INSTRUMENTATION & TECHNIQUES

A digital ultrasound recorder

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A system that continuously monitors and records rodent ultrasonic vocalizations over the frequency range 10-90 kHz is described. The system records vocal frequency, sound pressure, time, and duration of call. Data are stored digitally. The system was validated by a pilot study in which distress calls of rat pups were selectively activated while they were subjected to a cold environment.

Ultrasonic vocalizations are known to be part of the behavioral-response repertoire of many rodents (Allin & Banks, 1971; Anisko, Suer, McClintock, & Adler, 1978; Barfield & Geyer, 1972, 1975; Okon, 1971; Pye & Flinn, 1964; Sales & Pye, 1974). Thermal stimuli are, for example, known to influence the production of distress calls by infant rats and mice at ultrasonic frequencies (Allin & Banks, 1971; Okon, 1971). The correlation of ultrasonic calls with aggressive and submissive behaviors in rats and other small mammals has been observed (Sales, 1972). States of submission are generally indicated by the production of long pulses of ultrasound, whereas aggressive encounters are normally associated with short pulses. A relation between 22-kHz ultrasonic signals and the sociosexual behavior of rats has also been investigated (Anisko et al., 1978; Barfield & Geyer, 1972, 1975). Table 1 summarizes many of the ultrasonic calls of Wistar and Long-Evans rats and the circumstances under which such calls are detected.

The study of ultrasonic vocalization provides a novel approach to defining the behavioral-response patterns of laboratory rodents. Ultrasonic-signal analysis could be used as a basis to investigate the possible neurasthenic effects of chronic low-level microwave irradiation (Justesen, 1979). We have previously suggested this approach to stress-related behavioral analysis (Voss, Note 1; Voss, Paranjape, & Turner, Note 2), and our objective is to show that it is now electronically feasible.

To record the ultrasonic vocalizations of rodents, most investigators have relied on techniques developed by Pye and Flinn (1964) and Sales and Pye (1974).

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One technique is to record ultrasound directly by high-speed FM tape recorders. The low-speed replay of these tapes effectively converts high-frequency ultrasound into low-frequency audiosound. This technique has been used by a number of authors (e.g., Holman, 1981; Thiessen, Graham, & Davenport, 1978). A tape recorder and its associated equipment can give very detailed analyses, but the process is time consuming.

Another technique is to use an ultrasound converter, which provides a simple way to "hear" ultrasound. An ultrasound detector directly converts (by the heterodyne method) the original frequency of the sound signal into an audible frequency band. Complete frequency analysis is not possible, and amplitude variations cannot be determined. Only the presence of ultrasonic signals is indicated.

Automatic recording and counting devices for mouse calls, which have a smaller frequency range than those of the rat, have also been developed (Brain, Benton, Cole, & Prowse, 1980; Morgret, 1972; Clarke, Smith, Justesen, & Cushman, Note 3). These are capable of detecting vocalizations, but their limitation is that a restricted range of frequencies must be selected—vocalizations out of the test band are not monitored.

Harrison and Holman (1978) first attempted to convert ultrasonic calls into digital format and to use computer scanning for their analysis. The automatic recording device of Harrison and Holman monitors the ultrasonic-frequency spectrum across four test bands. If the relevant frequency component is present in an ultrasonic call, a digital state of "one" is registered at one of four parallel twin-T filter circuits. These four outputs are periodically scanned by a computer, and their levels are stored for future analysis (White, 1971). Since there are only four narrow test bands, calls that do not contain significant spectral components in the test bands are lost.

Analysis of murine vocalizations has been difficult because satisfactory broadband ultrasound detecting devices have not been available. Rodents are known to

Table 1
Ultrasonic Calls Reported for Wistar, Wild, and Long-Evans Rats

Study	Strain	Range of Frequencies*	Typical Duration**	Rate of Calling	Cause	Notes
Sewell (1968)	Wistar	40-90	5-65	Not given	Isolation from nest	Pups 4-16 days old (slightly lower frequency for pups > 16 days old)
Okon (1971)	Wistar	35-55	Not given	Calls per minute = 48,45, 25; Intensity = 3 micro-bar peak	Lower temperature (3°, 12°, 22°C)	Pups 0-22 days old (most vocal when homiothermy is developing)
Sales (1972)	Wistar	22-30	700-1000	Many calls per minute	Submission to stronger male	Male rats (calls may inhibit aggression; submissive call)
	Wild		300-600	Many calls per minute	Restrained by hand	
Sales (1972)	Wistar	40-70	3-65	Many calls per minute	Domination over weak male	Male rats (calls may indicate dominance; results from aggression)
	Wild				Restrained by hand	
Sales (1972)	Wistar	30-56	100-800	Many calls per minute	Heterosexual activity	Male or female
		34-120	2-40	Very sudden	Genital sniffing; mounting	
Anisko et al. (1978)	Long-Evans	22-25	1-3000	Not given	Postejaculation	Depression call deters female; male incapable of sexual activity

*in kHz. **in msec.

vocalize over the frequency range 15-150 kHz (Sales & Pye, 1974). Equipment capable of continuously monitoring signals across this frequency range is now possible because of recent advances in digital electronics. We have developed a broadband ultrasound detector that automatically monitors most of this frequency range. It is compatible with a large-scale computer analysis of the data collected. A microprocessor-controlled device, it can continuously monitor signals at frequencies from 10 to 90 kHz. The high-speed digital components of the system trigger data collection on detection of an ultrasonic call. Storage of null data is eliminated. The output of the system is automatically stored in a random-access-memory (RAM) buffer. For extended periods of data collection, information can be transferred to any digital, mass-storage device, such as a parallel printer, disk drive, or modem with connection to a mainframe computer. Subsequent, more detailed analysis of ultrasonic calls can be made.

THEORY OF OPERATION

The ultrasound recorder converts any 10-90-kHz call into a digital signal. Figure 1a, a block diagram, shows the recorder divided into five discrete units. When an ultrasonic call is received by the condenser microphone, it is directed to the analog (input-conditioning) unit (No. 1). This unit prepares the call for the digital-conversion unit (No. 2). The control unit (No. 3) directs all the system's functions: It triggers events in all sections and controls the flow of data among them. The

memory unit (No. 4) is a temporary storage depot; data are passed from it and through the output unit (No. 5) for final transfer to a device such as a computer or parallel printer. In this way, the components work together to detect, to digitize, to store, and, finally, to transmit data on the ultrasonic calls.

The control unit contains the system's central processing unit (CPU) and 8-bit microprocessor (MCM 6802). This processor executes programs stored in a set of erasable, programmable, read-only memories (EPROMs). The operating system is the largest program in this memory bank. When the operating system starts execution, it interrogates the user for the system-timer speed, the rate of data collection, and the method of data transfer. Once these basic parameters are defined, the system begins to monitor ultrasonic signals.

A flowchart of the system is presented in Figure 1b. During operation, the recorder constantly tests for ultrasonic signals. On detection of an ultrasonic call, the system begins data collection. It first reads the timer to find out when the call began and then measures the amplitude and frequency of the call. This information is stored in the local memory buffer. The recorder then waits for the timer to trigger it to start making further measurements of amplitude and frequency. This process continues for the duration of the ultrasonic call. When the call ends, the recorder returns to its earlier mode and begins testing for the occurrence of additional ultrasound.

The cycle of testing for calls and making readings continues until a time-out condition is reached. The

Figure 1. Components of the digital ultrasound recorder. (a) The block diagram of the recorder with the functions of its five major units specified. (b) The flowchart for the control unit. (c) The block diagram of the analog input-conditioning unit. Calls are split into three components. Line 'A' is set to 5 V when a call begins. The envelope detector on line 'B' traces the amplitude of the call. The voltage limiter on line 'C' controls the amplitude of the signal to a maximum of ± 15 V. (d) The block diagram of the digital-conversion unit. A Schmitt trigger improves the slew rate of the ultrasound detect line 'A.' The sample-and-hold (S/H) and analog-to-digital (A/D) converter on line 'B' converts the amplitude of the ultrasonic call into a digital signal. The frequency-to-voltage converter (F/V) on line 'C' generates a voltage proportional to the frequency of the ultrasonic call. This signal is converted into a digital signal by the S/H and A/D. A 5-V signal on line 'A' will cause the control unit to trigger conversions of the amplitude and frequency of an ultrasonic call.

time-out condition can be brought on by: (1) local memory overflow; (2) system-timer termination of the experiment; or (3) user selection of a continuous transfer mode of operation. The presence of a time-out condition results in some of the data's being transmitted out of the system by the output unit. After transfer has been completed, the time-out condition is automatically removed and the operating system continues its normal flow.

The analog input-condition unit of the recorder essentially guarantees that all signals directed through it to the digital-conversion unit are within prescribed tolerances. The block diagram in Figure 1c shows the output of the condenser microphone first amplified by a factor of 2,000. This improves the signal-to-noise ratio for the rest of the system, and also isolates that microphone's output from the digital network. The microphone and amplifier are a part of an S100 Bat detector (QMC Instruments, Ltd., Queen Mary College, University of London, London, England). The signal is then split into three parallel signals: A, B, and C. Signal A is compared with a user-specified noise level. Setting the appropriate value for this level is critical because, for the system to collect data, the sound pressure of the ultrasonic call must be greater than this noise level. The position of the noise level is determined by the following factors: (1) the level of ambient, interfering noise; (2) the proximity of the microphone to the experimental subject(s); and (3) the electronic noise generated by the system. The noise is centered at a frequency of 1 MHz, a frequency that cannot trigger the system. The amplitude of the noise could, however, mean that some low-intensity signals are not recorded: A variable resistor within the unit controls the threshold of the noise level and is set at 0.1 V. At this level, an ultrasonic signal detected by the microphone must have an amplitude of >5 mV in order to be registered. If the incoming signal is greater than the noise level, the output of a comparator is set to 5 V, which indicates to the control unit that a valid call is present; the output is labeled "the ultrasound detect line." The second parallel signal, B, is fed into a precision rectifier and envelope detector. Only the positive envelope of the original call passes through these components. It is a slow-moving signal and represents the amplitudinal variation and frequency modulation of the ultrasonic call. The third parallel signal, C, is tested to ensure that its voltage levels will not damage subsequent circuits. These three signals (A, B, C) are directed to the digital-conversion unit.

The primary task of the digital-conversion unit is to couple incoming analog signals to the CPU (in the control unit). The three analog signals of the previous unit are converted into digital signals by the processes shown in Figure 1d. The ultrasound detect line, A, is technically a digital signal when it leaves the analog unit. It has two states, 0 and 5 V. To add a small amount of hysteresis and to ensure that the transition between states is rapid,

the signal is fed into a Schmitt trigger. This signal can now be read by the CPU.

The amplitudinal variation of the ultrasound, line B, is fed into a sample-and-hold circuit (S/H), which is directly connected to an analog-to-digital (A/D) converter. The CPU triggers the S/H and the A/D to convert the input analog signal, B, to its equivalent digital signal at user-defined time intervals (this digital signal can be accessed by the CPU).

The frequency variation of the ultrasonic call, line C, is converted to a digital number by a frequency-to-voltage (F/V) converter. The module counts the number of zero crossings of the ultrasonic call. It is, therefore, a measure only of the fundamental frequency of the call. The number of zero crossings is translated into an analog voltage, which is fed into an S/H and an A/D, in a fashion similar to that of the previous signal. The CPU is able to simultaneously trigger a measurement of frequency and of amplitude. These three signals, which are directly related to the incoming ultrasonic call, are available to the CPU within 100 microsec of detection.

The CPU stores these digital numbers, which represent amplitude and frequency, in the local memory buffer as part of its normal execution of the operating system. The memory buffer can hold up to 10 KB of information (5,000 ultrasound measurements). Depending on the rate of animal vocalization and data collection, the memory buffer will be filled in anywhere from 2 min to the duration of the experiment. The buffer is made up of static RAM chips (MCM 2114), and the data stored can be maintained for any length of time (if the line current is not interrupted).

Transfer of data through the output unit must occur before useful information can be extracted from the recorder. Transfer will occur if the local buffer is filled. It will also occur if the user selects an active mode of data transfer. In this mode, the operating system gets a system time-out each time it collects data. An output program is called to test if the current rodent call is complete. If it is not, the recorder is returned to the control of the operating system and data collection continues. If the call is complete and no further measurements are forthcoming, the output routine begins to transfer data. It will transfer one entire call and then return control of the recorder to the operating system. The active-transfer mode can be used only if the recorder is transmitting data to a device at a fast transfer rate. If the transfer rate is not sufficiently high, the chances of data loss increase. That is, before adopting this method, the user must determine the average time required to transfer a call and to compare it with the rate of vocalization.

Eventually, data transfer must occur. During initialization, the user specifies the type of device (serial or parallel) to which data will be transferred. Data can be transferred to most digital mass-storage devices (parallel printer, cassette tape, modem to mainframe computer, or disk drive). By being connected to a mainframe

computer, data analysis can be simplified with statistical software packages. Frequency, amplitude, call duration, and time of call can be correlated with animal behavior or experimental conditions. Thus, with these facilities, it may be possible to relate, with high accuracy, behavioral patterns to distinct ultrasonic calls, and to investigate in a systematic way the abilities of rodents to communicate through ultrasound.

EXPERIMENTAL VERIFICATION

Two different approaches were employed to determine the effectiveness of the detection system described. The first was based on artificially produced ultrasonic signals to test the system's ability to reconstruct ultrasonic calls accurately. The second was based on monitoring the ultrasonic signals from rodents under conditions in which the type of calls they make had been documented previously.

Three test frequencies, 22, 50, and 70 kHz, were chosen to cover the broad range of calls made by rodents. A small amount of modulation was introduced so that amplitude variations could also be determined. The signals were fed directly into the recorder, bypassing the microphone, thereby eliminating the problem of ensuring the integrity of the modulation of the signal through a loudspeaker.

The results are shown in Figure 2. There is a significant similarity between the output of the recorder and the input ultrasonic signals. The uncertainty of the frequency measurement is less than 5%, and that of the amplitude measurement is less than 10%. (Any nonlinearities in the system can be determined by testing its output across the entire frequency spectrum. If data are being transferred to a computer for statistical analysis, this kind of error can be easily suppressed by multiplying a datum by the appropriate scaling function.)

The monitoring system was further tested by ultrasonic signals produced by pups of randomly bred Sprague-Dawley rats (*Rattus norvegicus*) from Bioscience Animal Services, University of Alberta, Edmonton, Alberta, Canada. The rats were housed in standard 21 x 21 x 45 cm cages in the Animal Unit of the Surgical-Medical Research Institute, University of Alberta. Food and water were available ad lib. The room temperature was maintained at 22°(±1.5°)C, and a light:dark schedule of 12:12 h was employed. The infant rats were maintained under maternal care, except for the duration of 5 min monitoring periods. The limited ability of the pups to move about allowed all measurements to be carried out upon an open pedestal, which minimized interference by echoes.

Previous work confirmed that isolated Wistar-strain rat pups under hypothermal stress will, before their thermoregulatory systems are fully developed, make ultrasonic calls in the frequency range of 35-55 kHz

Figure 2. Three sets of graphs of recorder output for known ultrasonic signals. Input signals are: (1) 22-kHz carrier with 4-Hz modulation and peak amplitude of 3.8 V, (2) 50-kHz carrier with 4-Hz modulation and peak amplitude of 1.6 V, and (3) 70-kHz carrier with 4-Hz modulation and peak amplitude of 1.5 V.

(Allin & Banks, 1971; Okon, 1971). These types of calls were induced in 6-10-day-old pups by removing them individually from their nests and placing them on a pedestal precooled to 2°-3°C—a ~36°C drop from normal nesting temperatures and a sufficient stimulus to elicit ultrasonic calls. The distress/discomfort calls were monitored by the detection system. A typical distribution of their calling patterns is shown in Figure 3.

DISCUSSION

Our detection system is significantly more complex in design than others previously reported and requires expertise in microelectronics for its fabrication. However, there are several advantages to its implementation. It is broadband, so that it can simultaneously monitor more than one type of call. As a result, it, as is the FM tape recorder, is also susceptible to noise. But noise signals are relatively easy to identify in the output because they are short in duration, have rapid amplitudinal variations, and tend to be lower in frequency.

Figure 3. Three sets of representative graphs of recorder output for ultrasonic calls from infant Sprague-Dawley rats. Rats were placed on a pedestal cooled to $2-3^{\circ}\text{C}$. Calls are within predicted 35-55-kHz range.

The monitoring system collects data only during vocalizations. It records the time when the call began and then, at regular intervals, records its frequency and amplitude. Unlike with the tape recording system, there are no "gaps" between calls. Automatic recording and counting devices do not collect null data (Brain et al., 1980; Morgret, 1972; Clarke et al., Note 3); they can only detect calls within specified frequency bands and therefore cannot simultaneously monitor more than one type of call. These devices indicate when a call is in the passband but do not define its precise frequency; they do not identify any frequency variations within the passband or indicate the amplitude of the vocalization.

The output of our digital recorder can be readily accessed by computer. This may allow researchers new approaches to analyzing and studying the complex ultrasonic signaling patterns known to occur in nature.

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