylcholine, a neurotransmitter, was implicated in cognitive processes and schizophrenia.

CNiFERs have high selectivity for a given neurotransmitter, but their response adapts on exposure to a constant concentration of agonist. This appeared as a phasic response that decayed to a persistent, tonic level over ~102 s. The phasic component did not depend on the external $[Ca^{2+}]$ and was consistent with a Ca^{2+} flux derived from the endoplasmic reticulum as part of IP3 receptor activation in the Gq protein cascade [150]. The transition from the larger amplitude of the phasic response to the smaller tonic response can result from a decrement in signalling at any point in the muscarinic Gq protein cascade. Past work implies that there is little desensitisation or internalisation of the M1 receptor [151]. In contrast, $[Ca^{2+}]$ mirrors [IP3] and IP3 signalling exhibits both phasic and tonic components [152], similar to the components that we observed with CNiFERs. Thus, a rate-limiting step in the generation of IP3, such as the availability of the intermediate phosphatidylinositol 4,5-

Table 1 Some biosensors for acetylcholine and choline and there detection limits

Sample number	Electrode	Targeted neurotransmitter	Detection limit	References
1	Carbon fibre	Choline (Ch)	6.1–2.7 mm	[131]
2	Platinum iridium	ACh and Ch	0.66–0.46 pm ACh and 0.33–0.09 pm Ch	[132]
3	Platinum working electrode	ACh (acetylcholine) and Ch	Ca. 100 nm for both Ach and Ch at the Ch–AChE electrode and ca. 40 nm for ACh at the Ch	[133]
4	Carbon fibre electrode	ACh and Ch	1 μm	[134]
5	Platinum disc electrode	ACh and Ch	0.1 ppb	[135]
6	Platinum electrode (amperometric biosensor)	ACh and Ch	12 and 27 fmol for Ch and ACh	[117]
7	Glassy carbon electrode	ACh	8.7 mmol/l	[136]
8	Platinum electrode	ACh and Ch	0.0×10^{-7}m for ACh and 5.0×10^{-7}m Ch	[137]
9	Mesoporous silica membranes	ACh	5.2 μm	[138]
10	Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS)	ACh and Ch	0.3 fmol/μl for ACh and 20 fmol/μl for Ch	[139]
11	Using ion-pair liquid chromatography with amperometric detection	ACh	0.40±0.12 nM	[140]
12	Nanoneedle based on multi-walled carbon nanotube	Dopamine and glutamate	Dopamine 100–1,000 pm by differential pulse voltammetry, and enzyme-modified nanoneedles were able to respond to glutamate in the 100–500-pM range by potentiostatic amperometry	[141]
13	Platinum microelectrode	ACh and Ch	63.0±3.4 nM for ACh and 25.0±1.2 nM for Ch	[142]
14	Trienzyme chemiluminometric biosensor, single line flow injection (FI) system	ACh and Ch	1–1,000 nM for Ch and 3–3,000 nM for ACh	[143]
15	Platinum electrode (Amperometric Sensor)	ACh and Ch	Detection limits were in the sub-micromolar range	[144]
16	Electrochemiluminescence (ECL) cadmium sulphide nanocrystals (CdS NCs) on the surface of multi-walled carbon nanotubes (MWCNTs)	Ch and ACh	0.8 and 1.7 mM	[145]

bisphosphate [153], may explain the adaptation of the CNiFERs. A complementary explanation for the adaptation is that the tonic response is maintained via calcium flux through the cytoplasmic membrane rather than internal calcium stores, as evidenced by the abolishment of the tonic signal in calcium-free media.

CNiFERs were used for the measurement of neurotransmitter that is not possible using microdialysis, electrochemistry or radioisotope tracers. CNiFERs indirectly report neurotransmitter release, but directly report receptor subtype activity. They provide a general approach for observing in-situ activation of G protein-coupled receptors by small molecules and peptides in all living animals. CNiFERs for Gq protein-coupled receptors, such as serotonin and prostaglandins, can be engineered as an immediate extension of the M1-CNiFERs and their Ca^{2+}-based response. On the other hand, CNiFERs for Gs and Gi/o protein-coupled receptors, such as those for the peptide transmitter's vasoactive intestinal peptide and somatostatin, respectively, can be based on changes in the concentration of cAMP. It is detected via a genetically encoded indicator for the activation of protein kinase A [154]. Alternatively, an endogenous indicator for Ca^{2+} can be used if this coexpressed with the promiscuous Gα1639 or Gα chimeras [155]. These G proteins allow receptors not normally linked with the Gq pathway to elicit cytosolic Ca^{2+}. Thus, CNiFERs may be used to detect any signalling molecule, of which neurotransmitters are a broad and important class that activates a G protein-coupled receptor.

Technical improvements have advanced neurochemical measurement to the real-time domain. One critical limitation of present methods is the highly invasive nature of implanting a recording device. It causes reaction to the brain tissue and neuro-inflammation. Because of this, sampled microenvironment altered and results in a diffusion barrier that encapsulates the probe and therefore restricts access to released neurotransmitters. Using radically different strategies, new approaches (CNiFERs) address this key hurdle for achieving the longstanding goal of chronic, real-time neurochemical monitoring.

Economic Values

The purpose of a biosensor is to obtain a number that can be relied on to make a decision. Why are so many people working on developing sensors for analytes where no sensor is needed or desired? Quite often there are alternative methodologies that are much cheaper per data point, much more reliable, specific and already commercially available in stable form, at very low prices relative to the high data rate, automation and analyte flexibility available. Examples include chromatography, MS and immunoassays. Some have even gone to Mars, but would not come back. It is fair to conclude that much biosensor work is done because ‘it can be done’ and not because ‘it needs to be done’. We have differences between the non-profit academic world and the taxpaying commercial world in attitude about labour vs equipment. In academics, we view labour as cheap and equipment as expensive. In the commercial world, it is the other way around. The widely mistaken view is that this has something to do with relative salaries in these different worlds. This is not the case except to a very small degree. This perception primarily comes from the fact that labour and physical overhead (buildings, electricity, vacations, medical expense, retirement plans, etc.) in academia are frequently paid for by taxpayers, students, endowments and grants, whereas labour in industry must pay taxes. It also derives from the concept of depreciation expense, which chemistry professors and government scientists do not always worry about.

An enormous amount of talented human capital is perhaps wasted by the way grants are awarded, unless we consider academic research only as a means to train students. This leads

to a focus on what looks like exceptionally low-cost science, such as biosensors, which in reality can be very high-cost science because much of it is not needed at all. This is slowly being corrected as we move more in the direction of academic centre grants, collaborations and general cost sharing of major instrumentations.

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References

1. Clausius, N., Born, C., & Grunze, H. (2009). *Neuropsychiatrie, 23*, 15–25.
2. Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). *Current Opinion in Pharmacology, 9*, 65–73.
3. Gunduz-Bruce, H. (2009). *Brain Research Reviews, 60*, 279–286.
4. Lewis, S. J. G., & Barker, R. A. (2009). *Journal of Clinical Neuroscience, 16*, 620–625.
5. Schultz, K. N., & Kennedy, R. T. (2008). *Annual Review of Analytical Chemistry, 1*, 627–661.
6. Wightman, R. M. (2006). *Science, 311*, 1570–1574.
7. Alwarappan, S., Erdem, A., Liu, C., & Li, C. Z. (2009). *Journal of Physical Chemistry, 113*, 8853–8857.
8. Alwarappan, S., Liu, G., & Li, C. Z. (2010). *Nanomedicine: Nanotechnology, Biology, and Medicine, 6*, e52–e57.
9. Alwarappan, S., Prabhulkar, S., Durygin, A., & Li, C. Z. (2009). *Journal of Nanoscience and Nanotechnology, 9*, 2991–2996.
10. Alwarappan, S., Butcher, K. S. A., & Wong, D. K. Y. (2007). *Sensors and Actuators, B, 128*, 299–305.
11. Hirano, A., & Sugawara, M. (2006). *Mini Reviews in Medicinal Chemistry, 6*, 1091–1100.
12. Andreu, V., Blasco, C., & Pico, Y. (2007). *Trends in Analytical Chemistry, 26*, 534–556.
13. Rodriguez-Mozaz, S., de Alda, M. J. L., & Barcelo, D. (2006). *Analytical and Bioanalytical Chemistry, 386*, 1025–1041.
14. Hamdi, N., Wang, J. J., Walker, E., Maidment, N. T., & Monbouquette, H. G. (2006). *Journal of Electroanalytical Chemistry, 591*, 33–40.
15. Chakraborty, S., & Raj, C. R. (2009). *Electrochemistry Communications, 9*, 1323–1330.
16. Ahuja, T., Mir, I. A., Kumar, D., & Rajesh. (2007). *Biomaterials, 28*, 791–805.
17. Lojou, E., & Bianco, P. (2006). *Journal of Electroceramics, 16*, 79–91.
18. Wilson, G. S., & Hu, Y. B. (2000). *Chemical Reviews, 100*, 2693–2704.
19. O'Neill, R.D. (1993). Analyst (Cambridge, U.K.,) 118, 433–438.
20. Ahmad, F., Christenson, A., Bainbridge, M., Yusof, A. P. M., & Ghani, S. A. (2007). *Biosensors and Bioelectronics, 22*, 1625–1632.
21. McMahon, C. P., & O'Neill, R. D. (2005). *Analytical Chemistry, 77*, 1196–1199.
22. Rutherford, E. C., Pomerleau, F., Huettl, P., Stromberg, I., & Gerhardt, G. A. (2007). *Journal of Neurochemistry, 102*, 712–722.
23. Koschwanez, H. E., & Reichert, W. M. (2007). *Biomaterials, 28*, 3687–3703.
24. Mitala, J. J., & Michael, A. C. (2006). *Analytica Chimica Acta, 556*, 326–332.
25. McAteer, K. & O'Neill, R.D. (1996) Analyst (Cambridge, U.K.) 121, 773–777.
26. Lin, L., & Guthrie, J. T. (2006). *Journal of Membrane Science, 278*, 173–180.
27. McMahon, C. P., Killoran, S. J., & O'Neill, R. D. (2005). *Journal of Electroanalytical Chemistry, 580*, 193–202.
28. Lowry, J. P., O'Neill, R. D., Boutelle, M. G., & Fillenz, M. (1998). *Journal of Neurochemistry, 70*, 391–396.
29. Boutelle, M. G., Stanford, C., Fillenz, M., Albery, W. J., & Bartlett, P. N. (1986). *Neuroscience Letters, 72*, 283–288.
30. Jung, C. C., & Hall, E. A. H. (1995). *Analytical Chemistry, 67*, 2393–2400.
31. Centonze, D., Losito, I., Malitesta, C., Palmisano, F., & Zambonin, P. G. (1997). *Journal of Electroanalytical Chemistry, 435*, 103–111.
32. Lowry, J. P., & O'Neill, R. D. (1992). *Journal of Electroanalytical Chemistry, 334*, 183–194.
33. Rocchitta, G. McMahon, C.P., Serra, P.A., Kirwan, S.M., Bolger, F.B., Lowry, J.P. and O'Neill, R.D. (2006). Monitoring molecules in neuroscience. In: Di Chiara, G., Carboni, E., Valentini, V., Acquas, E., Bassareo, V., Cadoni. C. (Ed.) Cagliari: University of Cagliari, p. 331.

34. Oldenziel, W. H., Dijkstra, G., Cremers, T. I. F. H., & Westerink, B. H. C. (2006). *Brain Research, 1118*, 34–42.
35. Garguilo, M. G., & Michael, A. C. (1996). *Journal of Neuroscience Methods, 70*, 73–82.
36. Hu, Y., Mitchell, K. M., Albahadily, F. N., Michaelis, E. K., & Wilson, G. S. (1994). *Brain Research, 659*, 117–125.
37. Burmeister, J. J., & Gerhardt, G. A. (2003). *Trends in Analytical Chemistry, 22*, 498–502.
38. Kulagina, N. V., Shankar, L., & Michael, A. C. (1999). *Analytical Chemistry, 71*, 5093–5100.
39. Oka, T., Tasaki, C., Sezaki, H., & Sugawara, M. (2007). *Analytical and Bioanalytical Chemistry, 388*, 1673–1679.
40. Oldenziel, W. H., Dijkstra, G., Cremers, T. I. F. H., & Westerink, B. H. C. (2006). *Analytical Chemistry, 78*, 3366–3378.
41. Kulagina, N. V., & Michael, A. C. (2003). *Analytical Chemistry, 75*, 4875–4881.
42. Schmidtke, D. W., & Heller, A. (1998). *Analytical Chemistry, 70*, 2149–2155.
43. O'Neill, R. D., Fillenz, M., Sundstrom, L., & Rawlins, J. N. P. (1984). *Neuroscience Letters, 52*, 227–233.
44. Bruno, J. P., Gash, C., Martin, B., Zmarowski, A., Pomerleau, F., Burmeister, J., Huettl, P., & Gerhardt, G. A. (2006). *European Journal of Neuroscience, 24*, 2749–2757.
45. Hu, Y. B., & Wilson, G. S. (1997). *Journal of Neurochemistry, 68*, 1745–1752.
46. Hu, Y. B., & Wilson, G. S. (1997). *Journal of Neurochemistry, 69*, 1484–1490.
47. Hocevar, S. B., Zivin, M., Milutinovic, A., Hawlina, M., Hutton, E. A., & Ogorevc, B. (2006). *Frontiers in Bioscience, 11*, 2782–2789.
48. Patel, B. A., Arundell, M., Parker, K. H., Yeoman, M. S., & Hare, D. O. (2006). *Analytical Chemistry, 78*, 7643–7648.
49. Schuvailo, O. M., Soldatkin, O. O., Lefebvre, A., Cespuglio, R., & Soldatkin, A. P. (2006). *Analytica Chimica Acta, 573*, 110–116.
50. Ferreira, N. R., Ledo, A., Frade, J. G., Gerhardt, G. A., Laranjinha, J., & Barbosa, R. M. (2005). *Analytica Chimica Acta, 535*, 1–7.
51. Dixon, B. M., Lowry, J. P., & O'Neill, R. D. (2002). *Journal of Neuroscience Methods, 119*, 135–142.
52. Fillenz, M., & Lowry, J. P. (1998). *Experimental Physiology, 83*, 233–238.
53. Kirwan, S. M., Rocchitta, G., McMahon, C. P., Craig, J. D., Killoran, S. J., O'Brien, K. B., Serra, P. A., Lowry, J. P., & O'Neill, R. D. (2007). *Sensors, 7*, 420–437.
54. Bartlett, P. N., Pletcher, D., & Zeng, J. (1997). *Journal of the Electrochemical Society, 144*, 3705–3710.
55. Cooper, J. M., Foreman, P. L., Glidle, A., Ling, T. W., & Pritchard, D. J. (1995). *Journal of Electroanalytical Chemistry, 388*, 143–149.
56. Wang, J., & Wu, H. (1993). *Analytica Chimica Acta, 283*, 683–688.
57. Malitesta, C., Palmisano, F., Torsi, L., & Zambonin, P. G. (1990). *Analytical Chemistry, 62*, 2735–2740.
58. Sasso, S. V., Pierce, R. J., Walla, R., & Yacynych, A. M. (1990). *Analytical Chemistry, 62*, 1111–1117.
59. Reyes-De-Corcuera, J. I., Cavalieri, R. P., Powers, J. R., Tang, J. M., & Kang, D. H. (2005). *Journal of Agricultural and Food Chemistry, 53*, 8866–8873.
60. Myler, S., Eaton, S., & Higson, S. P. J. (1997). *Analytica Chimica Acta, 357*, 55–61.
61. Ohnuki, Y., Matsuda, H., Ohsaka, T., & Oyama, N. (1983). *Journal of Electroanalytical Chemistry, 158*, 55–67.
62. Craig, J.D. and O'Neill, R.D. (2003) Analyst (Cambridge, U.K.) 128, 905–911.
63. Lowry, J. P., Miele, M., O'Neill, R. D., Boutelle, M. G., & Fillenz, M. (1998). *Journal of Neuroscience Methods, 79*, 65–74.
64. Lowry, J. P., McAteer, K., El Atrash, S. S., Duff, A., & O'Neill, R. D. (1994). *Analytical Chemistry, 66*, 1754–1761.
65. McMahon, C. P., Rocchitta, G., Serra, A. A., Kirwan, S. M., Lowry, J. P., & O'Neill, R. D. (2006). *Analytical Chemistry, 78*, 2352–2359.
66. Oldenziel, W. H., van der Zeyden, M., Dijkstra, G., Ghijsen, W. E. J. M., Karst, H., Cremers, T. I. F. H., & Westerink, B. H. C. (2007). *Journal of Neuroscience Methods, 160*, 37–44.
67. O'Brien, K. B., Killoran, S. J., O'Neill, R. D., & Lowry, J. P. (2007). *Biosensors and Bioelectronics, 22*, 2994–3000.
68. Rocchitta, G., Migheli, R., Dedola, S., Calia, G., Desole, M. S., Miele, E., Lowry, J. P., O'Neill, R. D., Serra, P. A., & Serra. (2007). *Sensors and Actuators B: Chemical, 126*, 700–709.
69. Serra, P. A., Rocchitta, G., Bazzu, G., Manca, A., Puggioni, G. M., Lowry, J. P., & O'Neill, R. D. (2007). *Sensors and Actuators B: Chemical, 122*, 118–126.
70. Killoran, S. J., Rai, D. K., & O'Neill, R. D. (2007). *Journal of Neurochemistry, 102*, 196–207.
71. Guyenet, P. G., Javory, A. F., Beaujouan, J. C., Rossier, B. J., & Glowinski, J. (1975). *Brain Research, 84*, 227–244.
72. Haubrich, D. R., Gerber, N., Pflueger, A. B., & Zweig, M. (1981). *Journal of Neurochemistry, 36*, 1409–1417.

73. Bullock, R., Butcher, S. P., Chen, M. H., Kendall, L., & McCulloch, J. (1991). *Journal of Neurosurgery, 74*, 794–802.
74. Murai, S., Saito, H., Masuda, Y., Itsukaichi, O., & Itoh, T. (1995). *Archives of Oral Biology, 40*, 663–668.
75. Izaki, Y., Hori, K., & Nomura, M. (1998). *Neuroscience Letters, 258*, 33–36.
76. Roisin, M. P., Brassart, J. L., Charton, G., Crepel, V., & Ben Ari, Y. (1991). *Journal of Neuroscience Methods, 37*, 183–189.
77. Yao, T. Y., & Handa, S. (2003). *Analytical Sciences, 19*, 767–770.
78. Martin, K. F., & Marsden, C. A. (1987). *Life Sciences, 41*, 865–868.
79. Tamiya, E., Sugiura, Y., Nepomuceno Navera, E., Mizoshita, S., Nakajima, K., Akiyama, A., & Karube, I. (1991). *Analytica Chimica Acta, 251*, 129–134.
80. Netchiporouk, L. I., Shram, N. F., Jaffrezic-Renault, N., Martelet, C., & Cespuglio, R. (1996). *Analytical Chemistry, 68*, 4358–4364.
81. Shram, N. F., Netchiporouk, L. I., Martelet, C., Jaffrezic-Renault, N., Bonnet, C., & Cespuglio, R. (1998). *Analytical Chemistry, 70*, 2618–2622.
82. McNally, M., & Wong, D. K. Y. (2001). *Analytical Chemistry, 73*, 4793–4800.
83. Venton, B. J., Troyer, K. P., & Wightman, R. M. (2002). *Analytical Chemistry, 74*, 539–546.
84. Rubianes, M. D., & Rivas, G. A. (2003). *Analytical Letters, 36*, 329–345.
85. Yao, T., Suzuki, S., Nishino, H., & Nakahara, T. (1995). *Electroanalysis, 7*, 1114–1117.
86. Larsson, N., Ruzgas, T., Gorton, L., Kokaia, M., Kissinger, P., & Csoregi, E. (1998). *Electrochimica Acta, 43*, 3541–3554.
87. Bowen, D. M., Smith, C. B., White, P., & Davison, A. N. (1976). *Brain, 99*, 459–496.
88. Wester, P., Eriksson, S., Forsell, A., Puu, G., & Adolfsson, R. (1988). *Acta Neurologica Scandinavica, 77*, 12–21.
89. Hanin, I. (1974). *Choline and acetylcholine: handbook of chemical assay methods*. New York: Raven.
90. Hanin, I. (1982). In S. Spector & N. Back (Eds.), *Modern methods in pharmacology* (pp. 29–38). New York: Alan R. Liss.
91. Potter, P. E., Meck, J. L., & Neff, N. H. (1983). *Journal of Neurochemistry, 41*, 188–194.
92. Meek, J. L., & Eva, C. (1984). *Journal of Chromatography, 317*, 343–347.
93. Tyrefors, N., & Gillberg, P. G. (1987). *Journal of Chromatography, 423*, 85–91.
94. Damsma, G., van Lammerts Bueren, G. D., Westerink, B. H. C., & Horn, A. S. (1987). *Chromatographia, 24*, 827–831.
95. Tyrefors, N., & Carlsson, A. (1990). *Journal of Chromatography, 502*, 337–349.
96. Gunaratna, P. C., & Wilson, G. S. (1990). *Analytical Chemistry, 62*, 402–407.
97. Haen, H., Hagenmaier, H., & Remien, J. (1991). *Journal of Chromatography, 537*, 514–519.
98. Mascini, M., & Moscone, D. (1986). *Analytica Chimica Acta, 179*, 439–444.
99. Marty, J. L., Sode, K., & Karube, I. (1990). *Analytica Chimica Acta, 228*, 49–53.
100. Morelis, R. M., & Coulet, P. R. (1990). *Analytica Chimica Acta, 231*, 27–32.
101. Campanella, L., Sammartino, M. P., & Tomasetti, M. (1991). *Annali di Chimica (Rome), 81*, 639–645.
102. Rouillon, R., Mionetto, N., & Marty, J. L. (1992). *Analytica Chimica Acta, 268*, 347–350.
103. Navera, E. N., Sode, K., Tamiya, E., & Karube, I. (1991). *Biosensors and Bioelectronics, 6*, 675–680.
104. Garguilo, M. G., Huynth, N., Proctor, A., & Michael, A. C. (1993). *Analytical Chemistry, 65*, 523–528.
105. Van der Zee, E. A., & Luiten, P. G. M. (1999). *Progress in Neurobiology, 58*, 409–471.
106. Boonyapisit, K., Kaminski, K. H. J., & Ruff, R. L. (1999). *American Journal of Medicine, 106*, 97–113.
107. Guan, Z. Z., Nordberg, A., Mousavi, M., Rinne, J. O., & Hellstron-Lindahl, E. (2002). *Brain Research, 956*, 358–366.
108. Wessler, I., Reinheimer, T., Kilbinger, H., Bittinger, F., Kirkpatrick, C. J., Saloga, J., & Knop, J. (2003). *Life Sciences, 72*, 2169–2172.
109. Coulson, F. R., & Fryer, A. D. (2003). *Pharmacology and Therapeutics, 98*, 59–69.
110. Nagele, R. G., D'andrea, M. R., Anderson, W. J., & Wang, H. Y. (2002). *Neuroscience, 110*(2002), 199–2011.
111. Tsai, T. H. (2000). *Journal of Chromatography B, 747*, 111–122.
112. Stavinoha, W. B., & Weintraub, S. T. (1974). *Analytical Chemistry, 46*, 757–760.
113. Ishimaru, H., Ikarashi, Y., & Maruyama, Y. (1993). *Biological Mass Spectrometry, 22*, 681–686.
114. Acevedo, L. D., Xu, Y. D., Zhang, X., Pearce, R. J., & Yergey, A. (1996). *Journal of Mass Spectrometry, 31*, 1399–1402.
115. Zhu, Y., Wong, P. S. H., Cregor, M., Gitzen, J. F., Coury, L. A., & Kissinger, P. A. (2000). *Rapid Communications in Mass Spectrometry, 14*, 1695–1700.
116. Reubsaet, J. L. E., Ahlsen, E., Haneborg, K. G., & Ringvold, A. (2003). *Journal of Chromatographic Science, 41*, 151–156.

117. Huang, T., Yang, L., Gitzen, J., Kissinger, P. T., Vreeke, M., & Heller, A. (1995). *Journal of Chromatography B, 670*, 323–327.
118. Tsai, T. R., Cham, T. M., Chen, K. C., Chen, C. F., & Tsai, T. H. (1996). *Journal of Chromatography B, 678*, 151–155.
119. Guerrieri, A., & Palmisano, F. (2001). *Analytical Chemistry, 73*, 2875–2882.
120. Yao, D., Vlessidis, A. G., & Evmiridis, N. P. (2002). *Analytica Chimica Acta, 462*, 1999–2008.
121. Tsafack, V. C., Marquette, C. A., Pizzolato, F., & Blum, L. J. (2000). *Biosensors and Bioelectronics, 15*, 125–133.
122. Emteborg, M., Irgum, K., Gooijer, C., & Brinkman, U. A. T. (1997). *Analytica Chimica Acta, 357*, 111–118.
123. Lapp, H., Spohn, U., & Janasek, D. (1996). *Analytical Letters, 29*, 1–17.
124. Hasebe, T., Nagao, J., & Kawashima, T. (1997). *Analytical Sciences, 13*, 93–98.
125. Valcarcel, M. and Luque de Castro, M.D. (1993) Analyst 118, 891–893.
126. Zhag, X. R., Baeyenes, W. R. G., Garcia-Campana, A. M., & Ouyang, J. T. (1999). *Trends in Analytical Chemistry, 18*, 384–391.
127. Abou-Enein, H. Y., Stefan, R. I., van Staden, J. F., Zhang, X. R., Garcia-Campana, A. M., & Bayers, W. R. C. G. (2000). *Critical Reviews in Analytical Chemistry, 30*, 271–289.
128. Kiba, N., Ito, S., Tachibana, M., Tani, K., & Koizumi, H. (2001). *Analytical Sciences, 17*, 929–933.
129. Kiba, N., Miwa, T., Tachibana, M., Tani, K., & Koizumi, H. (2002). *Analytical Chemistry, 74*, 1269–1274.
130. Khan, A., & Ghani, S. A. (2012). *Biosensors and Bioelectronics, 31*, 433–438.
131. Michael, G., Garguilo, Adrian, C., & Michael. (1996). *Journal of Neuroscience Methods, 70*, 73–82.
132. Kim, M., & Mitchell. (2004). *Analytical Chemistry, 76*, 1098–1106.
133. Guerrieri, G., Lattanzio, V., Palmisano, F., & Zambonina, P. G. (2006). *Biosensors and Bioelectronics, 21*, 1710–1718.
134. Schuvailo, O. N., Dzyadevych, S. V., El'skaya, A. V., Gautier-Sauvigne, S., Csoregi, E., Cespuglio, R., & Soldatkin, A. P. (2005). *Biosensors and Bioelectronics, 21*, 87–94.
135. Curulli, A., Dragulescu, S., Cremisini, C., & Palleschi, G. (2001). *Electroanalysis, 13*, 236–242.
136. Chen, J., Lin, X. Q., Chen, Z. H., Wu, S. G., & Qingwang, S. (2001). *Analytical Letters, 34*, 491–501.
137. Yang, M., Yang, Y., Yang, Y., Shen, G., & Yu, R. (2005). *Analytica Chimica Acta, 530*, 205–211.
138. Shimomura, T., Itoh, T., Sumiya, T., Mizukami, F., & Ono, M. (2009). *Enzyme and Microbial Technology, 45*, 443–448.
139. Persike, M., Zimmermann, M., Klein, J., & Karas, M. (2010). *Analytical Chemistry, 82*, 922–929.
140. Bundel, D. D., Sarre, S., Eeckhaut, A. A., Smolders, I., & Michotte, Y. (2008). *Sensors, 8*, 5171–5185.
141. Boo, H., Jeong, R. A., Park, S., Kim, K. S., An, K. H., Lee, Y. H., Han, J. H., Kim, H. C., & Chung, T. D. (2006). *Analytical Chemistry, 78*, 617–620.
142. Zhu, W., An, Y., Zheng, J., Tang, L., Zhang, W., Jin, L., & Jiang, L. (2009). *Biosensors and Bioelectronics, 24*, 3594–3599.
143. Kib, N., Ito, S., Tachiban, M., Tani, K., & Koizumi, H. (2003). *Analytical Sciences, 19*, 1647–1651.
144. Guerrieri, A., Benedetto, G.E.D., Palmisano, F. and Zambonin, P.G. (1995) Analyst, November 120, 2731–2736.
145. Wang, B. X. F., Zhou, Y., Xu, J. J., & Chen, H. Y. (2009). *Advanced Functional Materials, 19*, 1444–1450.
146. Clark, J. J., Sandberg, S. G., Wanat, M. J., Gan, J. O., Horne, E. A., Hart, A. S., Akers, C. A., Parker, J. G., Willuhn, I., Martinez, V., Evans, S. B., Stella, S., & Phillips, P. E. M. (2009). *Nature Methods, 7*, 126–129.
147. Nguyen, Q. T., Schroeder, L. F., Mank, M., Muller, A., Taylor, P., Griesbeck, O., & Kleinfeld, D. (2010). *Nature Neuroscience, 13*, 127–132.
148. Wilson, G. S., & Gifford, R. (2005). *Biosensors and Bioelectronics, 20*, 2388–2403.
149. Hume, R. I., Role, L. W., & Fischbach, G. D. (1983). *Nature, 305*, 632–634.
150. Scarr, E., & Dean, B. (2009). *Expert Review of Neurotherapeutics, 9*, 73–86.
151. Vögler, O., Bogatkewitsch, G. S., Wriske, C., Wriske, C., Krummenerl, P., Jakobs, K. H., & Koppen, C. J. V. (1998). *Journal of Biological Chemistry, 273*, 12155–12160.
152. Willars, G. B., & Nahorski, S. R. (1995). *British Journal of Pharmacology, 114*, 1133–1142.
153. Horowitz, L. F., Hirdes, W., Suh, B. C., et al. (2005). *Journal of General Physiology, 126*, 243–262.
154. Zhang, J., Hupfeld, C. J., Taylor, S. S., Olefsky, J. M., & Tsien, R. Y. (2005). *Nature, 437*, 569–573.
155. Coward, P., Chan, S. D., Wada, H. G., Humphries, G. M., & Conklin, B. R. (1999). *Analytical Biochemistry, 270*, 242–248.