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Noise Reduction in Single Fiber Auditory Neural Responses Based on Pattern Matching Algorithm

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Abstract: When recording single-unit responses from neural systems, a common problem is the accurate detection of spikes (action potentials) in the presence of competing unwanted (noise) signals. While some sources of noise can be readily dealt with through filtering or "template subtraction" techniques, other sources present a more difficult problem. In particular, noise components introduced by power supplies, which contain harmonics of the power-line frequency, can be particularly troublesome in that they can mimic the shape of the desired spikes. Thus, standard "template subtraction" techniques or notch-filtering approaches are not appropriate. In this study, we propose the use of a novel template-subtraction scheme that involves estimating the power-line noise waveform and using cross-correlation techniques to subtract them from the recordings. This technique requires two key steps: (1) cross-correlation analysis of each recorded waveform extracts a robust representation of the power-line noise waveform and (2) a second level of cross-correlation to successfully subtract that representation from each recorded waveform. This paper describes this algorithm and provides examples of its implementation using actual recorded waveforms that are contaminated with these noise signals. An improvement (reduction) in the noise level is reported, as are suggestions for future implementation of this strategy.

Key words: Single-unit recordings, Template subtraction, Cross correlation, Single fiber response

INTRODUCTION

Biosignals are often contaminated by noises from some sources which make the signal-to-noise ratio (SNR) low. Therefore, post- or pre-processing for removing noise and stimulus artifact is important in the biosignal processing [1]. The acquisition of single-unit (or single-fiber) action potentials from experimental animal preparations is typically complicated by several sources of electrical contaminants that can make spike (action potential) detection difficult. Physiological noise, such as unwanted EKG and EEG signals are typically present, although they usually present a tractable problem due to their relatively low amplitudes. Stimulus-induced noise can be a significant problem in studies that measure evoked responses. For example, in auditory research, radiated energy from an acoustic driver (earphone) can be picked up by the recording micropipette. Unwanted signals from earphone radiations, however, can often be reduced by techniques as simple as electrical isolation. In some cases, stimulus contaminants can be segregated from the desired responses simply from the fact that the response may be sufficiently delayed from the offset of the stimulus contaminants. This can be the case when relatively short-duration stimuli are employed. In those instances, however, it may be particularly important to avoid the use of restrictive, narrow-band, filtering that can cause significant temporal "smearing" and, thus, increasing the overlap of the stimulus artifacts with the response. Another common technique is the use of alternating-polarity stimulus waveforms and summation of the responses evoked by such stimuli, which should, in theory, result in the cancellation of stimulus artifacts. This approach is not always feasible, however. Also, in studies involving electrically-evoked responses, electrical

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stimulus artifacts can be particularly problematic and can necessitate the use of "template subtraction" schemes, such as that described by [2].

Noise-reduction schemes can be categorized in terms of "real-time" and "post-processing" techniques. Real-time approaches include electrical isolation, stimulus polarity alternation, filtering, and in some cases, the subtraction of a stimulus-artifact template. Post-processing schemes typically involve more computationally intensive procedures or the need for user decisions that make automated approaches problematic. However, some post-processing schemes, such as the one developed in the paper, could theoretically be implemented in real time, given sufficiently fast computational resources and algorithm optimization. Indeed, with faster computer resources, some "post-processing" schemes could be implemented in an "on line" fashion.

One particularly difficult form of electrical contamination is the presence of noise waveforms introduced by power supplies and laboratory equipment. The presence of digital (switching) power supplies and nonlinear processes such as rectification can introduce significant harmonic energy related to the a.c. waveform of the power line. While the fundamental frequency of this waveform (i.e., 50 Hz or 60 Hz) may not be a particular issue, the harmonics may contain spectral energy similar to that present in the desired (action potential) waveform. For that reason, standard filtering approaches [3, 4] are not appropriate. Furthermore, experimenters typically make special efforts to ensure that power-line noise is not synchronized with the stimulus, so that line-induced noise components do not appear to be causally linked to the stimulus. This, however, results in the somewhat random distribution of line noise components in the recorded signals. As neural responses may also be randomly distributed across time (e.g., in the case of spontaneously active neurons or in the case of neural responses to noise-like stimuli), the power-line harmonics may be difficult to distinguish against random neural responses.

The nature of noise components generated by line power therefore present a particular challenge that is the subject of this paper. We present a method of identifying unwanted signals that are related to laboratory equipment (typically, line-voltage power supplies) that is relatively computationally intensive and thus is implemented as a post-processing scheme. The procedures developed in this paper arose out of our laboratory's interest in recording single-unit responses from the electrically stimulated auditory nerve of mammals. Because many of our experiments employ electrical stimuli that approximate the stimuli delivered by cochlear prosthesis, our experimental preparation may be subject to an additional source of electrical contamination. In particular, as the tissue is excited electrically, there is a greater probability that the stimulation equipment will introduce power-line related noise components through difficult-to-control electric leakage paths.

The auditory neural responses were recorded from single nerve fibers of cats using monophasic current pulses delivered by a monopolar intracochlear electrode. Unlike some laboratories, which record spike "events", a standard procedure of our laboratory is to record and store all "raw" response waveforms to the mass storage device. As is typical for single-unit research, multiple (repeated) responses from a given fiber are obtained in order to assess response properties such as firing probability, mean spike latency, and spike jitter. The storage of the raw response waveforms facilitates experimentation of various post-processing schemes, such as that described here. The procedure to reduce power-line noise contaminants involves the use of cross-correlation techniques to both identify the noise signal and then to effectively subtract that signal from each template.

METHOD AND MATERIALS

Animal Preparation

For the purposes of developing this noise-reduction scheme, response waveforms from an acute cat preparation were used. Details of the methodology for animal preparation are previously described [2, 5]. All experimental procedures were done with the subject maintained at surgical levels of anesthesia. General anesthesia was induced with ketamine (30 mg/kg) and acepromazine (0.3 mg/kg). Atropine sulfate (0.04 mg/kg/12h) was given to reduce mucosal secretions. Core body temperature was maintained by a circulating water heating pad and drapes and vital signs (Heart rate, blood oxygen saturation, expired C02 pressure and rectal temperature) were monitored throughout the experiment. A standard posterior fossa approach was used to expose the auditory nerve and a cochleostomy was performed in the basal turn of the cochlea using a surgical drill to provide access to the scala tympani. A small Pt/Ir stimulating ball electrode was placed in the scala tympani to provide for the intracochlear delivery of electric current pulses.

Stimulus Presentation

Stimuli were generated by custom software controlling a 12 bit D/A converter. The positive output of the current source was connected to the monopolar stimulating electrode in the basal turn of the cochlea and the negative output was connected to a needle electrode placed in the forepaw. The stimuli were biphasic rectangular pulses (duration 40 μs/phase). The inter-pulse interval (IPI) was set to 4 ms. A train of 75 electric pulses (total duration 300ms) was presented in a sweep [5].

Single Fiber Neural Response Recording

Standard micropipette techniques were used to recording single-fiber responses [2]. The micropipette was connected to an Axon Instruments Axoprobe headstage and amplifier, which, in turn, was fed to a 6-pole low-pass filter (30 kHz cut-off frequency) and to an A/D converter (sampling rate 100,000 samples/s). The responses to a low-rate (250 pulses/s) train of biphasic current pulses were recorded for 350 ms, so that the each response waveform consisted of a 300 ms epoch with responses to the electric pulses and a subsequent 50 ms epoch without any electric pulses. This post-train interval provided a stimulus-free interval useful for evaluating the power-line noise characteristics. As is typical for single-unit work, multiple recordings were made in response to multiple presentations of the stimulus pulse train. While this is a standard means of obtaining neural response statistics, it also facilitates the implementation of our noise-reduction technique, as it provides multiple "copies" of the electrical contamination signal, enabling the algorithm to more effectively identify this signal. A schematic diagram of the stimulus pulse train and the recording epoch is shown in Fig. 1. All surgical and experimental procedures were approved by the University of Iowa Animal Care and Use Committee and complied with the standards of the U.S. National Institutes of Health.

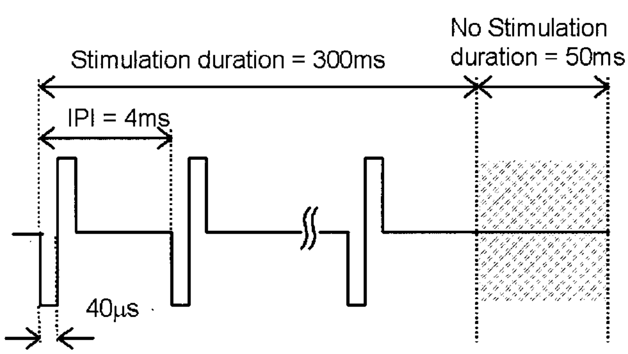

Fig. 1. Electric stimulation in each sweep. Total duration, 350 ms, included the stimulation dura tion and no stimulation duration. Inter-pulse interval is 4ms with 40 μs/phase biphasic stimulation.

Noise Reduction Protocol

General approach

The proposed reduction of electrical contaminants produced by line-voltage components is premised upon the notion that this unwanted signal is deterministic and therefore has a well-defined waveform shape. Thus, across the repeated recorded epochs, this noise component, while not necessarily synchronized across the multiple recorded epochs, can be identified by a well-defined waveform. If this is the case, the cross-correlation of two recorded epochs (each containing this contaminant) should produce an identifiable peak in the correlogram. By recording micropipette potentials relatively free of neural responses, it should therefore be possible to identify this noise waveform. Furthermore, by performing multiple cross-correlations across multiple recorded epochs, it should be possible to not only identify this waveform, but through the temporal alignment of their multiple instances, obtain a robust copy, or template, of this noise signal. Finally, this robust copy of the noise waveform can be aligned, by again using cross-correlation, to the instances of this noise that occur in each recorded sweep and then subtracted from each sweep.

Noise Template

As seen in Fig. 2, the recorded responses included spike (action potential) activity imbedded in a noisy background. Closer examination of the noise (see inset in Fig. 2. which shows greater temporal detail of the noise signal) reveals a complex waveform with harmonics related to the power-line (60 Hz) frequency.

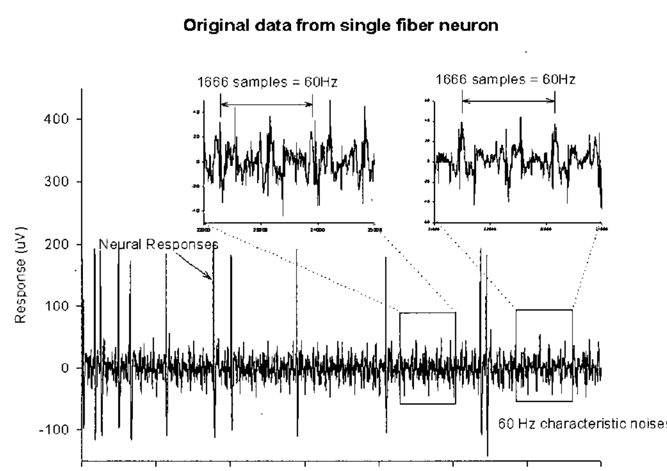

Fig. 2. The single fiber neural response in which the 60 Hz characteristic peak noises were included. Total recording duration is 350 msec (35,000 samples with 100,000 samples/sec). [0 msec ~ 300 msec] : Electric stimulus and [300 msec ~ 350 msec] : no stimulus

It could be analyzed using FFT and auto-correlation which are usually used in detecting hidden periodicity (Fig. 3). These noises had wide frequencies characteristics over the neural response's frequency range as well as 60Hz. These unwanted noises were presumably caused by a 60 Hz power supply and other electric experimental equipment. The wide spectral content of this noise signal makes it clear that standard filtering techniques are not the most appropriate means of reducing this noise. In this study, we developed and applied a template subtraction method to reduce this particular noise component.

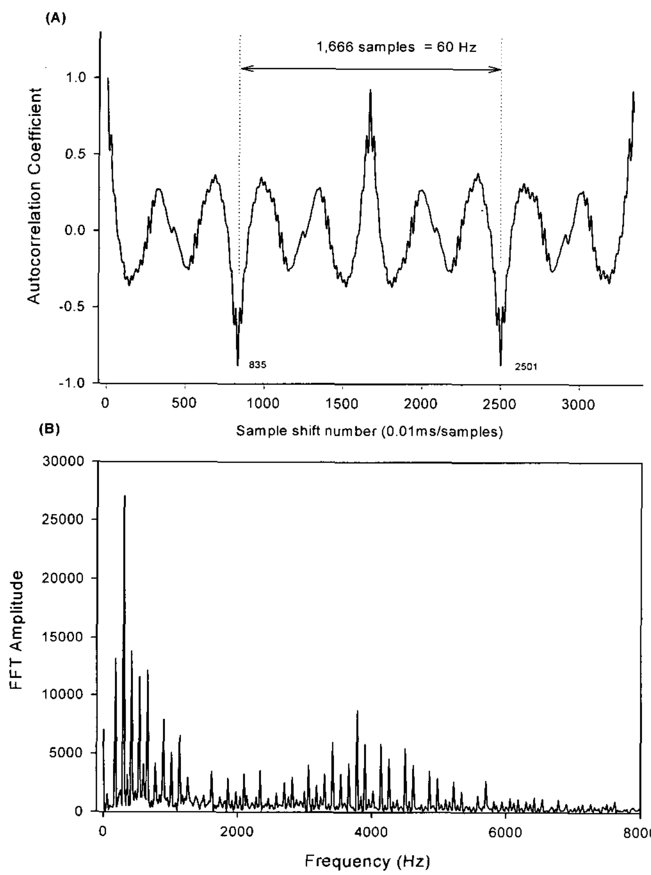

Fig. 3. Noise frequency analysis using (A) autocorrelation and (B) FFT. These show that noise is composed with complex waveforms which has wide frequency range as well as 60Hz.

To produce a template (i.e., a robust representation) of this noise signal, we treated it as a "desired" signal and applied signal averaging to extract it from other background noise components. This averaging approach was needed to ensure that our subsequent subtraction of the noise signal template did not result in an appreciable *increase* in background noise amplitude. The region of each sweep ranging from 300 ms to 350 ms (i.e., where no electric stimulus was presented) was used to extract this component. To obtain a "grand average" or robust template of the noise signal, repeated cross-correlations were performed iteratively to identify and "build" the grand-average.

The averaging process, shown schematically Fig. 4, was first performed between two sets of sampled points ($S_i^{(k)}$ and $S_{i+1}^{(k)}$) that were obtained from two sweeps (i and $i+1$) of the 35 acquired sweeps and from the iteration k. It is important to note that the desired noise signal in these two sets of samples are arbitrarily out of phase with each other, as they were not recorded in a manner that synchronized this noise signal with the recording epoch. It is thus necessary to shift (or lag) one of the waveforms relative to the other to align the noise signals prior to performing the average. Cross-correlation provides a means of determining the proper time shift. This process of aligning and averaging two sets of samples was repeated for other pairs of sets obtained from the 35 sets, *completing the first iteration* (k=1) *of averaging* and producing a total of 18 averaged sets (with the 35th, the last set, included as the 18th "average"). This process was then repeated for a second (k=2) iteration, further reducing the set to 9 averages, improving the representation of the targeted noise signal. This iterative process was performed a total of $K=\log_2(35)$ times until a single, grand, average was obtained.

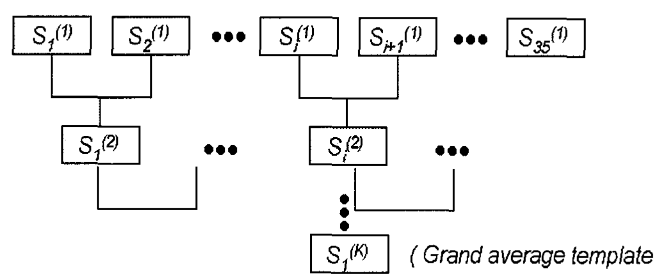

Fig. 4. Diagram showing the process of calculation of the grand average template using no stimulus intervals in each sweep. After K iterations of averages, $S_1^{(K)}$ could be calculated (see text for description).

The underlying computations of the above process are now described. The cross-correlation of the two sequences $S_i^{(k)}$ and $S_{i+1}^{(k)}$ was calculated according to one sequence shifted by $\Delta t_i^{(k)}$, Eq. (1). $\Delta t_i^{(k)}$ represents the amount of lag by which one sequence has been shifted to the left [6, 7].

$$\rho_{i,i+1}\left(\Delta t_i^{(k)}\right)=\frac{\sum_{n=0}^{N-1} S_i^{(k)}(n)\cdot S_i^{(k+1)}\left(\Delta t_i^{(k)}+n\right)}{\left[\sum_{n=0}^{N-1}\left(S_i^{(k)}(n)\right)^2 \sum_{n=0}^{N-1}\left(S_i^{(k+1)}(\Delta t_i^{(k)}+n)\right)^2\right]^{1/2}} \quad (1)$$

The maximum lag, $\left(\Delta t_i^{(k)}\right)_{\max}$, makes the cross correlation coefficient, $\rho_{i,i+1}\left(\Delta t_i^{(k)}\right)$, maximum [8]. With this maximum lag, the next iteration was calculated as:

$$S_i^{(k+1)}=\left(S_i^{(k)}(t)+S_{i+1}^{(k)}(t+(\Delta t_i^{(k)})_{\max}\right)/2 \quad (2)$$

Through this iteration process, all 35 data sets (from the 35 recorded sweeps) were used to finally obtain the grand average template $S_1^{(K)}$ shown in Fig. 5. (A). Through the process of waveform alignment, some samples (across the available 50 ms duration of each original epoch) were lost so that the averaging could be performed. In this case, the duration of the grand average was approximately 30 ms, reflecting some degree of loss.

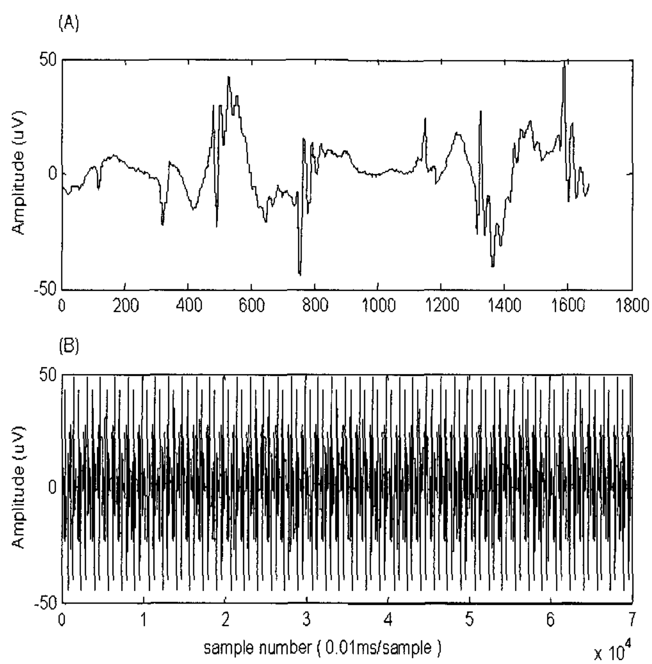

Fig. 5. (A) The result of a grand average template which was calculated by Eq. (2). (B) The whole template with duration of 700 msec was made using resampled template

As the duration of the grand average was insufficient to cover the entire 350 ms epoch of each original recorded sweep, necessitating the repeated concatenation of the grand average with itself to form a template long enough to extend across the 350 ms recording epoch. Spectral analysis of our grand average template, however, indicated that the period of the noise signal was not an integer number of samples, but instead had a period of 1,666.6667 samples. Proper concatenation (in which the fundamental period of the derived noise signal was not altered by this sampling mismatch, Fig.6) required resampling of the template such that

$$S_{resampling,j+1}(i)=\alpha\times S_{resampling,j(i)}+(1-\alpha)\times S_{resampling}(i+1)$$

$$where,\ S_{resampling,1}=S_1^{(K)}\ and\ \alpha=0.6667\ or\ 0.3334 \quad (3)$$

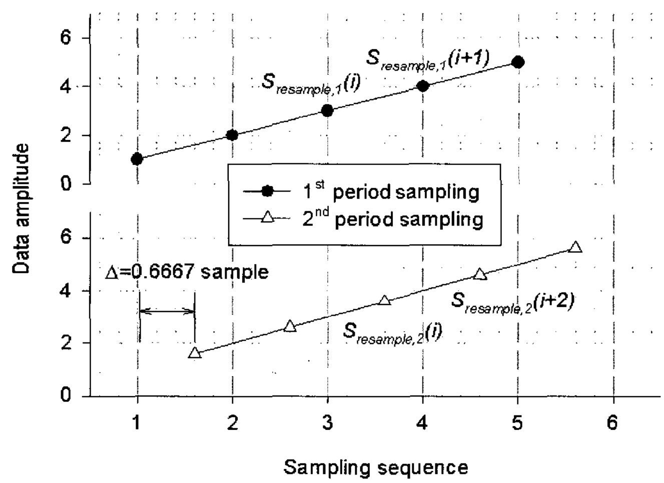

Fig. 6. Template resampling to make proper concatenation. In experiment, the second data shifts with 0.6667 sample time as the period of noise was not an integer number of samples.

In the next step, we made the whole template, Ω, which has a total 700 ms periods which is long enough to cover the any acquisition data set in our experiment environment. The copy method was applied using Eq. (4). According to the previous frequency analysis of noises, it shows that the noise is modulated in 60 Hz which is exactly f_{real} = 1,666.6667 samples in 100kHz A/D sampling. To avoid the non-integer period error, we used the period of template samples as f_1 =1,666 or f_2 =1,667 alternatively. If not so, the truncated sample intervals would have seen propagated.

$$\Omega(i+(f_1+f_2)\times j) = S_{resampling,j}(i) \quad where,\ i=1,2,\cdots,(f_1+f_2)\ and\ j=0,1,\cdots,32 \tag{4}$$

Template Subtraction Using The Cross Correlation

For the subtraction between the whole template and a response signal, we also should match the phases. It was performed at the interval 300ms~350ms between the response signal, $R_j(t)$, and the grand average template, $S_1^{(K)}$. The template $S_1^{(K)}$ was shifted one point in time along the response signal and the cross correlation coefficient was recomputed for the alignment. With this process, we found the best shift (or lag) value, Δt_j, for Ω to subtract a response signal with template.

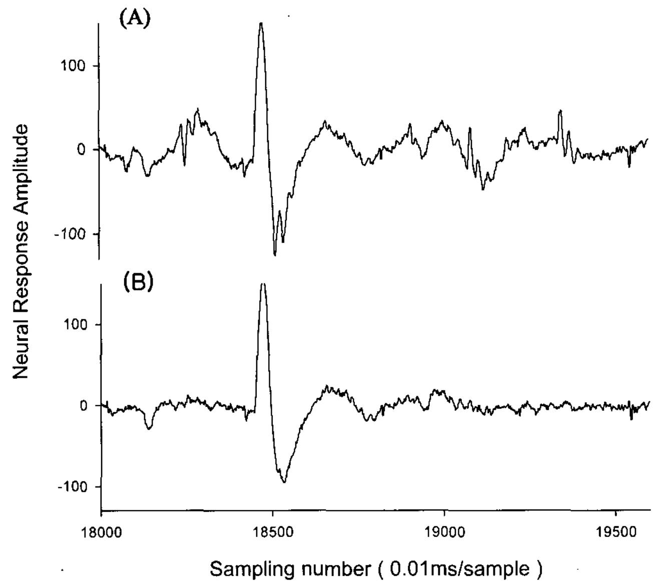

Fig. 7. A example of template subtraction. The original data is shown in (A) and the data after subtraction in (B). The noises were decreased and removed.

RESULTS

We made the whole template from the data set # 145 of Cat D41. The data set comprised 35 sweeps. To demonstrate the applicability of this template subtraction algorithm, we measured the performance of these template subtraction algorithms over the data set # 140, #141 and also #145 of Cat D41. Fig. 5 and Fig. 6 show the results of template subtraction. The attenuated noise power within the no stimulus interval (300 ms ~ 350 ms) was calculated by Eq. (5). This shows how the noises are reduced before and after.

$$(Attenuation) = 10\times\log[(Power_{ouput})/(Power_{input})] \tag{5}$$

The results are plotted in Fig. 9. The mean of the attenuated power was 10.15 ± 0.67 (dB). This result demonstrates the usefulness of the template subtraction method as applied to single fiber neural response and the efficiency of noise reduction which might come from laboratory equipments. Fig. 10 also shows the noise distribution before and after template subtraction method in whole response range which was applied to data set D41 #140 35 sweeps. The average level of noise was decreased from 37.0 to 30.8 and standard deviation from 16.2 to 10.0. The decrease of standard deviation can expect more efficient for a spike detection process.

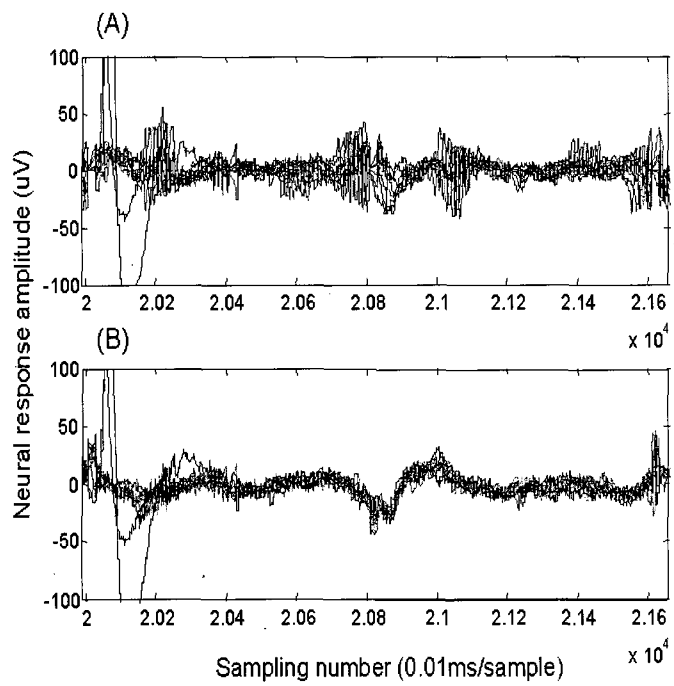

Fig. 8. (A) The original neural response data without subtraction and (B) the result after template subtraction for 10 sweeps (data set #140, D41). The neural spike responses were not changed. However, the overall noise level was decreased.

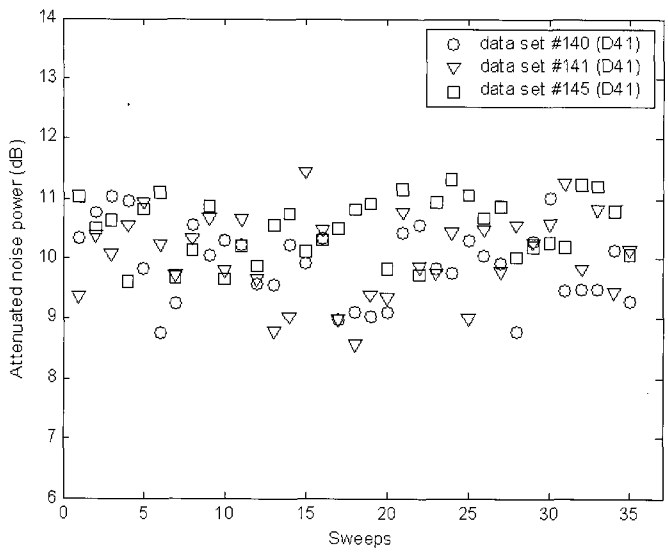

Fig. 9. An attenuated noise power after template subtraction applied to 105 data sets. The mean of attenuated power is 10 dB.

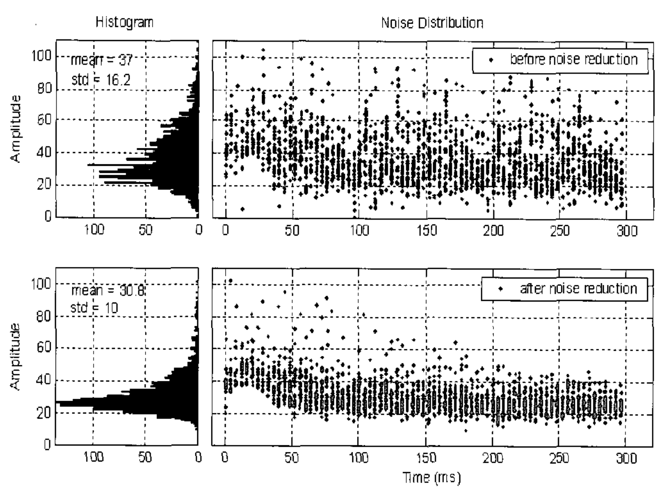

Fig. 10. Noise distribution before and after noise reduction in whole recording range in data set D41 #140. The mean level of noise fell off by around 20% and the standard deviation which is used in threshold level for spikes was decreased by 38%.

DISCUSSION

In this study, we have used template subtraction method to reduce the noises contaminated in single fiber auditory neural responses. The whole template which was calculated from the response data set #145 of Cat D41 was applied to other data set of Cat D41. The experimental results show that our template subtraction method using cross correlation is highly effective in removing the noises. Although a notch filter at a noise frequency or a low-pass filter at a relatively high frequency can decrease the stimulus artifact, it may also reduce and distort the shape of neuronal spikes. If the template itself may contain neural activity, then the template subtraction in noise reduction will eliminate the neuronal signal [9]. However, we could overcome this limitation by using the no stimulus interval for making the template.

In recent experiment, the amplitudes of noise level were not varied. As this reason, our algorithm could have a good performance. However, for the possibility of noise amplitude variation, we should make an additional algorithm which calculates the noise level automatically and fixes an overall amplitude-ratio of noise template. It will be studied in the next step. Eventually, we will apply this algorithm as pre-processing for spike detection which uses the parameter of background noise standard deviation. Application of this method enables one to calculate the firing rate with high reliability.

REFERENCES

[1] A. Choi, E. Woo, S. Park, and Y. Yoon, "*High Frequency Noise Reduction in ECG using a Time-Varying Variable Cutoff Frequency Lowpass Filter*", J. Biomed Eng. Res., Vol. 25, pp. 137-144, 2004.

[2] C. A. Miller, P. J. Abbas, B. K. Robinson, J. T. Rubinstein, and A. J. Matsuoka, "*Electrically evoked single-fiber action potentials from cat : responses to monopolar, monophasic stimulation*", Hearing Research, Vol. 130, pp. 197-218, 1999.

[3] P. S. Hamilton, "*A comparison of adaptive and nonadaptive filters for reduction of power line interference in the ECG*", IEEE Trans Biomed Eng, Vol. 43, pp. 105-109, 1996.

[4] R. Marshal, "*The auditory brainstem response recorded with 60Hz notch filters*", Ear Hear, Vol. 12, pp. 155-158, 1991.

[5] C. L. Runge-Samuelson, P. J. Abbas, J. T. Rubinstein, C. A. Miller, and B. K. Robinson, "*Response of the auditory nerve to sinusoidal electrical stimulation : effects of high-reate pulse trains*", Hearing Research, Vol. 194, pp. 1-13, 2004.

[6] E. C. Ifeachor and B. W. Jervis, Digital Signal Processing : A Practical Approach: Addison-Wesley Publishing Company, 1996.

[7] S. Levine, J. Gillen, P. Weiser, M. Gillen, and E. Kwatny, "*Description and validation of an ECG removal procedure for EMGdi power spectrum sanalysis*", J. Appl Physiol, Vol. 60, pp. 1073-1081, 1986.

[8] R. Wagner and H. L. Galiana, "*Evaluation of Three Template Matching Algorithms for Registering Images of the Eye*", IEEE Trans Biomed Eng, Vol. 39, pp. 1313-1319, 1992.

[9] T. Hashimoto, C. M. Elder, and J. L. Vitek, "*A template subtraction method for stimulus artifact removal in high frequency deep brain stimulation*", Journal of Neuroscience Methods, Vol. 113, pp. 181-186, 2002.