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Research Report

Acoustic measurement: A tutorial for molecular biologists*

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ABSTRACT

Although skilled in in vitro techniques, the molecular biologist may not understand the finer points of acoustical measurement. Measurement is necessary whenever the auditory system function is being measured using the auditory brainstem response (ABR) or distortion product otoacoustic emissions (DPOAE) or is being challenged by a noise exposure. While the theory of measuring an acoustic signal with a calibrated measuring microphone is simple, in practice, it can become complex. The present article presents guidelines for measuring acoustic stimuli which is within the abilities of a well equipped laboratory. It also presents a set of links for further information and some sources for procurement of equipment.

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Testing the functioning of the auditory system can be accomplished a number of ways. If the subject is human, the easiest technique is to ask the subject if they perceive the stimulus. This is the basis for tests administered by audiologists to determine hearing thresholds, for example. If the subject is non-human, a number of techniques again are available, but constraints of time and cost may limit the choices. For example, behavioral techniques may yield the most sensitive thresholds. But for screening purposes, the auditory-evoked potential (or auditory brainstem response, ABR) thresholds may provide all of the information that is necessary. The ABR is a measurement of synchronous activity in the lower auditory system evoked by a short acoustic stimulus—usually a few millisecond tone burst or click. This electrical activity is recorded from electrodes on the skin surface. In certain situations, tests of cochlear function may be made by testing the ability of the ear to generate the distortion product otoacoustic emission (DPOAE). DPOAEs are acoustical signals generated by the auditory periphery in response to two

different frequency stimuli played into the ear canal. They are measured in the ear canal. The DPOAE represents non-linear processes in the normal auditory periphery. For details on these procedures, the reader is directed to Patham et al. (2001).

One of the most common uses of ABRs or DPOAEs is to show changes in the auditory system due to some treatment. To obtain the maximum information from the sensory system, care must be taken to ensure that all parameters remain as equivalent after a treatment as before. This requires careful placement of electrodes, equipment calibration, and calibration of the acoustic measurement system. If the investigator is not experienced in acoustic measurement, it can be confusing.

The same concerns occur when exposing experimental animals to noise. Care must be taken to ensure that the sound stimulus remains constant from one exposure to another or at least is controllable. Also, the stimulus should not change during the course of an exposure.

The purpose of this article is to provide a tutorial and checklist for acoustic measurement in the case of ABRs,

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DPOAEs, and/or noise exposures. Once the basic principles are understood, it does not matter if you are measuring an Auditory Brainstem Response stimulus or a noise exposure stimulus, both are within the capability of the well-equipped laboratory.

This article is arranged to first discuss the acoustic stimulus, then the use of measuring microphones, monitoring microphones, measuring amplifiers, calibrators, stimulus frequency, calibrating the stimulus, calibrating the calibrator, a procedure, costs, and sound attenuating chambers. Appendix A contains a listing of suppliers that can be used as a starting point for procuring equipment.

Pearce et al. (2001) have produced a valuable article looking at various techniques for measuring the acoustic stimulus for the mouse. They found that the mouse measuring system can be simplified and produce meaningful estimates of acoustic levels in the ear.

1. The acoustic stimulus

The acoustic stimulus is a compression wave traveling through air as rarefactions and condensations. The compression wave has the dimensions of amplitude and frequency (or wavelength in the medium). Clicks, pulses, and noises are complex stimuli and are a summation of waves with differing frequencies and amplitudes. Many stimuli are defined in the time domain as an envelope which modulates some underlying waveform such as a tone or noise. The normal human ear responds to acoustic signals in the dynamic range of about 20 μPa (0 dB SPL, threshold) to 20 Pa (120 dB SPL, threshold of pain).

The young human ear can hear sounds ranging in frequency from 20 Hz to more than 20 kHz. Animal ears are not necessarily more sensitive to sounds but can often hear much higher frequency sounds (mice can hear to 80 kHz). Table 1 shows some animals commonly used in hearing experiments (including humans) and the lowest frequency

Table 1 – Common laboratory animals and the frequency range of their audiograms

Species	Lowest frequency	Highest frequency (kHz)	Greatest sensitivity (kHz)
Mouse (Mus musculus)	1 kHz	80	16
Rat (Rattus norvegicus)	500 Hz	76	8-24
Cat (Felis catus)	45 Hz	60	8
Human (Homo sapiens)	20 Hz	20	4
Guinea pig (Cavia procellus)	50 Hz	50	8–11
Rabbit (Oryctolagus cuniculus)	63 Hz	50	8
Gerbil (Meriones unguiculatus)	100 Hz	60	4
Chinchilla (Chinchilla laniger)	90 Hz	23	1–2
Chicken (Gallus gallus) 4 days old	125 Hz	7	1–2

Data taken from Fay (1988).

they can behaviorally detect, the highest frequency they can detect, and their most sensitive frequency or frequency range (Fav. 1988).

Standards have been developed over the years for measuring and describing acoustic energy. Because of the extreme dynamic range of acoustic signal magnitudes, professionals use the decibel as the measurement unit. The decibel (dB) is a relative unit based on the logarithmic scale. The decibel must be referenced to some physical value. In air the most common reference is 20 μ Pa (or 0.0002 dynes/cm², the same value in different units). The 20 μ Pa standard pressure is often called the reference "Sound Pressure Level" or "SPL." Some of the best sources for information on acoustics are the manufacturers of measurement systems (e.g., Brüel and Kjær, 1994, 1998).

Eq. (1) is the formula for determining dB_{SPL} for acoustic pressure.

$$L = 20 \, \log_{10}(P_1/P_0) \tag{1}$$

 P_0 is the reference acoustic pressure (20 μPa), P_1 is the measured acoustic pressure. L is the sound pressure level (dB SPL, referenced to 20 μPa). Note that a doubling of measured acoustic pressure is a 6-dB increase, a halving of measured acoustic pressure is a -6-dB decrease; a ten-fold increase is an additional 20-dB increase and a ten-fold decrease is a -20-dB decrease.

It is easy to become confused between acoustic *power* measurements and acoustic *pressure* measurements. Acoustic *power* refers to the energy emitted by a source—the analogy is a light bulb: light bulbs of different wattages (power) emit different levels of light. The wattage does not change as you move away from the light bulb. However, as you move away from the light bulb, the light intensity falls off in a predictable way. Think of the illumination falling on a surface as acoustic intensity which is related to the pressure squared for a plane wave. Acoustic *power* doubles in 3-dB steps and halves in -3-dB steps. Acoustic *power* increases ten-fold in 10-dB steps and decreases ten-fold in -10-dB steps. Further clarification can be provided by Vernon et al. (1976) and Kinsler et al. (2000).

2. Microphones

The recommended transducer for laboratory measurements of acoustic pressure is the precision, condenser microphone (Fig. 1). Measuring microphones are manufactured by a number of companies. Quality microphones are designed to be very stable and if treated with care, retain calibration within 1 dB over decades.

Condenser measurement microphones consist of two plates: the thin diaphragm which moves in response to acoustic pressure and a stiff, fixed backing plate (Brüel and Kjær, 1996). These two components are contained in the microphone cartridge. The diaphragm and backing plate must be polarized (typically to 200 V) either by a power

 $^{^1}$ Decibels used in measuring acoustic power=10 log₁₀ (W $_1$ /W $_0$) and are referenced to acoustic watts, 1×10^{-12} W.

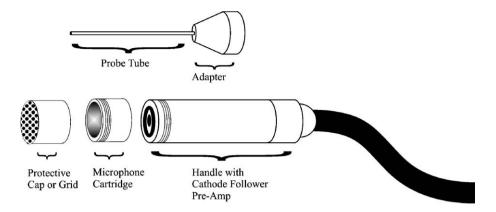


Fig. 1 – A typical measuring microphone consists of a microphone cartridge containing the fragile diaphragm which moves in response to acoustic energy and forms one plate of a capacitor. In addition, the cartridge contains a backing plate which forms the other plate of the capacitor. The diaphragm is protected by the protective cap or grid. The cartridge is screwed onto a handle which houses the preamplifier. The cartridge is polarized (typically to 200 V) either by an external power supply or may be prepolarized by an internal dielectric (electret). The protective grid may be removed and a probe tube substituted on the front of the microphone cartridge. This allows measurements to be made in tight or inaccessible areas. However, the probe tube and microphone must be recalibrated to determine their characteristics.

supply or by being prepolarized (i.e., an electret microphone). Microphone cartridges come in different diameters. Most common are 1 in., 1/2 in., 1/4 in. and 1/8 in. The larger diameter microphones are more sensitive to acoustic pressure but have a lower high-frequency cutoff. Because most human testing is 8 kHz or below, most human audiometric laboratories calibrate with a 1-in. microphone. A 1-in. microphone is an excellent choice for calibration in most laboratories. Animal laboratories require a 1/2-in. or 1/4-in. microphone to reach the upper cutoff frequency of most mammals (Table 1). Some species, e.g. bats, may require use of an 1/8-in. microphone to be able to measure very high frequencies. An 1/8- in microphone has a high-frequency cutoff of about 140 kHz.

Measurement microphones are very high impedance voltage sources. In order to conduct electrical signals any distance without electrical interference an impedance matching circuit must be placed between the microphone and the cable. This circuit usually does not amplify the signal but converts the signal to a lesser impedance which can drive long cables. This amplifier must be located close to the microphone cartridge and is usually built into the handle. A cable attaches the handle to a 200-V power supply. The power supply can also be internal to the piece of measuring equipment.

Quality microphones are an investment in the success of your laboratory. See Wong (1995a) for a discussion of their care.

For measuring in tight areas, microphones can be fitted with an optional probe tube (Figs. 1 and 3D). The probe tube is a metal tube 0.5 to 3 mm in diameter and up to 100 mm or more in length. The installation of a probe tube to the end of a microphone changes the relatively flat frequency response of the microphone to one with a number of resonances. The microphone fitted with the probe tube must be calibrated against a standard microphone to

characterize its frequency response at the frequencies of interest in the laboratory.

3. Monitoring microphones

When noise-exposing animals, it is important to continuously monitor the exposure stimulus. While the use of a Type 1 microphone is encouraged, a monitoring microphone need not be of the same quality. (Mice love to gnaw and for some reason the insulation on microphone cables is especially attractive.) Given the more precarious life in an exposure chamber, a less expensive (including entertainment quality) microphone may be used if first calibrated against a condenser microphone (Fig. 3). The easiest measurement technique uses an acoustic microphone calibrator (see Section 5).

As the frequency of the acoustic signal of interest increases, the physical wavelength decreases. As the acoustic wave reflects off surfaces, its echoes interfere constructively and destructively with the direct stimulus. These interactions produce standing waves. Standing waves are spatial variations of sound pressure. These can be detected as the condenser microphone is moved in and out slightly from a reflecting surface. It is commonly believed in the audiology community that frequencies above 8 kHz cannot be measured reliably and repeatedly due to standing waves. Part of this difficulty is related to the use of 1-in. diameter microphones (which begin to lose sensitivity above 10 kHz). As the wavelength decreases, the effect of the microphone on the sound field increases. Considerations about the size of the microphone are especially important for high frequency stimuli (>10 kHz). Standing waves can be a significant problem when measuring tones. Noise bands are not as affected by standing waves due to averaging over the frequency band. Standing waves can be minimized by making the environment anechoic. In a free field, the microphone may have to be

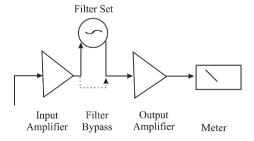
placed and replaced a number of times to sample the acoustic space of the stimulus. A stimulus value can be described as one of the common descriptors of central tendency (mean, median, or mode) and variability (range, standard deviation, or variance).

By sealing the microphone into a speculum sealed to an earphone or speaker, many of the problems of the free field can be eliminated. The microphone can be temporarily sealed into the exit of the speculum in place of the ear canal (using plastic tubing with a volume comparable to the animal's ear canal) and the acoustic stimulus can be calibrated.

Condenser microphones come in two styles based on how the space behind the diaphragm is vented-free field and pressure (Brüel and Kjær, 1994). Generally, free field microphones are used to measure acoustic pressure in front of speakers; pressure microphones are used in enclosed systems. That said, either style may be used in either configuration. In the free field environment, free field microphones should be faced directly at the speaker, pressure microphones should be faced at right angles to the speaker. The two styles look exactly alike, and the only way to tell them apart is to examine their data sheets.

4. Measuring amplifiers

The output of the microphone is an AC voltage proportional to the sound pressure. The least complex measuring instrument is a standard RMS voltmeter with a decibel scale or even an oscilloscope. Although a calibration curve can be determined using Eq. (1) to estimate acoustic pressure at the microphone, a better choice is to use a measuring amplifier which is designed to interface to a microphone. The signal from the microphone is amplified, filtered if desired, amplified again, and displayed on a meter (Fig. 2). Most measuring amplifiers directly display decibels on a linear meter. A number of companies make measuring amplifiers (see Appendix A) with a wide range of computer interfaces, special filters, and integration times. Most of these extras are unnecessary for



Block Diagram of Typical Measuring Amplifier

Fig. 2 – The typical measuring amplifier has two stages of amplification with attenuators associated with each stage. The microphone output is presented to the input amplifier. Once amplified, the output of the input amplifier can be presented to the output amplifier directly or directed to a filter set (or the typical A or C weighting network). Finally, the output of the output amplifier is presented to the meter which displays the value as decibel.

measurement and significantly add to the cost. Almost every piece of equipment designed for acoustic measurements has the option of frequency weighting by inserting special filters. Generally, frequency weighting (e.g., A-weighting) in animal research is not a good idea since the filters are designed to model the human auditory system. Acoustic signals in the laboratory should be measured in the "linear" mode.

The use of a computer-based spectrum analyzer can simplify the setup and measurement of the stimulus. It can be as simple as placing an acoustic calibrator on the microphone, connecting the microphone to the analyzer, entering the value of the calibrator on the analyzer screen and pushing the "calibrate" button.

Analog-to-digital computer interface boards can be connected to microphones to serve as the input to software-based measurement applications such as LabView. Recall that the use of the decibel requires common logarithms so the dynamic range of the analog-to-digital converter and onboard amplifiers needs to be able to accommodate those signal levels.

5. Calibrators

How does one reference the output voltage of the measuring microphone back to 20 μPa (the zero dB point)? All measurement microphones arrive with a factory calibration trace consisting of the microphone output for a constant acoustic input over the microphones specified frequency range. This graph is useful for determining the sensitivity of the microphone cartridge. (The sensitivity of the microphone cartridge is the output voltage per Pascal.) The tracing also allows one to correct results from the microphone at certain frequencies where the output is no longer in the flat portion of the frequency band—typically the highest frequencies for which the microphone is rated. However, the calibration curve is usually not very useful in the day-to-day functioning of a laboratory. Better is the use of an acoustic microphone calibrator. The acoustic calibrator is placed over the end of the microphone cartridge and it generates a tone. This tone is precisely generated by a moving piston or a speaker using a feedback circuit. These tones are generated at a high enough acoustic level to mask any environmental sounds-either 94 dB or 124 dB SPL at 1 kHz or 250 Hz. (If you do the math in Eq. (1), 1 Pa is equal to 94 dB SPL.) Utilizing a barometer to adjust for local atmospheric pressure the calibration tone can be corrected to one tenth or one hundredth of a dB (Wong, 1995b). For physiological and behavioral experiments measurement to one decibel is adequate. Periodically, measurement microphones, analyzers and calibrators should be checked against standard microphones and sources at an accredited testing laboratory (Brüel and Kjær, W. Caldwell, Modal Shop, Scantek, etc.).

The factory calibration curve gives a precise sensitivity of the microphone, for example, 50 mV/Pa. Knowing the sensitivity of the microphone, using Eq. (1) and an RMS voltmeter, one can calculate the predicted output voltage for any sound level. For example, while the acoustic calibrator (94 dB SPL at 1 kHz) is driving the microphone, the example microphone should be producing 50 mV of output. At 74 dB SPL (0.1 Pa), the microphone should be producing 5.0 mV of

output. At 114 dB SPL (10 Pa), the example microphone should be producing 500 mV.

An advantage of the use of the acoustic microphone calibrator is that any signal loss due to connectors and cables can be accounted for in the final measurement. Also, by acoustically calibrating at the beginning of the experiment and the end of the experiment, any changes in the system such as cables or batteries, etc. can be discovered and fixed.

The acoustic calibrator operates in a very low frequency part of the microphone frequency response band. The tone generated is in the flat region of most microphone pass bands. The reader will be making measurements at higher frequencies. The factory calibration curve provides information about the frequency at which the microphone begins to deviate from the flat.

Lower quality microphones may also be calibrated using an acoustic calibrator or may be calibrated, in situ, with a measurement microphone. They then can be connected to a measuring amplifier, voltmeter or spectrum analyzer to get "ball park" measures of noise during exposures. This protects your high-quality measuring microphones from the teeth of awake mice.

6. Measuring frequency

Because headphones and speakers can interact with their environment to emphasize certain frequencies and attenuate others ("color" their acoustical outputs) all complex signals should be measured acoustically. Frequency of sinusoidal stimuli can be measured using a microphone attached to an oscilloscope or a frequency counter (impulsive stimuli like ABR tone bursts are more easily calibrated with a continuous output, although triggered sampling using an oscilloscope permits amplitude measurements.)

Broadband noises or filtered bands of noise can be measured by a microphone in conjunction with a filter set and measuring amplifier or a spectrum analyzer. A spectrum analyzer measures the pressure amplitudes of the various frequency components of the stimulus. Most spectrum analyzers are specialized computers and use Fourier or narrowband analysis to decompose the time signal into the frequency spectrum. When using Fourier analysis, the window type applied to the time sample has an effect on the resulting spectrum (Oppenheim and Schafer, 1989).

Frequency can be measured by adding a filter set to a measuring amplifier. A filter set consists of a selectable set of filters in the auditory range that can be inserted between the 1st and 2nd amplifier stages (Fig. 2). By selecting different filters, noise bands can be measured and documented. Sequential measurement is more time consuming than a one-shot Fourier spectrum.

7. Measuring the stimulus

The first question the researcher must ask is where will the animal be located? In an exposure facility (Fig. 3A), the microphone must be moved around to sample the volume in which the animal can move. In the case of a speculum placed

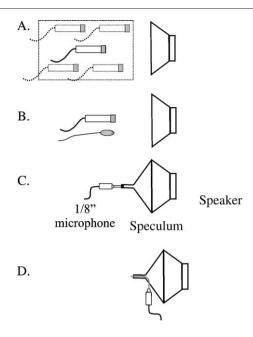


Fig. 3 - Typical setups for measurement purposes. (A) The dashed box represents the volume in which the animal may be present. This diagram indicates that the microphone must be moved systematically within the space the animal can inhabit in order to get a good idea of the levels to which they are being exposed. If too much variability is present, absorptive or reflective material can be added to equalize the area. (B) Other microphones may be calibrated against measurement microphones in the free field. Care should be taken to be sure that the diaphragms of the two microphones are in the same plane. This is how probe tube microphones can be calibrated, for example; or how monitoring microphones can be calibrated against a measuring microphone. (C) If the ABR (or DPOAE) is being generated by a headphone or speaker/speculum combination, an 1/8-in. or 1/4-in. microphone may be able to fit into the lumen of the speculum. A short piece of Tygon tubing may also be used to connect the output of the speculum to the microphone. All attempts should be made to place the microphone in the same relative position as the animal's ear or tympanic membrane. (D) A probe tube may be temporarily or permanently inserted into the lumen of the speculum to make measurements at the opening of the ear. While seemingly the most straightforward, the probe tube introduces additional problems. The microphone/probe tube combination must be calibrated at the frequencies of interest.

in an anesthetized animal's ear, the measurement will be at the end of the speculum (Fig. 3C). Placing the microphone just lateral to the opening of the speculum is the best place to calibrate the stimulus. Pearce et al. (2001) showed that reasonable stimulus measurements could be made by inserting an 1/8-in. microphone into a short piece of plastic tubing sealed to the end of the sound speculum.

If the animal is to be located in the open field for noise exposure, for example, the microphone should be located within the space the animal may move. Thus, if the animal is constrained to a smaller area, only a few points need be measured. If the area is more open, points at every centimeter

or so should be measured. Differences of 1 or 2 dB are not significant. If the animal will be anesthetized, the microphone may be placed where the animal's ear will be located.

For precise measurements in a speculum, a probe tube may be placed on the front of a microphone and the probe tube inserted down the bore of the speculum (Fig. 3D). Thus, the system may be calibrated while the anesthetized animal is in place. The probe tube adds to the complexity of the measurement system. Use of a probe tube at frequencies higher than about 20 kHz becomes problematic (Vernon et al., 1976).

One advantage of sealing the speculum into the subject's ear is a much more robust low frequency response from the transducer. Sealing involves using bone wax or paraffin to make a tight seal. Mice and rats may not benefit from sealing due to the higher low-frequency cutoff of their hearing.

8. Calibrating the measurement system

Although microphones do not change their calibrations, the same cannot be said for electronics. Generally, anything electronic should be placed on a calibration schedule with a National Institute for Standards and Technology (NIST) certified calibration laboratory. Some universities may have calibration capabilities in their Engineering School. Calibration can be done annually or semi-annually. Equipment should be sent out for calibration if measurement numbers begin to look significantly different or if equipment is dropped or stressed in any way. Certified laboratories will record and provide "as received" values which can be compared with the final calibrations. Quality equipment should not change more than a few tenths of a dB in the calibration process.

9. What now?

You've obtained a high-quality microphone, preamplifier, power supply, and a measuring amplifier. How do you actually do a measurement? Each laboratory should develop standard operating procedures for acoustic measurement. However, this procedure is a general outline based on a Brüel and Kjær (B and K) measuring amplifier which can be used as a starting point and modified for use.

Somewhere in your acoustic generation system is an attenuator. The attenuator is a precision resistor network that is labeled in dB steps. The attenuator is vital to the system because it allows you to add or subtract signal voltage to precisely adjust the resulting acoustical signal. If you are calibrating an ABR system, it is part of the ABR stimulus system. If you are calibrating a noise system, it may be part of the power amplifier or a separate attenuator between the noise generator and the power amplifier.

(1) Most analytical instruments have an internally generated calibration voltage. The B and K measuring amplifier has a 50- μ V calibration AC voltage which you can use to make sure the amplifiers are working correctly. Make sure the "GAIN" potentiometer is set to "CALIBRATED." Use a screwdriver in the "CALIBRATION" potentiometer to make the needle display 50 μ V on the meter. (Then remember to turn off the calibration voltage.)

- (2) Place the acoustic calibrator over the end of the microphone and turn it on. The calibrator has an approximate calibration associated with it that is listed on the side of the calibrator or in the paperwork (usually 94, 104 or 124 dB SPL). The precise calibration output level will be listed in the calibration report supplied with the calibrator.
- (3) Adjust the "GAIN" potentiometer until the needle now sits on the dB value for the calibrator (e.g., 94 dB). Your system is now calibrated with respect to the acoustic calibrator. Record the starting values of the attenuator knobs of the input and output amplifier stages of the measuring amplifier. In the case of the spectrum analyzer, pushing the "CALIB" button will cause the analyzer's internal computer to go through the same adjustments. Remove the microphone calibrator from the front of the microphone.
- (4) In theory, the output voltage of the microphone is now calibrated and can be read by adjusting the measuring amplifier input and output amplifier attenuator knobs and noting the meter reading. Each attenuator click is a 10-dB change in sensitivity. The two attenuator knobs add to one another so a 10-dB change on the input attenuator is equivalent to a 10-dB change on the output attenuator.
- (5) If possible, turn on the signal of interest and add or subtract attenuation in the stimulus generating system until it matches the value measured by the acoustic calibrator (e.g., 94 dB SPL). Note the value of the stimulus generation system attenuator. If you are not able to generate tones of that level, subtract attenuation in the measuring system until the meter needle becomes active and produces a valid reading. Some ABR software will allow you to modify the software so you can generate display values which match the acoustic values. If you are not able to change the display values, you must develop a set of constant values which must be added or subtracted from the display values to provide true measured values.
- (6) Note the attenuator setting of the stimulus generation system, the measuring amplifier attenuator settings and the meter reading you can measure any stimulus level in dB SPL. If the meter pegs (the pointer hits the right-hand stop), you must add attenuation to the measuring amplifier. If the meter goes under range (the pointer hits the left-hand stop), you must remove attenuation. Each change of attenuation must be accounted for in the total level of the signal.
- (7) By inserting a filter set into the path between the input amplifier and the output amplifier, you can measure sound levels in any frequency band of interest. A spectrum analyzer can automatically display the spectrum of a signal by use of Fast Fourier Transform (FFT).

Although this sounds difficult, once you have done it once or twice, it makes good sense.

All information associated with measurement should be included in a permanent logbook which can be referred to each time a system calibration is conducted. A simple block diagram of the setup helps in recalling and recreating the system next time. Calibrations should be conducted regularly. Daily calibrations or calibrations at the beginning and end of procedures are preferred, if possible. Any change in the stimulus generation system of more than a few decibels requires troubleshooting. The more automatic measurements become, the easier they are.

10. A word about costs

A good measurement system can cost a lot of money, especially for a new investigator. This a major outlay for equipment which may be used infrequently. A commitment should be made from day one to accurately measure acoustic stimuli. Good systems can be purchased on the used equipment market. These systems do not become technically obsolete and may be repaired and recalibrated indefinitely. If not needed on a daily basis, the measurement system may be rented from a vendor. Rental companies recalibrate measurement systems before delivery. Or if there is close physical proximity to a laboratory doing similar measurements, it may make sense to share one or more expensive instruments (e.g., spectrum analyzer.)

Finally, if possible, a backup microphone cartridge should be purchased and stored carefully away. This cartridge should only be used infrequently. Having a known good cartridge allows for troubleshooting measurement systems. Also, access to a good quality dissection or operating microscope will allow the investigator to examine microphone diaphragms for cracks, tears or dirt.

11. Sound attenuating chambers

Although having nothing to do with acoustic measurement, keeping sound out when doing auditory brainstem responses and sound in when doing noise exposures is a concern. Since the auditory system is being stimulated by a tone pip or click, every effort should be taken to isolate the animal from the external acoustic environment. Ideal is a double-walled sound-attenuating room for testing. However, more important is the isolation of the animal from noises and vibrations transmitted through the building to the animal. Such vibrations can move electrodes, generating false electrical noises. A sponge pad or even a tissue absorbent pad can decouple the anesthetized animal from the building. A quiet corner of the laboratory may be quiet enough to measure ABRs. One should pay particular attention to the electrical noise which can affect ABR measurements. On the other hand, DPOAEs require very quiet testing environments.

For noise exposures, some sort of sound attenuating room should be used to prevent the acoustic exposure of laboratory personnel. A sound attenuating room can consist of a closet in which the cracks around doors can be sealed. When working around an active noise environment of more than 85 dB SPL, personnel should be required to wear effective hearing protection (earmuffs or earplugs.) Davis and Franks (1989) describe a noise exposure box which may be used to sit inside of a room to reduce noise levels.

Appendix A. Suppliers

The author does not endorse any of the following suppliers. A search of the internet will turn up other suppliers. This is meant as a starting point for information about building your

measurement system. Supplier capabilities may change with time. Suppliers are in alphabetical order.

ACO Pacific specializes in measurement microphones. They provide microphone calibrators as well as everything needed up to the analysis instrumentation. http://www.acopacific.com.

Agilent Technologies, a division of Hewlett-Packard, is a U.S. company which manufactures test equipment suitable for acoustical measurement. http://www.agilent.com.

Brüel and Kjær is a Danish company known for their acoustic and vibration equipment. B and K can provide an entire start-up system from microphones to measuring amplifiers to spectrum analyzers. http://www.bkhome.com.

Etymotic Research specializes in DPOAE microphones and probe tube microphones as well as DPOAE systems. http://www.etymotic.com.

G.R.A.S. Acoustics and Vibration is a Danish company which manufactures microphones, microphone calibrators and signal conditioners. http://www.gras.us.

National Instruments is a software and hardware company which makes the popular LabVIEW application. Computerized measurement systems have been built upon this platform. http://www.ni.com.

The Modal Shop is a distributor of acoustic and vibration equipment. They sell, rent, lease and calibrate equipment. They also sell used equipment. http://www.modalshop.com.

Ono Sokki is a Japanese manufacturer of microphones, sound level meters, microphone calibrators and analyzers. http://www.onosokki.net.

Stanford Research Systems is an U.S. company which manufactures FFT analyzers for the acoustic measurement industry. http://www.thinksrs.com/index.htm.

Tektronix is a manufacturer of test equipment including oscilloscopes and spectrum analyzers. http://www.tek.com.

Tucker-Davis Technologies produces computer-based stimulus generation systems and ABR systems. They also sell high frequency transducers. http://www.tdt.com.

Tucker Electronics specializes in new and used analytical electronic equipment. If you contact them, they may have measuring amplifiers, power amplifiers, microphone calibrators, etc. available or may be able to find such equipment for you. http://www.tucker.com.

Scantek is a distributor of acoustical instruments. The company also provides acoustical calibration services. http://www.scantekinc.com.

West Caldwell Calibration Laboratories provides acoustical calibration services which are traceable to the U.S. National Institute for Standards and Technology (NIST). http://www.wccl.com/services.htm.

REFERENCES

Brüel, Kjær, 1994. Measurement microphones. Brüel and Kjær, Lecture Note: Measurement Microphones. Available at their website as item BA7216-15.

Brüel, Kjær, 1996. Microphone handbook. Volume 1. Theory. Available at their website as item BE1447-11.

Brüel, Kjær, 1998. Lecture Note: Basic Concepts of Sound. Available at their website. BA-7666-11.

- Davis, R.R., Franks, J.R., 1989. Design and construction of a noise exposure chamber for small animals. J. Acoust. Soc. Am. 85, 963–966.
- Fay, R.R., 1988. Hearing in Vertebrates: A Psychophysics Databook. Hill-Fay Associates, Winnetka, IL.
- Kinsler, L.E., Frey, A.R., Coppens, A.B., Sanders, J.V., 2000. Fundamentals of Acoustics. John Wiley and Sons, Inc., New York
- Oppenheim, A., Schafer, R., 1989. Discrete-Time Signal Processing. Prentice-Hall, New York.
- Patham, K., Sun, X.-M., Kim, D.O., 2001. Noninvasive assessment of auditory function in mice: auditory brainstem response and distortion product otoacoustic emissions. In: Willott, J.F. (Ed.), Handbook of Mouse Auditory Research: From Behavior to Molecular Biology. CRC Press, Boca Raton, FL, pp. 37–58.
- Pearce, M., Richter, C.-P., Cheatham, M.A., 2001. A reconsideration

- of sound calibration in the mouse. J. Neurosci. Methods 106, 57–67
- Vernon, J.A., Katz, B., Meikle, M.B., 1976. Sound measurement and calibration of instruments. In: Smith, C.A., Vernon, J.A. (Eds.), Handbook of Auditory and Vestibular Research Methods. Charles C. Thomas, Springfield, IL, pp. 306–358.
- Wong, G.S.K., 1995a. Handling, cleaning, and storage of condenser microphones. In: Wong, G.S.K., Embleton, T.F.W. (Eds.), AIP Handbook of Condenser Microphones. Theory, Calibration, and Measurements. American Institute of Physics, Woodbury, NY, pp. 287–291.
- Wong, G.S.K., 1995b. Comparison methods of microphone calibration. In: Wong, G.S.K., Embleton, T.F.W. (Eds.), AIP Handbook of Condenser Microphones. Theory, Calibration, and Measurements. American Institute of Physics, Woodbury, NY, pp. 215–223.