Telemetry System for Reliable Recording of Action Potentials From Freely Moving Rats

Emerson S. Hawley,[1] Eric L. Hargreaves,[1]
John L. Kubie,[2] Bruno Rivard,[2] Robert U. Muller[1,3]*

[1]*Departments of Physiology and Pharmacology, SUNY Health Sciences Center at Brooklyn, Brooklyn, New York*
[2]*Department of Anatomy and Cell Biology, SUNY Health Sciences Center at Brooklyn, Brooklyn, New York*
[3]*MRC Centre for Synaptic Plasticity, University of Bristol, Bristol, UK*

ABSTRACT: Recording single cells from alert rats currently requires a cable to connect brain electrodes to the acquisition system. If no cable were necessary, a variety of interesting experiments would become possible, and the design of other experiments would be simplified. To eliminate the need for a cable we have developed a one-channel radiotelemetry system that is easily carried by a rat. This system transmits a signal that is reliable, highly accurate and can be detected over distances of ≥20 m. The mobile part of the system has three components: (1) a headstage with built-in amplifiers that plugs into the connector for the electrode array on the rat's head; the headstage also incorporates a light-emitting diode (LED) used to track the rat's position; (2) a backpack that contains the transmitter and batteries (2 N cells); the backpack also provides additional amplification of the single cell signals; and (3) a short cable that connects the headstage to the backpack; the cable supplies power to the headstage amplifiers and the LED, and carries the physiological signals from the headstage to the backpack. By using a differential amplifier and recording between two brain microelectrodes the system can transmit action potential activity from two nearly independent sources. In a future improvement, two transmitters with different frequencies would be used to telemeter signals from four microelectrodes simultaneously. *Hippocampus 2002;12:505–513.* © 2002 Wiley-Liss, Inc.

KEY WORDS: place cells; complex-spikes; single unit; single cell; radiofrequency modem

INTRODUCTION

Although a wide variety of methods are valuable in efforts to understand the neural basis of behavior, electrical recordings from individual nerve cells provide exceptionally high spatial and temporal resolution measurements of brain activity. Single-cell recordings from surgically reduced or anesthetized or immobilized preparations have had enormous impact, but it is clear that the optimal way to learn about the normal functioning of nerve cells and their role in controlling behavior is to record from fully intact, freely moving animals.

To detect single-cell activity under natural or nearly natural circumstances, two problems must be solved. The first may be called "externalization," the process by which the electrical activity of neurons is made available somewhere on the body surface (generally, the head) by inserting into relevant brain structures microelectrodes whose other ends terminate in electrical connectors. Externalization has been achieved using many different techniques, one of which, the movable electrode array designed by Kubie (1984), is adopted in this study. Regardless of the details of the externalization method, however, it is obvious that current technology permits recordings only from cells whose inputs, outputs, or internal structure are disturbed by the nearby electrodes. Just how these disturbances modify or obscure normal activity patterns of targets cells has hardly been addressed and will not be considered further here, where we focus on a solution to what may be called the "communication" problem.

By the communication problem, we mean the process of transferring the electrical signals at the animal's body surface to the recording system itself. Here there are two possibilities, namely, the use of a cable (Strumwasser, 1958; Hubel, 1958, 1959, 1960) or of telemetry via broadcast electromagnetic radiation. Cables are easier to use, but unavoidably hinder the animal's motions. Kinking of cables due to turning by the animal is a form of hindrance but is solvable by using a slip-ring assembly or commutator in the electrical path. As the animal turns, the torque created at the bottom of the cable is transferred to the top of the cable so that the center of the slip-ring assembly turns. In this way, the cable turns equally with the rat, preventing kinking. Electrical continuity is achieved by at-

Grant sponsor: National Institutes of Health; Grant number: R01-NS37150; Grant number: NS20686.
*Correspondence to: Robert U. Muller, Department of Physiology and Pharmacology State University of New York (SUNY) Health Sciences Center at Brooklyn, Box 31, 450 Clarkson Avenue, Brooklyn, NY 11203.
E-mail: bob@fasthp.hippo.hscbklyn.edu
Accepted for publication 7 October 2001
DOI 10.1002/hipo.10040

taching each wire in the cable to its own conducting slip-ring which in turn is contacted by its own stationary brush.

In addition to kinking, cables hinder or interact with the animal's motions in several ways that are harder or impossible to solve. First, the tendency of cables to snag on fixed objects means that recordings can be made only the simplest environments. Second, the length of the cable limits the size of the recording environment. Long cables, with reel or counterweight mechanisms to maintain only a certain amount of slack are possible but are mechanically complex and leave the cable vulnerable to chewing if they fail to operate correctly. In addition, the cable acts as a visual and kinematic distraction, tending to tug on the animal in different ways in different places. It is therefore clear that radiofrequency or infrared telemetric methods are better suited to permit full freedom of motion inside spaces chosen for other considerations.

Ideally, a telemetry system would transmit signals from a large number of electrodes, as many as is possible with a cable. Up to now, however, difficulties with transmission fidelity and range have limited practical use of telemetry even with only one (Beechley and Lincoln, 1969; Edge et al., 1969; Eichenbaum et al., 1977; Yamamoto et al., 1984) or two (Grohrock et al., 1997) channels, and no attempts at multiplexing a large number of channels for small animals have been reported. Here, we describe a new one-channel, analog telemetry system that we have used to record single-cell activity from freely moving rats. This system consists of a headstage amplifier that plugs into the recording electrode array on the rat's head, a backpack containing batteries, amplifiers, and a transmitter and a 4-cm cable that connects the headstage to the backpack. One advantage of this system over previous implementations is the use of an instrumentation amplifier mounted on the head as the input stage; this amplifier, when connected to two microwires, reduces noise from the brain due to its great common mode rejection. The second and major advantage of our new system is the use of a radiofrequency modem transmitter/receiver pair to carry the signal. The modem pair is crystal controlled, eliminating almost all drift. Moreover, the transmitter signal is very powerful, overcoming signal dropout difficulties (dead areas) experienced with previous systems.

To demonstrate the properties of our telemetry system, we show recordings of hippocampal neurons under the same conditions in which the communication problem has been previously solved with cables (Muller et al., 1987). The results indicate that action potential waveforms are transmitted with the same fidelity seen with cables, regardless of the rat's position inside a 76-cm-diameter cylinder. Possible improvements to this system including decreased weight, increased battery life and increased channel count are described in the discussion.

MATERIALS AND METHODS

Different aspects of the telemetry system are shown in Figures 1 and 2. Figure 1A is a cartoon of the main elements of a system that sends two measurements of the state of a freely moving rat to a stationary data acquisition computer with no mechanical connection between the rat and the outside world. The location of the rat in the environment is detected by tracking a red light emitting diode (LED) with an overhead video camera and a special purpose analog-to-digital converter (tracker); this signal pathway is shown in red. The location of the rat in the horizontal plane is sent at 60 Hz to the computer as two 8-bit numbers; the only unusual aspect of this process is that the LED is powered from the batteries on the backpack, and not via a cable.

The second signal sent from the rat is an amplified version of the voltage difference between 2 of the 10 electrodes that were inserted in the rat's brain; this signal pathway is shown in green. The low amplitude waveforms generated by single hippocampal neurons (generally <500 μV) are amplified first on the head, a second time at the backpack to increase the signal sufficiently to modulate the transmitter and a third time between the receiver and the data acquisition computer. As will be demonstrated, the use of a radiofrequency modem transmitter/receiver pair provides excellent fidelity for electrophysiological signals including single unit spikes.

The portions of the telemetry system carried by the rat are directly visible in the photograph of Figure 1B. The electrode assembly includes a circular female connector embedded in dental acrylic. The electrode assembly in turn is mounted on the rat skull with white dental adhesive; it can be driven into the brain by turning three screws held in the dental acrylic (Kubie, 1984). Additional information about the electrode assembly and surgical implantation is given below. In the photograph, an active connector that terminates one end of the recording cable has been inserted into the mating connector on the headstage. The active connector contains a pair of operational amplifiers and the red tracking LED. The other end of the cable goes to a backpack worn by the rat. The backpack contains two small batteries (N cells) to provide 3-V power, amplifiers and the radiofrequency modem transmitter. The backpack is held on the rat's shoulders with a halter cut from a Silastic surgical glove. The halter goes over the front legs and has glued to it a piece of Velcro that sits between the shoulder blades; the mating piece of Velcro is glued on the bottom of the backpack. Also visible in the picture are the batteries and the red wire of the vertical helical antenna.

A detailed schematic diagram of the part of the system carried by the rat is shown in Figure 2. For electrical recordings, a reference voltage is provided by the animal ground which is an uninsulated 26-gauge hypodermic tube through which the microelectrode wires are threaded (see Kubie, 1984). Amplifiers 1 and 2 are configured as gain-of-one followers that couple the high-input impedance microwires to the cable. The outputs of these amplifiers, the animal ground and the ± 3 V power provided by the backpack batteries, are coupled from the headstage to the backpack via the 5 conductor cable.

The backpack itself consists of a series of three amplifiers, the modem transmitter, the batteries and the antenna. Amplifier 3 is an instrumentation amplifier fitted with matched ($<1\%$) resistors. The amplified ($\times 9$) difference of the outputs of amplifiers 1 and 2 is further multiplied by amplifier 4 ($\times 15$) and amplifier 5 (variable; range is $\times 1$–$\times 15$). The modulation range of the transmitter is $\pm$ 1.0 V. When the overall gain of the amplifier series is set at $\sim$1400,a good-sized waveform of 250 μV produces an output of

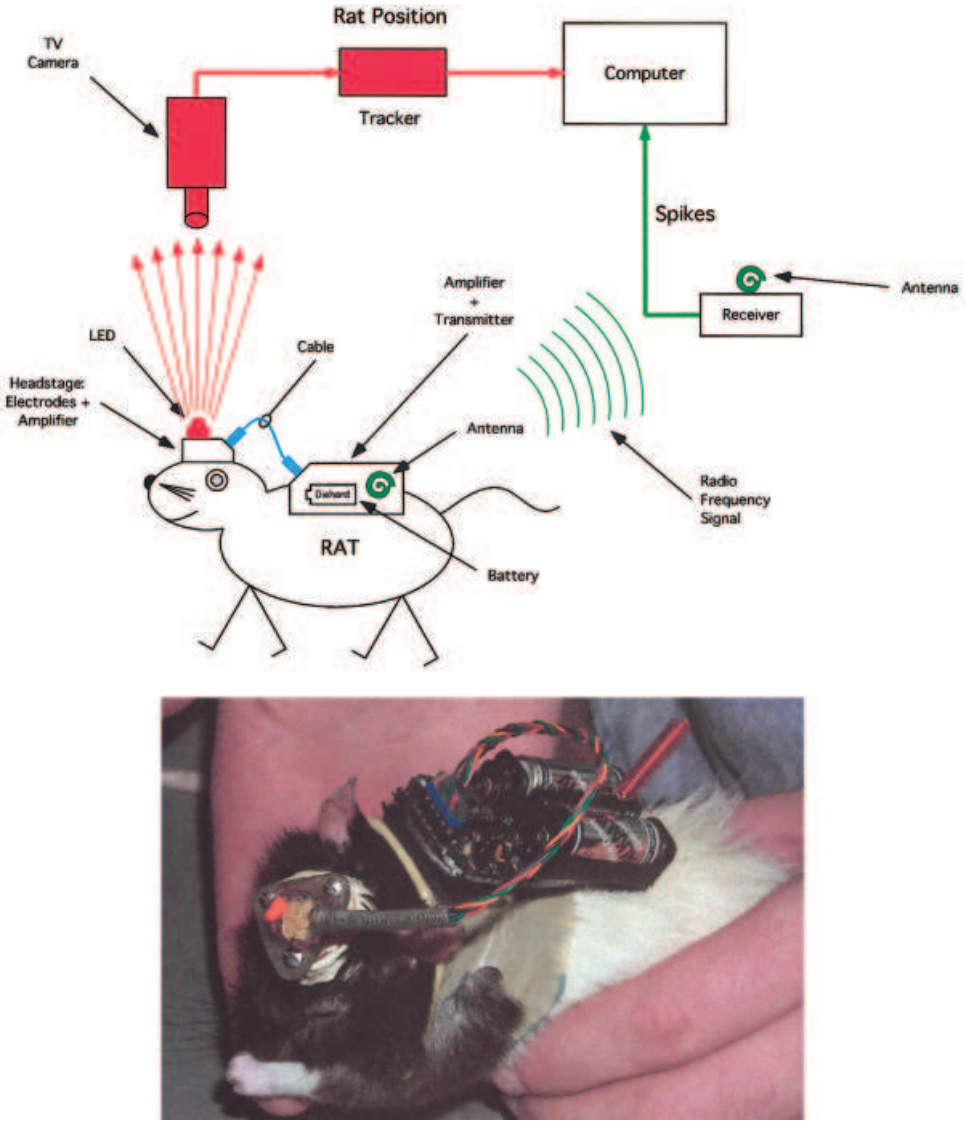

FIGURE 1. Summary of the telemetry system. A: Cartoon of the main components of telemetry system. Except for the radiofrequency link that replaces the standard recording cable, the system is virtually unchanged from a cable-based setup (Muller et al., 1987). Circuit details are given in Fig. 2. B: A rat wearing the telemetry system. The electrode array is terminated in a 10 pin female connector whose top is flush with surface of the rounded triangular drive mechanism; the three small screws allow the electrode array to be advanced through the brain. The headstage is plugged into the electrode connector; the tracking LED is visible, as is the spring-protected origin of the cable, which terminates at the backpack. The forelegs of the rat fit through a latex harness cut from a disposable rubber glove. The transmitter backpack is attached to the harness with Velcro. Easily seen on the backpack are the batteries and the helical antenna. The harness has no apparent effect on the rat's behavior during recording sessions.

~350 mV, enough to modulate the transmitter without fear of saturation.

Training

Behavioral and single-unit recording methods are similar to those used by Muller et al. (1987). In brief, rats are trained to forage for randomly dropped 20-mg food pellets in a cylindrical chamber. The cylindrical recording chamber is 76 cm in diameter, 51 cm high, and painted gray with a white cue card of the same height, spanning 100 degrees of arc pasted to the inner cylinder wall (Muller et al., 1987). The recording cylinder is set concentrically inside a dark circular curtain that runs from floor to ceiling; for additional isolation, the cylinder plus curtains is inside a 3 m × 3 m room whose door was closed during recordings. Light is provided by four 25-W bulbs mounted overhead on the corners of a square. At the completion of training, rats spend most of their time running inside the cylinder searching for pellets, so that their behavior is quite homogeneous is position and time.

Electrodes

The electrode assembly is adapted from the design of Kubie (1984). It consists of a bundle of ten 25-μm, Formvar-insulated microwires, such that each wire is attached to one pin of a 10-pin connector (1058-1G34; Thomas and Betts, Memphis, TN) built into a movable electrode drive. The wires are threaded through a length of 26-gauge stainless steel tubing whose bottom end enters the brain and whose top is anchored to the bottom of the Augat connector; this uninsulated tube serves as animal ground. Before implantation, the wires are cut square so that each protrudes about 500 μm below the end of the guide tube.

Surgery

All surgical procedures conform to institutional and NIH guidelines. To implant the electrodes, rats are first given subcutaneous injection (0.75 mg) of methylatropine to reduce respiratory problems caused by the anesthesia. Next they are anesthetized with

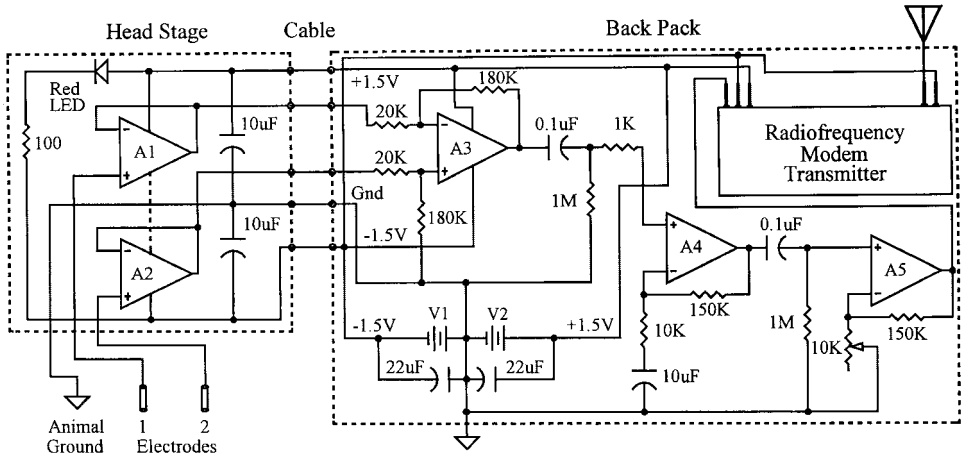

FIGURE 2. Schematic diagram of the headstage and backpack. The headstage is built onto a circular 10-pin connector (1058-1G34; Thomas and Betts, Memphis, TN), which plugs into an electrode assembly surgically implanted into the hippocampus of a rat. The output from the two selected two electrodes are each buffered with an FET input follower amplifier (A1 and A2 are TLV2262; Texas Instruments, Dallas, TX). These amplifiers were chosen for their low-input bias current (1 pA), low noise (12 nV/MHz) and their ability to operate with a 3.0-V supply. Also built into the headstage is a wide-angle LED (MV5777C; QT Optronics, Goleta, CA) used to track the rat. The headstage weighs 3.7 g and consumes 7.6 mA. The backpack consists of driver amplifiers (A3, A4, and A5 are Texas Instruments TLV2264), a battery pack (2 N cells) and a radiofrequency modem transmitter. The headstage is connected to the backpack with a 4-cm cable. A3 differentially amplifies the outputs of A1 and A2 with a gain of 9. A3 is AC coupled (1.6Hz hi pass) to A4 and A4 is AC coupled (1.6-Hz hi pass) to A5. The gain of A4 is 15; the gain of A5 is adjustable at 1–15. When the total gain is 2100, a 400-μV signal from a hippocampal neuron modulates the transmitter maximally. The transmitter is a crystal controlled, 418-MHz radiofrequency modem (TX2; Radiometrix, Watford, UK; the stationary receiver is an RX2; Radiometrix, Watford, UK). The transmitter and receiver antennas are 26 turn helices, 2.0 cm long and 0.5 cm in diameter. The weight of the backpack (batteries included) is 34.9 g; the batteries weigh 18.4 g. With the LED, amplifiers and transmitter in use, battery life is somewhat more than 24 h. Receiver (not shown) The RX2 receiver was modified by disabling a digital switch to eliminate a switching artifact. The signal from the receiver goes to a differential AC amplifier (model 1700; A-M Systems, Carlsborg, WA).

pentobarbital (50 mg/kg intraperitoneal) and mounted in a Kopf stereotactic instrument in the position specified by Paxinos and Watson (1998). The scalp is incised at the midline and retracted. Holes are drilled for anchoring the electrode assembly and for inserting the electrode bundle into the brain. The microwire bundle is lowered with the stereotactic manipulator so that the tips were in the alveus of the right hippocampus. The stereotactic coordinates were −3.3 mm posterior to and 2.4 mm lateral to the bregma and 1.7 mm below the brain surface.

Recording Procedures

After 10 days of recovery from surgery, the microwires are screened daily for discriminable units during 5–10 min sessions. Signals from single microwires are amplified 10,000 times and band pass filtered between 300 Hz and 10 kHz. The signals are viewed on oscilloscopes and digitized for storage at 30 kHz. If during screening no waveform of sufficient amplitude (>250 μV) is found on any wire the bundle is lowered by 40–80 μm, and the rat returned to its home cage.

Once a discriminable unit is located it formal recordings are made. An initial session using the cable is done to document the existence of one or more well-isolated units. The same day, a pair of 16 min recording sessions is, the first using the cable and the second using the telemetry system; these sessions are always done within 30 min of each other. In all, two interneurons (theta cells) and seven complex-spike cells (pyramidal cells) were recorded from three rats in 5 sets of 3 sessions. Theta cells and complex-spike cells were identified using criteria stated by Ranck (1973) and Kubie et al. (1989). Simultaneous recordings of multiple units were made in three sets, one for each rat. Simultaneously recorded waveforms are separated into individual time series presumably generated by single cells, using software from DataWaves (Longmont, CO).

In addition to separate cable and telemetry recordings, a fourth rat was run in a single 16-min session, during which both systems were used to monitor simultaneously the same electrode. Continuous sweeps were manually triggered to capture spontaneous unit activity via both paths during this session. In this way it was possible to compare the same events captured by the two routes directly.

RESULTS

The Telemetry System Did Not Affect the Behavior of the Rats

Each rat was trained using only the cable and never wore the halter plus backpack until the first telemetry session. Despite this lack of preexposure, each rat ran normally and continued to re-

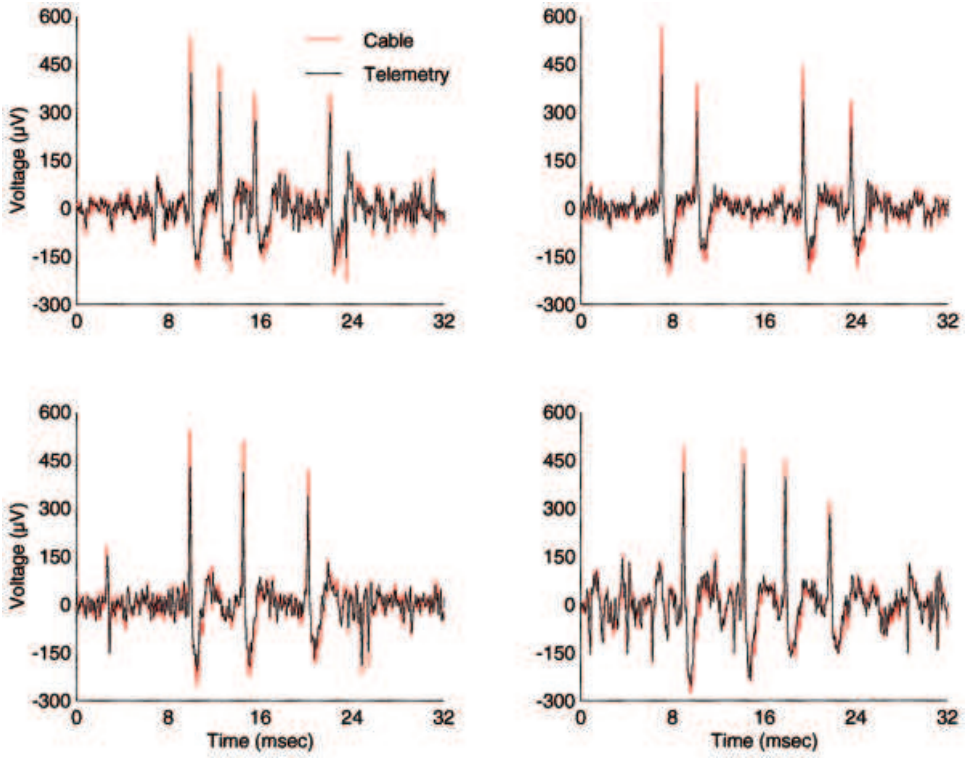

FIGURE 3. Simultaneous recordings of neuronal activity recorded with the cable (red trace) and telemetry (black trace) links. Each part shows continuous recordings digitized at 40 kHz. Each of the 32-ms segments were selected because it shows a complex-spike recorded from a hippocampal pyramidal cell. The small discrepancies between the cable and telemetered traces are due to a somewhat lower high-frequency cutoff in the telemetry channel.

trieve food pellets. Moreover, we saw no evidence of fatigue later in the 16-min recording session. We conclude that the mechanical properties and weight of the halter and backpack do not affect performance in the simple pellet chasing task.

Comparisons of Single-Cell Waveforms Were Captured With the Telemetry and Cable Systems

The fidelity of the telemetry link is assessed in Figures 3 and 4. Figure 3 shows examples of high-gain continuous recordings made simultaneously with the cable (red trace) and telemetry (black trace) systems. The examples were chosen for the presence of the "complex spikes" that are characteristic of extracellular recordings from hippocampal pyramidal cells; each complex spike consists of a high-frequency burst whose components are of decreasing amplitude. The near-coincidence of the traces between and during action potential activity shows that the signal paths through the telemetry and cable systems are almost identical. The lack of exact correspondence between the traces is due to filtering differences in the two signal paths; the high-frequency cutoff is nominally 10% lower for the telemetered signal, which results in some attenuation during spikes.

The fidelity of the telemetry system is shown in a second way in Figure 4, where each panel shows, for a single cell, an average of 200 randomly selected waveforms recorded with the cable (red line), an average of 200 randomly selected waveforms recorded with the telemetry system (green line) and a rescaled version of the telemetry average (black line). The scaling factor is selected by lining up the first points of the cable and telemetry recordings and

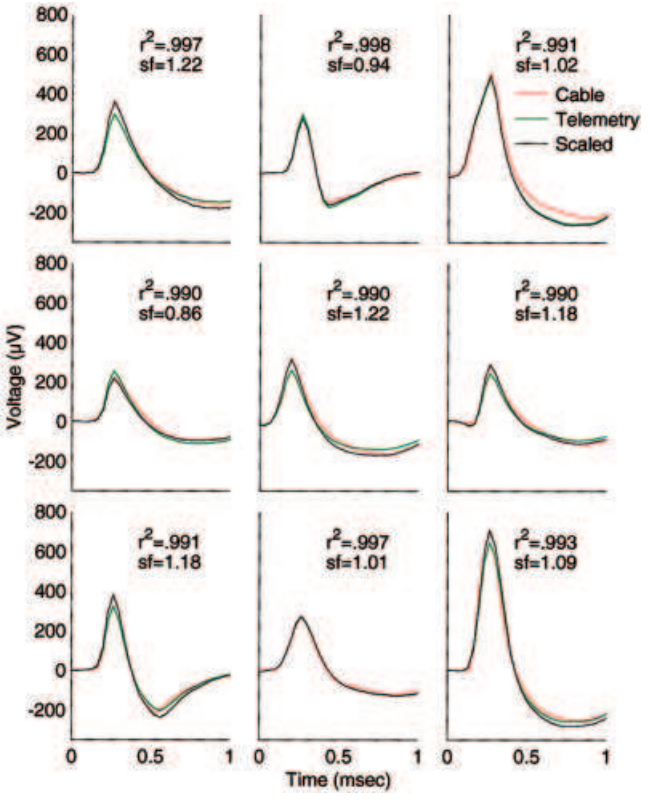

FIGURE 4. Single-cell waveforms recorded with the cable (red trace) and telemetry (green trace) links. Each panel shows the average of 200 waveforms from a single cell. The black trace shows the telemetry average scaled so that its maximum is equal to the peak of the cable average.

then multiplying all the points such that the peak of the telemetered average coincides with the peak of the cable average. The scaling factor and the square of the correlation (r^2) between the telemetered and cable averages is shown in each window.

The great resemblance between the cable and the raw telemetered waveforms for each cell in Figure 4 is visible by inspection of the raw averages and this impression is reinforced by the very high r^2 values. We also found that scaling the telemetered average does little to improve the similarity of averaged waveforms collected with the two methods. We calculated for each cell the mean absolute difference between the cable waveform and the raw telemetered waveform (cable–raw) and between the cable waveform and the scaled telemetered waveform (cable-scaled). Across the cells, the average cable–raw difference is 15.99, and the average cable-scaled difference is 14.65. A paired t-test shows that the average raw-scaled difference is not reliably smaller ($t = 0.868$; $df = 8$; $P(t \geq 0.868) = 0.411$). The lack of improvement by scaling suggests that plugging and unplugging the rat between sessions caused little movement of the electrodes relative to the cells. The averaged waveforms in Figure 4 imply that the telemetry system transmits signals as well as the cable.

Averaging the telemetered waveforms might conceal noise sources that would be evident in single sweeps with high temporal resolution. Accordingly, we obtained single waveforms with both methods and analyzed the variance at each of the 32 data points. The 200 randomly selected waveforms for unit C1P2 recorded with the cable (red) and the telemetry system (black; adjusted) are shown in Figure 5A, where it seems clear that the dispersion is about the same for both methods. To confirm this we calculated first the variance at each of the 32 data points for each set of waveforms (Fig. 4B) and then the F-statistic (ratio of the variances calculated such that the ratio is ≥ 1.0) at each point (Fig. 4C). The small deviations of the F-statistic from 1.0 suggest that the variances are equal. Thus, the raw F-statistic approaches or is greater than the 0.05 probability level only at 3/32 points, a result in keeping with a random sampling from an F-distribution whose mean is 1.0. This result is typical of the cell sample. We conclude that the telemetry system introduces no extra noise into the signal.

The near-equivalence of waveforms recorded with each method implies that spike separation techniques will work equally with either data path. To test this, we used both methods to record 3 units on one wire and another unit on a second wire. We recorded differentially between the wires and passed the original signal to one channel of an A/D converter and an inverted version of the same signal to a second A/D channel. By setting a threshold of the same polarity for the direct and inverted channels, we could independently trigger data acquisition for the three waveforms on one wire and the single waveform on the second wire.

After data collection, spikes were sorted into sets of similarly shaped waveforms using values extracted from each waveform. In this case, three spike features, namely, the peak value, the minimum value and the time interval from peak to minimum, sufficed to separate the spikes into clear "clusters." The results of this sorting process are shown in Figure 6. The near-equivalence of the two

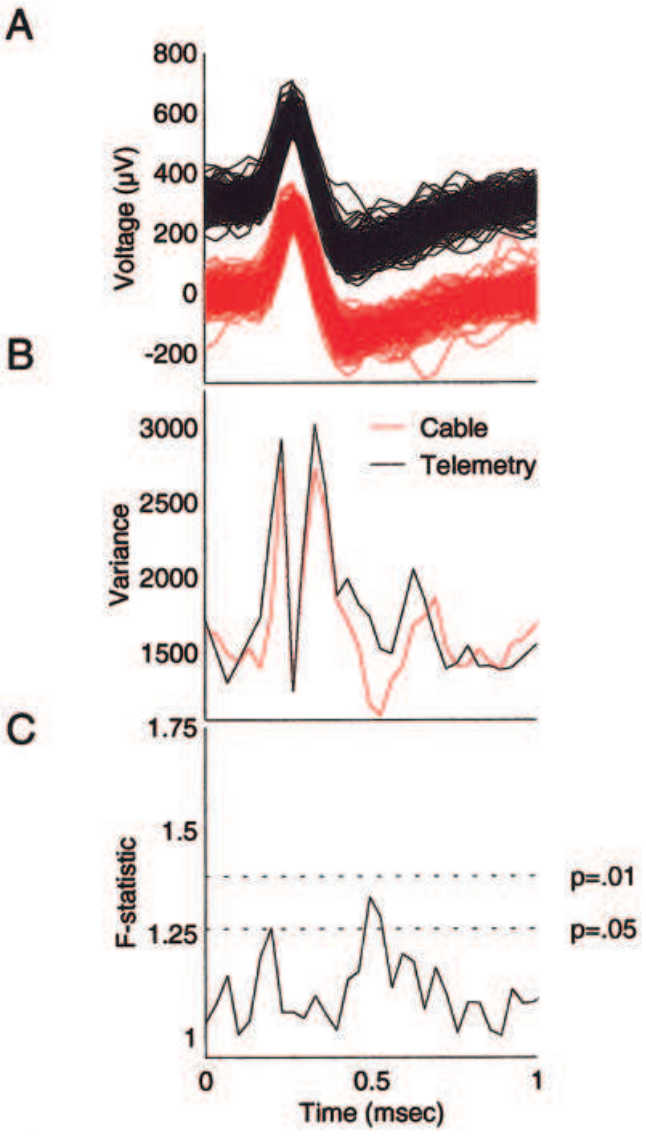

FIGURE 5. The telemetry system does not increase the variance of waveforms. A: 200 randomly selected spikes from the same cell recorded via the cable (black traces) and telemetry (red traces) links. B: The variance of the voltage at each of the 32 time points is plotted for the cable (red trace) and telemetry (black trace) links. C: The line shows the ratio of the variances at each time point (F-statistic). The 0.05 and 0.01 probability levels are shown. The 0.05 level is exceeded at 3 points, or in about 10% of the cases, suggesting that the variances of voltage are the same for the cable and telemetry systems.

methods is seen directly from the waveforms in Figure 6A1 for the cable recording and Figure 6A2 for the telemetered recording. The clustering of points in three-dimensional (3D) feature space is evident for both the cable (Fig. 6B1) and telemetered (Fig. 6B2) recordings. The separations among the clusters suggest that the points included in each are generated by a different source in the brain. The similarity of the cable and telemetry recordings are reflected in the similar positions of the clusters in the 3D feature space.

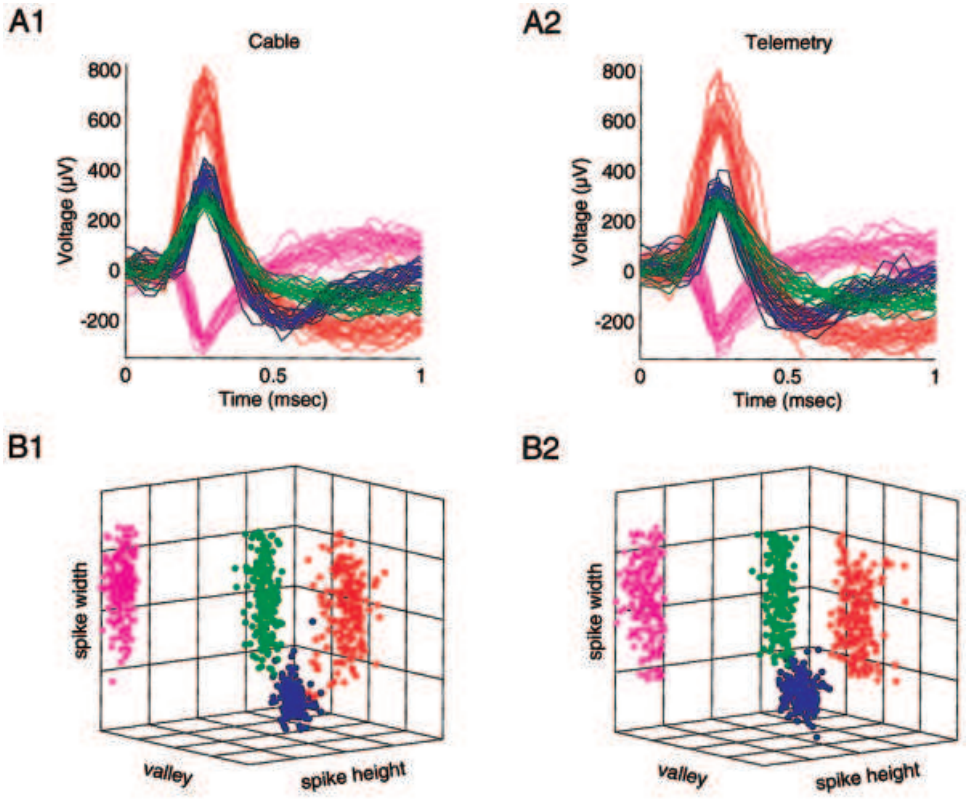

FIGURE 6. Separation of multiple waveforms from two microwires using the cable and telemetry methods. A: Waveforms from four putative units recorded differentially between two microwires. The waveforms in A1 are from the cable system and those in A2 from the telemetry system. The downgoing waveforms in both panels are from the wire connected to the inverting input of the recording system; the other three are from the wire connected to the direct input. B: Separation of the waveforms in three-dimensional parameter space. The points in parameter space fall into clusters that correspond to the waveforms in A1 and A2. Note that in addition to the clustering that the positions of the clusters in parameter space are extremely similar for the two recording methods.

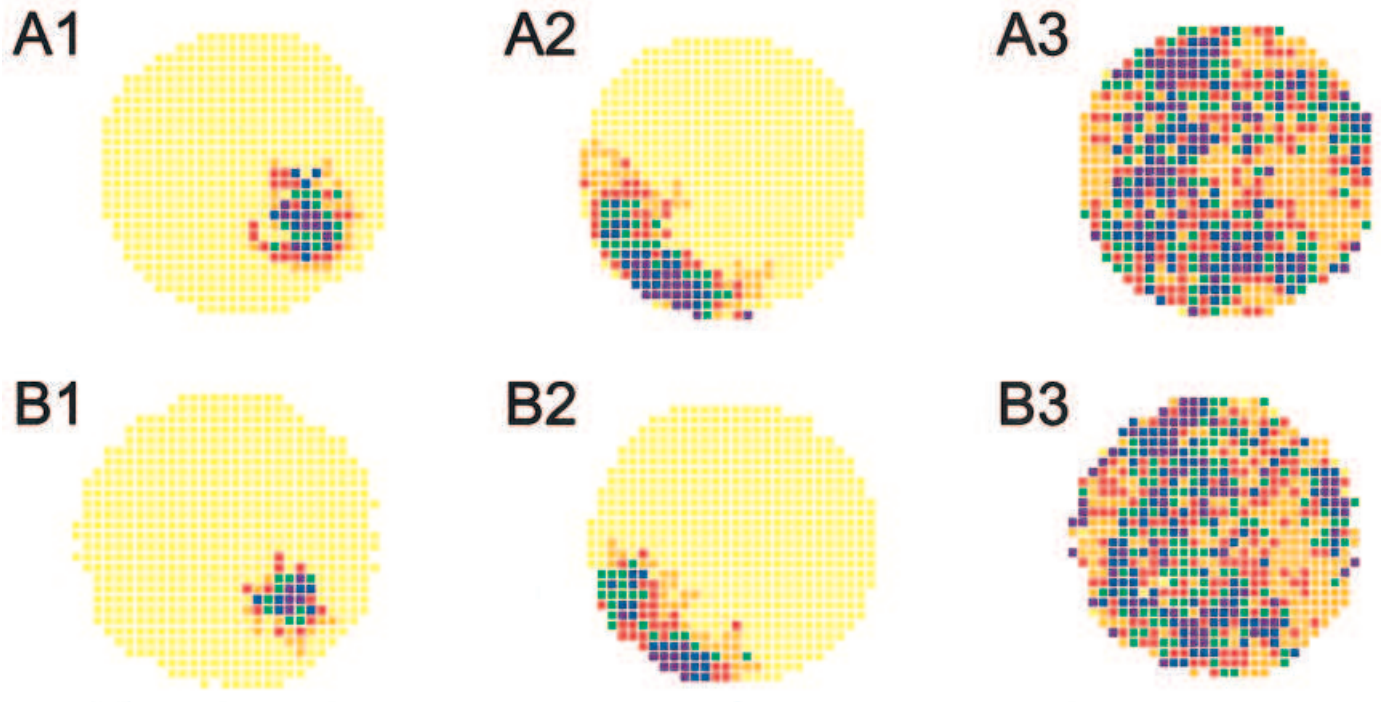

FIGURE 7. Firing rate maps for two place cells (A, B) and for a theta cell (C) recorded with the cable and telemetry systems. The increasing firing rates are coded in the order: yellow, orange, red, green, blue, purple. Yellow represents a rate of exactly zero. The dark regions in the maps for the place cells are the firing fields; out-of-field firing has been suppressed. The similarity of the firing fields recorded via the cable (row 1) and telemetry systems (row 2) is unmistakable. In addition, the positional firing pattern for the theta cell (C) is much the same using both methods; thus, the location and shape of low rate regions (orange) are very similar. Median firing rates in the highest (purple) color category (spikes/s): A1: 11.7, B1: 9.5, A2: 28.8, B2: 26.1, A3: 39.3, B3: 41.1.

Place Cell Recordings With the Telemetry System

Although the telemetry system has many potential uses, our immediate application is to record the location-specific activity of hippocampal cells from untethered rats. In one test, we generated positional firing patterns for individual cells using the cable and telemetry system. Figure 7 shows color-coded firing rate maps for three cells with the cable recordings on top (Fig. 7A1, A2, and A3) and the telemetry recording below (Fig. 7B1, B2, and B3); the first two pairs of maps are for place cells and the final pair is for a theta cell (interneuron). By inspection, it is clear that the positional firing pattern of each cell is very similar across the two recording methods. This visual impression is borne out by a numerical analysis in which the correlation is calculated for a pair of firing patterns on a pixel-by-pixel basis as one pattern is rotated by 1° steps against the other. The z-transform of the greatest of these 360 correlations measures the similarity of the firing pattern. In addition, the angle of the maximal correlation is expected to be 0° if the recording methods yield the same results. The rotation angle for maximum similarity is 0° and −1° for the two place cells and −1° for the interneuron, showing that the firing patterns superimpose as expected if the two recording methods yield equivalent results.

In a second test of suitability of the telemetry system for measuring location-specific activity we took advantage of the fact that interneurons discharge at an appreciable rate (>10–20 spikes/s) everywhere in a small enclosed environment such as the cylinder. Thus, we extracted waveforms that were detected when the rat was in five different regions of the environment as shown in the diagram of Figure 8A. When averages of 200 spikes from each region are plotted on the same axes they virtually superimpose (Fig. 8B), showing that waveform properties are invariant to position.

Finally, to determine the range of the transmitter we progressively increased its separation from the receiver. We found no reduction in signal strength at distances less than 20 m (data not shown). This distance is well short of the 200 m expected when using helical antennas of the specified characteristics. There are probably main two reasons for this shortfall. First, the power supply voltage was only 2.8 V, whereas ≤12 V can be used. Second, the transmitter and receiver were in a relative narrow corridor (2 m in width) with metal walls, providing less than optimum conditions for transmission. Nevertheless, the 20 M range is sufficient for many purposes and could be extended by increasing transmitter voltage or recording in a more suitably shaped area.

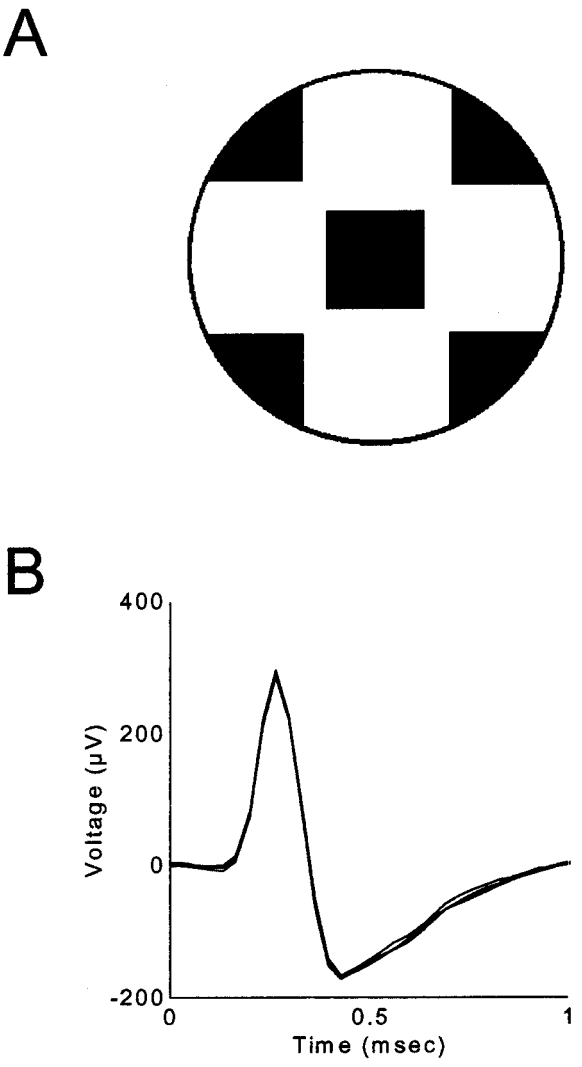

FIGURE 8. Demonstration of the reliability of the telemetry system as a function of location. A: Because theta cells (interneurons) discharge at a relatively high rate everywhere inside a small recording apparatus, used masks to select spikes fired in the five parts of the cylinder shown in black. B: Superimposed averages of 200 waveforms from the five regions. The positional invariance of firing is clear from the almost perfect overlap of the averages.

DISCUSSION

The one-channel telemetry system described here performs extremely well and is a practical method of recording limited amounts of single-cell activity from freely behaving rats. The telemetry system introduced no extra noise into the recordings and produced equally strong signals regardless of the animal's position inside a 76-cm-diameter cylinder. In operation, we found it possible to record three to five individual cells that were simultaneously present on a single electrode wire. Moreover, by recording differentially between two wires we believe it would be possible to record an additional 2–4 units for a respectable upper limit of about 8 units. This count depends on the rather low firing rates of hippocampal place cells and on the fact that place cells present on single wires often have widely separated firing fields. Both properties greatly reduce the probability of near-simultaneous spikes from two or more cells so that the production of composite waveforms should not seriously interfere with the discrimination process. In contrast, the higher firing rates of interneurons and their tendency to fire everywhere in space means that it would be difficult to

record more than two at the same time before overlap of action potentials and consequent problems in spike sorting became objectionable.

How does the method presented in the present report differ from previous one-channel telemetry systems for rats? We think there are several advantages. First, the strong signal means that recordings are invariant to both the orientation and position of the rat's head in the environment. Pioneering work by Eichenbaum et al. (1977) described a system whose signal was constant as the animal rotated at a certain location. This system, however, did not perform ideally as the animal moved around. Instead, there were dead areas inside relative small environments from which no signal could be received (Kubie, unpublished observations). Second, battery life is long. We find that two alkaline N cells are sufficient to power the transmitter, amplifiers, and LED for $\leq$12 h. Lithium batteries of similar size could extend a recording time of $\geq$36 h. Continuous experiments of this length would likely reveal important new features of pyramidal cell activity during locomotion and several stationary states (quiet alertness and different forms of sleep). Third, the use of differential recording means that it is possible to send action potential activity from two wires over a single channel.

Several improvements to the current system would be relatively easy to implement. First, by using a printed circuit board, surface mount chips and lithium batteries, it should be possible to reduce the weight of the system to about 20 g. Second, it may be possible to add a second transmitter/receiver pair, thereby allowing action potential activity from two additional wires to be recorded. In the same vein, the four wires could be arranged as two "stereotrodes" (McNaughton et al., 1983) by recording differentially between one wire of each stereotrode on each telemetry channel.

Having demonstrated that the modem-based telemetry system reliably transmits strong signals, we close by listing some experiments that become possible with even a single channel of telemetry. First, the size and complexity of the recording environment can be increased. Larger environments make it possible to have different subregions in which the animal can eat, drink or sleep. If the animal moves among such subregions, cells can be studied along linear paths set up by the animal's motivational state and not by physical constraints imposed by ramps and tracks. The distribution of discharge among subregions would make it possible to look for patterns of multiple firing field occurrence. The absence of a cable to bite and the long battery mean that very long, unsupervised sessions are feasible.

A second class of experiment possible with a telemetry system is to make a 3D environment such that the animal can go under or climb on top of a platform. Using an infrared LED and infrared-transparent materials would allow tracking whether the animal was on or below the platform. In this way, we could determine how the same X,Y position is represented at two different heights. The absence of a cable also means that recordings can be made in mazes with vertical walls. A third line of investigation would be to record unit activity from two different rats during social interactions using transmitter/receiver pairs operating at different frequencies. Finally, by mounting the receiver on the animal and making the transmitter stationary it would be possible to control the animal's behavior by stimulating the lateral hypothalamus, possibly as a function of position detected with the tracking system.

REFERENCES

Beechley P, Lincoln DW. 1969. A miniature FM transmitter for radiotelemetry of unit activity. J Physiol (Lond) 203:5P–6P.

Edge GM, Horn G, Stechler G. 1969. Telemetry of single unit activity from a remotely controlled microelectrode. J Physiol (Lond) 204:2P–4P.

Eichenbaum H, Pettijohn D, Deluca AM, Chorover SL. 1977. Compact, miniature microelectrode-telemetry system. Physiol Behav 18:1175–1178.

Grohrock P, Hausler U, Jurgens U. 1997. Dual-channel telemetry system for recording vocalization-correlated neuronal activity in freely moving squirrel monkeys. J Neurosci Methods 76:7–13.

Hubel D. 1958. Cortical unit responses to visual stimuli in nonanesthetized cats. Am J Ophthalmol 46:110–122.

Kubie J. 1984. A drivable bundle of microwires for collecting single-unit data from freely-moving animals. Physiol Behav 32:115–118.

Kubie JL, Muller RU, Bostock E. 1990. Spatial firing properties of hippocampal theta cells. J Neurosci 10:1110–1123

Kubie J, Muller RU, Hawley ES. 1997. Single-cell recording in awake behaving rats. In: Lynch MA, O'Mara SM, editors. Neuroscience. Labfax, p 269–281.

McNaughton BL, O'Keefe J, Barnes CA. 1983. The stereotrode: a new technique for the simultaneous isolation of several single units in the central nervous system. J Neurosci Methods 8:391–397.

Muller RU, Kubie J, Ranck J. 1987. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J Neurosci 7:1935–1950.

Paxinos G, Watson C. 1997. The rat brain in stereotaxic coordinates. 3rd ed. San Diego, CA: Academic Press.

Ranck JB Jr. 1973. Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. Exp Neurol 41:461–555.

Strumwasser F. 1958. Long-term recording from single neurons in brain of unrestrained mammals. Science 127:469–470.

Yammato T, Oomura Y, Aou S, Nakano Y, Nemoto S. 1984. A telemetry system for single neuronal discharge recording from behaving monkey. Brain Res Bull 12:129–132.