12 In Vivo Voltammetry with Telemetry

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CONTENTS

INTRODUCTION

Overview

A new instrument for the monitoring and control of neural activity is described in this chapter. The novel characteristic, wireless communication, confers several advantages. Most revolve around freeing the animal from the cable tether linking animal and recording equipment in traditional, hardwired set-ups. The new instrument, called real-time animal telemetry or RAT, was initially developed to support fast-scan cyclic voltammetry (FSCV) at a carbon-fiber microelectrode (CFM). Hence, a large portion of the background section of this chapter is dedicated to this voltammetric microsensor technique for chemical monitoring. We are also working towards the goal of incorporating other microelectrode approaches into RAT, such as, electrophysiology for measuring bioelectric signals and potentiometry for measuring ions. In theory, RAT could accommodate any sensing technology that transduces the input signal into a voltage signal. While RAT was also developed to support electrical stimulation for focally controlling neural activity, it should be possible to incorporate other methods for this purpose as well. Before the actual description of the wireless instrument, a brief primer on telemetry is presented, along with an abbreviated review of existing wireless instruments for neural monitoring and control.

Monitoring Neural Activity

As described in Figure 12.1, neural activity in the brain is fundamentally composed of chemical and bioelectrical signals. Consequently, one approach for characterizing brain function is to sample these signals. Several techniques have been developed over the past decades to achieve this important goal. Among the most widely used is microdialysis [1–3], which removes chemicals from the brain for subsequent analysis by methods, such as, electrochemical detection, fluorescence, capillary electrophoresis, and mass spectroscopy. Two general microelectrode techniques for monitoring neural activity, voltammetry and electrophysiology, are also well established. Shown diagrammatically in Figure 12.1, voltammetry monitors the chemistry of the brain, whereas electrophysiology measures its bioelectrical activity. Like microdialysis, voltammetry and electrophysiology have been applied to animals that are awake, rendering these techniques well suited for investigating the neural substrates of behavior. Also shown diagrammatically in Figure 12.1 is potentiometry. This electrochemical technique is used with ion selective microelectrodes [4].

As described in detail in the "Principles of FSCV at a CFM" and the "Applications of FSCV at a CFM" sections of this chapter, we are working towards the goal of incorporating voltammetry, electrophysiology and potentiometry into a wireless instrument. The rationale is that combined, the use of these microelectrode techniques affords a broad-based, integrative study of neuronal activity in the fast time and small space domains in which neurons function during important types of behavior. The initial thrust of instrument development was focused on FSCV at a CFM. Wightman establishes four criteria for sampling brain chemistry faithfully [5]: specificity, sensitivity, temporal resolution, and spatial resolution. It is our conviction that this voltammetric microsensor technique meets these stringent criteria for chemical monitoring in the brain quite well under suitable experimental conditions (see "Voltammetry" and "Principles of FSCV at a CFM" sections of this chapter).

Developing an instrument for neural monitoring begs the question of how the study of brain function could be enhanced. The primary answer is rooted in the nature of the connection between the animal and recording equipment in existing set-ups: the cable tether. Clearly, hardwire set-ups have afforded elegant studies investigating neural substrates of behavior, with the cable tether providing a robust connection. However, the cable tether is also restrictive, as the animal is never completely free to behave, and recordings in natural environmental settings, such as in a

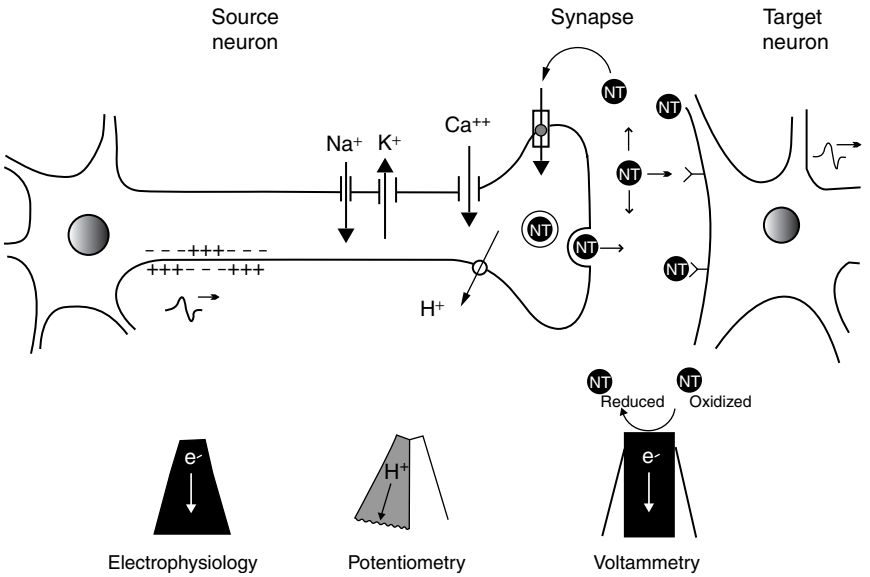

FIGURE 12.1 Monitoring neuronal activity. The function of the brain is to control behavior, and the functional cells of the brain are called neurons. (From Kandel, E. R., Schwartz, J. H., and Jessell, T. M., *Principles of Neural Science*, McGraw-Hill, New York, 2000; Zigmond, M. J., Bloom, F. E., Landis, S. C., Roberts, J. L., and Squire, L. R., *Fundamental Neuroscience*, Academic Press, San Diego, CA, 1999.) These cells are highly specialized for rapid signaling over large distances. During neurotransmission, an electrical signal called an action potential is conducted along the length of the axon to the synapse, where it elicits the release of a chemical neurotransmitter from the source neuron. Released neurotransmitter diffuses in brain extracellular space to a target neuron. Although shown immediately across the synaptic cleft, the target neuron may also be several microns away from the source neuron, when the neurotransmitter signals via non-synaptic communication. (From Vizi, E. S., *Pharmacological Reviews*, 52 (1), 63–89, 2000; Zoli, M., Torri, C., Ferrari, R., Jansson, A., Zini, I., Fuxe, K., and Agnati, L. F., *Brain Research Reviews*, 26 (2–3), 136–147, 1998.) Regardless of the spatial arrangement, subsequent binding of the neurotransmitter to a specific protein receptor elicits a change in the physiological status of the target neuron. Receptor binding often manifests as an alteration in the generation of action potentials. Mediating many of the processes of neurotransmission is the flux of ions between intracellular and extracellular compartments. For example, Na^+ and K^+ underlie the ion fluxes associated with the action potential, and Ca^{2+} is intimately involved in exocytosis, the mechanism releasing neurotransmitter into the extracellular compartment. Intracellular pH, which changes as a result of neural activity, is tightly controlled via several mechanisms transporting H^+ across the neuronal membrane, including the amiloride-sensitive Na^+/H^+ exchanger. Playing an integral role in neuronal activity is the brain cell microenvironment, the physical and chemical milieu immediately surrounding neurons. (From Nicholson, C. and Rice, M. E., *Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission*, Raven Press, New York, 1991.) In addition to an information pathway, the dynamic brain cell microenvironment maintains ionic homeostasis and provides a conduit for metabolic substrates supporting the proper functioning of neurons.

social housing condition or in an enriched environment that demands species-specific responses (such as crawling in or around obstacles), are impossible. Yet, it is in these settings that we are most likely to gain the most insight into behaviorally relevant neuronal operations. Another limitation related to investigating social interaction is monitoring in a single animal only. Indeed, monitoring neural activity in more than one animal, simultaneously, would result in a tangled mess of tethers. In short, the answer to the question posed above is a wireless connection, which would offer an elegant solution to the limitations imposed by the cable tether.

Voltammetry

Voltammetry directly monitors chemicals in brain's extracellular fluid. Although many different methodologies have been developed, the basis of each is that the chemical is either oxidized or reduced at the sensor surface by an applied potential. The measured faradaic current is then proportional to the concentration of chemical in the region of the sensor [6–8]. Several characteristics make voltammetry attractive for chemical monitoring. These include the presence of important, easily oxidized neurotransmitters in the brain (e.g., dopamine, norepinephrine, epinephrine, and histamine), fast kinetics and measurement of redox reactions, and ready fabrication of voltammetric microsensors. Voltammetric monitoring of dopamine has clearly received the most attention. This neurotransmitter is found in high concentrations in the brain and subserves important functions related to motor control, cognition, motivation, and endocrine control [9–11].

Early attempts to monitor brain dopamine voltammetrically, pioneered by Adams and co-workers in the 1970s [12,13], were hindered by the high concentrations of electroactive interferents in brain extracellular fluid [8]. Even today, selectivity represents the most contentious issue of the voltammetric technique, especially for measurements in the behaving animal [14–16]. Second generation techniques utilized modified electrodes and more complex waveforms to improve selectivity [7,8,17], enabling reliable measurements of neurotransmitter metabolites in ambulant animals with minute temporal resolution [18–20]. Continued advances have resulted in the development of three in vivo voltammetric techniques for the real-time monitoring of neurotransmitters: high-speed chronoamperometry [21], FSCV [22–24] and amperometry [25,26]. These techniques sample dopamine with sub-second temporal resolution at a CFM.

No attempt is made here to contrast the various voltammetric techniques developed and applied to investigate brain function. Such comparisons are available in the literature [6,8,17,27] and, if interested, the reader is encouraged to pursue this direction. Rather, this chapter focuses on FSCV at a CFM, which is incorporated into the wireless instrument. The rationale for incorporation is straightforward: this technique affords millisecond temporal and micron spatial resolution for chemical monitoring, along with provision for identifying the analyte detected in the form of a background-subtracted cyclic voltammogram. Moreover, FSCV at a CFM has been used recently in significant neurobiological experiments; thus, appears to be well positioned for contributing prominently to neurobiology research for many years to come. In the following two sections, the principles and applications of this voltammetric microsensor technique are described in more detail.

Principles of FSCV at a CFM

Figure 12.2 describes the technique of FSCV at a CFM. Typically, a triangle waveform is applied intermittently to the CFM at 100 ms intervals. In the example shown in Panel A, voltage is linearly ramped from a resting potential of −400 mV to a peak of 1000 mV and back. At a rate of 300 V/s, the duration of the voltage scan is approximately 9.3 ms. This scan rate supports monitoring at a temporal resolution (100 ms) that is suitable for characterizing brain dopamine dynamics, as the scan interval should be about ten times the scan length. The approximately 90 ms in between scans also allows ample time for dopamine to adsorb to the CFM, thus increasing sensitivity. During the time in between scans, the CFM rests at −400 mV. A single triangle waveform is shown in Panel B (top). Immediately below (middle) are two recordings obtained at the same CFM during single voltage scans. The black line denotes current collected in the striatum of an anesthetized rat, in the absence of electrical stimulation. Notice the large background or charging current that changes direction at the switching potential of the scan. The red line denotes current collected at the same CFM, but during electrical stimulation of dopaminergic fibers to elicit dopamine release. Notice that two additional, but smaller, features appear on top of the background current around 4 and 8 ms, corresponding to approximately 750 and −100 mV, respectively. Because the background current is stable, it can be removed by simple subtraction to reveal these smaller features more

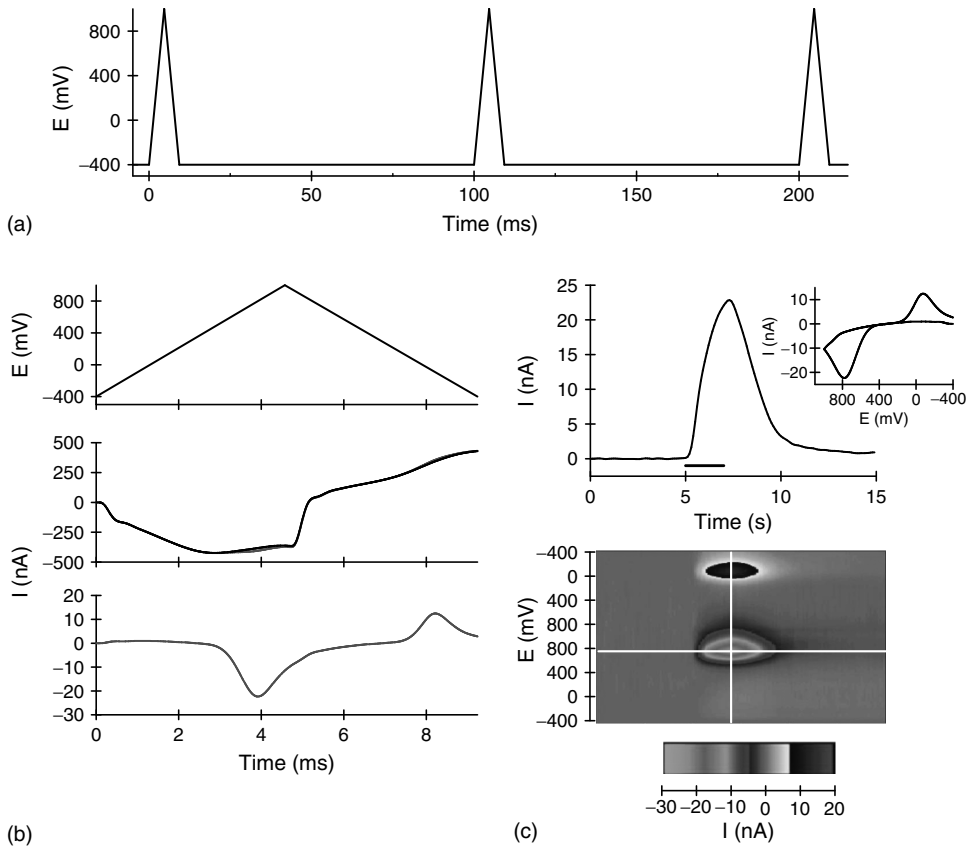

FIGURE 12.2 (**See color insert following page 272.**) Fast-scan cyclic voltammetry at a carbon-fiber microelectrode. (a) application of the triangle wave form at 100 ms intervals; (b) current monitored at a CFM during a single voltage scan; (c) voltammetric recording of electrically evoked dopamine release in the striatum of a urethane-anesthetized rat.

clearly (bottom). These features are due to faradaic current, when dopamine oxidizes to a quinone (the early negative current) and when the electroformed quinone is reduced back to dopamine (the later positive current).

The faradaic current collected during a scan is typically shown as a voltammogram, which is a plot of current as a function of applied potential. Because charging current is removed, the voltammogram shown in Panel C, INSET, is called a *background-subtracted* voltammogram. This voltammogram was constructed from the recording shown in Panel B (bottom). Notice the same negative and positive peaks around 750 and −100 mV, respectively. These peaks are indicative of dopamine; thus, the background-subtracted voltammogram is used as a chemical signature for identifying the analyte detected. Additionally, the change in dopamine-per-time is determined by plotting the current obtained for each scan at the oxidation potential for dopamine. For example, Panel C (top) shows the current monitored at approximately 750 mV in the rat striatum before, during, and after electrical stimulation. The stimulus train is demarcated by the solid line underneath the voltammetric recording, between 5 and 7 s.

While informative, the limitation of the voltammogram is that it represents a change in analyte(s) between only two time points. In the present case, pre-stimulus voltammograms were averaged and subtracted from the average of voltammograms collected around the peak of the electrically evoked signal. However, the entire electrochemical record can be viewed by

successively plotting all of the background-subtracted voltammograms with time [28]. Such a plot is shown in Panel C (bottom), with time as the *x*-axis, applied potential as the *y*-axis, and current (in color) as the *z*-axis. Notice the same two, prominent features displayed in the color plot at around 750 and −100 mV, coincident with electrical stimulation. Indeed, a plot of the current recorded at approximately 750 mV versus time, and corresponding to the horizontal white line, yields the current versus time trace shown in the top of Panel C. Moreover, a plot of the current recorded at approximately 7 s versus applied potential, and corresponding to the vertical white line, yields the voltammogram shown in the INSET at the top of Panel C. The color plot clearly demonstrates that dopamine is the only analyte detected at the CFM under these conditions.

Applications of FSCV at a CFM

Carbon-fiber microelectrodes were originally used for electrophysiological measurements (see Figure 12.4) [29], and FSCV was originally developed for calibrating the iontophoretic application of dopamine with a CFM [30,31]. Iontophoresis uses current to apply charged chemicals directly into the brain via a micropipette. However, the first use of FSCV at a CFM for monitoring endogenous dopamine was in the anesthetized rat during electrical stimulation [32]. Although a relatively simple signal (e.g., see Figure 12.2, top), the evoked measurement is replete with information about the fundamental mechanisms regulating extracellular dopamine levels in the brain, such as, release, uptake, and diffusion [22,33,34]. Hence, the anesthetized experiment with electrical stimulation, and its analogous experiment in vitro [35–37], have proven to be workhorse approaches for investigating dopamine neurochemistry and neuropharmacology.

A twist on the evoked experiment with FSCV at a CFM is, instead of using electrical stimulation, focally applying pharmacological agents to alter brain dopamine levels [38,39]. Evaluation of the factors regulating dopaminergic tone, the ambient concentration of dopamine in extracellular fluid, is possible with this later approach, which is a prospect not considered amenable to real-time voltammetric techniques. More recently, coupling FSCV with a CFM to principal component analysis [40] has been used to quantify the increase in basal dopamine levels following cocaine administration [41].

Towards the end of the 1990s, FSCV at a CFM was applied to unanesthetized animals, paving the way for a new era of more behaviorally oriented experiments. The first measurements in freely moving animals were dopamine evoked by electrical stimulation [42]. This development enabled pharmacological experiments much like those performed in the anesthetized animal, but without the concern for anesthesia artifacts, and accompanied by the simultaneous assessment of drug-altered behavior [43–45]. It was also possible to investigate the role of dopamine in the classic paradigm of intracranial self-stimulation on a sub-second temporal level [46–50].

Perhaps the most impressive application of FSCV at a CFM in unanesthetized animals is the measurement of behaviorally evoked changes in brain dopamine levels [51]. The evolution of this application has been driven by steadily improving detection limits, achieved through advances in instrumentation, sensors and waveforms [52–56]. As a result, it is now possible to monitor the sub-second dopamine changes presumably elicited by burst firing of dopaminergic neurons during phasic signaling [57,58] and associated with sociosexual interaction [59,60], and food [61] and drug [62,63] seeking.

Figure 12.3 shows a recording collected by FSCV at a CFM in an unanesthetized animal. The trace described by the dotted line in the left panel was electrically evoked by a pulse train applied at 5 s to the medial forebrain bundle. Animals lever-press to obtain this same pulse train (60 Hz, 0.4 s, 125 μA) during intracranial self-stimulation [46,47]. The black line in the INSET at the top is the background-subtracted cyclic voltammogram obtained at the peak of the evoked signal. The large, downward (i.e., oxidative) peak around 700 mV and smaller, upward (i.e., reductive) peak around −300 mV clearly indicated dopamine. Underneath the evoked trace is the color plot showing all of the voltammograms collected during this time. Coincident with the electrical stimulation and the

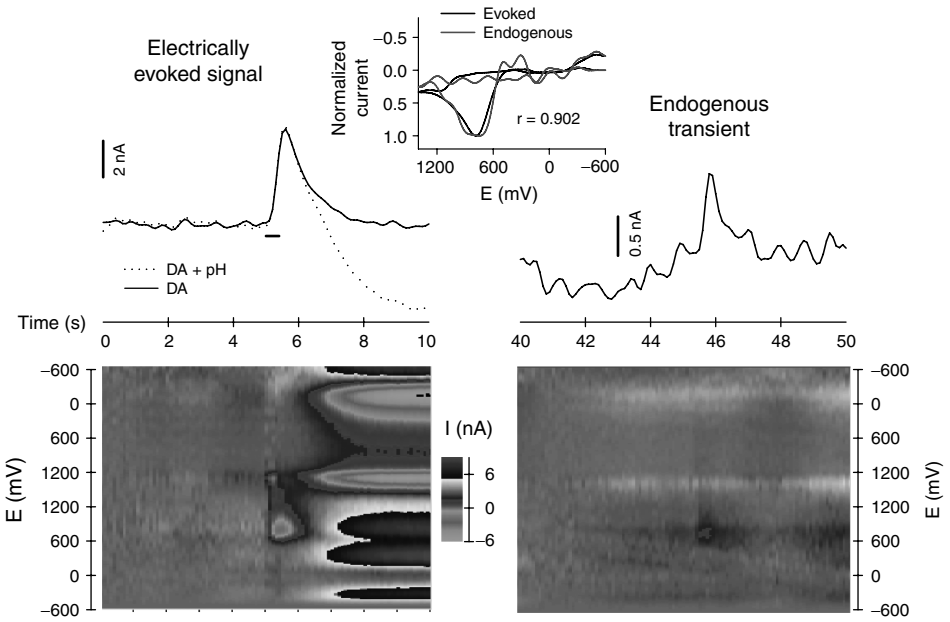

FIGURE 12.3 (**See color insert following page 272.**) Voltammetric measurement of dopamine in a freely moving rat. For this recording, the CFM was placed in the dorsomedial caudate–putamen, and the stimulating electrode was placed in the medial forebrain bundle. Voltammograms shown in the INSET were normalized to peak oxidative current.

increase in the evoked trace is a greenish dot at approximately 5 s and 700 mV, corresponding to the oxidative peak in the dopamine voltammogram. Also notice several features emerging in the color plot just after the dopamine peak. These features reflect changes in brain pH associated with the electrical stimulation [64,65] and cause the evoked trace (dotted line) to plummet between 7 and 10 s. However, the pH interference is readily removed by differential subtraction, resulting in a pure signal describing the dopamine changes (solid line).

A 10 s portion of the same recording, but beginning 35 s after the electrical stimulation, is shown in the right panel. The same CFM location at which an evoked dopamine signal was recorded also yielded a non-electrically evoked, transient signal. Although smaller in amplitude, the endogenous signal exhibited comparable dynamics to the electrically evoked dopamine recording, suggestive of similar origins. The INSET at the top overlays the background-subtracted cyclic voltammogram obtained from electrical stimulation (black line) and the endogenous transient (red line). Voltammograms are similar ($r = 0.902$), and indicate that the endogenous transient is a dopamine signal. Indeed, the color plot below shows a clearly demarcated purplish dot at about 45 s and 700 mV, corresponding to the transient signal and the oxidative peak of the voltammogram. As identified above, this transient dopamine concentration spike is thought to result from a burst of action potentials during phasic dopaminergic signaling [16,66].

Carbon-fiber microelectrodes also serve as excellent sensors for electrophysiological measurements [29]. Figure 12.4 compares extracellular single units (i.e., action potentials) recorded in the striatum of an anesthetized rat by a saline-filled glass capillary (A) and a CFM (B). At least in our hands, CFMs perform as well as or exceed glass capillaries for this purpose [67]. The ability to measure both single unit activity and dopamine was exploited by Millar and co-workers in developing quasi-simultaneous electrophysiology and voltammetry at a CFM [68,69]. In this elegant technique, the CFM is switched in the time between voltage scans from a current-to-voltage transducer circuit for measuring voltammetry to a voltage-follower circuit for measuring

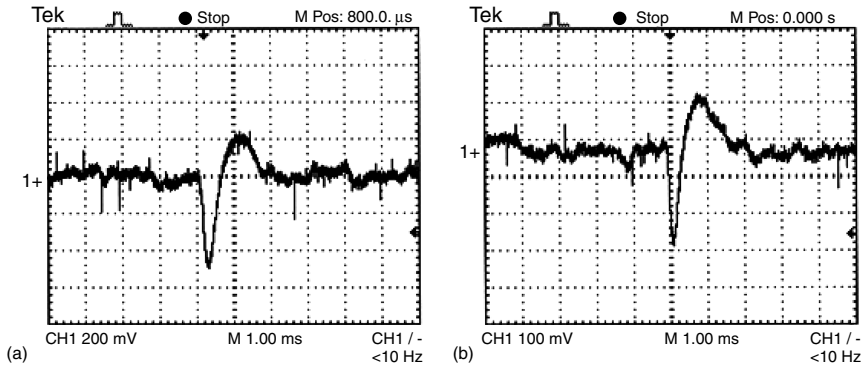

FIGURE 12.4 Comparison of electrodes for measuring electrophysiology. (a) Saline-filled glass micropipette; (b) CFM. Both recordings were collected in the dorsomedial caudate–putamen of a urethane-anesthetized rat.

electrophysiology. The beauty of this technique is that dopamine and the target cell response to dopamine are measured at approximately the same time and in approximately the same vicinity. More recently, quasi-simultaneous electrophysiology and voltammetry at a CFM has been applied to the freely moving rat [70]. Although not attempted yet, it should be possible to incorporate this dual sensing technique into a wireless instrument.

PRINCIPLES OF TELEMETRY

Resources

Several telemetry resources are available for both the technically and less technically inclined. For the more general reader, two excellent texts are *The Essential Guide to RF and Wireless* by Carl Weisman (2002, Prentice Hall) [71] and *The Essential Guide to Wireless Communications Applications* by Andy Dornan (2002, Prentice Hall) [72]. Focusing primarily on consumer oriented wireless devices, both provide readable reviews of basic telemetry principles and technologies. Much of the information provided in the next two sections was obtained from these two texts. Although some information is understandably dated now, it nevertheless is interesting to compare the various predictions as to the direction it was believed wireless technology would take, with the direction it has actually taken, particularly regarding Wireless Fidelity (Wi-Fi) and Bluetooth, as both have enjoyed rapid developments in the last three years. More up to date information about wireless devices is found in *PC Magazine Wireless Solutions* by Neil Randall and Barrie Sosinksy (2005, Wiley) [73].

Theory and Basic Instrumentation

Radio waves, a form of electromagnetic radiation, allow telemetry devices to transmit data wirelessly [72]. Typically conceptualized as waves, electromagnetic radiation is characterized by frequency (which is the number of cycles per second and inversely related to wavelength) and amplitude (which describes the power of transmission). The term "radio" was originally given to electromagnetic radiation used in communication, although modern communication technologies also use waves with much greater frequencies, such as, microwaves and infrared. Radio waves range in frequency between a lower limit of around 5 kHz to an upper limit of around 300 GHz, where they overlap with the lower end of microwaves. The higher the frequency, the shorter is the range. This is due to the greater attenuation (i.e., loss of energy due to collisions) of higher

TABLE 12.1
Example Radio Frequencies (in Hz)

AM radio	530,000
FM radio	88,000,000
TV	746,000,000
Wireless LAN	2,400,000,000
Satellite	4,200,000,000
Radar	9,000,000,000

Source: From Weisman, C. J., *The Essential Guide to RF and Wireless*, Upper Saddle River, Prentice Hall, NJ, 2002.

frequency waves. The amount of data transmitable, hence the speed of data transmission, is also related to frequency. More specifically, the larger the bandwidth, the frequency range or channel allocated to transmission, the greater the amount of information that can be sent. Example frequencies of radio applications are shown in Table 12.1.

Telemetry devices share common principles (Figure 12.5) [71]. The original data signal is converted by a transmitter from an electrical signal into an electromagnetic wave, which is sent through the air. If everything goes smoothly, the receiver then converts the airborne wave back into the original data signal. The transmitter is comprised of several components including an amplifier, a filter and an antenna. Two additional and critical components are the oscillator and mixer. During wireless transmission, the data signal is not sent directly by the transmitter. Rather, the data signal is "piggy-backed" onto a carrier signal, e.g., a sine wave of a particular frequency and generated by the oscillator. The data signal is added to the carrier signal by the mixer in a process called modulation. The receiver operates in the opposite fashion, removing the original data signal from the carrier signal in a process called demodulation.

Telemetry is distinguished on the basis of several characteristics, including the frequency of the carrier wave, the type of modulation, and whether the original data signal is analog or digital [71]. Example frequencies for various radio devices were described above in Table 12.1. Two general types of modulation are amplitude modulation (AM) and frequency modulation (FM). During AM,

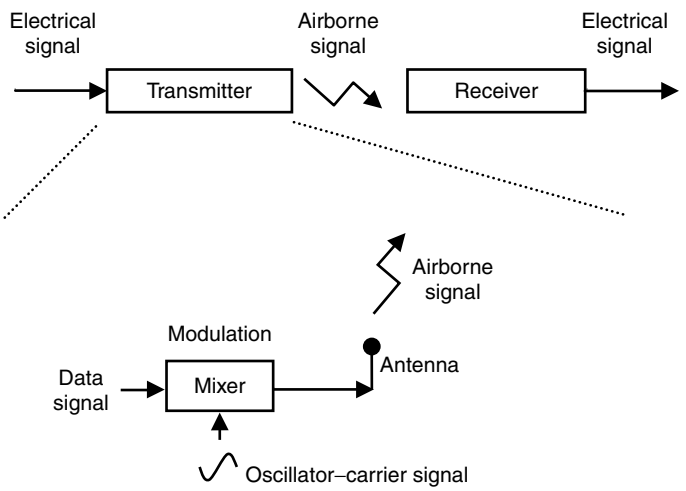

FIGURE 12.5 Principles of telemetry. (Modified from Weisman, C. J., *The Essential Guide to RF and Wireless*, Prentice Hall, Upper Saddle River, NJ, 2002.)

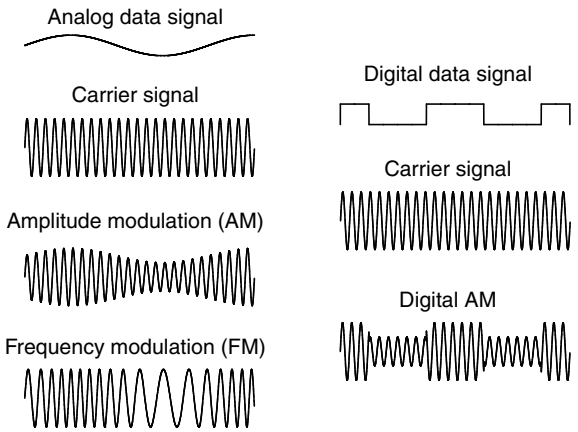

FIGURE 12.6 Modulation. (Modified from Weisman, C. J., *The Essential Guide to RF and Wireless*, Prentice Hall, Upper Saddle River, NJ, 2002.)

the amplitude of the carrier signal is modified by the data signal, whereas frequency is modified during FM. The examples found in the left panel of Figure 12.6 show AM and FM transmitting an analog, or continuous, data signal. More recently, digital, or binary, signals have been sent wirelessly. The zeroes and ones of a digital signal are readily observed on a carrier wave as "highs" and "lows" after digital AM in the stylized example shown in right panel of Figure 12.6. Phase modulation, which adds the original signal onto the carrier wave by adjusting its phase, is commonly used in digital applications. There are various forms of amplitude, frequency and phase modulation and, as described in more detail in the next section, additional modulation is used when different signals share the same carrier frequency.

Although analog signals were the first to be sent wirelessly, the subsequent rise in digital telemetry and the great fanfare surrounding its introduction, suggest important advantages to the wireless transmission of digital signals. Indeed, digital telemetry confers several key advantages [72]: noise reduction, reliability, transmission efficiency, security, and timing. Perhaps the easiest advantage to conceptualize is noise reduction. Whereas a receiver cannot distinguish between noise and signal in an analog wave, the highs and lows of a digital signal are distinct states, more easily separated from noise (see Figure 12.6). Reliability is enhanced, because digital signals are encoded with extra data permitting error correction. Although error correction reduces capacity, the increased reliability compensates by affording more efficient use of the same bandwidth. Increased spectral efficiency is also achieved by the compression of digital data. Security has always plagued the wireless transmission of analog signals, and while possible, encryption is unreliable. On the other hand, digital signals are readily encrypted to various degrees and without the loss of capacity during transmission. Finally, because digital signals are easily stored, they can be divided into packets and sent at any time to enhance efficiency. The internet is based on timing in the form of packet-switching.

Are there any advantages to analog telemetry? Yes, in fact, there are several. For example, analog devices are cheaper, simpler, and smaller. Consequently, because they are sufficient for many applications, analog telemetry can be the right choice.

Digital Telemetry at 2.4 GHz

Two types of digital telemetry have gained prominence recently, Wi-Fi and Bluetooth [73]. Both operate in the internationally allocated band of 2.4 GHz. While this unlicensed band is only one of several designated by the International Telecommunications Union for industrial, scientific, and

medical (ISM) purposes, it is the only ISM band available in every country. Wireless fidelity has emerged as the standard in the United States for wireless local area networks (LANs). Although sometimes confused as a competitor, Bluetooth was developed to support personal area networks (PANs), characterized by mobile access points (compare with the fixed access point in LAN), and piconets, a collection of several Bluetooth devices. Interestingly, another 2.4 GHz technology, called HomeRF, was developed specifically for the home networking and entertainment market [71]. This technology combines features of Wi-Fi and cordless phones, and was originally designed to be more affordable and simpler to implement than Wi-Fi. Since then, Wi-Fi has become relatively inexpensive, and many home networks are now based on this technology. Table 12.2 compares salient features of Wi-Fi and Bluetooth.

Wireless fidelity is based on the Institute of Electrical and Electronic Engineers (IEEE) 802.11 standard [73]. To date, the two most successful protocols are 802.11b and 802.11g. Back compatible, 802.11g is newer and faster (11 versus 54 Mbps). As with any protocol operating in the unlicensed 2.4 GHz band, Wi-Fi must use a second type of modulation, in addition to piggybacking the data signal onto a carrier wave, to combat interference from other signals existing at the same time and in the same place [71]. One such general technology is spread spectrum, which essentially hides the signal in the noise, so it becomes undetected. This is accomplished by "spreading" the carrier wave across a wider frequency spectrum. For example, the direct sequence spread spectrum (DSSS) used by 802.11b multiplies the digital signal by a pseudo random noise (PN) code, effectively converting the signal into apparent noise. The signal is readily uncovered again if the PN code is known. The newer 802.11g uses a modulation called orthogonal frequency division multiplexing (OFDM), which breaks the bandwidth into separate and distinct sub-bands. More complex than DSSS, OFDM is especially valuable for minimizing multipath (i.e., indirect signals caused by reflections).

Like the first 802.11 protocol, Bluetooth uses frequency hopping spectrum spread (FHSS) to navigate the crowded 2.4 GHz airwaves [71]. In this spread spectrum technology, the frequency of the carrier wave is jumped between 79 1 MHz bands, again according to a PN code. In contrast to the FHSS of the original 802.11, the dwell time (the time at which the wave rests at one frequency) is very short, as Bluetooth hops frequencies at a rate up to 3200/s (compare with 2.5 hops/s). The fast hopping rate makes Bluetooth well suited for crowded 2.4 GHz environments and relatively resistant to multipath. On the downside, it limits the data transmission rate (see next paragraph) and can interfere with other 2.4 GHz signals. For this reason, Bluetooth has been called a "rude radio" [72].

Although transmitting more slowly (less than 1 versus greater than or equal to 11 Mbps) and across shorter distances (9 versus 90 m maximally) than Wi-Fi, Bluetooth enjoys several advantages, including reliability (see preceding paragraph), small size, low power requirements and low price [71]. As described below in the "Real-Time Animal Telemetry" section of this chapter,

TABLE 12.2
Comparison of Wi-Fi and Bluetooth

Characteristic	802.11b	802.11g	Bluetooth
Frequency	2.4 GHz	2.4 GHz	2.4 GHz
Application	LAN	LAN	PAN, piconet
Technology	DSSS	OFDM	FHSS
Range	90 m (300 ft.)	90 m (300 ft.)	9 m (30 ft.)
Data rate	11 Mbps	54 Mbps	1 Mbps

Source: From Weisman, C. J., *The Essential Guide to RF and Wireless*, Prentice Hall, Upper Saddle River, NJ, 2002; Dornan, A., *The Essential Guide to Wireless Communications Applications*, Prentice Hall, Upper Saddle River, NJ, 2002.

similar advantages make Bluetooth well suited for developing a wireless instrument supporting FSCV at a CFM. As one might expect, these advantages are also consistent with the original goal of Ericsson (Sweden) in developing Bluetooth: a protocol supporting PANs and piconets. In a PAN, one wireless device becomes the access point for several other wireless devices. A Bluetooth piconet, in turn, enables eight devices to be linked together, with one device serving as the master to the other seven slaves. Bluetooth piconets are scalable, as a slave can be a master to a different set of slaves. To make the concept of PANs and piconets work most successfully, it is speculated that Ericsson must provide Bluetooth devices at the low cost of $5 or less [72]. Dating back to 1994, Bluetooth was initially received as the "next best thing." Although applications were slow in coming to the marketplace, which led many industry analysts to wonder whether the technology was a bust, it appears now that Bluetooth products are everywhere, from the computer mouse and key board, to the camera, the PDA, the cell phone and even the car [73]. The next generation of Bluetooth technology is predicted to send signals at 20 Mbps to distances of up to 90 m, transmission characteristics similar to 801.11b [71].

WIRELESS NEURAL MONITORING AND CONTROL

Scope

No attempt is made here to exhaustively review the literature on wireless neural monitoring and control; the use of telemetry for electrophysiological recording and electrical stimulation in experimental animals is not new; analog systems supporting these applications have been available for decades [74–77] and similar systems, but based on digital technology, have been introduced in the past few years [78–81]. Rather, this section will highlight the most recent developments related to our wireless instrument. On a related front, it should be mentioned here that the use of wireless devices in medicine is rapidly expanding [82–85] and that this phenomenon will, no doubt, have a great impact on the future development of wireless neural monitoring and control. The availability of a turnkey, commercial system will ultimately drive more widespread applications. Such wireless instruments, though limited to the monitoring of physiological parameters, such as, heart rate, blood pressure, ECG, EEG, temperature and locomotor activity, are currently available from Data Sciences International (St Paul, MN) and Minimitter (Sunriver, OR).

Electrophysiology

Analog FM technology has a suitable data transmission rate for capturing single unit activity in the brain. For this reason, several FM-based systems have been developed over the years for the wireless monitoring of electrophysiology in freely behaving animals, including the rat [86], rabbit [86], toad [87], monkey [88], song bird [89], and owl [90]. As mentioned above in the "Theory and Basic Instrumentation" section of this chapter, analog devices are readily miniaturized, hence their suitability for smaller laboratory animals. Although stereo-FM has been also used to record single unit activity from two implanted electrodes simultaneously [90], there is a general trend in electrophysiology to move towards multiwire array electrodes in order to record tens of units at once [91–94]. Three wireless devices have been described recently to support these state-of-the-art, multiwire array applications. Nicolelis and co-workers have developed a device based on 802.11b to record single unit activity in the monkey from 16 electrodes sampled at 30 kS/s with 12-bit resolution [95]. This system weighs 260 g and measures $14 \times 8.3 \times 4$ cm, about the size of a young adult rat. However, a smaller wireless array system has been specifically developed for the rat. This analog device, available commercially from Triangle BioSystems (Durham, NC, U.S.A.), comes in a 7- and 15-channel package and is compatible with Plexon (Dallas, TX, U.S.A.) hardware and software [96]. Plexon makes hardwired systems for multiwire electrophysiology. A smaller digital system is also in the developmental stages [97]. This device operates at a frequency of

433 MHz using frequency-shift keying modulation and samples 88 channels simultaneously, with 10-bit resolution at 15 kS/s. It is worth noting that this device also uses a 2.64 MHz inductive link to power the unit.

Voltammetry

Perhaps related to the fact that there are a smaller number of users of voltammetry compared with the number of users of electrophysiology, there are fewer descriptions of wireless voltammetry. A slow scan electrochemical technique called *differential normal pulse voltammetry*, which samples electroactive neurotransmitter metabolites with minute temporal resolution, has been coupled with infrared telemetry [98–101]. This example highlights another advantage of wireless for microelectrode applications: reducing electrical cross talk when combining voltammetry with other techniques such as electroencephalography, electrooculography, and electromyography. This optoelectronic system was later modified to support direct current amperometry (i.e., pulsed amperometry or chronoamperometry) for sub-second measurements of the neurotransmitter serotonin at a CFM [102]. A company called Pinnacle Technology (Lawrence, KS, U.S.A.) now offers a digital wireless instrument supporting chronoamperometry [103,104]. This same company has also developed a wireless instrument for an enzyme-linked microsensor for monitoring the neurotransmitter glutamate [105]. Because glutamate is not electroactive, an enzyme, glutamate oxidase, is used to produce hydrogen peroxide from glutamate, which can then be detected amperometrically (i.e., at a constant potential). The sampling rates for the infrared device supporting direct current amperometry (5 S/s) and for both Pinnacle Technology devices (0.5 S/s) are far too slow to support FSCV, which requires sampling rates in excess of 20 kS/s [106].

Electrical Stimulation

As with electrophysiology, analog systems for wireless electrical stimulation have been available for decades [75]. In fact, electrophysiology, in some form, and electrical stimulation are often combined into the same device [76,77]. Some wireless devices for electrical stimulation are powered inductively [87,107]. Most appear to stimulate with constant voltage. However, by correcting for the different impedances of stimulating electrodes, constant current is preferable. One of the more interesting applications of wireless electrical stimulation appeared in the journal *Nature* in 2002. In this so called robot rat example, stimulating electrodes implanted bilaterally in the somatosensory cortex were wirelessly controlled and used to steer a rat in real time across a variety of terrains [108]. Another stimulating electrode was implanted in the medial forebrain bundle for activating the brain reward system, similar to the intracranial self-stimulation experiment described above in the "Principles of FSCV at a CFM" section of this chapter. This rewarding stimulation was used to train animals to turn in the appropriate direction depending upon whether the right or left somatosensory cortex was activated. The wireless device for electrical stimulation is described in more detail in a later paper [109]. Constant voltage stimulation was controlled by an onboard micro-processor and remotely triggered by a UHF (approximately 420 MHz) transmitter. The device also supported intracranial self-stimulation, with a delay between lever press and pulse train application of only 45 ms.

REAL-TIME ANIMAL TELEMETRY

Instrument Overview

We are developing wireless instrumentation, called Real-time Animal Telemetry or RAT, for both monitoring and controlling neuronal activity in laboratory animals [106]. As described later in "Future Directions," the ultimate goal is a wireless instrument supporting a wide variety of microsensors. The initial strategy was to implement FSCV at a CFM. The rationale is that, because FSCV

is technically more challenging than electrophysiology or potentiometry, for example, expanding RAT functionality to include these other sensing technologies at a later time would not be as difficult once FSCV is established. The general design for the RAT instrument supporting FSCV at a CFM is shown in Figure 12.7 [106]. RAT consists of a remote unit, which is affixed to the animal, and a home-base unit, for interfacing with the experimenter. The remote unit contains three components: (1) voltammetry analog front-end, i.e., a miniature potentiostat for controlling electrochemistry; (2) micro-processor, for controlling the potentiostat, data acquisition and telemetry; and (3) transmitter–receiver, for two-way communication with the home-base unit. A desktop PC computer and transmitter–receiver comprise the home-base unit. Front-end software for this unit has provision for initiating data collection, storing data to a file, and data viewing and analysis.

Wireless communication between home-base and remote units is performed by Bluetooth digital telemetry. Bluetooth was selected for several reasons, in particular fidelity, small size and low power requirements. The small size and low power requirements of Bluetooth are essential for developing a wireless device supporting measurements in small laboratory animals such as rodents. Efforts by the IEEE to develop an industry standard based on Bluetooth (802.15) and the success of Bluetooth applications in the marketplace will drive continued development of this wireless technology. Therefore, one additional advantage is that upgrading the wireless capabilities of RAT will be relatively straightforward by incorporating the latest Bluetooth offering. The selection of a digital telemetry to support FSCV at a CFM is also vital, because the voltammogram requires high-resolution acquisition and is very susceptible to transmission artifacts. Indeed, the rapid conversion of an analog signal to digital decreases the chance of degradation in signal because of noise and loss of signal integrity, as further signal conditioning stages are used (e.g., amplifiers, external antenna noise adding to signals, modulation/demodulation, filtering, etc.). In contrast to an analog system, signal integrity can be maintained beyond 10–12 bits with digital telemetry, a necessity for the color plot of FSCV (Figure 12.2 and Figure 12.3) [28].

The transmitter–receivers of the home-base and remote units are identical. This Bluetooth module, a developmental version (ROK 101008/21, Ericsson, Stockholm, Sweden), is accessed via a high-speed serial or USB interface. The former was chosen, because of our expertise and the

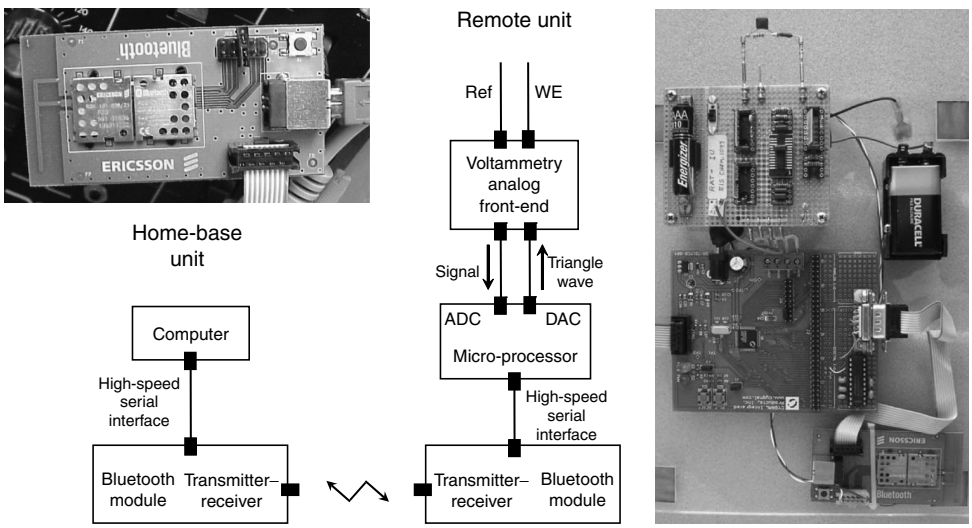

FIGURE 12.7 Prototype RAT. This large prototype was used to establish proof of principle for the wireless transmission of FSCV at a CFM using RAT. Abbreviations: Ref (reference electrode); WE (working electrode or CFM).

serial-communication support built into LabVIEW (National Instruments, Austin, TX, U.S.A.), the platform for the home-base, front-end software. While the USB interface is faster, the data transmission rate (460 Kbps) of the serial interface (232PCI1A, B&B Electronics, Ottawa, IL, U.S.A.) is sufficiently matched to Bluetooth (approximately 700 Kbps) such that additional speed is not necessary. Of course, a USB, PCI or other interface would be required to exploit fully proposed improvements that would increase Bluetooth's transmission rate to 20 Mbps. Whereas we initially developed our own driver, LabVIEW now supports Bluetooth. The left photograph in Figure 12.7 shows the home-base Bluetooth module with a serial connection (lower right). A USB connection (center right) is also shown, but it is only used to provide 5 V for powering the unit.

The micro-processor plays a key role in RAT, by generating the triangle waveform for FSCV via a digital-to-analog converter (DAC), digitizing the microsensor signal via an analog-to-digital converter (ADC), and directing wireless transmission. To perform these functions, we selected the C8051F007DK micro-processor from Silicon Laboratories (Austin, TX, U.S.A.), which features a 12-bit ADC with configurable gain and multiple inputs, two 12-bit DACs, and a high-speed serial interface. Software stored in FLASH memory generates the voltage scan, controls data acquisition, and communicates with the Bluetooth module. An approximately 15 MHz crystal supports digitization at a rate of 100 kS/s. Digitized data, stored temporarily in internal RAM, is wirelessly sent to the home-base unit during the time in between voltage scans. Overall, RAT characteristics for data acquisition are very similar to hardwired systems, which typically digitize at rates between 20 and 100 kS/s at a resolution of either 12 or 16 bits [106].

The analog circuit of the voltammetry front-end contains three amplifiers and is configured such that the reference electrode is held at circuit common potential, while the working electrode (i.e., the CFM) is scanned. The operational amplifier serves as a current-to-voltage converter with gain. One differential amplifier subtracts the voltage scan from the measured signal, and the second differential amplifier is used to generate potentials for the subtraction and reference electrode. The potentiostat circuit is operated in *two-electrode mode* (i.e., a working and a reference electrode), as there is no provision for an auxiliary electrode. However, this mode is made possible by virtue of the small currents recorded at the CFM, thereby minimizing IR voltage errors.

Establishment of Proof of Principle

A prototype RAT instrument was built to establish the wireless transmission of FSCV at a CFM [106]. Although too large to be affixed to a laboratory rat, the size of the prototype expedited construction, modification and testing. The potentiostat electronics are shown at the top of the right picture in Figure 12.7. A dummy cell, a resistor and capacitor circuit mimicking the electrical properties of the CFM, is connected to the working and reference electrode inputs. The printed circuit board in the middle of the picture contains the micro-processor, the component near the center. A serial cable connects the micro-processor board with the Bluetooth transmitter–receiver. The prototype is powered by standard 9.0 V radio and 1.5 V AAA batteries.

Several dry and wet tests were performed on the prototypte RAT instrument [106]. Dummy cell recordings were sent wirelessly for distances up to 16 m with line of sight, in real time, without data loss, and with high fidelity and stability. Wireless measurements of dopamine in the brain of an anesthetized rat during electrical stimulation and collected at a CFM with flow injection analysis, compared favorably to a conventional hardwired voltammetry system. Collectively, these data establish proof of principle for the wireless transmission of FSCV at a CFM. Of considerable interest was that measurements collected by the prototype RAT were largely immune to ambient electrical noise, while measurements with a conventional hardwired system need to be performed inside a grounded Faraday cage to minimize external interference, typically from 60 Hz noise generated from power lines and AC-powered equipment. Because only the analog signal is affected by the ambient noise, we reasoned that the reduced interference was due to digitization of the

analog signal near the measurement site. This finding indicates that FSCV at a CFM can be performed outside of the Faraday cage, another advantage of digital telemetry for neuronal monitoring.

Preliminary Findings in Freely Moving Animals

To support measurements in freely moving animals, a miniature RAT instrument was built based on the successful design establishing proof of principle (Greco et al., in preparation). The miniature RAT, shown in Figure 12.8, consists of four components: (1) custom printed circuit board housing the micro-processor and voltammetry circuit, (2) transmitter–receiver, (3) battery, and (4) analog circuit for electrical stimulation. Each module is roughly the same size (approximately 4.0×2.0 cm). When sandwiched together, the height is 1.5 cm with a weight of 17 g. The micro-processor and Bluetooth module are identical to those used in the large prototype RAT. A marked reduction in overall size was achieved by the use of surface mount electronic components, careful design of the printed circuit board, and stripping away unnecessary components on the large prototype.

In contrast to the main board, the stimulus generator was constructed on breadboard with conventional electronic devices. It also uses a separate, isolated power supply. The stimulus generator creates biphasic stimulus pulses, which are applied in between voltage scans to minimize stimulus artifact. The operational amplifier in the circuit is configured for constant-current output. Digital outputs from the micro-processor generate positive and negative phases of the pulses, which are optically isolated. The stimulus generator circuit is passive and can be detached without altering the function of the other components. After detachment, the remaining components of the miniature RAT weigh 10 g.

Tests similar to those described above establishing proof of principle were also performed on the miniature RAT instrument. Overall, the miniature RAT compared favorably with the large prototype RAT and a hardwired voltammetry system (Greco et al., in preparation). Due to a shorter antenna, the transmission distance of approximately 8 m was less than the large prototype, but near the Bluetooth specification of 9 m. As anticipated, the miniature RAT was as insensitive to ambient electrical noise as the large prototype. Figure 12.9 compares the functions of FSCV and electrical stimulation for the miniature RAT and a conventional hardwired system. Panel A shows evoked

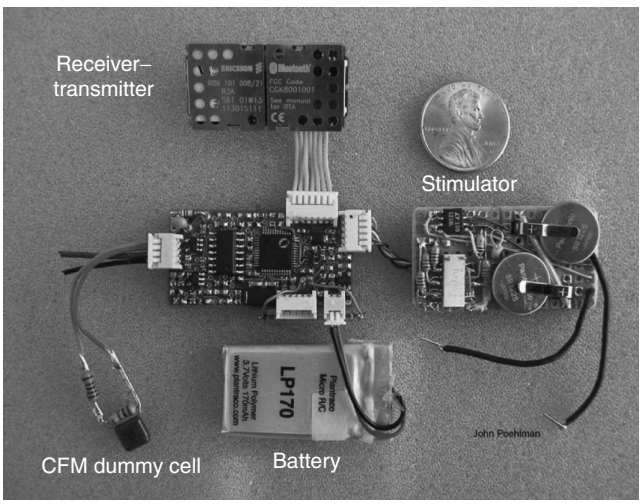

FIGURE 12.8 Miniature RAT. This small RAT device was used for monitoring electrically evoked dopamine in a freely moving rat.

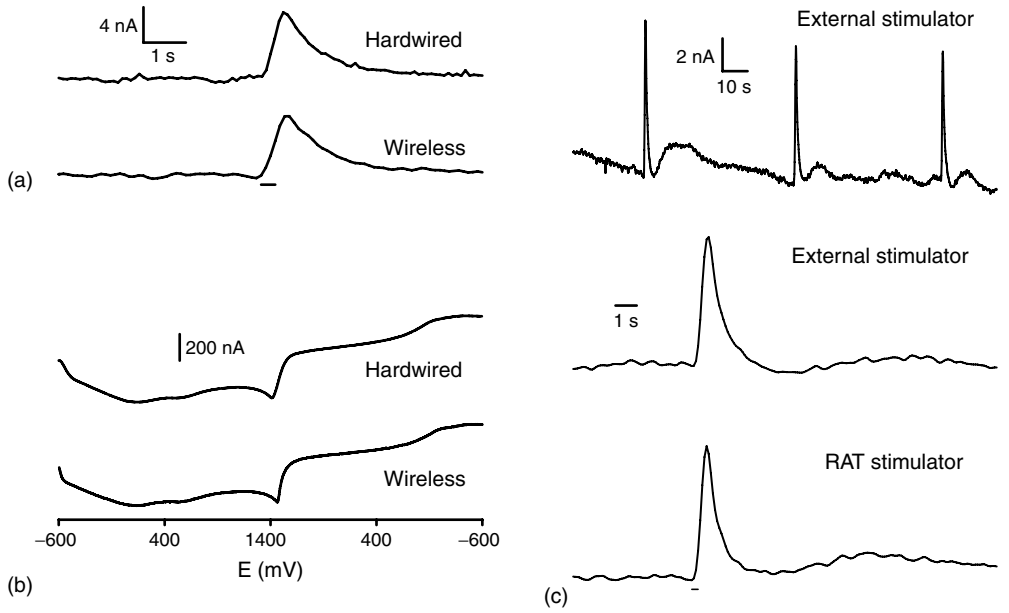

FIGURE 12.9 Comparison of hardwired and wireless systems for FSCV at a CFM and electrical stimulation: (a) dopamine levels were recorded at the same CFM by either the hardwired or wireless system (electrical stimulation was computer controlled and provided by the hardwired system as indicated); (b) background cyclic voltammograms were recorded at the same CFM by either the hardwired or wireless system as indicated; (c) dopamine levels were measured at the same CFM by the miniature RAT, but evoked either by the wireless device or an external stimulator (S88 Grass Stimulator, Grass-Telefactor, West Warwick, RI, U.S.A.) as indicated. All wireless measurements and electrical stimulation were performed by the miniature RAT shown in Figure 12.8. All recordings were collected in a urethane-anesthetized rat. The CFM was implanted in the dorsomedial caudate–putamen, and the stimulating electrode was implanted in the medial forebrain bundle. Electrical stimulation consisted of a 0.4 s, 60 Hz pulse train delivered at 125 μA.

traces recorded in the striatum of an anesthetized rat at the same CFM and collected by either the hardwired system (top) or the miniature RAT (bottom). Electrical stimulation, which elicited the signal increase, was generated by the computer of the hardwired system. Background currents for these recordings are found in Panel B. Overall, responses recorded by the hardwired system and miniature RAT are very similar. Panel C shows recordings collected in another anesthetized rat and at the same CFM by the miniature RAT. In this case, electrical stimulation was provided either by a stand alone unit (i.e., external stimulator) or the stimulus generator module of the miniature RAT. Again, similar responses were recorded by the two stimulation units.

The miniature RAT was also tested in a freely moving rat. The four components of this device were bundled together, wrapped in parafilm® (American National Can, Chicago, IL, U.S.A.), and attached using Velcro® tape to a home-made vest worn by the animal. This vest fit over the front paws, akin to a backpack. Animals were previously habituated to the vest and a mock RAT made with Play-Doh® (Hasbro, Pawtucket, RI, U.S.A.), of comparable size and weight. Figure 12.10 shows an electrically evoked trace recorded in the striatum of an ambulatory rat using the miniature RAT. A 60 Hz, 24 pulse train, applied to the medial forebrain bundle at the arrow, elicits a robust increase in the voltammetric signal. The background subtracted voltammogram (INSET) and color plot (below) identify the signal increase as originating from dopamine. The quality of the signal recorded with the miniature RAT is comparable to a hardwired voltammetry system (e.g., see Figure 12.4). Taken together, these results support the utility of RAT for the wireless monitoring of FSCV at a CFM and control of electrical stimulation in freely moving animals.

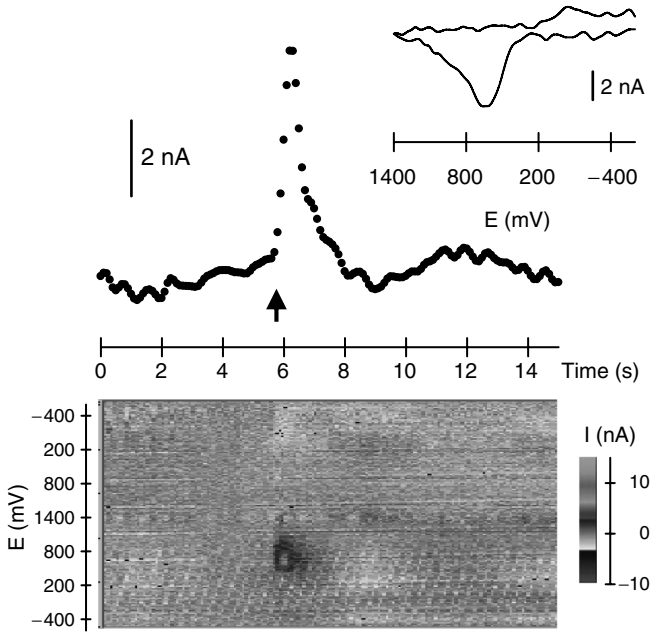

FIGURE 12.10 (**See color insert following page 272.**) Wireless measurement of electrically evoked dopamine levels in a freely moving rat. The recording was collected by the miniature RAT shown in Figure 12.8. The CFM was implanted in the dorsomedial caudate–putamen, and the stimulating electrode was implanted in the medial forebrain bundle.

FUTURE DIRECTIONS

The Smaller the Better

While the miniature RAT is manageable for an adult laboratory rat, a reduction in size will be beneficial by making the instrument less intrusive to the animal. Reduced size is additionally necessary for applying RAT to smaller rodents, such as the hamster and mouse. Hamsters are well suited models for studies on circadian rhythms [110], aggression [111], and sexual behavior [112]. Development of a wireless instrument for use in mice would be significant, because of the capability for generating transgenic animals. Indeed, transgenic mice have revolutionized biomedical research, making the mouse the model system of choice in several fields of neuroscience [113]. Currently, all electronic components on the main board and the Bluetooth module are now available in a profile approximately half the size. In addition, the newer Bluetooth devices have lower power requirements. Because the transmitter–receiver is the most power hungry of the electronic components on RAT, the size of the battery can be reduced as well. Another space saving modification is moving the stimulus generator circuit to the main board and using surface mount components for its fabrication. Thus, further miniaturizing RAT is imminently possible, even without a major change in engineering strategy.

Multifunctional Wireless Instrument

The ultimate goal of RAT is a multifunctional wireless instrument accommodating several technologies for neuronal monitoring and control. A module instrument is one strategy for realizing this goal. In such a design, the main module would consist of the micro-processor and Bluetooth telemetry. Plugged into the main module, and depending upon the application, is second

a module supporting a microsensor(s). This design is based on the notion that, regardless of the sensing technology, the measured signal is eventually converted into voltage and digitized. Front-end software for the home-base unit would also be developed for conditioning, viewing and analysis of each data type. In addition to the stimulus generator of the miniature RAT, it may also be possible to develop an iontophoresis module for focal delivery of drugs. Iontophoresis is routinely coupled with electrophysiology [114,115] and to a lesser extent, voltammetry (see below) [69,116].

As a first step to a multifunctional wireless instrument, we have already built, on the main board of the miniature RAT, a voltage-follower circuit with gain and filtering for extracellular recording of unit activity (Figure 12.8). The two black wires just above the dummy cell are the leads, and electrophysiological signals could be digitized by a free ADC channel on the micro-processor. In this manner, the miniature RAT could be used to measure voltammetry and electrophysiology simultaneously at separate microelectrodes. Additional switching circuitry between the voltammetry and electrophysiology circuits would support quasi-simultaneous monitoring of chemical and bioelectrical signals at the same CFM [68,69].

The impedance of the voltage-follower circuit is also sufficiently high to accommodate ion selective microelectrodes. Software changes for incorporating the latter sensing technology are straightforward to implement. Because changes in extracellular ion concentrations are relatively slow, a measurement would only need to be collected once every voltammetric scan (i.e., 10 Hz or every 100 ms). The data packet format used to send the voltammogram wirelessly easily accommodates this additional datum, thus affording simultaneous voltammetric and potentiometric measurements at separate microelectrodes.

Portable Microsensor Measurement System

In addition to measurements in freely moving animals, RAT will uniquely support other types of applications by functioning as a portable microsensor measurement system (PMMS). Ideally, the so called PMMS is a mobile instrument that is readily moved from place to place for operation. In this capacity, RAT confers at least four distinct advantages. First, the small size of the instrument permits convenient incorporation into existing, instrument-crowded set-ups. Second, RAT comes with its own data acquisition system. Thus, beyond the home-base Bluetooth module and serial card, no additional hardware is required. Our experience also indicates that the serial card does not interfere with the operation of standard data acquisition and control boards interfacing to the PCI-bus of the same computer, suggesting that RAT is compatible with most set-ups. If not, RAT can be simply run by a separate computer. The latter alternative, moreover, highlights another advantage of RAT: wireless communication allows the home-base unit to be situated several feet from the remote unit, further supporting convenient incorporation into existing set-ups. In the age of massive tangles of cables, even for home computer operations but especially in the laboratory, wireless connections are most welcome. Third, the relative insensitivity to ambient electrical noise permits applications of RAT in experiments that do not utilize a Faraday cage or are difficult to perform in a Faraday cage. And fourth, some applications, perhaps due to harsh reaction conditions, may require physically separating the sensor from other components of the set-up, and wireless communication may be the best way to achieve this separation.

Although there are several potential applications for RAT as a PMMS, two are discussed here. Figure 12.11a shows an electrophysiological record of extracellular unit activity in the striatum of the anesthetized rat (Sandberg et al. 2005). These multiple units were activated by glutamate and then inhibited by dopamine, both administered by iontophoresis. Because the units were recorded by a CFM (e.g., see Figure 12.4), it was possible to measure the iontophoresed dopamine profile subsequent to the electrophysiological record using RAT (Figure 12.11b). Such a determination is critical for calibrating iontophoresis, as the same applied current may yield different dopamine concentrations depending upon pipette fabrication. In this example, RAT was incorporated into a very crowded electrophysiology set-up containing various instruments, including an amplifier,

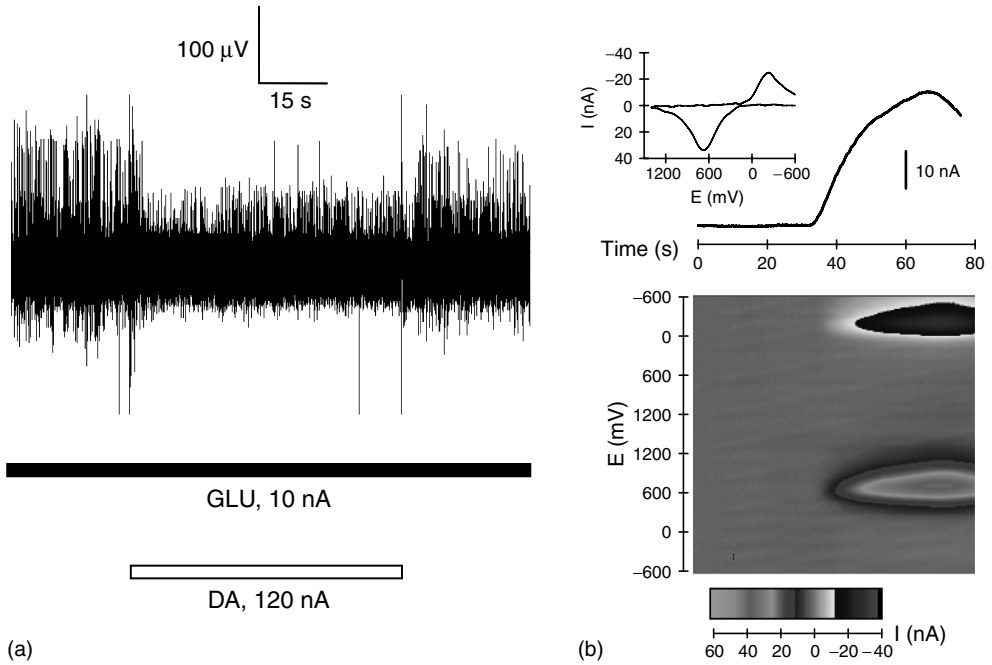

FIGURE 12.11 (**See color insert following page 272.**) Electrophysiological and voltammetric measurements at the same CFM. The CFM was implanted in the dorsomedial caudate–putamen of the urethane-anesthetized rat. Glutamate and dopamine were administered by iontophoresis. Electrophysiology was recorded by a standard hardwired set-up (negative voltage up), and dopamine was monitored by FSCV using RAT.

a filter, an iontophoresis unit, a stimulus generator, and an oscilloscope. RAT signals were also collected by a separate computer, for the simple reason that installing the home-base serial card in the computer interfaced to the electrophysiology set-up was inconvenient. Of course, an alternative and more elegant approach is quasi-simultaneous electrophysiology and voltammetry as discussed above. However, this approach requires special hardware and software and may not be absolutely necessary for all experiments.

A second application of RAT as a PMMS is shown in Figure 12.12. This figure shows a RAT measurement of dopamine in the striatum of an anesthetized rat during cerebral ischemia [117]. Focal ischemia was caused by the intraluminal thread model of middle cerebral artery occlusion [118]. In this model, a fine nylon thread is inserted into the internal carotid artery and advanced until the middle cerebral artery, which feeds the striatum and adjacent cortex, is unilaterally occluded. In agreement with previous work [119,120], ischemia elicited a massive increase in extracellular dopamine. The advantage of FSCV in these ischemia experiments is the better temporal resolution compared to the microdialysis and voltammetry approaches used earlier.

As a PMMS, the ischemia example highlights another advantage of RAT: measurements outside of a Faraday cage. Indeed, the ischemia experiment requires a microsurgical scope and accessory instruments, e.g., for blood pressure and heart rate monitoring and temperature control, that are difficult to fit inside a Faraday cage. Moreover, the rat lies in a supine position during experimental ischemia, but pronely for voltammetric measurements in a stereotaxic apparatus. To overcome this latter problem, the animal was first surgically prepared for dopamine monitoring under freely moving conditions (see Figure 12.3 and Figure 12.10). For the subsequent ischemia experiment, the rat, with an implanted CFM and stimulating electrode, was then placed supinely on a raised platform with a cutout portion through which the hardware mounted on the head could

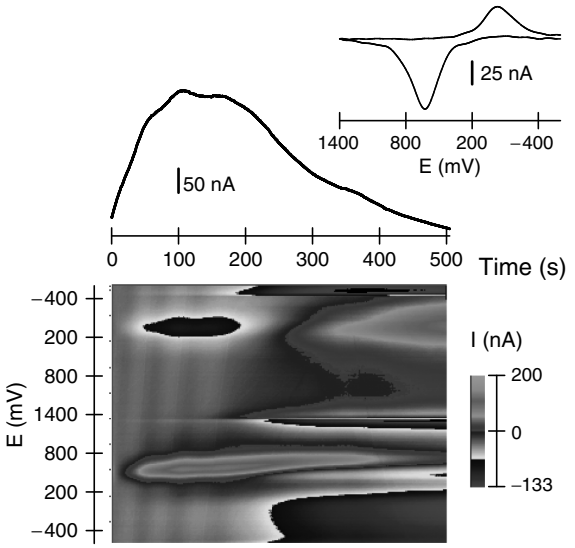

FIGURE 12.12 (**See color insert following page 272.**) Voltammetric measurement of dopamine during focal cerebral ischemia. The CFM was implanted in the dorsomedial caudate–putamen of the isoflurane-anesthetized rat. The middle cerebral artery was occluded for 20 min, beginning approximately 120 s prior to the onset of the voltammetric recording.

safely pass. The implanted electrodes were then connected to RAT, which transmitted data to the home-base unit located several feet away from the crowded ischemia set-up.

CONCLUSIONS

We describe a new instrument called RAT, developed for wireless neural monitoring and control. Presently, RAT supports FSCV at a CFM and electrical stimulation, and is suitably sized for adult rats. Extending the functionality of the existing RAT to electrophysiology and potentiometry should be straightforward. Further miniaturization of RAT for use in smaller rodents such as hamsters and mice should be possible with available smaller components and without major re-engineering. In addition to experiments with freely behaving animals, RAT also has utility as a PMMS.

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