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### **Basic Neuroscience**

## A novel device for the calibration of sonic and ultrasonic recording transducers

## Matthew S. McMurray<sup>a,\*</sup>, Devin K. Hubbard<sup>b,1</sup>

<sup>a</sup> Department of Psychology, University of Illinois at Chicago, 1007 W. Harrison Street. M/C 285, Chicago, IL 60607, USA
<sup>b</sup> Department of Biomedical Engineering, University of North Carolina, 401 Taylor Hall CB# 7575, 109 Mason Farm Road, Chapel Hill, NC 27599, USA

#### HIGHLIGHTS

- No devices currently exist to calibrate the amplification of vocalization recordings.
- Calibration Unit for Recording Transducers (CURT) are designed to fill this need.
- It emits a tunable frequency and amplitude in human-sonic and -ultrasonic ranges.
- It is highly portable and fits a variety of microphone connector types.
- We demonstrate the reliability of the unit and show the improvement in data quality.

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#### ABSTRACT

Recently, there has been an increase in the analysis of animal vocalizations in behavioral neuroscience as a social cue or indicator of neurological integrity. Despite the multitude of researchers examining vocalizations in a variety of species, no inexpensive, tunable devices currently exist to calibrate the amplification applied to such vocalizations before data are collected. Many commercially available recording systems have analog adjustments for gain, but such methods are notoriously unreliable and highly variable. Without a consistent level of gain, the amplitudes of recorded acoustic signals cannot be reliably compared. Here, we describe an apparatus designed to fulfill this need, which we have labeled the Calibration Unit for Recording Transducers (CURT). To maximize application to various fields, its emitted frequency and amplitude are tunable to output frequencies in both human-sonic (20 Hz–20 kHz) and human-ultrasonic ranges (20 Hz–100 kHz). Additionally, it is a portable (weighing approximately 180 g), customizable, stand-alone unit, and fits a variety of microphone connector types. The CURT is also relatively low cost to build (under 250.00 USD), thereby making such a device available to as many researchers as possible in animal behavior and neuroscience.

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NEUROSCIENCE Methods

#### 1. Introduction

The study of animal vocalizations in behavioral and neurobiological research has become increasingly popular over the last decade. Prior to the year 2000, the term "vocalization" was found by Pubmed in only 3979 papers. Between then and the year 2012, this number has effectively doubled, with over 4100 additional papers added. This increase in interest by the scientific community may be the result of numerous factors, but perhaps specifically due to the relevance of vocalizations in countless species as both a social cue and an important biological signal (Zeskind et al., 2011). Given its translational nature and our general understanding of the physiological mechanisms of cry production, one can assume that

<sup>1</sup> Tel.: +1 919 843 6076.

the popularity of vocalizations will continue to increase. However, further adoption of this endpoint must be tempered by the development of accurate tools to assess the various spectral parameters, including frequency, amplitude, harmonics, duration, repetition rate, and waveform complexity (shape), and melody.

The accurate quantification of spectral characteristics in any species requires accurate recording, amplification, digitization, and analysis. For many labs, this can be accomplished via commercially available systems, such as the popular Computerized Speech Lab (KayPentax, Montvale, NJ) used in human speech research, or the UltraSoundGate (Avisoft Bioacoustics, Berlin) used in rodent, birdsong, and bat research. These are turn-key systems that provide ease of utility, but at the cost of reduced flexibility and/or specialization. Other labs, including our own, have resorted to custom-built solutions, thereby granting flexibility or specialization, but at the cost of increased system development and training costs. No matter the tool selected, it is important to understand its limitations and constraints at each level of acquisition. For example, sampling rates in analog to digital converters should be taken into

<sup>\*</sup> Corresponding author at: Tel.: +1 312 996 5370.

E-mail addresses: mcmurray@uic.edu (M.S. McMurray),

dhubbard@email.unc.edu (D.K. Hubbard).

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**Fig. 1.** The relationship between voltage and sound amplitude (relative decibels) in our recording equipment. This data is reflective of the typical exponential relationship between the two measures. Details of recording equipment are listed in text.

consideration. Sampling at too high of a rate will cause aliasing artifacts and sampling at too low of a rate will prevent accurate reconstruction of the entire waveform. To determine the appropriate sampling rate, a popular heuristic is the Nyquist-Shannon Theorem, (Nyquist, 1928; Shannon, 1949) which states that one must record at a minimum of twice the input signal frequency (i.e. one must sample a 2 Hz signal at a minimum of 4 Hz to be able to capture the signal correctly); however, many consider the Nyquist frequency to be the *minimum* acceptable sampling rate. Similarly, when recording ultrasonic vocalizations (>20 kHz), common to rodent and bat species, using a microphone with a frequency response range lower than 20 kHz would not be appropriate, since sampling sounds outside the frequency response range of a microphone results in dramatic attenuation of the signal due to non-linear frequency response of microphones.

The primary function of a microphone is to convert the physical movement of air into a change in electrical potential (voltage) that can be measured or recorded. This conversion is accomplished via a transducer and results in minute changes in voltage (mV range). In our equipment, there is an exponential relationship between voltage and amplitude (dB, see Fig. 1), with the range and shape dependent upon the specific hardware used. Such small changes in signal typically require amplification before they can be effectively digitized, especially when sounds occur at low volumes. Amplification (i.e. recording volume adjustment in audio systems) is one often overlooked limitation of many recording systems. Variations in amplification can result in inaccurate quantification of a sound's amplitude, thus biasing measurements of the sound duration (due to clipping) and frequency. On most commercial systems, amplification (gain) is accomplished via an analog "volume knob". In some commercial systems, this knob has numeric feedback to inform the user of the approximate level of amplification, but more often than not there is no objective feedback available. Without accurate feedback of the level of amplification, it is likely that amplification levels will vary between recordings, potentially confounding results. Additionally, when using multiple microphones/amplifiers, it is critical to ensure each system is representing amplitude in the same manner. Thus, calibration of amplification systems is of paramount importance to ensure measurement is as accurate as possible, especially when the level amplification is set via an analog mechanism.

Despite the multitude of researchers currently examining vocalizations, no calibration device has been widely adopted for use in the field. The two commercial recording systems mentioned above (Computerized Speech Lab and UltraSoundGate) both have analog adjustments for gain, but only Avisoft (manufacturer of UltraSoundGate) supplies an inexpensive calibration device (Model 60105), and this device has a fixed frequency output (40 kHz), limiting its usage in other fields. Other calibration devices, such as the G.R.A.S. Sound and Vibration, Sound Calibrator Type 42AB, can produce a calibration tone in a variety of frequencies, but are limited in range (<10 kHz). Given the increasing adoption of vocalization measures, a need exists to develop a calibration tool for a variety of frequency and amplitude ranges. Here we describe an apparatus, which we have labeled the Calibration Unit for Recording Transducers (CURT), designed to fulfill the needs described. The CURT was designed to maximize utility in a number of fields, and can be easily customized to suit the specific needs of most labs. The emitted frequency and amplitude are tunable to output frequencies in both human-sonic (20Hz-20kHz) and human-ultrasonic ranges (20 Hz-100 kHz), up to a maximum frequency of approximately 100 kHz. Additionally, it is designed to have the capability of being a highly portable, stand-alone unit that fits a variety of microphone connector types. The CURT is also very low cost to build (under 250.00 USD), thus enabling adoption by many researchers.

#### 2. Circuit design

The CURT is composed of two main portions: the internal driving circuitry (enclosed within a custom made ABS and acrylic enclosure), and the external calibration source (enclosed in a custom mounting bracket tailored specifically to the transducer to be calibrated). The entire apparatus weights 180 g, and the driving electronics fit into a box that is 107 mm  $\times$  70 mm  $\times$  56 mm.

The basic flow-diagram of the circuit is provided in Fig. 2 (circuit diagram provided in Supplementary material). The microprocessor used was a PIC18F4550 (Microchip Technology Inc., Chandler, AZ). This chip was selected to provide the basic functionality required of the calibration unit, while providing the possibility of USB connectivity in the future. The circuit derives its clock from an external crystal oscillator clocked at 20 MHz  $\pm$  600 Hz (30 ppm), allowing for a high resolution, high-frequency pulse-width modulated (PWM) square wave signal to be generated as the primary pre-amplified source for the emitted sound. In this case the duty cycle is fixed at 50% (i.e. the output signal spends 50% of the period at a high amplitude and the other 50% low). The PWM amplitude (volume) is adjustable via a 10 k $\Omega$ , 10-turn, rotary potentiometer with stops (i.e. a dial with fine "clicks"). Frequency is modified in software and stored in program memory based on application, while the amplitude is adjusted by running the PWM output through a passive voltage-divider circuit, allowing for smooth, analog adjustment. For applications requiring adjustable frequency, a 10-turn potentiometer can be added to provide external frequency control (resolution depending on the frequency). The divided PWM signal is then passed through a buffering amplifier (TL074, Texas Instruments, Dallas, TX) prior to stimulating an externally connected piezoelectric element (base resonant frequency at 4.4 kHz). The piezoelectric crystal is mounted into a custom bracket to ensure proper orientation and distance to achieve a physiologically relevant, accurate and repeatable response in the microphone (transducer) of interest. A custom bracket can be made to suit any microphone setup desired. It is important to note that the mounting bracket used to connect the transducer to the CURT was custom made to fit our transducers (the CM16/CMPA40-5 V [Avisoft-Bioacoustics, Berlin, Germany]), and would need to be customized to fit other transducers. The housing holds the transducer face parallel to, and approximately 1 cm from, the surface of the piezoelectric element, which was secured to the base of the acrylic bracket using cyanoacrylate glue. The calibration bracket containing the piezoelectric element was connected electrically to the driving circuit through a standard 1/8 in. Download English Version:

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