Coping with Complexity: PCs in Neurobiology

The PC, together with devices such as analog-to-digital converters, is helping scientists at the University of Illinois study nerve cell plasticity in marine mollusks.

Understanding how the mammalian brain functions is one of the last frontiers of biology. The human brain's level of complexity for information processing is staggering. It contains roughly $10^{11}$ nerve cells (or neurons), each capable of interacting with hundreds of thousands of others. Perhaps the most significant technological advance in neurobiological research during the past few years was the introduction of computers to help cope with the tremendous complexity involved. Because of their versatility and power, microcomputers are becoming critical pieces of laboratory hardware in neurobiology.

Neurobiological research entails probing the mechanisms by which nerve cells function and interact with one another to produce behaviors and store and manipulate information. Even with help from a PC, mammalian brains are so complex that neurobiologists are often forced to start with a simpler system. Molluscan neurons are tens to hundreds of times larger than their mammalian counterparts, yet they number only in the thousands. And, in spite of their relative simplicity, mollusks display discrete, quantifiable behaviors and are capable of simple forms of learning.

Studying the giant nerve cells of mollusks can offer significant insights into the functioning of nerve cells in more complex organisms.

Drs. Martha and Rhanor Gillette and I are investigating mechanisms of nerve cell plasticity in the marine mollusk Pleurobranchaea. Through our research, we have identified an ionic current, stimulated by an endogenously occurring compound, cyclic 3'5' adenosine monophosphate (cAMP), and regulated by calcium ions. The presence of cAMP induces this current and changes the excitability of the neuron. The two main avenues of our research are the mechanisms of cAMP induction of inward current and the regulation of cAMP activity by intracellular enzymes.

We use an IBM PC with a Tecmar 12-bit analog-to-digital (A/D) converter. A/D conversion allows the collection and analysis of data while an experiment is in progress. It is a great time saver, since collected data does not have to be transferred to a computer or calculator for analysis.

In addition, on-line collection is often the most accurate method of data collection. Most importantly, collecting data on-line allows continuous monitoring of the progress of the experiment, and immediate analysis of experimental results means more efficient use of time and resources.

At the heart of the A/D converter is an integrated circuit that transforms the in-
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coming voltage into a TTL (transistor-to-transistor logic) signal that the computer can handle. This process compares an unknown input voltage against some form of internally produced reference voltage. The reference voltage is changed until the difference between the two voltages falls below the resolution of the chip.

Integrated circuits can utilize several different methods of A/D conversion. The most common—as well as the fastest and most accurate—method is successive approximation, whereby the input voltage is compared bit by bit and complete conversion is accomplished in \( n \) steps for an \( n \)-bit converter. The dual-ramp (or slope) method, which is commonly found in most digital panel and multimeters, is also very accurate but has a much slower conversion time. The ultra-high-speed parallel technique is used for very-high-speed conversion, but its resolution is limited.

A/D and D/A packages can be assembled in several ways. Discrete components allow one to mix and match for the optimum system, but they generally require a better working knowledge of the whole system. The popular hybrid (multichip) converters are comprised of a ready-to-use package of components. Monolithic (single chip) converters are very inexpensive but generally not as good as hybrid or discrete systems. Successive-approximation hybrid A/D converters are probably the most appropriate choice for biomedical research.

Resolution is another consideration when choosing an A/D system. The selection is generally between 8, 12, 14, or 16 bits, and the choice depends on the desired speed, accuracy, and cost. Systems using 8-bit A/D converters are the most economical, easiest to use, and fastest, but the accuracy is only 1/256 of the full voltage range. Twelve bit A/D converters have a resolution of 1/4096 of the full voltage range and are still fast enough for most biological phenomena. A/D converters of 13 to 16 bits are available and are not too much slower than 12-bit converters, but the increased resolution is marginal.

Once the type of A/D converter and number of bits have been determined, a couple of other considerations remain. An A/D chip can convert only a limited range of input voltages. The most common input voltage ranges are 0 to 5, 0 to 10, and -10 to +10 volts. The proper range depends on your specific needs, but since negative potentials are frequently encountered in neurobiology, the -10 to +10 volt range is best.

It’s useful to have several counters on your A/D board. If your instrumentation puts out a TTL-compatible signal, they can function as straight counters, and they can be cascaded to handle very large numbers. Alternatively, the counters can be programmed as clocks to trigger conversion to real-time data collection.

Signal conditioning is often necessary prior to data collection. Input signal gain should be scaled to take full advantage of the converter’s input voltage range. Variable amplifiers to take care of this are simple to construct and work quite well. Even better, many AID boards feature software-programmable gains that are easier to manipulate during an experiment. Other signal conditioning devices include active filters to minimize unwanted noise prior to data collection and variable low- and high-pass filters to produce a narrow “window” of acceptable frequencies.

Our Technic Lab Master 12-bit A/D converter uses the 40 kHz switching frequency and programmable gain options to record membrane currents during patch and voltage clamping. Patch...
clamping is a new technique that allows electrical isolation and recording of a single-membrane protein channel. With this method, discrete openings and closings of ionophores can be monitored. We use this technique to examine the effects of cAMP and synergistically and antagonistically acting compounds on membrane current. The current flowing through each protein channel is roughly 1–2 picoamps (10–12 amps), so a good deal of amplification is required. Mean open time for each channel varies in the range of 0.1 to 10 milliseconds, so it is clearly necessary to sample as quickly as possible. We read the A/D signal from the port, immediately assign the value to a memory location, and then repeat the process until all usable RAM is filled. With this method, we can sample every 40 microseconds, with sampling periods of longer than 15 seconds. After the RAM is filled, the number in each memory location is read, converted to a voltage, and analyzed. We sample several 15-second bins to establish a baseline channel activity prior to experimental manipulation, and we then sample after the treatment to discern the change in channel activity.

Whole-cell voltage clamping involves monitoring the current flowing across the entire cell’s membrane while holding the neuron’s voltage at a desired potential. The current recorded is the summation of all the single-channel activities. The events of interest occur much more slowly (20 milliseconds to 60 seconds) than during patch clamping, so a slightly different A/D paradigm is required. Our voltage clamp experiments frequently involve intracellular injection of compounds or step changes in voltage, so A/D conversion is externally triggered to precisely coincide with the manipulation, and the voltage is sampled at regular intervals following the triggering stimulus.

The general types of research problems and methods of analysis we encounter in neurobiology are analogous to those encountered in many different areas of biology. With minimal effort, the PC and XT can be adapted to facilitate research in these other areas.

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