Miniature stereo radio transmitter for simultaneous recording of multiple single-neuron signals from behaving owls

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Received 26 March 2000; received in revised form 6 June 2000; accepted 8 June 2000

Abstract

Wireless radiotelemetric transmission of neuronal activity is an elegant technique to study brain-behavior interaction in unrestrained animals. In the current study, a miniature FM-stereo radio transmitter is described that permitted simultaneous recordings from two microelectrodes in behaving barn owls. Input from two independent channels is multiplexed to form a stereo composite signal that modulates a radio frequency carrier. The high quality of broadcasted extracellular signals enabled separation of single units based on differences in spike waveforms. Recording several single cells from different electrodes allows the possibility of investigating correlations between small, distributed neuronal ensembles. Multi-channel radiotelemetry that meets the demands of modern electrophysiology might open a new perspective for combined behavioral/neurophysiological approaches in freely-behaving animals. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Barn owl; Multi-channel telemetry; Awake behaving bird; Single-unit recording; Visual forebrain

1. Introduction

The most direct approach to bridge the gap between brain activity and behavior is to record electrical activity of single cells in awake behaving animals. Methodological advances — such as high-impedance microelectrodes, subminiature microdrives, multi-channel recordings and spike-sorting algorithms — opens up a new realm of possibilities to address questions about brain mechanisms with behavioral feedback (e.g. Phillips, 1973; Wilson and McNaughton, 1993; Vaadia et al., 1995; Nicolelis, 1999). Physical restraint of animal subjects is generally inherent to technically-demanding electrophysiological studies. For some neuro-ethological experiments with highly mobile animals such as birds, however, it would be extremely desirable to have the animal free from the encumbrance and restriction of connecting wires. In these cases, the use of telemetry could be very advantageous and might enable a new perspective for combined behavioral/neurophysiological approaches.

Radiotelemetry, wireless transmission of data via radiowaves, has fascinated researchers in bio-medical sciences for several decades (review by Mackay, 1968; Kimmich and Vos, 1972) and is widely applied to convey bioelectric potentials like electrocardiograms, electromyograms or electroencephalograms. Compared to the transmission of such potentials, telemetry systems broadcasting activity of single nerve cells have to meet specific demands. Action potentials are fast (ca. 1 ms) and exhibit small amplitudes in the range of 100 µV when recorded extracellularly. Several transmitters suitable for broadcasting neuronal unit activity have been designed in pioneering studies (e.g. Skutt et al., 1967; McElligott, 1973; Eichenbaum et al., 1977; Pinkwart and Borchers, 1987), but continuous improvements of electronic components and the application of highly-integrated circuits nowadays offers the possibility for easy construction of high-quality, miniature systems with more than one transmission channel.

Single-channel radiotelemetric transmission of neuronal activity has proven to be a valuable tool for recording from freely-moving songbirds in previous studies (Nieder and Klump, 1999a,b). Here, a new...
2. Methods

Behaving barn owls (*Tyto alba*) served as subjects for these experiments. Care and treatment of the owls were in accordance with the guidelines for animal experimentation as approved by the Regierungspräsidium Köln, Germany. Details about the electrophysiology, behavioral task and visual stimulation are given elsewhere (Nieder and Wagner, 1999, 2000).

Briefly, high-impedance tungsten microelectrodes (10 MΩ, FHC) were used to record single units and small clusters of neurons. Electrodes were glued to an array of microdrives to allow manipulation after implantation. Microdrives were derived from drives used for chronic recordings in rats by McNaughton and co-workers (e.g. Wilson and McNaughton, 1993). A similar design has been described in detail by Nichols et al. (1998) and Gaese (1998).

Owls were given valium (1 mg/kg) for sedation and were anesthetized with ketamine (15 mg/kg per h). The skin on the dorsal surface of the skull was opened along the skull’s midline and a hole was drilled in the skull to expose the dura. Three to four microdrives supplied with one or two microelectrodes were fixed to the skull with dental acrylic. The electrodes were aligned to penetrate the visuotopically-organized visual forebrain perpendicularly. Two tungsten wires inserted into the posterior forebrain served as indifferent electrodes. Recordings started five or more days after implantation.

Neuronal activity was transmitted by the FM-stereo transmitter that was attached to the bird’s head. Spike signals were filtered (bandpass 480–7200 Hz, slopes 12 dB/octave), amplified, and monitored on an oscilloscope and audiomonitor. Recorded signals were digitized at a sampling rate of 32 kHz and stored to a PC equipped with a Datawave Technologies Discovery system. To separate single-unit activity, graphical cluster cutting was performed. A maximum of up to three well-defined units were separated per electrode. Time of spike occurrence was written to ASCII files and further analyzed with commercial software.

The owls were trained to perform a standard visual fixation task while they stood on a perch 57 cm in front of a CRT-screen. During the fixation task, a variety of visual stimuli were displayed on the screen to characterize the neurons response properties. Stereoscopic image presentation was performed by means of a stereoshutter (NuVision) placed in front of the CRT-screen that polarized light of the monocular images (Nieder and Wagner, 2000). In addition, owls wore filter glasses to allow the passage of the right-eye image to the right eye only while blocking it for the left eye, and vice versa. Behavioral performance and visual stimulation was controlled and monitored by custom-written software running on a Silicon Graphics workstation that also delivered the visual stimuli.

3. FM-stereo radio transmitter

3.1. Principles of FM-stereo broadcasting

The transmitter system exploits the principles of commercial stereo radio broadcasting, which will be described briefly in the following. In general, the audio signal (or low-frequency signal, respectively) is conveyed by causing a very high frequency carrier signal (radio frequency, RF) to vary in accordance with the audio waveform. In radio broadcasting, the RF-carryer (the radio station’s frequency) is in the range 88–108 MHz. Frequency modulation (FM) is used to modulate the RF-carryer, i.e. the RF-carryer deviates in frequency in accordance with the amplitude of the modulating low-frequency signal.

In monophonic FM transmission, the highest audio frequency transmitted is 15 kHz, which corresponds approximately to the upper human hearing range and is well above the upper spectral components of neural action potentials. Standard radio systems, however, permit modulating frequencies up to 75 kHz. The spectrum of modulating frequencies between 15 and 75 kHz that is void in mono-transmission is used for multiplexing two independent audio channels in FM stereo broadcasting (e.g. Beuth et al., 1996). Combining multiple signals onto one composite signal in such a way that the original signals can be reconstructed by the receiver is called multiplexing.

In FM stereo transmission, the signals of the left (L) and right (R) channels are fed into a summing and differential amplifier to get a L + R signal (monaural reproduction of the original input) and a L − R signal (the difference between the two channels). The L + R signal occupies the range between 0 and 15 kHz (Fig. 1(A)). The L − R signal is shifted higher up in the modulating frequency bandwidth by using it to amplitude modulate a second, subcarrier frequency at 38 kHz. Mixing the L − R signal in a balanced modulator with the 38 kHz subcarrier (Fig. 1(B)) produces a so-called double sideband suppressed carrier signal (the 38 kHz subcarrier is actually suppressed). These sidebands occupy the spectrum between 23 and 53 kHz (Fig. 1(A)). Finally, a 19 kHz pilot tone (38 kHz/2) is
containing information of the L – R signal, and the 19 kHz pilot tone are subsequently mixed to form a stereo composite signal (Fig. 1(B)). This stereo composite signal is then used to frequency modulate the RF-carrier.

After transmission, the stereo decoder in the receiver extracts the L + R and L – R signals and the original left and right signals are obtained by appropriate combination: (L + R) + (L – R) = 2L; (L + R) – (L – R) = 2R. (The reason L + R and L – R signals are encoded rather than L and R is that a mono receiver can just demodulate the L + R signal for mono radio while ignoring the rest of the composite signal.)

3.2. Stereo transmitter design

The preamplifier that is directly connected to the recording electrode has to measure the voltage generated by the neuron(s). In order to work effectively, the preamplifier needs a high-impedance input (several GΩ) and a low input noise characteristics. Very low voltage supply and small size are additional requirements for a non-wired telemetry system. The dual Operational Amplifier TLV 2262 (Texas Instruments Inc.) fulfills these requirements. This chip is fully specified for single-supply 2.7-V operation and available as a surface mount device (SMD). Two 1.4-V batteries provided a symmetric supply voltage. The applied circuit is shown in Fig. 2. Here, the preamplifier is principally used to amplify current with a twofold voltage gain.

The core of the transmitter (Fig. 2) consists of the integrated circuit BA1404 (Rhom Electronics Inc.).
Fig. 3. Photograph of the ready-to-use stereo-transmitter. The head-stage (Pre-Amp) is close to the pins that contact the recording electrodes (Ch1 and Ch2) and the reference electrode (Gnd) in order to reduce noise caused by long connections. The oscillator coil can be seen at the transmitter’s top. Scale in cm.

MHz (license from local telecommunication authorities is obligatory). A trimmer capacitor (5–20 pF) allowed to align the RF-carrier to a certain wavelength remote from powerful radio stations to minimize interference. The 38 kHz crystal generated the subcarrier.

The ready-to-use transmitter (Fig. 3) was covered with dental acrylic for stabilization and powered by a hearing-aid battery of 1.4 V. Physical and electronic specifications of the transmitter are summarized in Table 1. With large-capacity hearing-aid batteries, the transmitter works for several days. Distributors for components of transmitter system are listed in Table 2.

The transmitter output was received by a 2 m copper-wire antenna placed near the animal. Alternatively, a dipole antenna with integrated signal amplifier was used. The received data were then fed to a commercial FM-stereo receiver (Grundig Fine Arts) that demodulated the signal.

3.3. Transmitter performance

In electrophysiological recordings, both high frequency and low frequency signals outside the spectrum of action potentials need to be attenuated. Bandpass filter characteristics of the stereo-transmitter were adjusted to the main spike bandwidth and ranged from about 200 Hz to 5000 Hz (–3 dB point). The high-pass cut-off at about 5 kHz was determined by the de-emphasis network of commercial receivers. The spectrum of transmitted signals could easily be increased by either applying a pre-emphasis network at the transmitter input, or by removing the de-emphasis network of the receiver.

A potential problem inherent to multiplexing systems is channel crosstalk, i.e. the signal transmitted on an individual channel is reflected on another, independent channel that carries a different signal. While crosstalk in stereo-multiplexing systems can hardly be eliminated completely, the quality of a stereo transmitter can be estimated from channel separation, which is an estimate for the amount of crosstalk attenuation. For the stereo-transmitter presented here, channel separation was about 30 dB, which corresponds to a 32-fold attenuation. In Fig. 4(A), a sinusoid was fed to channel 1 while channel 2 was shunted to ground at the transmitter input and, thus, should not carry any signal (both channels with identical amplification). Crosstalk caused by encoding and decoding the stereo composite signal for this condition was negligible. This holds true also for the real recording situation. Activity of visual neurons at two different electrodes that were broadcasted simultaneously from a behaving barn owl with the stereo transmitter did not exhibit crosstalk (Fig. 4(B)). Full information about disparity-tuning properties of neurons recorded simultaneously at two electrodes is given in Nieder and Wagner (2000).
3.4. Isolation of single unit: waveform separation

To allow a more reliable isolation of the activity of single neurons, spike sorting techniques are commonly used that separate single units according to waveforms characteristics. An necessary prerequisite to enable waveform analysis is high-quality, low noise recording, and a reliable transmitter system has to meet this demand. The stereo-transmitter enabled unit recordings comparable in quality to direct-wire recordings (Fig. 4) and permitted detailed waveform analysis. The Datawave recording package used in the current study performed so-called ‘cluster cutting’. Cluster cutting was based on several parameters (e.g. spike amplitude, spike height, spike width) calculated from the spike waveforms. Waveforms tended to form clusters in a plot where two such parameters were plotted against each other. Waveforms from each cluster were attributed to individual neurons.

Fig. 5 displays an example of a recording in a behaving owl made with the current system (and 10 MΩ tungsten electrodes, FHC). In this case, three well-defined units could be separated from one individual electrode. Signals were digitized at a sampling rate of 32 kHz and stored to disk. All spike waveforms that exceeded a given threshold level were digitized for 1 ms. Fig. 5(A)–(C) show original plots of some essential waveform parameters. Each black dot represents an action potential assigned to one of the three spike clusters (1–3). Separated waveforms derived from cluster cutting are displayed in Fig. 5(D).

Isolation of single units is obligatory in cases where adjacent neurons prefer different stimulus attributes. The three isolated units shown in Fig. 5 exhibited strikingly different response characteristics to horizontal disparity. Each of the three single cells, although recorded at the same electrode and, thus, in close spatial vicinity, exhibited very different preferred disparities (Fig. 5(E),(F),(G)).

4. Discussion

The current article describes design and application of a two-channel transmitter system that was used successfully to record single-unit activity and small clusters of neurons from the visual forebrain of behaving barn owls. The system is capable of broadcasting two analog-signal sources simultaneously. It is characterized by miniature size, light weight, low power consumption, low noise and minimum channel crosstalk. High-quality transmission of spike activity meets the demands of modern electrophysiology and enables both short-term and long-term recordings in behaving animals.

4.1. The benefits of radiotelemetry in birds

Radiotelemetric transmission of neuronal activity in behaving birds offers several advantages compared to recordings with awake but immobile or directly-wired animals. First, it allows a maximum of freedom both for the animal and the experimenter. In principle, there is no need to restrain the animal as cellular responses can be collected at any location by placing the receiving antenna near the animal. Isolation of single-units by manipulating the microdrive is possible while the animal is remote form the recording setup and there is no need to touch the electrode contacts again prior to the recording session, which reduces the risk of loosing a unit by causing small movements of the electrode. The second important advantage of radiotelemetry is the reduced stress to the animals. In principle, birds can move or even fly freely during recording sessions (Nieder and Klump, 1999a,b). With radiotelemetry, stimulation and behavioral protocols become the restraining factor rather than the recording per se. Third, wireless transmission of neuronal activity permits various motor actions to be monitored so that behavioral feedback can inform experimenters of the animal’s perception of conditioned stimuli. Owls, for example, were trained to use pecking keys to respond to stimuli (Nieder and Wagner, 1999, 2000). With a small trans-
mitter attached to the head it is even possible to train birds to fly from one perch to another to indicate the detection of a stimulus (Scharmann, 1996).

4.2. Multi-channel radiotelemetry

One might think that the easiest way of performing telemetric multi-channel recordings would be to assemble several separate one-channel transmitter units. Dual-channel telemetry systems developed for unit recordings so far put two separate oscillator stages together (e.g. McElligott, 1973; Grohrock et al., 1997). The most problematic aspect of such an approach — apart from a multiplication of equipment needed to transmit and receive signals — are induction effects between multiple coils in close vicinity (which is inevitable in small animals) that cause extensive crosstalk between independent channels (personal observation).
To avoid these disadvantages, multiplexing telemetry systems have been designed in the past (Kimmich and Vos, 1972; Fischer et al., 1996). Most of these systems use some type of time-multiplexing, i.e. channels are sampled sequentially at alternating time stamps. Clock speed in such systems is suitable to encode relatively slow potentials (EMG, ECG, EEG) that allow sampling with relatively low rates. The waveforms of action potentials, however, have to be sampled at much higher rates (32 kHz in the current study) to enable reliable cell separation. Precise synchronization between transmitter and receiver would be very demanding for time-multiplexing of spike signals.

To overcome both problems, principles of FM stereo radio broadcasting were exploited for two-channel transmission in the present approach. Here, the differential L – R signal is multiplexed onto the main RF-carrier by using it to modulate a subcarrier located at 38 kHz. The major advantages of this approach is that, first, analog signals with optimal temporal resolution are conveyed and, second, high-fidelity radio-components can be used to receive and demodulate the signals. As shown in Section 3, signal quality (signal-to-noise ratio in the range of 1:4 to 1:8; see Fig. 4(B)) and channel separation of the stereo-transmitter were excellent.

The advantages of a two-channel telemetry system are three-fold: First, data collection gets faster since two channels instead of one were transmitted at a time. In addition, the probability of maintaining a stable recording at least at one of both electrodes increases. Second, so-called stereotrodes (McNaughton et al., 1983) — two closely spaced electrodes whose signals are compared for spike separation — might be used to enable reliable spike separation in certain brain regions (Wilson and McNaughton, 1993). And third, discharge of simultaneously recorded units could be compared to examine interactions between neuronal ensembles (e.g. Singer and Gray, 1995; Vaadia et al., 1995; Grün et al., 1999; Laubach et al., 1999).

5. Conclusion

Simultaneous telemetric transmission of single-unit activity of multiple neurons can serve as an elegant tool to study brain–behavior interaction in freely behaving animals. Of course, radiotelemetry is not a prerequisite to enable recordings from behaving and largely un-restrained animals. Especially when fifty or more channels are analyzed simultaneously (Nicoletis et al., 1997), there is no alternative to direct-wire recording. But radiotelemetry might enable neuro-ethological experiments that would otherwise be impossible and could prove important particularly in those situations where it is desirable to interfere with an animal as little as possible.

Acknowledgements

I am particular grateful to Georg M. Klump for introducing me to radiotelemetry. I thank my thesis advisor, Hermann Wagner, for his support through every phase of this project. Many thanks is also due to Bernhard Gaese for help with the Datawave system. Hermann Wagner, Bernhard Gaese and Barrie Frost provided helpful comments on the manuscript.

References
