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Sampling variation caused by A/D cards due to external trigger

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Abstract

In electrophysiological recording, a microcomputer-based analog-to-digital (A/D) card is an indispensable instrument for signal acquisition and analysis. In our studies, evoked responses sampled by our A/D card showed variation among different cycles. If several cycles had been averaged, the resultant waveform would have a smaller peak amplitude and a longer duration. To explain this phenomenon, a simulated sampling model of compound action potential was proposed. Our experimental data agreed very well with the prediction of the simulated model. The long and varied delay time between the external trigger and the first sampling in each cycle by our A/D card might be the main cause of such variation. This problem could not be solved by any post-sampled programming. Hence, for those electrophysiological laboratories which sampled evoked responses, to buy a new A/D card might be the most straightforward solution to the problem.

Keywords: Data acquisition; Analog-to-digital conversion; Evoked response; Signal averaging

1. Introduction

Microcomputers are widely used in neuroscience research for signal handling and processing due to their increasing capability and decreasing cost. An analog-to-digital (A/D) converter card is mandatory to convert the analog signal into digital form. However, many older version A/D cards do not synchronize well with the external trigger and will cause signal distortion if the sampled evoked responses are averaged.

We noticed this problem in the recording of compound action potential (CAP) of nerves (Jaw et al., 1993). To increase the signal-to-noise ratio, several cycles of CAP were digitized and then averaged. Usually, the averaged waveform had a smaller peak amplitude than the CAPs displayed on the screen of our analog storage oscilloscope. On the screen of the oscilloscope, all the CAPs had the same shape and overlapped like a single trace.

This was an interesting problem although we had not found it being described. To understand this problem, CAPs from a rat's dorsal root were sampled and examined in detail. As a result, it was found that the delay time (DT) between the external trigger and the first conversion of each cycle was long and unfixed. Based on the technical information and through model simulation, it was suggested that sampling variation was caused by the long and unfixed DT.

2. Methods

An isolated dorsal root of a rat was used for the recording of the CAPs. The animal preparation and the recording setup were the same as those described in our previous paper (Jaw et al., 1991).

The CAPs of the A fibers were digitized by a 12-bit A/D card (AT-MIO-16F-5 of the National Instruments) at 20 kHz. Six cycles of CAPs were sampled 1.6 ms each. A CAP was also sampled by a digital oscilloscope (Tektronix TDS 520A) for waveform hardcopy and comparison. The synchronous output of the stimulator was used to trigger the acquisition of the A/D card and the oscilloscope.

The sampled CAPs were imported into a worksheet software (Microsoft Excel) for data analysis and plotting.

3. Results

The sampled CAPs are shown in Fig. 1. The waveform of the CAPs sampled by the A/D card was quite similar to

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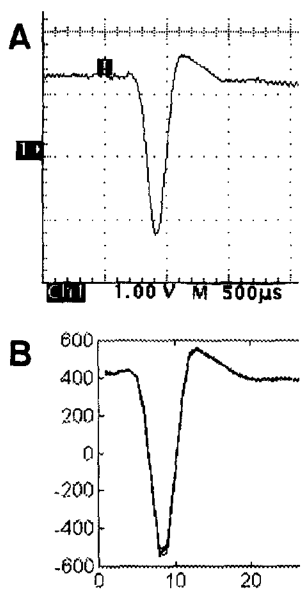

Fig. 1. The sampled CAPs. A: CAP sampled by the digital oscilloscope. The vertical sensitivity and horizontal time base are shown in the bottom. B: CAPs sampled by our A/D card. The numbers of the abscissa are the sequence of the sampled data. For example, the number 20 represents 1 ms (20×50 μs). The numbers of the ordinate are the digital code equivalent of the amplitude. Because the input range of the A/D card is ±5 V, the actual voltage of the amplitude is equal to the code number multiply by 2.44 mV.

that by the oscilloscope although only linear interpolation was used. Therefore, the 20 kHz sampling rate was adequate for the CAPs of the A fibers, the highest frequency component of which was below 3 kHz (Jaw et al., 1991). There was, however, a small variation near the peak of the CAPs (Fig. 1B). To examine such variation in more detail, the waveform of the CAPs was enlarged without interpolation as shown in Fig. 2. In the rising and falling phases of the waveform, the sampled values among different cycles at the same time instance were quite different. Such phenomenon could not have been clearly seen if the data had been linearly interpolated. The variation was so large that the quantization error of the A/D card and the noise interference of the recording system along could not be used to explain it. The reason is that while both the quantization error and the noise interference should affect all the data points similarly, however, the data points before the 5th and after the 13th showed no such interference in the system.

For numerical comparison, the data in Fig. 2 were partitioned into 2 groups. The first group composed of the 2nd, 3rd, 4th, 14th, 15th, and 16th data points was considered as the background noise group. The data from the 6th to the 11th time sequences were classified as the waveform group, where a large variation existed. The averaged standard deviation of the first group was 3.06 ± 0.85, and that of the waveform group was 13.49 ± 2.46. Owing to the same recording system, the same nerve, and the same CAPs, the paired t test was used to examine whether the difference was significant. As a result, the t value was 9.82 ($P < 0.0001$). Therefore, it was concluded that the background noise would not introduce such a large variation at the waveform group.

Another interesting phenomenon was that the variation seemed cycle dependent. The data in the series-4 were more negative than the other series (cycles) in the falling phase of the waveform, while they seemed more positive in the rising phase. On the other hand, the series-2 had the reverse order.

On the screen of the analog oscilloscope, however, there was no large variation and cycle dependence of the CAPs. Hence, these problems suggested that there must be some problems in the sampling process of our A/D card.

4. Discussion

To understand the variation we must know the timing information during the sampling process of our A/D card. In the external triggering mode, an unfixed DT is usually required for older commercial A/D cards to start conversion after being triggered. According to the information in the technical manuals, the DT variation is variable but within 1 sampling interval (Anonymous, 1992a, b). For

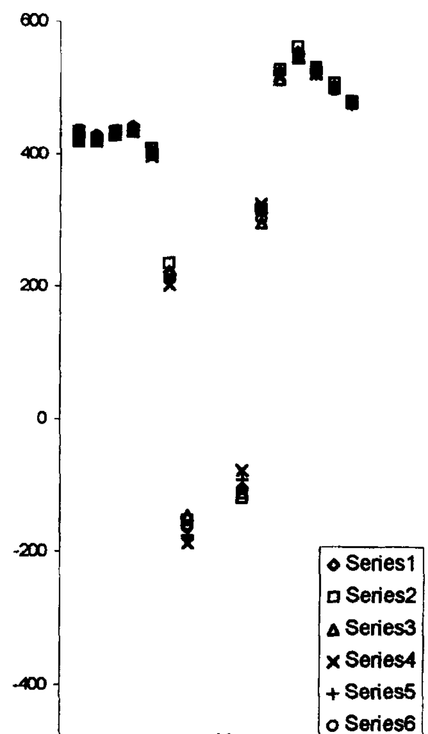

Fig. 2. Six cycles of the sampled CAPs in enlarged view.

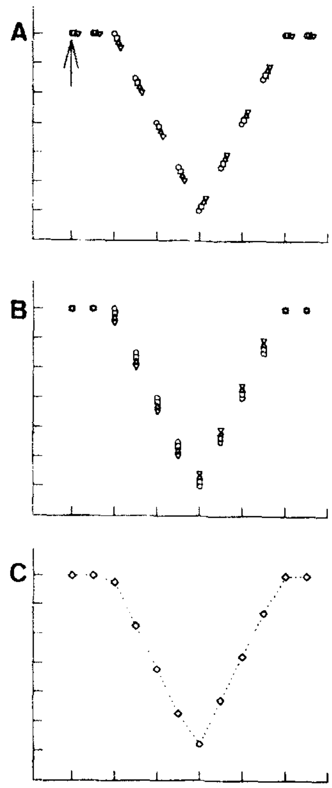

Fig. 3. Simulated sampling process of CAPs. A: time and amplitude relationship among the 4 sampled CAPs. The arrow represents the external trigger. The delay time between the triggering and the first sampling points of the 4 cycles are 0 (○), 0.1 (□), 0.2 (△), and 0.3 (▽) ms, respectively. B: time and amplitude relationship of the CAPs data in the memory of a microcomputer. C: signal distortion caused by averaging the CAPs. For visual inspection, dashed line is used to interpolate the averaged result linearly. Horizontal scale: 2 ms.

example, if the signal is sampled at 1 kHz, the variation range among each cycle is between 0 and 1 ms. Such variation in time might affect the data points sampled among different cycles.

To analyze this problem, a simulated model of the sampled CAPs is shown in Fig. 3. For simplicity, triangular waves are used to represent the CAPs. We assume that 4 cycles of the CAPs are sampled at 1 kHz. The timing and amplitude relationship of the sampled CAPs is shown in Fig. 3A. Because a crystal-based clock is usually used to control the sampling intervals of an A/D card, the time interval between two consecutive points in each cycle is always fixed. Thus, if the DT of cycle A is shorter than that of cycle B, the corresponding data points in cycle A would precede those in cycle B. From Fig. 3A we know that the amplitude differences among different cycles were larger in the middle (or fast) portion of the CAPs. The first 2 and the last 2 data points showed no amplitude difference although the time difference existed. While these data were sampled by the A/D card, the timing information among them was lost. In other words, they were aligned (in the computer memory) as those shown in Fig. 3B. Thereafter, we had no information about the DT of each cycle. This was the reason why the 6 CAPs data sampled by our A/D card looked like those in Fig. 2.

Ideally, if there had been no DT between the trigger and the first conversion, all the cycles sampled would have been exactly the same as what we have seen on the screen of an analog storage oscilloscope. Practically, owing to the asynchronous nature between the triggering signal and the pacing timer, which is used to control the sampling rates of the A/D card, a variable DT cannot be avoided. Hence, if many cycles of data are averaged, the waveform might be distorted. The averaged result of the 4 CAPs is shown in Fig. 3C. Evidently, it had a smaller peak amplitude but a longer duration than the original triangular wave.

A possible way of reducing this distortion would be to increase the sampling rate of the A/D card, that is, to decrease the DT variation. For example, considering a 'pure' sine wave of 100 Hz, the minimal sampling rate would be 200 Hz to meet the Nyquist frequency (Stanley et al., 1984). If we had increased the sampling rate to 10 kHz (an impractical high speed), the DT variation among different cycles would have been within 0.1 ms. For a 100 Hz sine wave, a 0.1 ms sampling variation means 6.28% of the maximally possible difference ($2\pi * 100/10k$) of the sampled value. This difference is much larger than the quantization error of a low resolution A/D card. In comparison, the quantization error of an 8-bit A/D card is only 0.4% (1/256). Clearly, increasing the sampling speed of an A/D card is not a practical method, especially in the studies where many channels of recording are required.

The older commercially available A/D cards which do not critically lock in time with the external trigger will cause sampling variations among cycles. Many scientists do not know this problem, and may use their A/D cards to gather evoked responses as usual. In this case, it should be careful while several cycles of the sampled signals are to be averaged. Otherwise, the resultant waveform might have a smaller peak amplitude and a longer duration. The long DT between the external trigger and the start conversion has been eliminated by some new A/D cards. Perhaps, to buy a new A/D card is a good solution to overcome this difficulty.

Acknowledgements

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