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# A multi-channel telemetry system for brain microstimulation in freely roaming animals

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**Abstract**

A system is described that enables an experimenter to remotely deliver electrical pulse train stimuli to multiple different locations in the brains of freely moving rats. The system consists of two separate components: a transmitter base station that is controlled by a PC operator, and a receiver-microprocessor integrated pack worn on the back of the animals and which connects to suitably implanted brain locations. The backpack is small and light so that small animal subjects can easily carry it. Under remote command from the PC the backpack can be configured to provide biphasic pulse trains of arbitrarily specified parameters. A feature of the system is that it generates precise brain-stimulation behavioral effects using the direct constant-voltage TTL output of the backpack microprocessor. The system performs with high fidelity even in complex environments over a distance of about 300 m. Rat self-stimulation tests showed that this system produced the same behavioral responses as a conventional constant-current stimulator. This system enables a variety of multi-channel brain stimulation experiments in freely moving animals. We have employed it to develop a new animal behavior model ("virtual" conditioning) for the neurophysiological study of spatial learning, in which a rat can be accurately guided to navigate various terrains.


*Keywords:* Telemetry; Electrical stimulation; Stimulator; Multi-channel; Rat; Navigation; "Virtual" conditioning

## 1. Introduction

As a fundamental tool in neurophysiology (Ranck, 1975; Tehovnik, 1996), electrical stimulation has long been used extensively in brain research (for a historical review, see Yeomans, 1990), but such experiments can sometimes be constrained by the physical necessity of using electric cables to connect brain-implanted electrodes to an external stimulator. While in anesthetized animals cable connections are generally adequate, in awake animals they not only limit the subject's freedom of movement, but also may distract its attention or produce emotional distress. Even though small animals such as rats tolerate the physical presence of skull-affixed cables, these animals' behavioral repertoire and maneuverability is restricted to small spaces, typically simple mazes or experimental chambers. At times, they can also impose visual and kinematic distractions, and the cables are often bitten or chewed. Moreover, these problems get worse in experimental protocols that require stimulation through large number of electrodes in the brain, which requires increasingly massive and unwieldy cable connections.

These problems might be alleviated by a multi-channel telestimulation system small enough to be carried by the animal in a backpack. This would enrich the scope of investigable behavioral paradigms and would specifically enable brain stimulation experiments in animals moving freely in large and complex three-dimensional (3-D) environments.

Pioneering work on telestimulation devices began in the 1930s (Light and Chaffee, 1934; Loucks, 1934), and continued for many years thereafter (Delgado et al., 1975; Gengerelli, 1961; Greer and Riggle, 1957; Lafferty and Farrell, 1949; Warner et al., 1968). These devices have limitations, however, that tend to prevent them from fulfilling the requirements of modern neurophysiological

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investigations: (1) Most provided only a single channel of stimulation allowing only one brain site to be excited at a time. (2) The fidelity of the transmission was usually poor. For example, the stimulus intensity tended to be dependent on the amplitude of the received analog signal, which varies with transmission fidelity. (3) Even though investigators put much effort into reducing the size and weight of the receiver that was implanted or mounted on the animal, the excessive size and weight of the stimulus generators and transmitters, plus the high power required to maintain transmission fidelity, confined the use only to specific laboratory locations. (4) Most of the systems generated monophasic pulses, which can cause electrolytic tissue injury and electrode damage (Lilly, 1961). This is less desirable than modern devices that use charge-balanced biphasic pulses.

Little progress has been made over the last two decades in developing miniaturized multi-channel brain telestimulation devices for small animal research. Here, we describe the development of a novel miniaturized digital telestimulation system that has enabled us to remotely deliver stimulation to multiple brain sites of freely moving animals (rats). The system was designed to enable development of new behavioral models, based solely on brain stimulation, for studying the neural correlates of spatial learning in freely moving animals. We used brain stimulation to generate both cues and rewards, and arranged reinforcement contingencies so that a human operator was able to accurately guide rats remotely, over arbitrarily defined routes and over varied 3-D terrains (Talwar et al., 2002). Built using a commercially available radio modem and microprocessor, this telestimulation system provides a small, light, efficient and reliable multi-channel telemetry microstimulation platform, with maximum flexibility for experimental configurations.

## 2. Methods

### *2.1. Overview*

We describe the system in the context of our experiments. It delivers brief trains of electrical stimulation to three or more brain locations, each implanted with a pair of electrodes. The system consists of two main components: a transmitter connected to a laptop PC through its serial port and an integrated receiver-microprocessor backpack mounted on the rat's shoulders and connecting to the implanted electrodes. Fig. 1 shows these two elements.

A program running on a laptop PC specifies the brain-stimulation parameters. The transmitter sends out digital commands to the receiver and microprocessor on the rats backpack. The microprocessor executes the incoming command, resulting in an output of a train of biphasic TTL pulses to the specified brain location.

### *2.2. PC program and transmitter base station*

The PC program, written in BASIC, configures the serial port to output stimulation-commands encoded by specific keystrokes (keystrokes “j”, “k” and “l” specified which of the three implanted brain locations needed to be stimulated). For each brain location, the parameters of a stimulus train—the duration of each pulse, the inter-pulse interval that determines frequency, and the number of pulses in the train—can be specified. The commands are sent as an ASCII string, at 2400 baud, from the PC to a transmitter via the serial port. It contains a short header, a safety code, the ID of the channel to be stimulated, and the three stimulation parameters. The short header (e.g. “U”, “U”) is necessary to first quiet inter-transmission noise and then establish timing.

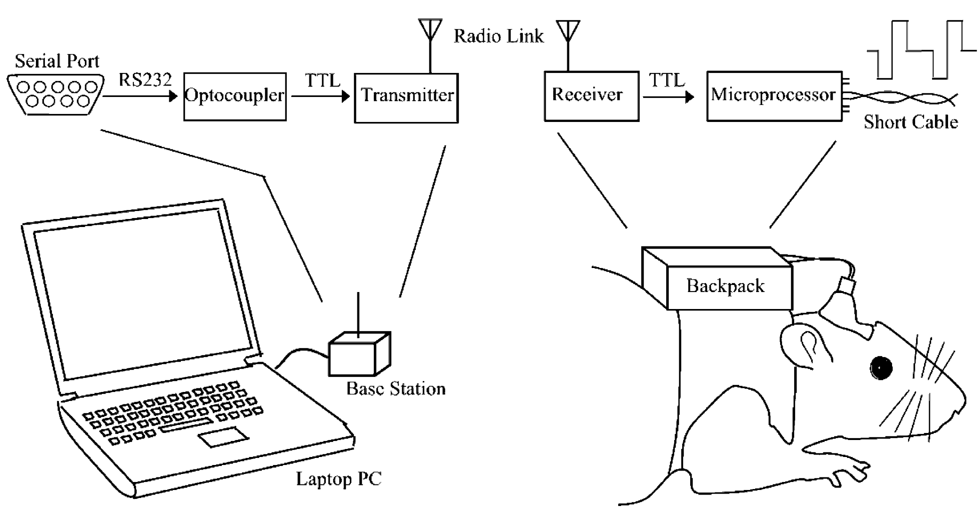



Fig. 1. Overview of the multi-channel telestimulation system. The main components of the system as well as the signal flow are shown. See text for details.

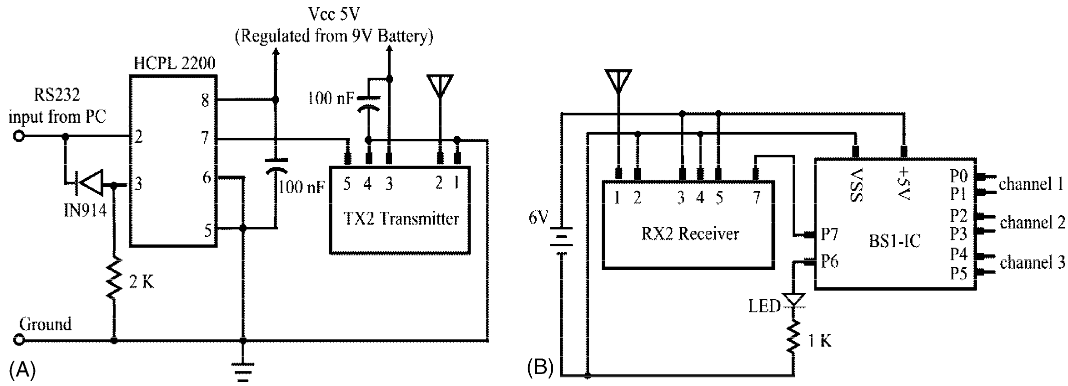



Fig. 2. Schematic diagram of the transmitter base station (A) and the backpack (B).

Fig. 2A shows the transmitter circuit, which was built around a UHF transmitter (TX2, Radiometrix, Watford, UK) powered by a 5 V supply regulated from a 9 volt battery. First, the serial port's RS232 signals are converted to TTL level signals using an Agilent HCPL 2200 optocoupler and then sent to the TX2 transmitter. A 1/4 wavelength whip antenna broadcasts the RF. The circuit is contained in an aluminum enclosure, which serves as circuit ground and RF ground plane. The TX2 module is a two stage surface acoustic wave (SAW) controlled, FM modulated transmitter that transmits at up to 40 kbps. It is available in 433 and 418 MHz versions, both of which we have employed at the same time, with no cross talk.

### 2.3. Backpack

Fig. 2B shows the backpack circuitry in detail. The backpack was assembled on a printed circuit board. Its main components are a receiver (RX2, 5V version, Radiometrix) and a microprocessor (Basic Stamp BS1-IC, Parallax Inc.) powered by a 6 V, 160 mAH lithium battery (2CR-1/3N). The receiver uses a helical antenna (as described in the RX2 documentation). The backpack includes an LED that provides direct visual verification of pulse delivery when the animals are freely moving. The input/output (I/O) pins of the microprocessor are connected to a skull-top adapter that housed the electrode ends by short flexible detachable cables. Under load (15 mA total), without regulation, the 2CR-1/3N battery puts out 5.5 V. The microprocessor ceases working when the battery voltage falls to about 4.5 V.

The programmable BS1-IC microprocessor has eight digital I/O pins: one of these is set to input the remotely received stimulation-command string and another for output to the LED indicator. The remaining six pins are paired to actuate three stimulus channels with biphasic pulse trains (thus each channel used two I/O pins to stimulate its respective electrode pair). In later experiments, we have also employed the basic stamp 2 module (BS2-IC) that has 16 I/O pins and two additional serial communication pins. With the BS2-IC, we are able to specify eight bipolar stimulating channels or up to 15 monopolar channels. These I/O pins could be set to either input mode with high impedance ($Z \cong 1\,\mathrm{M}\Omega$) or output mode with low impedance ($Z \cong 20\,\Omega$). The other major components of BS1-IC include an interpreter chip, an EEPROM (electrically erasable programmable read-only memory), a 4 MHz resonator and a 5 V regulator. A built-in timer is used for timing functions within the interpreter.

The microprocessor was pre-loaded with a PBASIC program that controlled stimulation. The program waits for ASCII command from the backpack receiver. As soon as a valid message is received, it branches to the stimulation routine. When not in use all I/O pins are left in input mode to prevent cross talk between electrodes. During stimulation, the requested pair of pins is opened for output and 5 V is alternately applied first to one and then the other, according to the regimen for that channel as specified in the PC command. Namely, 5 V is applied to each I/O pin for the specified pulse duration, and then the program pauses to complete the specified inter-pulse interval. This process repeats for the specified number of pulses. Thus, the microprocessor's interpretation of ASCII commands results in a train of pulses of specified duration, frequency and number. Since the backpack is electrically floating, applying a voltage to the first pin and then to the other results in a biphasic pulse. After stimulation, the pins are reset to input mode, and the program goes back to wait for the next ASCII command.

The backpack measures 48 mm × 23 mm × 19 mm and weighs 28 g. It is held to a harness (Harvard Apparatus, Holliston, MA) worn by the rat by means of mating Velcro pieces. Fig. 3 shows a picture of a rat wearing the backpack.

### 2.4. Surgery

Under anesthesia, two teflon-coated stainless steel microwire electrodes (100 μm diameter), 1 mm apart, were stereotaxically implanted in the medial forebrain bundle (MFB) and the whisker barrel fields of the left and right somatosensory cortices (SI) of five female Long–Evans rats (approximately 275 g). Stimulation experiments commenced 5 days after implantation.

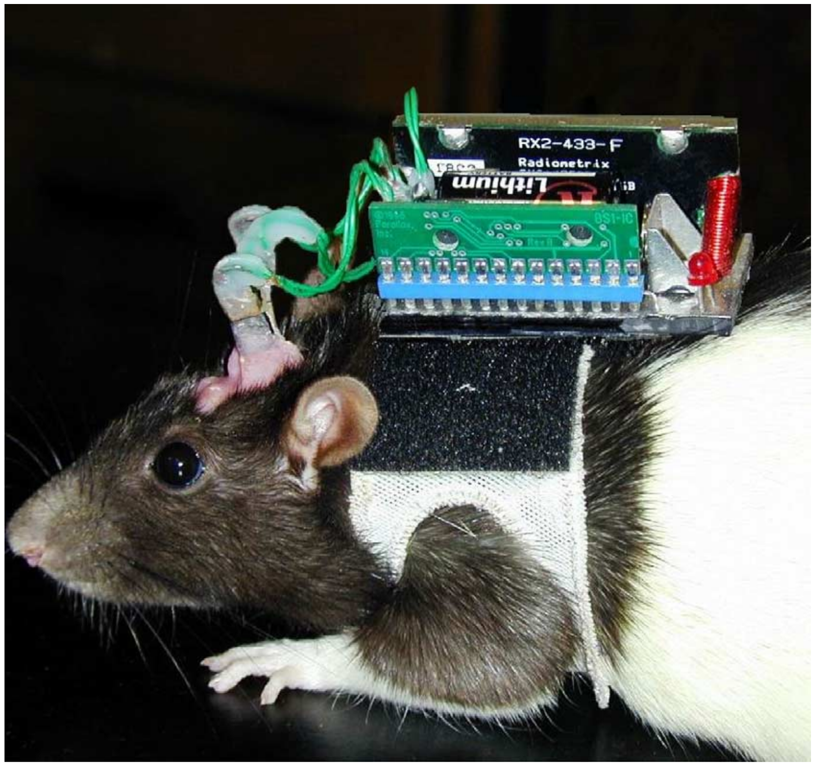



Fig. 3. Rat wearing a backpack under telemetric control.

## 3. Results

Fig. 4 shows the oscilloscope trace of an ASCII command string and the resulting train of biphasic pulses delivered to one stimulation channel. The telestimulator followed remotely received commands with high fidelity. The microprocessor can deliver arbitrarily specified stimulus trains from distances as much as 300 m (line of sight). The 6 V, 160 mAh lithium backpack battery system survives about 7 h of continuous stimulation (test stimulation consisted of

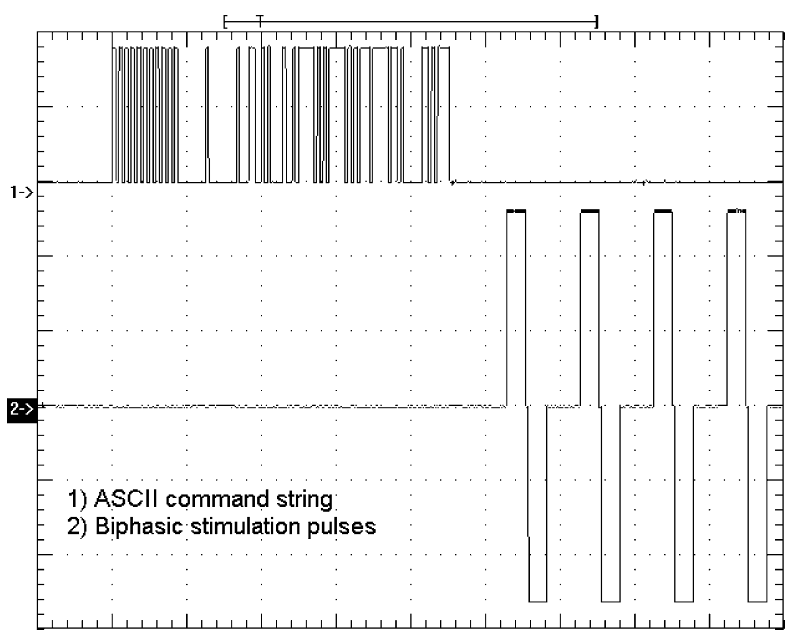
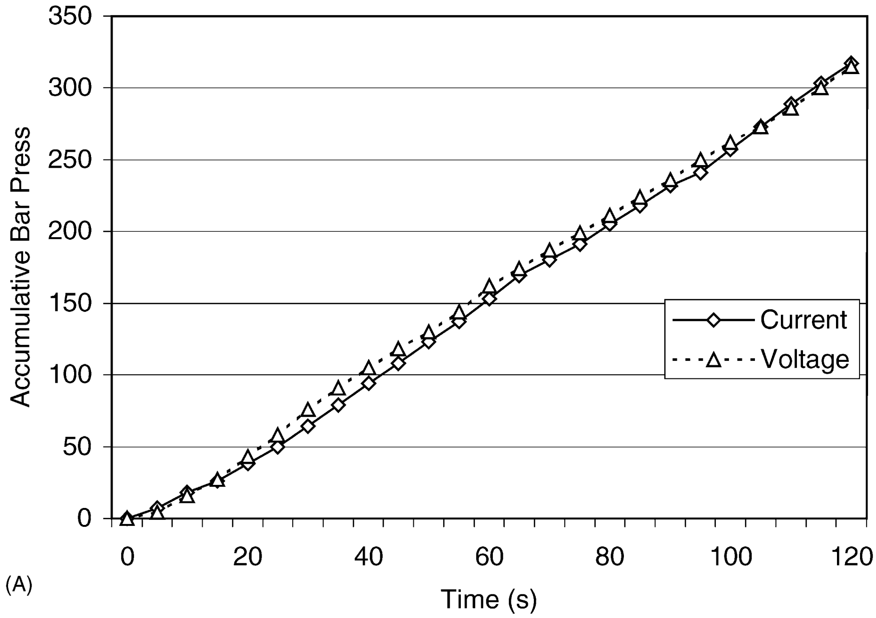



Fig. 4. ASCII stimulation command string (1) and output stimulation waveform (2) taken from the oscilloscope (TDS 210, Tektronix). Channel 1 shows the TTL command on transmitter input and channel 2 shows the biphasic stimulation waveform on the specified channel. Unit: 2 V, 10 ms. It took about 52 ms to transmit and execute a command. The stimulation waveform accurately followed the specified parameters (pulse number: 4, duration: 2.5 ms, and frequency: 100 Hz).

stimulus trains delivered at 0.2 Hz; each train had five biphasic pulses at 100 Hz with pulse duration 0.5 ms). The transmitter is able to work for several days (>7) using a 9 V lithium battery. It weighs 268 g and can easily be carried, along with the laptop, by the operator.

We next investigated the functional effectiveness of the system. Specifically, our goal was to evaluate the behavioral effectiveness of brain-stimulation delivered by the direct 5 V TTL output of the microprocessor. This was done by observing predictable behavioral responses consequent to stimulation of the MFB. Such stimulation is known to be rewarding and can be used to condition animal behaviors such as lever pressing (Olds and Milner, 1954). Connections were made between the microprocessor outputs and the implanted electrodes, and the rats were placed in a lever-equipped operant chamber in which a train of biphasic pulses to the MFB followed each lever press. Each train consisted of 2–10 pulses delivered at 100 Hz with pulse duration of 0.3–0.8 ms. Under this reinforcement schedule, all subjects lever-pressed continuously, reaching pressing rates



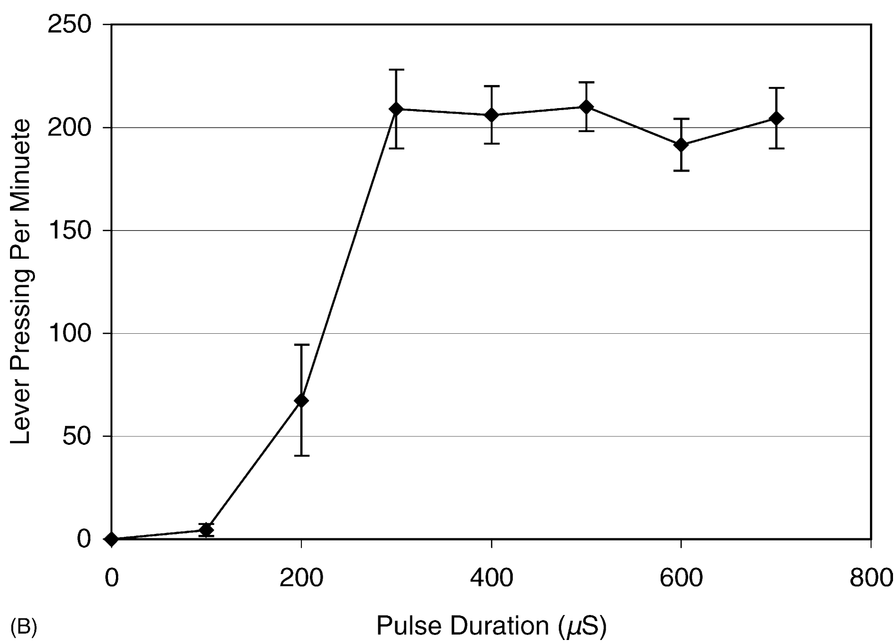




Fig. 5. Rat self-stimulation response. (A) Rat (no. N9) self-stimulation using the constant-voltage telestimulator compared to that using a constant-current stimulator. Voltage: 5 V and current: 100 μA. Each stimulation train consists of eight pulses at 100 Hz, with pulse duration of 0.7 ms. (B) Pulse duration–response of rat self-stimulation rate using the constant-voltage telestimulator (rat no. N4). Each stimulation train consists of eight pulses at 100 Hz, with varying pulse durations. The average rate and standard deviation were calculated from four separate 2-minute sessions.

as high as 200/min over a 20-min period. Using an oscilloscope hooked across a resistor placed in series with the rat brain, we measured electrode impedance (at 100 Hz) to be around 50–100 KΩ. Thus, we estimated that the 5 V TTL train delivered current amplitudes of around 50–100 μA in the behaving animal.

We compared the functional efficacy of the constant-voltage 5 V source in generating MFB stimulation rewards to that of a conventional constant-current source which was set to deliver a comparable pulse train at 100 μA (pulse duration: 0.7 ms, frequency: 100 Hz, eight pulses). Fig. 5A compares two cumulative plots of successive lever presses made by one rat over separate two-minute periods using our constant-voltage telestimulator and a constant-current stimulator (WPI 601 bipolar stimulator). The plots obtained using these two techniques are similar in that they almost overlap (correlation coefficient $r = 0.9988$, $P < 0.0001$). We concluded that the reliability and stationarity of reward stimulation produced by the telestimulator was equal to that of the constant-current stimulator set at 100 μA. Fig. 5B shows bar pressing rates, in a single subject across four sessions, as a function of varying MFB stimulation along a single dimension (pulse duration), demonstrating the versatility of the telemetric system (similar behavioral changes were seen by varying pulse number and pulse frequency).

The telemetric stimulator enabled us to develop a new behavioral paradigm that required rats navigate freely in large open ground and various 3-D terrains (Talwar et al., 2002). The animals were first trained to move forward continuously to obtain periodic MFB stimulation. Thereafter, stimulation of SI cortex (five pulses delivered at 100 Hz with pulse duration of 0.5 ms) served as directional cues, in that the animals learned to turn left or right depending on which SI cortex was stimulated. Under this basic reinforcement contingency, we found that the rats could be accurately guided over arbitrarily specified 3-D routes at considerable distances away, showing that both cues and rewards were reliably delivered by the telestimulation system. The rats moved comfortably with the backpack and worked without sign of fatigue for periods up to a 1-hour test limit.

## 4. Discussion

The telestimulation system described here overcomes some of the shortcomings of previous remote stimulators by providing multiple output channels in a single package. It allows virtually simultaneous bipolar or monopolar stimulation of multiple brain sites and it is both reliable and robust as a brain-stimulator. A special feature of the system is that it accomplished this task using conventional TTL pulses. The use of logic circuitry to generate biphasic stimulus pulses allows an experimenter to stimulate chronically over long time periods while avoiding the electrolytic injury caused by unidirectional currents. Another feature of the system is that the backpack containing the receiver, microprocessor and battery is small, light and power efficient, allowing it to be carried by small animals over relatively long time periods. In addition, there is no pulse generator or high power needed for this digital telestimulator. The laptop PC and transmitter can be easily carried by an operator, which allows stimulation experiments take place in a wide variety of environments. These advantages are attributable to the relative simplicity of the device, which uses a commercially available microprocessor to provide well-controlled multi-channel stimulus patterns. In our case, the backpack microprocessor was programmed to carry out specific stimulation patterns, but simple reprogramming allows almost any pattern to be specified.

In our experiments, the telestimulation system was used to develop new behavioral models for neurophysiological studies in freely moving animals. Somatosensory stimulation was used to create percepts that were conditioned to act as cues in a behavioral task reinforced by rewarding MFB stimulation. From the point of view of generating sensory percepts and rewards the fact that system output was a straightforward 5 V TTL pulse train was not a limitation. The effect of a stimulus pulse train at any given brain location depends on pulse amplitude, pulse duration, pulse frequency, and the total number of pulses delivered. We showed changing one or more of the three programmable parameters (pulse duration, frequency, and number of pulses) could create the desired magnitude of stimulation (see Fig. 5B). This is consistent with the findings that within certain windows these parameters sum linearly (for a review, see Gallistel, 1983). In our study, these stimulus parameters were arranged for optimizing the magnitude of stimulus percepts and rewards (reward magnitude of MFB stimulation was estimated by bar pressing rates in response to parametric variation).

Though the use of a 2400 baud serial communication system resulted in a transmission delay (about 45 ms, see the oscilloscope trace 1 in Fig. 4), this is not a factor in our experiments. If necessary, however, one can reduce the delay by increasing the baud rate. In the future, major advantages will be obtained by integrating a general-purpose transceiver with a microprocessor capable of handling multi-channel stimulation and also higher order I/O protocols. Such a wireless platform could incorporate the systems that serve to obtain neurophysiological recordings from the behaving animal (Hawley et al., 2002; Obeid et al., 2003). This would enable full duplex wireless transmission enabling the delivery of stimulus commands to the animal as well as receiving incoming sensor data if needed (Delgado et al., 1975). Such a scheme, for example, would be highly useful in studying the neural correlates of navigation while directing freely moving animals.

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