Microcontroller-Based Wireless Recording Unit for Neurodynamic Studies in Saltwater

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***Abstract*—This paper presents the design of a biocompatible implantable neural-recording unit for *Aplysia californica*, which is a common sea slug. Low-voltage extracellular neural signals ($< 250\ \mu$V) are recorded using a high-performance low-power low-noise preamplifier that is packaged with programmable digital data acquisition and control, and frequency-shift keying (FSK) telemetry that provides 5-kb/s wireless neural data through 18 cm of saltwater. The telemetry utilizes an 8-cm electric-dipole antenna matched to 50 Ω by exposing the ends of the antenna to the saltwater. A custom 27-MHz receiver has been developed using commercially available ICs. A clock data recovery algorithm is implemented in a microcontroller to synchronize the received data. A 3-V lithium-ion battery (160 mAh) allows 16 h of recording. Neural data obtained using extracellular nerve electrodes and a wired interface to this unit exhibit a 2.5-mV-rms noise, which is comparable to a commercial neural-recording equipment. Neural data were also collected through the wireless link, demonstrating the feasibility of low-power transmission through saltwater.**

***Index Terms*—Data acquisition, implantable, low-noise amplifier, low-power circuits, neural sensor, telemetry.**

I. Introduction

NERVOUS SYSTEMS of marine animals, particularly invertebrates, can serve as model systems for the study of neuroscience. A nervous system is an adaptable robust information processor that can transform sensory information into advantageous behavior through a bodily interface to the outside world. By studying more tractable systems, one can gain some insight into mechanisms of neural computation on the level of individual neurons [1]. This is not only scientifically and clinically significant, but may also have broad implications for engineers, particularly in the area of circuit and algorithm design [2], [3]. While more tractable nervous systems are widely studied [4], access to these systems is limited by available technology. Consequently, there is a growing demand for neural-recording units with which to observe the electrical activity of neural circuits without significantly disturbing their function. A recording unit must fulfill several requirements. First, it must have a very low noise, since extracellular neural signals are often in the microvolt range. Second, it must provide multiple channels of data, since a nervous system will have hundreds to billions of neurons. Third, it must be as noninvasive as possible. Ideally, neural recordings would be obtained from a freely behaving animal during the course of its normal behavior.

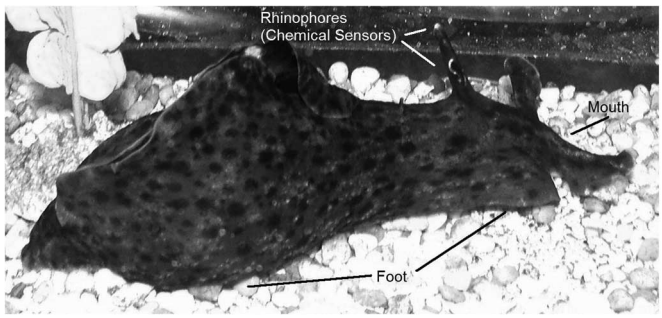

Fig. 1. Common sea slug *Aplysia californica* in an aquarium environment.

The common sea slug *Aplysia californica*, which is pictured in Fig. 1, has a particularly tractable system with approximately 20 000 neurons [5]. In this system, neural activity can be recorded *in vivo* and directly correlated with behavior. For example, Morton and Chiel demonstrated that one could quantitatively predict the feeding behavior of the animal (ingestive or egestive) from patterns of neural activity recorded from nerves [6], [7]. It has also been demonstrated that this animal is capable of simple forms of learning, such as distinguishing the taste of edible and inedible material [8]. However, studies of learning take place over many days and require a cooperative healthy animal. The current approach to *in vivo* neural recording involves stainless-steel hook electrodes glued to the animal's nerve or ganglion [9]. However, without wireless capability, the animal is tethered by transcutaneous electrode leads, which

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can significantly adversely affect its behavior. Also, these leads are a significant source of noise in an already weak signal. For long-term neural recording during learning tasks, a new wireless approach is required.

Implantable neural-interface units currently take many forms. In the area of neural stimulation, the most common in present use is the cardiac pacemaker [10]. Functional electrical stimulation can also be used to assist patients in grasping, standing, or urination [11]–[14]. Deep brain stimulation is an effective treatment for Parkinson's disease [15], [16]. Neural prostheses are commercially available for treating deafness in children [17], [18], while visual prostheses have had some preliminary success in creating sensations of vision [19], [20]. Neural-sensing technology is somewhat less common. However, multielectrode arrays are currently used in humans to determine the locus of epileptic seizures before surgery [21]. Neural recordings are also being studied as possible control signals for artificial limbs. It has been shown that a monkey can move a cursor to a target on a computer screen, or control a robot arm with its neural activity alone, recorded from the motor cortex [22].

In our application, which is the wireless neural sensing for scientific studies, there has been a considerable previous work. Large-scale multichannel wireless neural-recording units have been developed at the University of Michigan [23]–[29]. These include a complete system described by Mohseni and co-workers, having a low noise of 7.8-μV rms and power dissipation of only 2.2 mW. Unfortunately, the integrated probes for these units, which are developed primarily for primates, include comblike arrays or sieve electrodes and would be difficult to use with discrete ganglia. Also, the wireless links are not optimized for underwater use, which involves heavy signal attenuation through many centimeters of saltwater. The unit described here is designed specifically for discrete ganglia deep within a saltwater medium. Several smaller scale implantable recording units have been designed that incorporate a high-gain differential amplifier for neural signals and a wireless telemetry link from a similar environment [30]–[40]. Units developed for primates are simply too large for small animals [39], [40]. The noise in the remaining units, which is from 8–150-μV rms, is unfortunately too high for use in *Aplysia*. The design presented here offers two improvements. First, we use a low-noise (2.2-μV rms) CMOS bioamplifier, which is described in detail in [41]. Second, we add a microcontroller with integrated A/D conversion to digitize neural signals close to the source, with a consequent overall noise of 2.8-μV rms.

This paper presents a biocompatible implantable neural-recording unit for use in *Aplysia californica* that can transmit neural data wirelessly from within a freely behaving animal in an aquarium environment. It discusses the design and implementation of this unit and provides preliminary neural data. It also discusses the design of a custom wireless receiver for this application. The performance of this system is comparable to the standard conventional laboratory equipment for neural-signal acquisition. The basic approach can be scaled to produce an implantable wireless data acquisition unit for large-scale multichannel invertebrate studies of discrete ganglion. In addition to scientific benefits, a similar unit may have clinical applications in humans, particularly in the control of discrete ganglia in the autonomic nervous system [42]. A preliminary version of these results has been presented at a conference [49].

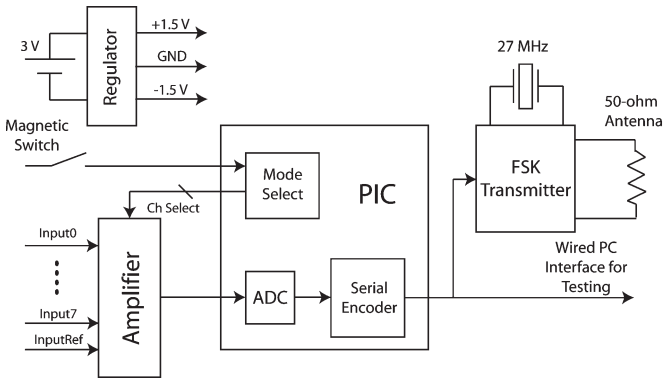

Fig. 2. Overall block diagram of the implantable wireless recording unit.

II. System Architecture

The specific application addressed here is neural recording in freely behaving *Aplysia californica* sea slugs. For live recordings, the input signal is obtained from an implanted stainless-steel wire glued to a nerve or above a particular neuron on the ganglion [6], [7], [9]. The neural-action potentials themselves range from 10 to 300 μV in amplitude with frequency content primarily between 100–500 Hz, with a small contribution from the firing frequency that may be 0.5–100 Hz. In addition to 60-Hz interference, the low-frequency noise is particularly challenging, since saltwater electrodes are associated with very large voltage drift on the order of hundreds of millivolts.

Fig. 2 shows an overall block diagram of this implantable sensor. The input electrodes attach to a high-gain differential amplifier with a bandpass filter. The single-ended output of this amplifier is digitized using an 8-bit microcontroller, which arranges the 8-bit data into a serial stream. Channels can be selected from outside of the animal using a magnetic switch. The microcontroller sends this data either directly to the PC through a wired RS-232 interface or to a commercially available frequency-shift keying (FSK) transmitter. It is then transmitted out of the animal using an electric-dipole antenna that can be implanted along the length of the animal's foot. The receiving antenna must be located inside the aquarium. A customized commercial receiver demodulates the incoming FSK data and recovers the sampling clock from the data using an algorithm on a microcontroller before passing it on to the PC via an RS-232 interface. A wired interface is also available for testing the device without the transmitter.

III. Preliminary Experiments

Since saltwater severely attenuates electromagnetic signals, several preliminary experiments were conducted to determine the feasibility of a saltwater telemetry system. Optical communication was not an option since the system is fully implantable without any transcutaneous element, and the skin of *Aplysia* attenuates IR. Far-field electromagnetic was also not a good option since high carrier frequencies are severely attenuated by

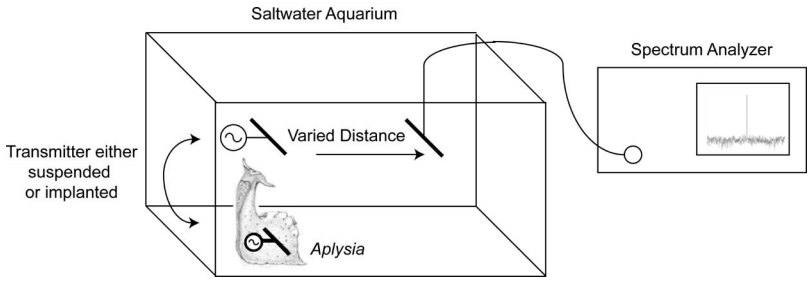

Fig. 3. Experiment setup for antenna testing in an aquarium. Transmitting antenna is either submerged or attached to a moveable pipe or implanted in a live animal (*Aplysia* sketch from [43]).

the saltwater. Thus, the primary options considered were near-field magnetic and electric.

Several antenna configurations were evaluated: an 8-cm perimeter, two-turn loop inductive antenna (magnetic dipole), as well as an 8-cm electric-dipole conductive antenna with ends exposed to the saltwater, with the receiving antenna in each case both inside and outside the tank. Testing was done in a 60-cm 15-gallon saltwater aquarium. A commercially available transmitter provided a 1-mW 27-MHz source, and a spectrum analyzer measured the signal strength at the receiving antenna. Fig. 3 shows the experimental setup. The transmitter must be completely submerged for accurate measurement of the attenuation. If any part of the transmitter was not submerged, a significantly larger signal was recovered at the receiving antenna in any location.

While there was severe attenuation, the 0-dBm transmitted signal produced a receive signal > -75 dBm for distances as large as 18 cm for both electric and magnetic dipoles. An electric-dipole conductive antenna with ends exposed had a comparable performance to a magnetic loop inductive antenna of comparable size provided that the receiving antenna was located within the tank, as illustrated in Fig. 4. A magnetic loop antenna with an outside receiver did produce a signal larger than the electric dipole by 8 dB.

Magnetic loop antennas are commonly used in implantable units [44]. However, in our application, the invasiveness of the implantation is probably the most important factor, since behavioral experiments require healthy cooperative animals. Therefore, we chose the electric-dipole antenna because the long thin wire can be more easily implanted through a small incision into the open space in a sea slug's foot. This will become particularly important in future smaller versions of this design.

It is important to note that both the electric and magnetic-dipole antennas had significant directional dependence. Fig. 5 shows the received signal as a function of the angle between the receiving and transmitting antennas at a fixed distance of 8 cm. Since the animal does stay attached to the walls of the aquarium, the possible orientations are somewhat restricted. However, the experimental setup will require multiple receiving antennas in several orientations to cover the entire aquarium, which is 60 cm in length.

Animals were tested and found to tolerate unit implantation. On one occasion, the transmitter only (encapsulated in silicone glue) was implanted into a 400-g animal through a 3-cm incision near the head. The circuit was maneuvered into the relatively large open space above the animal's foot. We verified that the signal strength observed at the receiving antenna matched the data shown above. The animal ate and behaved normally after the implantation. On another occasion, we implanted a blank PCB of equal size to our prototype (including the battery) using similar encapsulation. That animal also ate and behaved normally. From these two experiments, it seems that *Aplysia* is sufficiently robust for implantation.

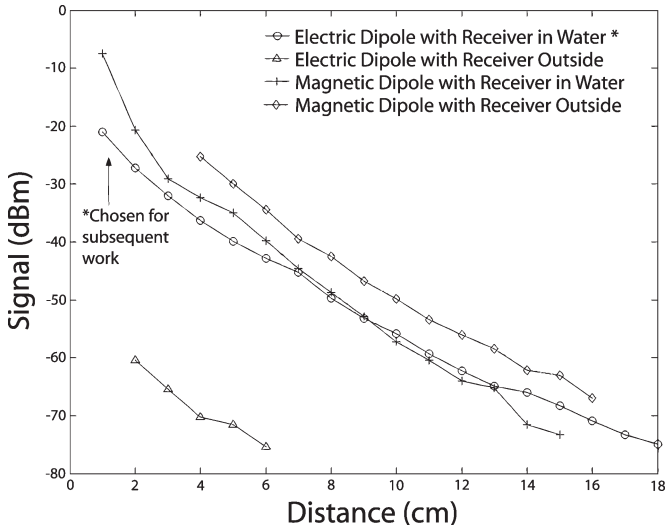

Fig. 4. Measured received signal strength as a function of distance for electric and magnetic-dipole antennas with receive antenna inside and outside water. The transmit signal is supplied by a 0-dBm source.

IV. Detailed Design

A. Bioamplifier

The extracellular electrodes attach directly to the differential inputs of a custom bioamplifier IC having core circuits that are described in [41] (see Fig. 6). It is designed for a first-stage gain of 100, with C1 set to 20 pF and C2 set to 200 fF. The gain of the second stage was set to 39 using external resistors, with $R2 = 390$ kΩ and $R1 = 10$ kΩ. Due to the very large low-frequency voltage drift associated with stainless-steel electrodes in saltwater, it was necessary to use an extra high-pass filter in the second stage. Unfortunately, since the second-stage high-pass filter is shared by the multichannel first-stage amplifier, the switching time between channels is several hundred milliseconds.

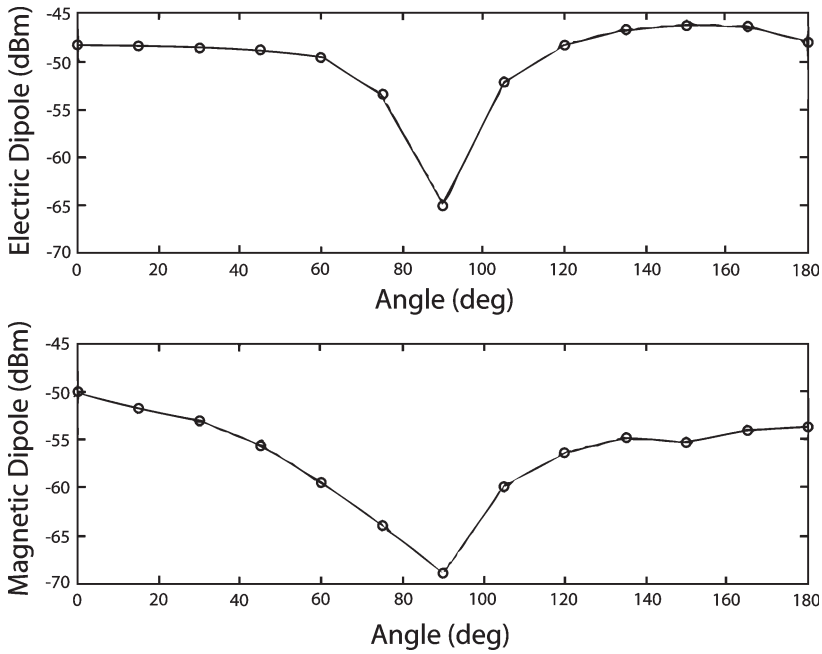

Fig. 5. Measured orientation dependence of electric and magnetic-dipole antennas. Data indicate the received signal strength as function of angle between the two, which is at a distance of 8 cm.

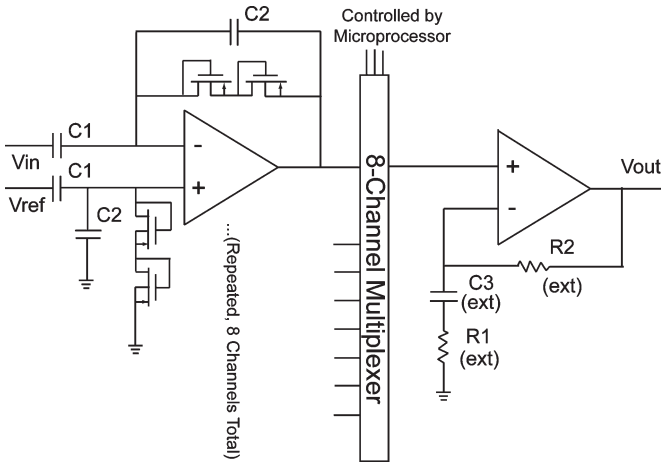

Fig. 6. Schematic of the eight-channel bioamplifier.

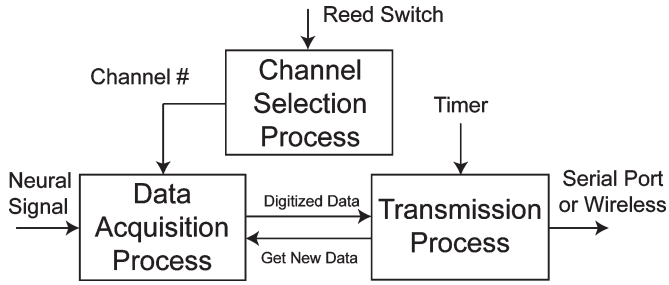

Fig. 7. Block diagram illustrating microcontroller processes in the wireless recording unit.

With C3 set to 1 μF, the high-pass and low-pass cutoff frequencies of the overall frequency response are 100 Hz and 7 kHz, respectively, for which the previously reported input-referred noise is 2.2-μV rms. Each of the eight first-stage amplifiers can be selected with a multiplexer, which has three associated control lines set by the microcontroller.

B. Microcontroller

For the microcontroller, we chose the PIC18F1320. This integrated circuit is low-power (450 μW), compact and inexpensive. It includes a built-in 8-bit A/D converter and RS-232 port. The microcontroller operates three parallel processes: channel selection, data acquisition, and data transmission (Fig. 7). The channel-selection process is activated using a contactless magnetic reed switch, which advances the acquisition mode with each pass of the magnet. Depending on the mode, a single channel, or preprogrammed subset of the eight channels, is scanned and successively sampled, which is subject to the limitation described above. Similar to the T1 communications protocol, a frame alignment bit (FAB) is added to the end of each data sample to form a slot. Twelve such slots are followed by a 4-bit channel number to form a frame, and the 12 FABs and 4-bit channel number are collectively referred to as the "frame alignment word" (FAW). The FAW is used to align the data word at the receiver end. When testing the wireless link, the microcontroller was programmed to send known varying output data.

C. Wireless Link

For the wireless transmitter, we chose a commercially available integrated circuit, which is the Tricome T86. It modulates the digital data using FSK with a central frequency of ~27-MHz, which is an FCC-allocated frequency for applications having a relatively low carrier frequency and wide bandwidth (320 kHz). The relatively low carrier frequency has less attenuation in saltwater than higher frequencies, and the large bandwidth will permit FSK bit rates up to 80 kb/s in future work. The maximum throughput for the present unit is 5 kb/s.

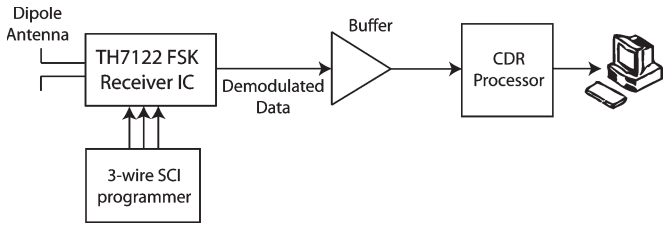

Fig. 8. Block diagram of the 27-MHz FSK receiver including serial control interface (SCI).

The impedance of the electric-dipole antenna is determined by both its geometry and the resistivity of the saltwater to which its ends are exposed. Antenna impedance was measured with a network analyzer while antennas were submerged. Varying the length, wire gauge, and amount of insulation removed from the tips fortuitously yielded a combination close to 50 Ω, which is the output impedance of the T86 transmitter.

The final antenna was formed using 8 cm of 26 gauge copper wire. Prior to implantation, the tips of the wire were coated with silicone glue to prevent the sharp wires from irritating the animal.

D. Receiver

A 27-MHz FSK wireless receiver was designed using the TH7122 Melexis transceiver IC [45] and other commercially available components, as illustrated in Fig. 8. This circuit can receive digital FSK data with a frequency deviation as narrow as 6.6 kHz, as is provided by the transmitter IC using a single-conversion superheterodyne architecture. The receiver uses the phase-coincidence principle for demodulating the FSK signal [45]. Since the frequency deviation in this application is very narrow, the discriminator used for phase-coincidence had to be adjusted for a narrow frequency-versus-voltage (F–V) characteristic. This was achieved by using a high resistance in parallel with the discriminator.

A varactor diode and 230-MHz inductor were used to generate a mixing frequency near 37.8 MHz and then tuned for an intermediate frequency (IF) of 10.7 MHz by programming the required ratio of reference and feedback divider factor. The reference divider is set to its maximum value to allow small tuning steps in the range of several kilohertz. An external buffer at the demodulated output was used to make the output level compatible with a CMOS digital input. This demodulated signal was provided to a receiver microcontroller that performed clock/data recovery (CDR) and also provided the programming interface between the PC and receiver IC.

E. Clock-Recovery Circuit

In order to sample the demodulated output, a clock was recovered using a Hogge phase detector [46] that was implemented in software using the receiver microcontroller. The algorithm is as follows.

1) Generate a clock at the nominal frequency of the data using a counter (Tcount).
2) Record the time of each falling edge of the clock (Tf) and each data transition (Td).

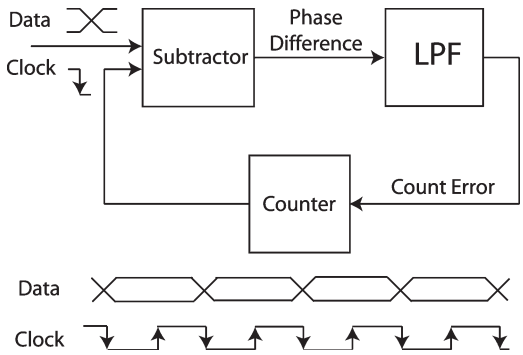

Fig. 9. Block diagram of CDR algorithm used in the receiver microcontroller including low pass filter (LPF).

3) Calculate the phase difference (Pdiff) between the two using the following equations:
   a) If $\mathrm{Td} - (\mathrm{Tf} + \mathrm{Tcount}) > 0$ {i.e., data transition is lagging the falling edge}

$$\mathrm{Pdiff} = \mathrm{Td} - \mathrm{Tf}.$$

   b) Else {i.e., data transition is leading the falling edge}

$$\mathrm{Pdiff} = \mathrm{Td} - (\mathrm{Tf} + \mathrm{Tcount}) - \mathrm{Tcount}.$$

4) Filter Tdiff using a low-pass filter (first-order infinite impulse response) to compute the error.
5) Add this filtered phase error to the counter, which is scaled by an appropriate relaxation factor, to compute an updated clock frequency.
6) Repeat to maintain lock.

The block diagram for this process is shown in Fig. 9. This algorithm is similar to a digital PLL, but uses a stepwise sequential implementation and provides a low-cost CDR for low data-rate applications. The sampled data are transferred to a PC using the serial port where a Matlab program is used to align to the FAW and strip FABs.

F. Testing Methods

In experiments that involved submerging the unit, the circuit board was covered with General Electric Silicone II glue and left to dry for 24 h. The battery was added by cutting away a flap of the silicone, resealing it once the battery was placed into the unit and waiting an additional hour before use. If the unit was intended for implantation, it was cured in running water.

The neural data shown in this paper were obtained with an *in vitro* preparation. The animal was anesthetized with 50% of its body weight (milliliters per gram) of MgCl2. The feeding musculature was then dissected out, and the buccal ganglion, which includes ∼2000 neurons responsible for controlling the feeding behavior, was carefully removed from the muscles. This ganglion was pinned out in a Sylgard-covered Petri dish. Buccal nerve 3, which contains the axons of many large motor neurons, was suctioned into a thin polyethylene tube filled with saltwater, and an Ag/AgCl wire was inserted into the solution. The end of this wire was attached to the input of our unit for testing.

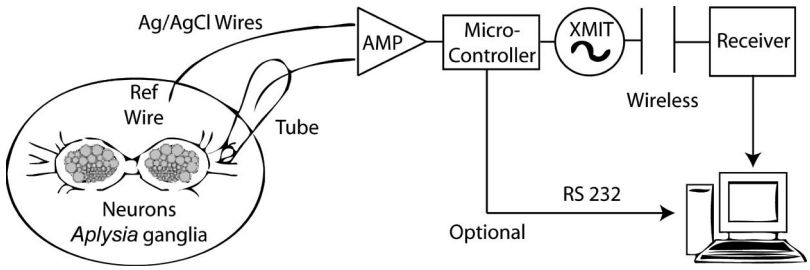

Fig. 10. Experimental setup for *in vitro* neural tests.

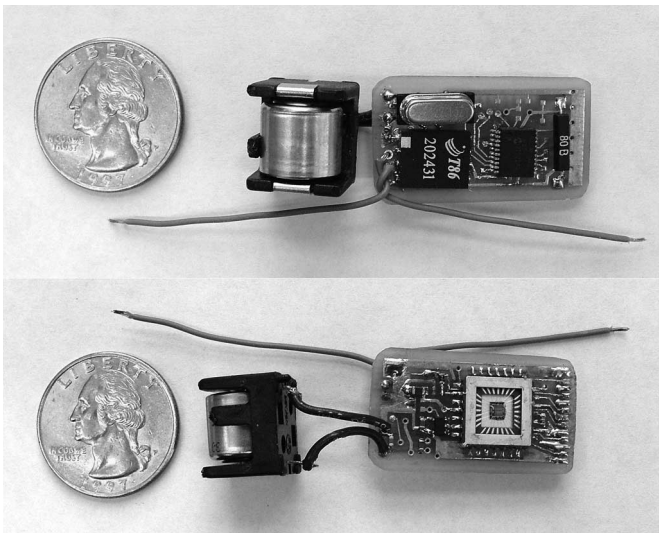

Fig. 11. Photograph of the wireless recording unit.

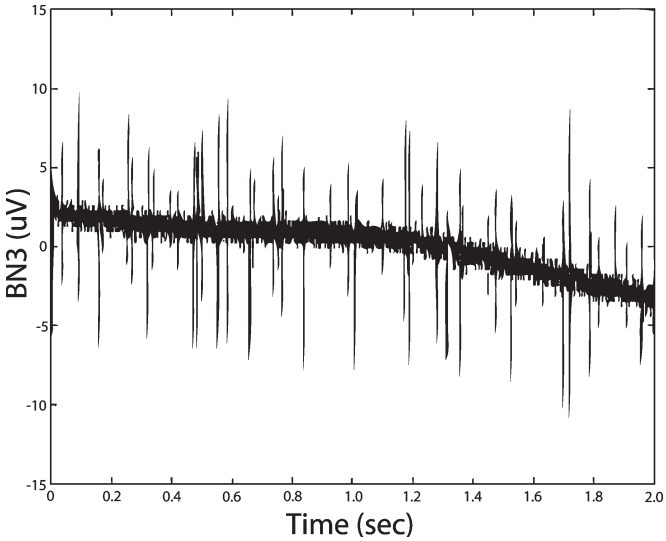

Fig. 13. Measured *Aplysia* neural data illustrating the challenge of low-frequency drift.

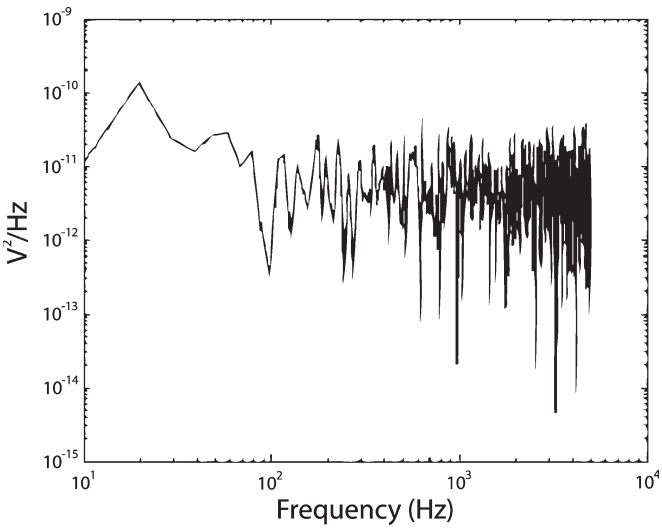

Fig. 12. Measured input-referred noise spectral density of the wireless recording unit.

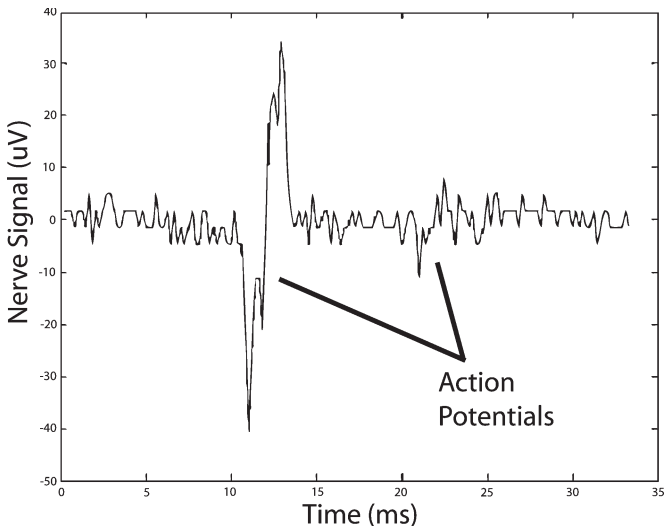

Fig. 14. Measured midsized and small-sized action potentials recorded from *Aplysia* nerve using wired 4.8-ksps interface.

Fig. 10 shows a diagram of this setup. While only one channel at a time was tested in our validation, this *Aplysia* preparation and similar *in vivo* techniques are routinely used to record from multiple nerves and could be used as a test bench for several channels.

V. Experimental Results

A. Fabrication

The custom amplifier IC was fabricated in the AMI ABN 1.5-μm two-metal two-poly CMOS process and packaged in an LCC28. Each of the eight amplifier circuits uses 0.16 mm^2 of

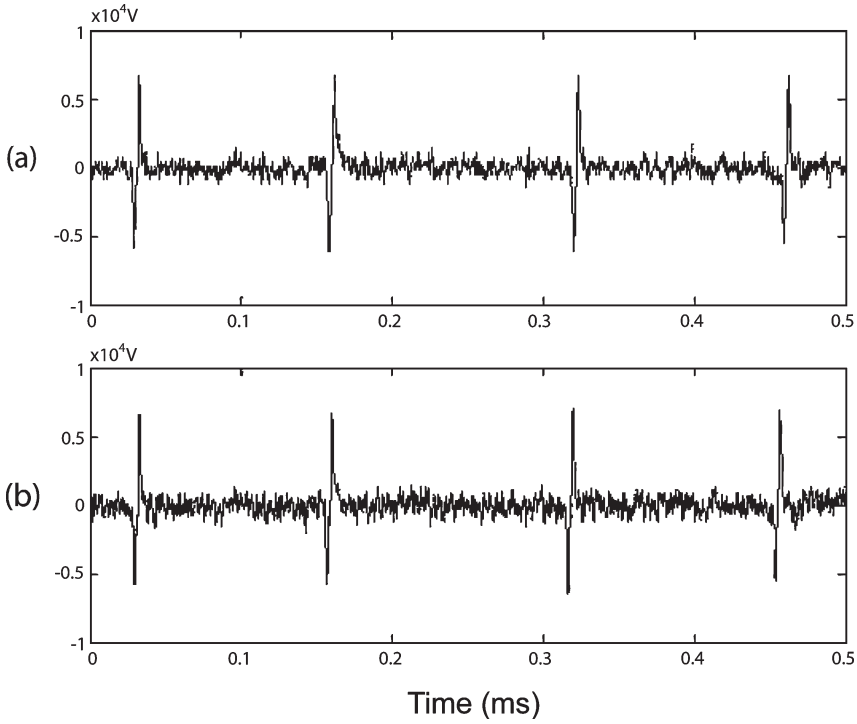

Fig. 15. Measured action potentials using (a) wired connection from the implantable recording unit and (b) commercial rack-mount amplifier.

silicon area, and 67% of this area is taken up by capacitors. All the components discussed above were assembled on a double-sided PCB (Fig. 11) measuring $1.2'' \times 0.6''$ powered by a 3-V size 1/3N 160-mAHr lithium-ion battery. This unit can be continuously operated for 16 h using the wireless interface and 90 h using a wired interface.

B. Noise Performance

We tested the noise performance of this prototype inside a full Faraday cage used for neuroscience recordings. The unit was initially tested using the wired interface with optocoupler to avoid the noise injected from the leads (Fairchild P/N H11L1M). The measured gain was 3480. The amplifier itself has a measured noise of 2.5-μV rms, which is comparable to the 2.2-μV rms reported in [41]. The addition of the input wires, the microcontroller, and the transmitter did increase the noise slightly to 2.8-μV rms. The noise spectral density is shown in Fig. 12, which is adequate for recording very small action potentials.

The noise, however, increased when the electrodes were placed into a saltwater solution, particularly in the low-frequency range. Even with the addition of a high-pass filter on the second stage, the DC value tends to drift with an amplitude up to 260 μV. The frequency is too low, which is < 1 Hz, to show up accurately in the logarithmic plot presented above. However, Fig. 13 shows a recording from our device of *Aplysia* action potentials on this drifting baseline using Ag–AgCl electrodes *in vitro*. Note that without high-pass filtering, these drift amplitudes would be hundreds of millivolts and somewhat worse for stainless-steel electrodes and *in vivo* recording. High-frequency noise tended to vary significantly from its base value of 2.8- to 4.1-μV rms presumably due to outside interference. The unit also began picking up ground artifacts that resembled "burst" noise, with discrete jumps from state to state [47], [48].

C. Measured Neural Data Using Wired Connection

With the second-stage high-pass filter, we were consistently successful in measuring the neural activity *in vitro*. Fig. 14 shows an example of a midsized and small-sized *Aplysia* action potential as recorded by the prototype using the wired interface for increased sampling (4.8 ksps). The quality of this data is comparable to the commercial rack-mount instruments. The unit is convenient to use even without implantation since it can be easily attached to a micromanipulator to provide reduced noise by digitizing neural data close to the source.

Fig. 15 shows the performance of the microcontroller including the A/D converter and the serial data link. The trace on the bottom [Fig. 15(b)] shows *Aplysia* action potentials at the output of the amplifier digitized by a conventional commercially available unit, which is the Axon Instruments Digidata 1322A using AxoScope 9.0.2 software. The top trace Fig. 15(a) shows the same data digitized by the microcontroller and sent via RS-232 interface to the PC (acquired in Matlab). One can see that the microcontroller is accurately digitizing and transmitting the input signals.

Practically speaking, the interference noise must be reduced for live animal recordings, since the amplitude of *in vivo* signals tends to be somewhat lower. In preliminary *in vivo* results, only the largest action potentials can be distinguished, as shown in Fig. 16. Also, since a high-pass filter is used at the input of the second stage, the offset difference between channels combined with the settling time at the second stage makes it difficult to scan through the channels quickly. In future versions of this system, we will either raise the cutoff frequency of the first

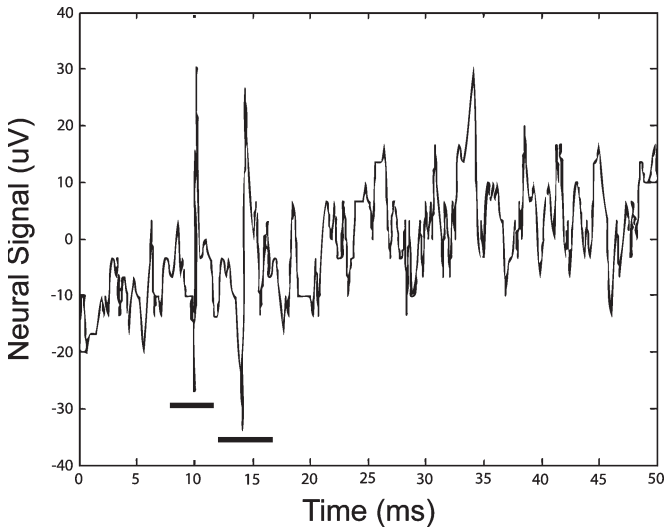

Fig. 16. Measured action potentials using *in vivo* preparation with stainless-steel nerve hook electrodes and the wired connection. Black bars denote identifiable action potentials while the remaining activity is noise.

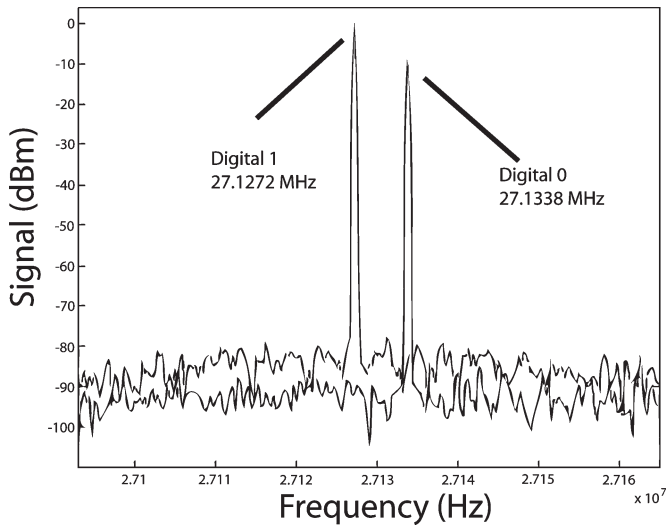

Fig. 17. Measured FSK spectra from the recording unit using antennas in close proximity and constant binary inputs.

stage or add a dedicated second-stage amplifier to each channel to facilitate a scanning mode.

D. Wireless Transmission

The commercial transmitter successfully modulates the digital data with a bandwidth of 6.6 kHz and a central frequency of 27.1305 MHz. Fig. 17 shows the resulting frequencies for digital inputs of zero and one. The signal strength was measured at a distance up to 18 cm with the unit implanted inside of the animal.

The receiver was tested with the silicone encapsulated unit and receiving antenna immersed in a saltwater aquarium. Data were faithfully transmitted to the receiver up to a distance of 10 cm where the signal strength at the receiving antenna was $\sim$50 dBm. While the receiver is usually capable of much higher performance, the Tricome T86 provides a very low FSK frequency deviation: 6.6 kHz at 27.135 MHz, which accounts for this required signal strength. Our future custom transmitter will use a wider bandwidth. The CDR algorithm is able to lock into $+/-$ 10% of the data rate at the nominal rate of 5 kb/s.

Neural data were obtained using an *in vitro* preparation and the custom receiver with both antennas submerged in a small saltwater container. A single action potential is shown in Fig. 18. While this unit only allows for one undersampled channel of transmission ($\sim$555 sps), it provides proof of concept for a future custom FSK transmitter with a larger bandwidth. Similarly, this transmitter's high power consumption (30 mW) is a limiting factor in this design, but the efficiency can be significantly improved in future custom designs. The next-generation transmitter will be capable of an 80-kb/s data rate, delivering a 1-mW signal with 80% efficiency.

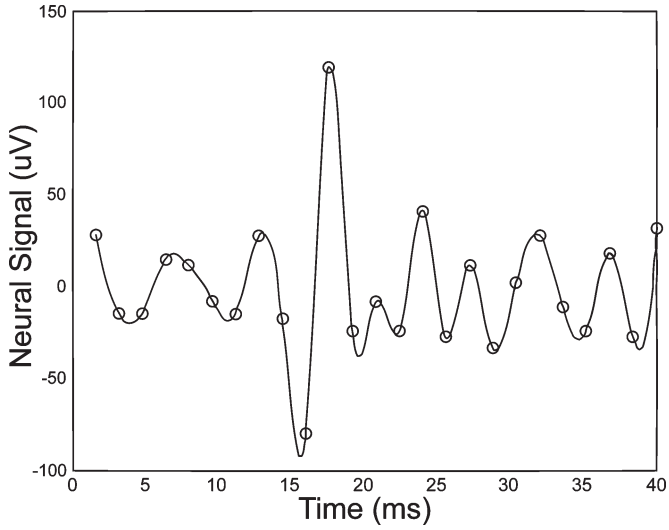

Fig. 18. Measured *Aplysia* action potential using 5-kb/s ($\sim$0.555 ksps) wireless interface. Smooth line shows spline interpolation of the undersampled data.

VI. Conclusion

In its current state, this prototype can be used for neurodynamic studies of *Aplysia in vitro* preparations. Also, some preliminary testing has been done with freely behaving animals. Using what we have learned from this iteration, we have fabricated and begun testing the next-generation prototype. We have reduced the size of the implantable unit by fully integrating the electronics and added stimulation capability as well as recording. For the wireless link, the power consumption of our custom-designed FSK transmitter (1 mW) is dramatically lower than the commercially available transmitter we used in this prototype (30 mW). Also, the bandwidth was raised to 80 kb/s to allow a sufficient sampling rate for multiple channels.

Since the smaller tractable multineuron circuits found in invertebrates such as *Aplysia* resemble those used for simulated and integrated-circuit neural networks, multichannel invertebrate studies could provide a wealth of information for this field. Also, due to the geometrical similarity of invertebrate ganglion to sympathetic ganglia located in the human autonomic nervous system, interface units designed for invertebrate systems could possibly be used to restore function in humans.

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