A device to enable the automatic recording of rodent ultrasonic vocalizations

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The difficulty of recording rodent ultrasonic vocalizations simultaneously with other behavior patterns has been overcome by the use of the apparatus described. The electronic circuitry automatically digitally codes specified frequencies of rodent ultrasounds on a behavioral recording device. A reliability study of its performance in recording vocalizations of copulating Mongolian gerbils, during experimental tests, is also described.

Rodent ultrasonic vocalizations are produced in a wide variety of behavioral situations: maternal (mice—Noirot, 1966), aggressive (rats—Sales, 1972), sexual (rats—Barfield & Geyer, 1972; mice—Whitney, Coble, Stockton, & Tilson, 1973). After detection by microphone, ultrasonics can be observed by examination of oscilloscope tracings (Griffin, 1958), by recording ultrasounds on magnetic tape and replaying them at lower speeds so that they are made audible (Sales & Pye, 1974), or by using heterodyne electronic circuits that produce audible sounds when ultrasonic signals are received (Pye & Flinn, 1964). Unfortunately, these methods of observation do not easily permit close temporal correlation of ultrasonic calls with ongoing behavior patterns. The electronic circuitry described below filters various frequencies and responds to any selected frequency producing a pulse that can be recorded on digital behavioral recording devices simultaneously as other motor behavior patterns are being recorded. The behavioral recording device used in the reliability study described below was a computer-based recording and transcribing system (WRATS) designed by White (1971). The device records key depressions from a keyboard on magnetic tape; the states (off and on) of each key are assessed once every 100 msec. If each key represents a pattern of behavior, then the frequency, latency, and duration of each pattern and their original temporal patterning are preserved. Therefore, when the frequencies that comprise a set of ultrasounds of a rodent have been categorized into physical types (e.g., by producing sound spectrograms of vocalizations), the filters can be calibrated for frequencies that are dependent on each vocalization.

CIRCUIT DESIGN AND OPERATION

The circuit diagram of the encoder is shown in Figure 1. The signal from the high-frequency microphone amplifier is relayed to the input of the first

stages of four dual-operational amplifiers. The feedback network of each of these stages consists of a standard TWIN-T filter, tuned to give maximum impedance and, consequently, maximum stage amplification at any desired frequency (i.e., on this encoder, 5, 26, 33, and 39 kHz). The general circuit of a TWIN-T filter is given in Figure 2, and the component values C and R may be found from the formula, $f_c = 1/2\pi CR$, where f_c is the desired filter center frequency in hertz, C is the capacitance in farads, and R is the resistance in ohms.

The signal can then be rectified and smoothed by the diode-resistor-capacitor network to give a dc voltage proportional to the signal voltage at the output of the filter (Figure 1). This voltage is then applied to the input of the second active stage, connected as a voltage comparator, the reference voltage of which may be varied by the potentiometer, thus controlling the trigger level at which the output changes state.

In theory, as the frequency of the input signal approaches the tuned frequency of a particular channel, the voltage input to the comparator rises; if the trigger level is adjusted correctly, the output of the comparator will decrease from +12 V to -12 V as the frequency input equals the tuned frequency. In practice, however, due to practical limitations in the accuracy of setting the trigger level and the hysteresis inherent in the circuit, the comparator is triggered over a small band of frequencies centered on the filter-tuned frequency. This sensitive band is approximately 10% of the filter center frequency, which is adequate for the channel spacing of this particular device. For more densely packed filter channels, the bandwidth can be considerably reduced by the use of higher tolerance trimming components and operational amplifiers with higher slewing rates. The represented circuit functions well up to approximately 100 kHz; however, this value may be increased by the use of higher bandwidth operational amplifiers.

To interface the encoder with the behavioral recording system (WRATS), it is only necessary to replace the keyboard switch by a normally switched

Figure 1. Circuit diagram of the ultrasonic encoder.

Figure 2. Circuit diagram of a TWIN-T filter. Letters are referred to in the text.

“on” transistor. Therefore, the outputs of the comparators are connected to the bases of four 2N3704 NPN transistors (Figure 1) whose open collector outputs may be connected directly to four WRATS inputs. When a particular channel is triggered, the comparator output falls to -12 V, thus switching the transistor off at the input of the associated WRATS channel.

The 33-kHz channel of the encoder differs in one respect from the others in that a 100-msec “one-shot” module is included between the comparator and the output transistor. The reason for this is that the duration of the 33-kHz vocalization, described below, is less than the 100-msec scan time of the WRATS; the one-shot circuit is used to increase the comparator pulse output to a detectable duration.

The remaining part of the encoder circuit is included to aid the calibration of the system. It consists of four 2N3702 PNP transistors connected to the comparator outputs so that when a particular channel is triggered, the PNP transistor is switched on and current flows through a light-emitting diode. This provides a visual indication of the presence of a signal of the correct frequency for that channel.

The open collector outputs of the encoder may be connected via resistors to any voltage supply up to $+30$ V. Therefore, the encoder may operate a variety of other recording devices, such as chart recorders, counters, paper-tape punches, and so on, or a digital interface for real-time monitoring by computer.

EXPERIMENTAL RELIABILITY

Method

The encoder was tested under experimental conditions using the Mongolian gerbil as the ultrasound-producing animal. The male emits a number of ultrasounds (Holman, 1977) during sexual behavioral tests with estrous females. The least intense (median = 49.5 dB SPL) of these ultrasounds is an upsweep vocalization emitted by the male during the pre-mount period. The call has an unmodulated first section of 30-kHz frequency and subsequently increases up to 34 kHz (Figure 3A). The median total length of the call is 19 msec. The call can be produced in bouts, each call separated by approximately 90 msec (Figure 3A). This call was chosen to make reliability

tests because it is the least intense call, and calibration of the encoder is most difficult with low-intensity sounds. The bandpass frequency to which the encoder responded was set at 33 kHz, the frequency through which the end of the upsweep vocalization travels. Ultrasounds were recorded during the pre-mount period produced by copulating pairs in an enclosed semianechoic observation chamber (Holman, 1977) measuring $35 \times 40 \times 26$ cm. Ultrasounds were detected by a Brüel and Kjaer .25-in. condenser microphone (Type 4135) and a Brüel and Kjaer preamplifier (Type 2603) fitted with a Krohn-Hite filter (Number 3550) set between 20 Hz and 200 kHz. The resultant signals were relayed to the encoder, a Lockheed tape recorder (Model 417), running at a tape speed of 15 ips and visually displayed on a Telequipment cathode-ray tube (CRT) oscilloscope.

The CRT screen and the light-emitting diode of the encoder channel, which responded during 33-kHz signals, were observed. The intensity levels of the encoder were adjusted so that the diode and most of the low-intensity traces of calls on the CRT screen responded together. On 95 different occasions, vocalizations from 19 pairs of gerbils were simultaneously recorded by the encoder and the tape recorder. Each recording period lasted 2 min. After each test, the tape was replayed at 25% speed and the number of upsweep vocalizations were counted.

Results and Discussion

Figure 3B shows that the frequency of upsweep calls recorded on magnetic tape and the frequency with which the 33-kHz channel on the encoder was activated were significantly proportional to each other. That is, the slope of the frequency of vocalizations plotted against the encoder frequency had a regression coefficient of .52, which was significantly different from zero [$t(s) = 19.7$, $df = 93$, $p < .001$]. Also, a significant portion

Figure 3. (A) Sound spectrogram (Kay spectrograph Model 6061B) of a bout of pre-mount male vocalizations. (B) The plot of the encoder frequency of the channel calibrated for the vocalizations shown in Figure 3A and the frequency of this vocalization as recorded on magnetic tape.

of the variance of the encoder frequency was explained by the regression on recorded frequency [$F(93) = 387.1$, $p < .05$]. Thus the encoder records approximately 50% of all of the low-intensity vocalizations. There are two reasons why the encoder would not be expected to record all vocalizations. First, as calls can succeed each other within intervals of less than 100 msec, some calls occur while the 100-msec one-shot output is operating after being triggered by the previous call. Therefore, the later calls are not recorded separately; only the bout in which they occur is recorded. Second, the electronic noise levels of the detecting apparatus, at the settings used during reliability tests, was 48 dB. Calls falling below this intensity were not filtered and, hence, were not detected by the encoder. Furthermore, noise levels were accentuated by high-frequency sounds produced by the gerbils' moving about in the observation chamber. As noted previously, the calibration of the encoder was set to detect calls that were easily distinguishable on the CRT screen, so that no false positive sounds were recorded.

However, all high-intensity vocalizations are recorded by this system. For example, male gerbils produce a 26-kHz, unmodulated, postejaculatory tone (Median intensity value = 67.2 dB SPL), approximately 145 msec in length (Holman, 1977). The light-emitting diode of a channel of the encoder, which was set at 26 kHz, always responded when the high-intensity postejaculatory vocalizations were observed on the CRT screen during copulatory tests with gerbils.

The accuracy of the measured frequency of

vocalizations by the encoder was therefore limited, to some extent, by the use of the behavioral recording apparatus (White, 1971). However, an accurate temporal relationship between vocalizations or bouts of vocalizations and other behavioral motor responses can be provided with this apparatus.

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