GUIDE TO INTERPRETING SPECIFICATIONS

General

Following are terms commonly used in electrophysiological measurements.

Thermal Noise

All resistors generate thermal noise. Thermal noise can be represented by a mean square voltage generator (e_n^2) in series with a noiseless resistor.

$$e_n^2 = 4kTRB$$

where k = Boltzmann's constant (1.138 X 10^{-23} VC/°K)
T = temperature in °K
R = resistor value in ohms
B = bandwidth in Hertz

The noise specified by this equation has a constant power spectral density within the bandwidth and is zero outside the bandwidth. When noise is measured through a low-pass filter having -3 dB bandwidth f_{-3}, the noise will be greater than the amount predicted because real filters continue to pass signals at frequencies > f_{-3}.

The noise of a 10 MΩ resistor measured through various filters is shown in Table I-1.

Bandwidth	1-Pole RC	2-Pole Butterworth	4-Pole Butterworth	Infinitely Sharp
DC-100 Hz	5.07	4.27	4.11	4.06
DC-1 kHz	16.0	13.5	13.0	12.8
DC-10 kHz	50.7	42.8	41.1	40.6
DC-100 kHz	160	135	130	128

Table I-1. Noise of a 10 MΩ Resistor in Microvolts rms

rms Versus Peak-to-Peak Noise

It is equally valid to specify noise as rms (root mean square) or peak-to-peak. The ratio of the zero-to-peak value to the rms value is called the crest factor. The value of the crest factor depends on the type of noise. For white noise the crest factor is about 4. Thus the peak-to-peak noise is about 8 times the rms noise.

Bandwidth — Time Constant — Rise Time

For an exponential response, there are simple relationships between the -3 dB bandwidth (f_{-3}, Hertz), the time constant (τ, seconds) and the 10-90% rise time ($t_{10\text{-}90}$; seconds)

$$f_{-3} = \frac{1}{2\pi\tau} \approx \frac{1.1}{\pi t_{10-90}}$$

$$t_{10\text{-}90} \approx 2.2\tau$$

Filters

Low-pass filters are commonly used in electrophysiology to reduce noise. The important parameters of a low-pass filter are the -3 dB frequency, the type and the order.

The **-3 dB frequency** (f_{-3}) is the frequency where the voltage response falls to $\sqrt{2}$ (0.707). Some manufacturers specify the "cutoff" or "bandwidth" based on the phase response or an asymptotic approximation to the high and low frequency amplitude response. Therefore when using a new filter you should check that the specified settings refer to the -3 dB frequency.

The three **types** of low-pass filters commonly used in electrophysiology are the Bessel, Butterworth and multiple coincident pole (RC) types. The most important differences are:

(1) When driven by a step voltage, the RC filter has no overshoot, the Bessel filter has < 1% overshoot and the Butterworth filter has around 10% overshoot.

(2) When driven by a noisy source, RC filters show the least rejection of the noise at frequencies above f_{-3}, Bessel filters show moderate rejection and Butterworth filters show the most.

The **order** of a filter refers to the number of poles. Each resistor-capacitor section contributes one pole (inductors are rarely used for bandwidths < 100 kHz). Thus 4th-order, 4-pole and 4 RC sections all refer to the same thing.

The attenuation of high-frequency noise increases with order. At frequencies well above f_{-3}, the attenuation increases at 20 dB/decade (6 dB/octave) for each pole. Thus a 4-pole filter rolls off at 80 dB/decade. Note: 20 dB attenuation corresponds to a drop in voltage amplitude to one tenth.

Microelectrode Amplifiers

Noise magnitude is very sensitive to the measurement technique. Specifications should state:

1) -3 dB bandwidth and order of the filter used in the measurement circuit. Typically the noise will look 20-30% better if a fourth-order low-pass filter is used instead of a first-order low-pass filter.

2) Microelectrode bandwidth. The capacitance neutralization should be adjusted so that the -3 dB bandwidth of the microelectrode is the same as the -3 dB bandwidth of the measurement circuit.

Voltage-Clamp Noise

Almost impossible to specify. Depends on cell mode, electrode model, capacitance neutralization setting, electrode interactions, electrode resistances, current measurement techniques, bandwidth of measurement circuit and clamp gain.

10-90% Rise Time ($t_{10\text{-}90}$)

The time for the response to go from 10% to 90% of the final value.

If a voltage step is applied to a microelectrode headstage via a resistor, the resistor should have a very low stray capacitance. Otherwise the stray capacitance couples the step directly into the headstage input and artificially fast rise times are measured. A better estimate of the rise time can be made by passing a current step out of the headstage into a load resistor.

For consistent comparisons, rise times should be measured with the capacitance neutralization adjusted for zero overshoot.

1% Settling Time (t_1)

When microelectrodes are to be used in a discontinuous (switching) single-electrode voltage clamp, the more relevant specification is t_1, the time taken for the response to go from 0% to 99% of the final value.

Input Capacitance

In a circuit using capacitance neutralization and other compensation techniques, it is meaningless to specify a value for the input capacitance. The efficacy of the neutralization circuit depends on the magnitude of the electrode resistance and the measurement technique.

For an electrode with resistance R and an exponential response to a step input, the *effective* input capacitance can be estimated from

$$C_{in} = \frac{0.45 t_{10-90}}{R} = \frac{0.22 t_1}{R}$$

For example, if $t_{10\text{-}90}$ = 10 µs and R = 10 MΩ, then C_{in} = 0.45 pF.

Input Leakage Current

Both well designed and badly designed headstages are normally adjustable to zero leakage current. This ability in itself is not an important test of quality. The correct measure of quality is the sensitivity of the input leakage current to temperature changes which occur after the adjustment is made. Changes of ±10°C can be expected in a typical laboratory environment.

Patch-Clamp Amplifiers

Bandwidth

The bandwidth of a patch-clamp headstage is usually measured by injecting current into the headstage input through a small capacitor. No holder or electrode is attached to the input.

The bandwidth during an experiment is usually much lower. It is typically limited by the electrode resistance and membrane capacitance, as well as by stray capacitances. For example, if a 5 MΩ electrode is used to clamp a whole cell having 20 pF of membrane capacitance, the time constant for measuring membrane currents is 100 µs (5 MΩ x 20 pF). This corresponds to a -3 dB bandwidth of 1.6 kHz, far below the 100 kHz that may be specified. 80% series-resistance compensation would improve this to 8 kHz. Nevertheless, it is still better to have a headstage with very wide bandwidth because in some cases series resistance compensation may work better.

Noise

The ideal RMS current noise in selected bandwidths is shown in Table 2 for various feedback resistors. These figures assume ideal resistors (which only have thermal noise) and noiseless electronics.

BANDWIDTH	**500 MΩ**	**1 GΩ**	**5 GΩ**	**10 GΩ**	**50 GΩ**
DC-1 kHz	0.182	0.128	0.057	0.041	0.018
DC-3 kHz	0.331	0.234	0.099	0.074	0.033
DC-10 kHz	0.574	0.406	0.181	0.128	0.057

Table I-2. rms Current in Picoamps

No patch-clamp amplifier can meet these ideal specifications. The difference between a value in the table and the actual value is the excess noise. The excess noise is always proportionately worse in the wider bandwidths.

Warning: The specified noise in a 10 kHz bandwidth will be misleadingly low if the headstage does not have a 10 kHz bandwidth. If the manufacturer only specifies the 10-90% rise time or the time constant, use the conversion factors given in the *General* section to verify that the bandwidth is sufficient to make the noise specification meaningful.

TELECOMMUNICATIONS AND THE PERSONAL COMPUTER

Over the last 10 years the personal computer has become an important tool in the laboratory for controlling experiments, acquiring and analyzing data, and for producing reports and graphs. Another increasingly popular use for personal computers is communications. Through computer bulletin boards and computer networks, a scientist may conduct research through computerized databases and archives; communicate with members of "user's groups," which are user-support groups set up for users of specific software or hardware products or for people with special interests; and find non-commercial and "shareware" programs that may be of interest.

To prepare a personal computer for communications it must be equipped with a *modem* and communication software. A modem is a device that allows computers to communicate using ordinary telephone lines. A communication program manages the use of the modem and helps automate repetitive tasks. The end of this appendix will provide information for choosing a modem and communication software. First, here are descriptions of two types of popular communication systems that are commonly open to the public.

Bulletin Board Systems

A Bulletin Board System (also referred to as "BBS") is typically one or several computers set up by an individual who manages the BBS as a hobby. Most BBS systems focus on particular topics and provide the means for people to communicate with each other about the featured topics and to exchange files. Many bulletin boards are free to use, but some may charge a fee. Listings of BBS phone numbers may be found in computer publications and even on some BBS systems.

An increasing number of bulletin boards are operated by companies who wish to provide up-to-date product and technical information. A software company might provide advice, bug-fixes or utilities to support or complement their main products. A computer hardware company might provide the latest software "drivers" to support its hardware products.

Axon Instruments provides a bulletin board to help support users of our software. The Axon Instruments BBS provides some general information for customers and a forum for software users to share tips and techniques by exchanging messages and programs. To connect with the Axon Instruments BBS, set your communication parameters to 8 data bits, 1 stop bit, and no parity. If this is unfamiliar to you, read the last section of this appendix. The phone number of the Axon Instruments BBS is (415) 571-6680.

Networks

A computer network consists of hardware and software which allow users to transfer information between two or more computer systems. Researchers often use networks to permit data or program file access across several computers of possibly different types that may be distributed over a large area such as different rooms of a laboratory.

Laboratory computer networks offer several benefits. For example, a higher performance IBM-PC or Macintosh computer can be used to retrieve and then analyze data files from the lower performance acquisition computers. Data file backup can be made to the host, either to magnetic tape or hard disk if the host is a larger computer, or to optical disk or cartridge tape in the case of a smaller host. Commercial network software can also allow the sharing of a resource among several users, such as a high-performance printer.

There are several ways to implement a network. In many research facilities, a central computer located in the vicinity (but may be up to miles away) acts as a "host," and each laboratory computer is connected to the host by a data communications line. The host computer is typically a mainframe or large minicomputer managed and paid for by an institutional facility, but could be a higher performance IBM-PC or Macintosh computer. Each user employs communication software to gain access ("log in") to the host system and to transfer files back and forth. The communications line may only be capable of low data transfer rates, *e.g.*, using a serial line connected to the serial port in an IBM-PC or Macintosh computer, with rates of 1,000 to 10,000 characters per second. This approach is minimally invasive in that no extra hardware is needed besides possibly a modem. An alternative with greater performance is the high-speed line, supported by conventions such as Ethernet, capable of transferring a million characters per second. This approach requires a slightly bigger investment in hardware and involves placing the Ethernet interface card on the computer bus, where it could conflict with other hardware and software. In general, however, this approach provides excellent service.

In some laboratories, the network consists merely of a number of personal computers connected to a local personal computer that acts as a host. In this case the software consists of a shareware or inexpensive program that is run when file transfer is desired. A simple switch box is used to select the computer that the host will communicate with, and the user starts the program in each computer. In more sophisticated installations, the network software is always resident in all computers, so that a user of any computer on the network can transfer files at any time without having to walk over to the other computer to start the file transfer program. Note however that network-originating file transfer might not be desirable during data acquisition, and the experimenter might wish to disable the locally running network software during this time. This type of network software is usually obtained from commercial sources such as Novell, Inc.*, and may require the attention of an expert to install or maintain the network. Novell, Inc., Artisoft, Inc.* (LANtastic network), Invisible Software, Inc.* and other network developers supply "peer-to-peer" network hardware and software. These networks are in many ways ideal for laboratories because they are relatively inexpensive, allow easy networking between a small number of

* See address at the end of this appendix.

computers, and require no host so that no computer needs to be dedicated for this purpose. Many of the network programs can be loaded into High DOS Memory (above the 640 kb limit) so they do not interfere much with normal operation, although this requires the use of memory manager software and usually seems to require more effort during installation.

The most trouble-free method for implementing a simple network is to use a larger local computer as a host for backup and retrieval purposes. Contact your local computer center for advice.

Another potential benefit to using a network connected to a larger machine host is the availability of electronic mail (e-mail) services. These services allow users to interchange text messages of up to a moderate size with other users around the world connected to a variety of other networks. Part of e-mail's usefulness lies in effectively hiding complexities, such as message transfer protocols and different ways of representing characters in different computers. Furthermore, the messages are always "stored and forwarded," meaning that they are held in a local moderate-to-large computer until the user runs a local program to retrieve them, so that the user's computer need not remain on and receptive all the time.

A number of networks have no other purpose than the transmittal of electronic mail. Many of these networks were created to transfer data at high speed between groups of computers, and eventually "gateways" were established to other networks to convert text messages between the respective required standard formats. BitNet is one of the largest networks for use by members of academic institutions. Member networks include BitNet in the U.S.A. and Mexico, CA*NET in Canada, and EARN in Europe, some countries in Africa and the Middle East. Internet is another large network in the U.S.A., and it maintains a gateway to BitNet. Some commercial dial-up services such as CompuServe, MCIMail and AppleLink provide e-mail gateways to Internet. Academic BitNet users can therefore easily communicate with users of corporate or private networks.

A member of an academic institution can usually gain access to one or more of these networks through the local computer center free of charge or for a very modest fee. A corporate user should contact a commercial dial-up service; the cost is usually quite modest. CompuServe, for example, provides easy-to-use software for use with IBM-PC or Macintosh computers to create, send, retrieve and file e-mail.

E-mail addresses can be cryptic. The most common address form, used by Internet, is **user@host.domain**. The domain describes the institution. Outside the U.S.A., the domain includes the country name and possibly the city. The host describes the department or host computer in the domain, and the user gives the user's name or account number. For descriptions of addressing and networks around the world consult the book **!%@:: A Directory of Electronic Mail Addressing and Networks** by D. Frey and R. Adams (O'Reilly and Assocs., Sebastopol, CA, 1990).

Mail traveling on Internet must be in plain text of no more than 50,000 characters. Larger files can be chopped up and sent separately, and shareware programs that convert binary (non-text) files to text can be found. Mail can take up to a couple of days to deliver, though transit times of as short as an hour or less are common.

Shareware, Viruses and Other Terms

There are some concepts that have evolved with the growth of bulletin board communications. *Shareware* is commercial software with a non-traditional distribution system. Instead of the product being distributed in stores, shareware is distributed mainly on bulletin boards. An interested person can download a shareware program from a BBS and try it free of charge for an evaluation period, and then either stop using the program or send the author a payment to become a registered (licensed) user. Shareware programs often rival the power of store-distributed software, but because of the low-cost distribution they are usually less expensive.

Shareware should be distinguished from *freeware* and *public domain* software. Freeware is software that is copyrighted like any other commercial software, but is explicitly free of charge. Anyone may use freeware, but no one may sell freeware or incorporate a freeware program into a bigger collection of software for sale. Public domain software is released by the author for completely free, unrestricted use. Anyone may use public domain software or sell it.

A growing problem in communications is the proliferation of computer "viruses." A virus is generally a program that places itself onto floppy disks and hard disks in a manner undetectable by the user. Every time the computer is turned on, the virus code is executed and sits dormant until an event occurs, such as the computer's internal clock getting to a particular date. Then the virus activates and usually does something destructive like destroying data on the hard disk. Viruses spread by placing themselves onto floppy disks placed in the floppy drive, or by attaching themselves to executable files (programs). When the infected program is run on an uninfected computer, the virus places itself on the computer's hard disk. Since a computer bulletin board contains many files that are exchanged by many people, this is a good way for viruses to spread. Downloading or copying an infected program onto your disk does not infect your computer per se. The only way to infect a computer is to boot up the computer with a floppy disk that has an infected boot-up program, or to run (execute) a program that has been infected. For anyone who downloads programs from bulletin boards or gets files on floppy disks from other sources, there now exists "anti-virus" software that checks hard and floppy disks for the presence of known viruses and deactivates them.

Modems and Communication Software

The minimum requirements for computer communications are a modem, communication software and a phone line. The modem and regular telephone can share the same phone line, although not at the same time. The choice of features in a modem is very important. The choice of software usually depends just on its price and ease of use.

Modems are rated by their speed in bits per second (bps). The most common, economically priced modems operate at 2400 bps. Increasingly common are 9600 bps modems, which are becoming more affordable. Other than speed, there are two additional features that increase the performance of the modem. An **error-correcting** modem automatically checks all data that is transferred from one modem to another, so transmissions are virtually error-free. A modem with **compression** encodes the data so fewer bytes are transferred, thus saving time. There are two popular types of error correction. MNP 4 is the original popular error-correction protocol. Many 2400 bps modems have this protocol. Many 9600 bps modems have V.42 error-correction. V.42 encompasses both MNP 4 and another error-correcting protocol called LAPM. Modems with MNP 4 error correction usually have MNP 5 data compression, which can compress data in a ratio of up to 2 to 1. V.42 modems usually have V.42*bis* data compression. This protocol will compress data as much as 4 to 1. A modem with V.42/V.42*bis* is versatile because it can fall back to MNP 4/MNP 5 if the other modem only supports those protocols. An ideal modem is a 9600 bps modem with V.42/V.42*bis.* However, a 2400 bps modem with MNP 4/MNP 5 would also suffice for computer communications.

Communication software features are mostly a matter of convenience. A scripting feature helps to automate logging into a system. A "dialing directory" might automatically configure the software and dial frequently-called numbers. One important feature is the list of file-transfer protocols supported by the software. For error-correcting modems, the Ymodem/G protocol is the most popular. This protocol does not do any error-correction when transferring files, which would be redundant on an error-correcting modem. For a non-error-correcting modem, the Ymodem protocol is popular. There are many other protocols, but your software should support at least the most popular protocols, which are Xmodem, Ymodem and Ymodem/G. A very popular full-featured communication program is ProComm Plus from DataStorm Technologies, Inc.[*] It is easy to use, and suits the needs of most people.

Addresses of Producers of Network Programs

Artisoft, Inc.
691 East River Road
Tucson, AZ 85704
1-800-846-9726

DataStorm Technologies, Inc.
P.O.Box 1471
Columbia, MO 65205
314-443-3282

Invisible Software, Inc.
1142 Chess Drive
Foster City, CA 94404
415-570-5967

Novell, Inc.
122 East 1700 South
Provo, UT 84606
800-346-7177
801-429-5900