Sound calibration for rodent behaviour experiments

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Here we present a relatively cheap and simple setup that will allow you to play pure tones from 1kHz to 21kHz with the same dB SPL level, overcoming the standard-speakers’ innate lack of precise equalization.

Two things should be kept in mind: firstly, this protocol is designed for an experimental setup dedicated to head-fixed rodents; secondly, it does not deal with sounds above 21kHz: indeed common commercially available speakers cannot efficiently handle frequencies above this boundary, neither can affordable calibrated microphones.

Materials

Bpod

https://sanworks.io/shop/viewproduct?productID=1001

EMM-6 Electret Measurement Microphone

<http://www.daytonaudio.com/index.php/emm-6-electret-measurement-microphone.html>

AudioBox iOne

<http://www.presonus.com/products/AudioBox-iOne/features>

TrueRTA Real Time Spectrum Analyser

<https://www.trueaudio.com/rta_down.htm>

LogitechSpeakers Z150

<http://www.logitech.com/en-us/product/multimedia-speakers-z150>

Teensy 3.2 and Audio Adaptor Board

<https://www.pjrc.com/teensy/teensy31.html>

<https://www.pjrc.com/store/teensy3_audio.html>

SD Card 8GB class 10

https://www.sandisk.com/home/memory-cardsCalibrationTeensy function

CalibrationTeensyHB.m

Setup

As almost all calibrated microphones, the EMM-6 needs a phantom power source to work: we adopted the iOne board, which is in turn connected to the computer. This microphone works with 48V, so the 48V button on the front of iOne case should be switched. Set the input gain for the mic just one click below the maximum. Place the tip of the microphone where the head of the animal will be positioned.

On the TrueRTA interface, select iOne as the input source in the Audio I/O options, and click on the SPL button to visualise dB SPL, because as default it shows dBu.

Connect the assembled Teensy device to your Bpod following the instructions on the wiki (<https://sites.google.com/site/bpoddocumentation/bpod-user-guide/function-reference/teensysoundserver>). Plug the speakers to Teensy and place them where they will be situated during your experiment. For a precise and optimal performance of the membranes, turn their knob to the maximum volume.

Save the CalibrationTeensy Matlab function in the Bpod Protocols directory in a homonymous folder.

Procedure

Launch the function from the Bpod interface selecting the CalibrationTeensy protocol. This protocol will create calibrated pure tones from 1kHz to 21kHz and store them as 2 second long wav files in your Teensy SD card. In the beginning you will be asked to choose the frequency resolution for calibration in this range: 1kHz, 0.5kHz or 0.25kHz. The first option will generate one tracks for every 1000Hz from 1kHz to 21kHz (1kHz, 2kHz, 3kHz, 4kHz etc..), the second tracks for every 500Hz from 1kHz to 21kHz (1kHz, 1,5kHz, 2kHz, 2,5kHz etc..), the third tracks for every 250Hz from 1kHz to 21kHz (1kHz, 1,25kHz, 1,5kHz, 1,75kHz etc..). So, according to your decision, you will end up with 21, 41 or 81 audio files coding pure sinewave pitch.

Therefore, the program will create and write on the SD Card a first set of sinewaves with the same arbitrary amplitude (0.3). Once the upload is complete, the True RTA interface and an excel file named Calibration will be opened: use the former to observe the sound volume (dB SPL) generated at each pitch and enter it in the latter, below the corresponding kHz value marked in the first row. The tones will be produced using the Bpod interface: LED2 will light up, and after one second a tone is played: to move to the next one use the poke button 3, to repeat it the poke button 1. The frequencies will be played progressively, i.e. from the lowest to the highest.

After saving and closing the xlsx file, you will have to choose the target dB SPL for your tones. Sine amplitudes will be adjusted accordingly, and a new set of wav files will be created. Next you have to repeat reading the SPL values for each pitch and update the Calibration.xlsx files as instructed. Amplitudes will be adjusted once more, and you will have to check the final result. Generally this is good enough for the frequencies to be played at the same SPL.

Subsequently, you will have the option to continue and create a white noise sound, or to stop the protocol. In the former case a white noise wav file will be loaded and played with the same Bpod interface procedure. You should read the dB SPL values for each 1kHz, 2kHz, 5kHz, 10kHz and 20kHz octave and report them below the newly appeared headers in the Calibration Excel file. Again, save and close the xlsx and wait for the new calibration: the new white noise track will then be played. However, don’t expect the sound to have an equal volume on all the frequencies (as the definition of white noise states): due to software and hardware limitations that’s what we could achieve at the moment. Nonetheless, the dB SPL distribution is acceptable for most purposes.

Additional Notes

It’s important to note that, whenever speakers or mouse locations are changed, speakers should be re-calibrated.

Whenever the 48V button is pressed, the input gain knob should be previously positioned on the minimum level: this will ensure a longer life to the microphone.

Bpod, Teensy 3.2 and Audio Adaptor Board are the only pieces of hardware that are essential to run this program: all other components are substitutable with other analogous alternatives.

If you already possess a software capable of reporting dB SPL levels, you don’t need TrueRTA: simply modify the path in the code.