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Estimating membrane potential in human motoneurons using a peri-stimulus time histogram

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Abstract: Although it is useful to learn how alpha motoneurons integrate motor and sensory inputs in humans to understand the motion control mechanism and to study the neuronal diseases, it was considered that the identification of after-hyperpolarization (AHP) is impossible in humans because it is unethical to record membrane potentials at the cell body in vivo. In this paper, we propose a novel method to estimate AHP based on peri-stimulus time histogram (PSTH) analysis. The response properties of PSTHs, where a stimulus is delivered at a fixed delay time after the previous discharge of a motor unit, provide the characteristics of the membrane potential at the cell body of motoneurons. We investigated the responses of 21 single motor units to electrical common peroneal nerve stimulation in the right tibialis anterior (TA) muscle of 5 healthy subjects. The stimulus was applied through a bipolar stimulating electrode placed distal to the neck of the fibula at an intensity of 83–93% of the motor threshold to only activate Ia afferents of the TA. We obtained 6 PSTHs with different delay times (5, 15, 25, 35, 45, and 55 ms) in a single recording session. To validate the estimation, we investigated the relationship between the mean interspike interval and the slope of the estimated AHP, and observed a significant inverse relationship ($p < 0.02$). We concluded that the relationship between the firing response and the delay of the stimulus represents the transition of the motoneuronal AHP.

Key words: After-hyperpolarization, Peri-stimulus time histogram, Tibialis anterior muscle, Motor unit

1. Introduction

The discharge patterns of tonically firing motoneurons are influenced by the characteristics of their presynaptic input and their intrinsic properties. The regularity of motoneuronal discharges is thought to reflect their prominent post-spike after-hyperpolarization (AHP). When a motoneuron fires at a steady mean rate, the distribution of interspike intervals (ISIs) is determined by the amplitude and frequency of synaptic noise together with the decrease in excitability following a spike due to AHP [1] [2].

Such knowledge about motoneuronal discharge patterns is largely based on intracellular recordings from deeply anesthetized animals. In these experiments, the motoneurons fired in response to an injected current; these kinds of experiments, of course, cannot be reproduced in humans. Fortunately, there is a one-to-one relationship between the discharge of a motoneuron and the discharge of the motor unit potential (MUP); therefore, recordings of human MUPs offer a unique opportunity to study intact motoneurons responding to physiological activation.

Change in the MUP discharge pattern in response to a stimulus are commonly detected by logging the occurrence of discharges relative to the stimulus. If the stimulus is repeated several times during the activation of a motor unit (MU) by gentle voluntary contraction, a cross-correlation between the stimuli and the motoneuronal spikes can be performed. Such a cross-correlogram, usually called a peri-stimulus time histogram (PSTH) [3], yields information for changes in the motoneuronal firing probability related to the stimulus and is therefore regarded as a tool to detect stimulus-induced motoneuronal excitation and inhibition [4].

Recently, Powers et al. proposed a method to estimate the AHP of a motoneuron with a discharge pattern using computer simulation [1]; however, this method was only available in simulation because it required the recording of MUPs over an extended time period as a prerequisite. It is well known that actual MU recording is easily stopped by minute changes of the electrode position or changes in the recruitment of the MU. The conventional method only uses the information generated from the MUP discharge pattern. If information about the change of the discharge

pattern can be used, the recording time required to estimate AHP can be reduced; therefore, in this study, we propose a novel estimation method using PSTHs and validate the method using MU recording in human subjects.

2. Materials and Methods

2.1 Data acquisition

We recruited a total of four participants, none of whom had a history of neuro-muscular disease. All subjects were 21–27-years-old right-handed males. The experimental procedures given below were described to the participants, who each gave informed consent. The procedures were approved by the local ethics committee of the Graduate School of Science and Technology, Keio University, Kanagawa, Japan. During the experiment, the subjects were comfortably seated in an armchair, and the examined leg was loosely fixed with the hip semi-flexed (110°), the knee slightly flexed (120°), and the ankle plantar-flexed (100°). This position was fixed using a knee-ankle-foot orthosis to prevent movement of the needle electrodes.

Single MUPs were recorded from the right tibialis anterior (TA) muscle using a disposable bipolar needle electrode (NM-030T; Nihon Kohden, Tokyo, Japan). The potentials were amplified and band-pass filtered (100–5,000 Hz) using an EMG amplifier (Neuropack MEB-2200; Nihon Kohden, Tokyo, Japan). The signals were digitized at 10 kHz (12-bit AD converter, PCI-6071E; National Instruments Corp., Austin, TX, USA) and stored on a personal computer.

During the experiment, the subjects slightly and isometrically contracted the TA muscle and continued the contraction to allow us to measure a single MUP. Stimulus to the ipsilateral common peroneal nerve (CPN) was applied through a pair of stimulating electrodes (Ag/AgCl disk electrodes with a diameter of 9 mm and an inter-electrode distance of 3 cm) placed distal to the neck of the fibula. The current-controlled stimulation was applied by an electrical stimulator (SEN3301; Nihon Kohden, Tokyo, Japan) with a mono-phasic and 1-ms rectangular wave (Fig. 1). Delivery of the stimulus was triggered by firing the MU with a constant delay.

2.2 Peri-stimulus-time histogram

After a few sessions to train the subject to isolate single MUs and to fire the MUs at constant intervals,

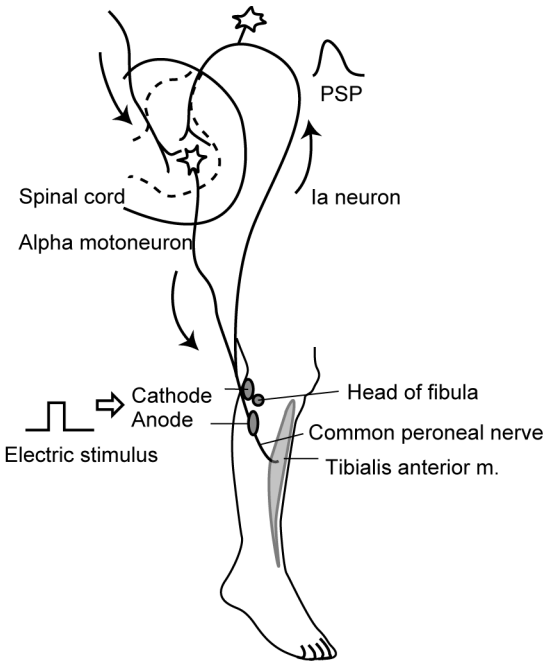

Figure 1. Neural connection in the spinal cord and stimulating electrode positions. The post-synaptic potential (PSP) is evoked by the direct electrical stimulation of Ia neurons from primary spindle endings in mixed nerves.

which varied from 80–180 ms, with the aid of visual and auditory feedback, we conducted a session to generate PSTHs. The firing interval was chosen such that it could be easily maintained at a more or less constant rate during a long sequence.PSTHs for a voluntarily activated MU were constructed for the period following a test stimulus. From the naturally occurring spike train, this process detects changes in the firing probability that are time-locked to the stimulus [4]. The particular method used in this study is fully described elsewhere [5] and will only be briefly summarized here.PSTHs delivered a test stimulus triggered by the discharge of a MU and measured the time interval between the stimulus and the next discharge. The measurement time ranged from 1–200 ms and the bin width was 1 ms; therefore, PSTHs contained 200 bins. In the same session, a histogram of the naturally occurring spike trains (without stimulation) was also constructed (inter-spike interval histogram; ISIH) to obtain the background firing probability. We finally obtained 1 ISIH and 6 PSTHs for each different delay time (5, 15, 25, 35, 45, and 55 ms) in the single recording session. As a result, we observed that the AHP response to stimulation at

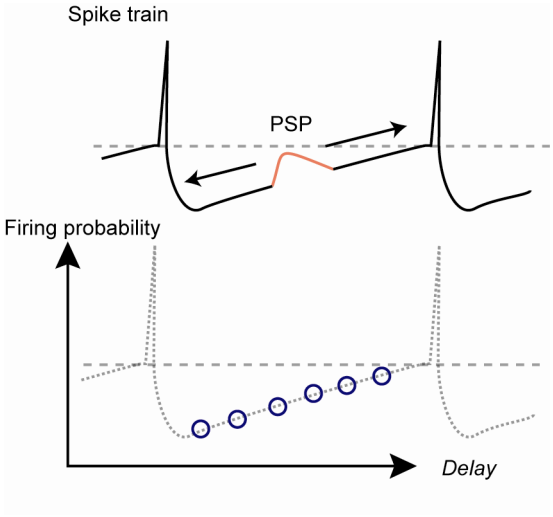

Figure 2. Scheme of the AHP estimation method.

the 6 time points depended on the stimulus delay time in 2 motoneuronal discharges (Fig. 2).

Triggers for the ISIHs and PSTHs were obtained alternately to achieve the same background firing probability between these histograms. So the total number of ISIH triggers was six times larger than for the PSTHs. Six test situations with different delay times were triggered by MU discharge in a random order. The intensity of the stimulation is generally expressed in multiples of the threshold intensity of the TA's direct motor wave (× MT). The value was fixed during a session and ranged from 0.86–0.96 × MT. This intensity was determined at the beginning of a session, given the experimental conditions and the sensitivity of the subject to the electric stimulation.

2.3 Weighted cumulative sum test

An efficient method to analyze PSTHs is given by the cumulative sum (CUSUM) derived from the PSTH [6]. The CUSUM is a timewise integration plot of the residual frequency at each bin between the PSTH and the ISIH, when the total number of triggers for both is the same. Regarding the statistical test for CUSUMs in the PSTH, where a stimulus is delivered

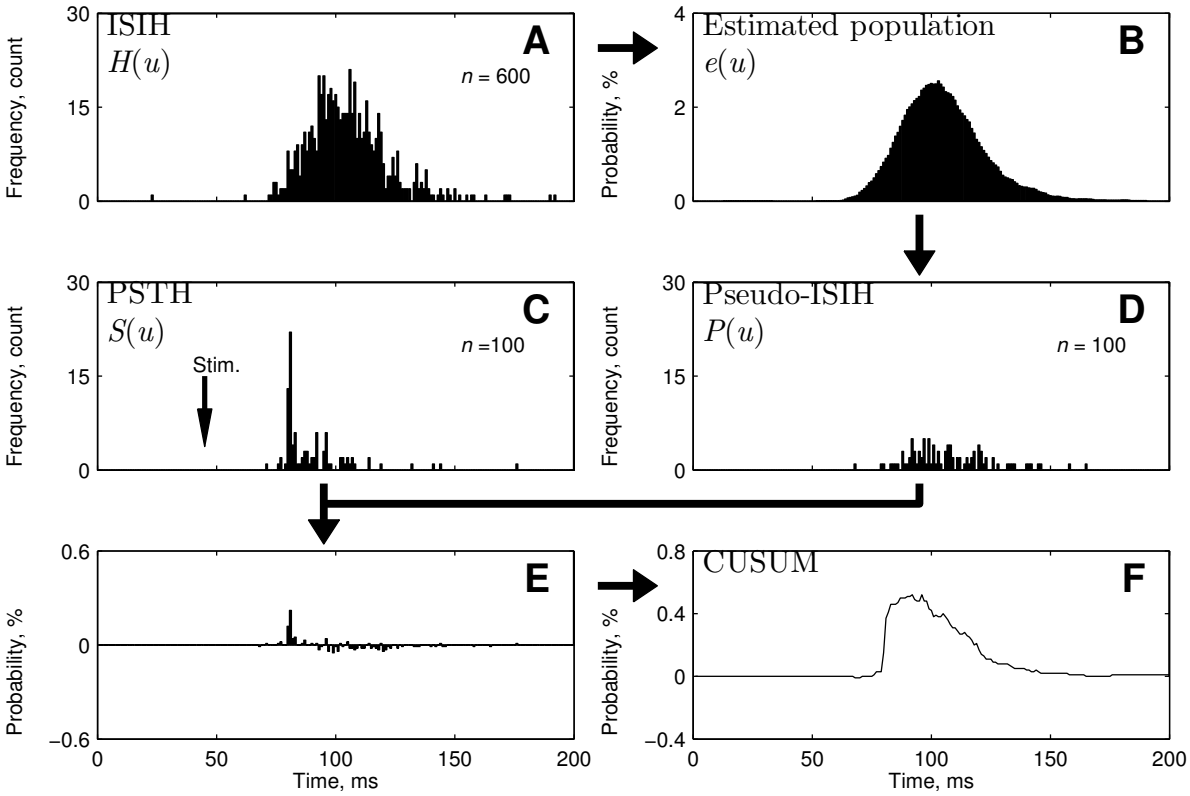

Figure 3. Scheme of the conventional CUSUM test. (**A**) Interspike interval histogram (ISIH). (**B**) Estimated population of background firing probability obtained by applying the moving average technique to the ISIH. (**C**) Peri-stimulus time histogram. (**D**) Pseudo-ISIH simulated by the Monte Carlo method. (**E**) PSTH subtracted from the pseudo ISIH at each bin. (**F**) CUSUM curve (time-wise cumulative summation of **E**).

at a fixed delay time after the previous discharge of a motor unit, a previously developed statistical test for the original PSTH method can be applied [7]. However, the delay time should be the same as a prerequisite for comparing PSTHs; therefore, we modified the CUSUM using a weighted function (named "wCUSUM").

A summary of the CUSUM test is also stated here. To statistically determine the hypothesis that a test stimulus does not affect motoneuronal AHP, an estimate of the population of background firing probabilities from ISIHs is initially required. A moving average technique is applied to eliminate the statistical scattering of ISIHs (see Figs. 3A and 3B). The averaged histogram is also normalized by the total number of triggers (the estimated population of background firing probabilities). On the basis of the estimated population, a pseudo-ISIH with the same total number of triggers in a PSTH (Fig. 3C) is simulated using the Monte Carlo method (Fig. 3D). After subtracting the pseudo-ISIH from the PSTH at each bin (Fig.3E), its cumulative summation is calculated to produce final CUSUM shape (Fig. 3F). We also used these conventional CUSUM procedures as a preliminary step in our wCUSUM procedure (Fig. 4).

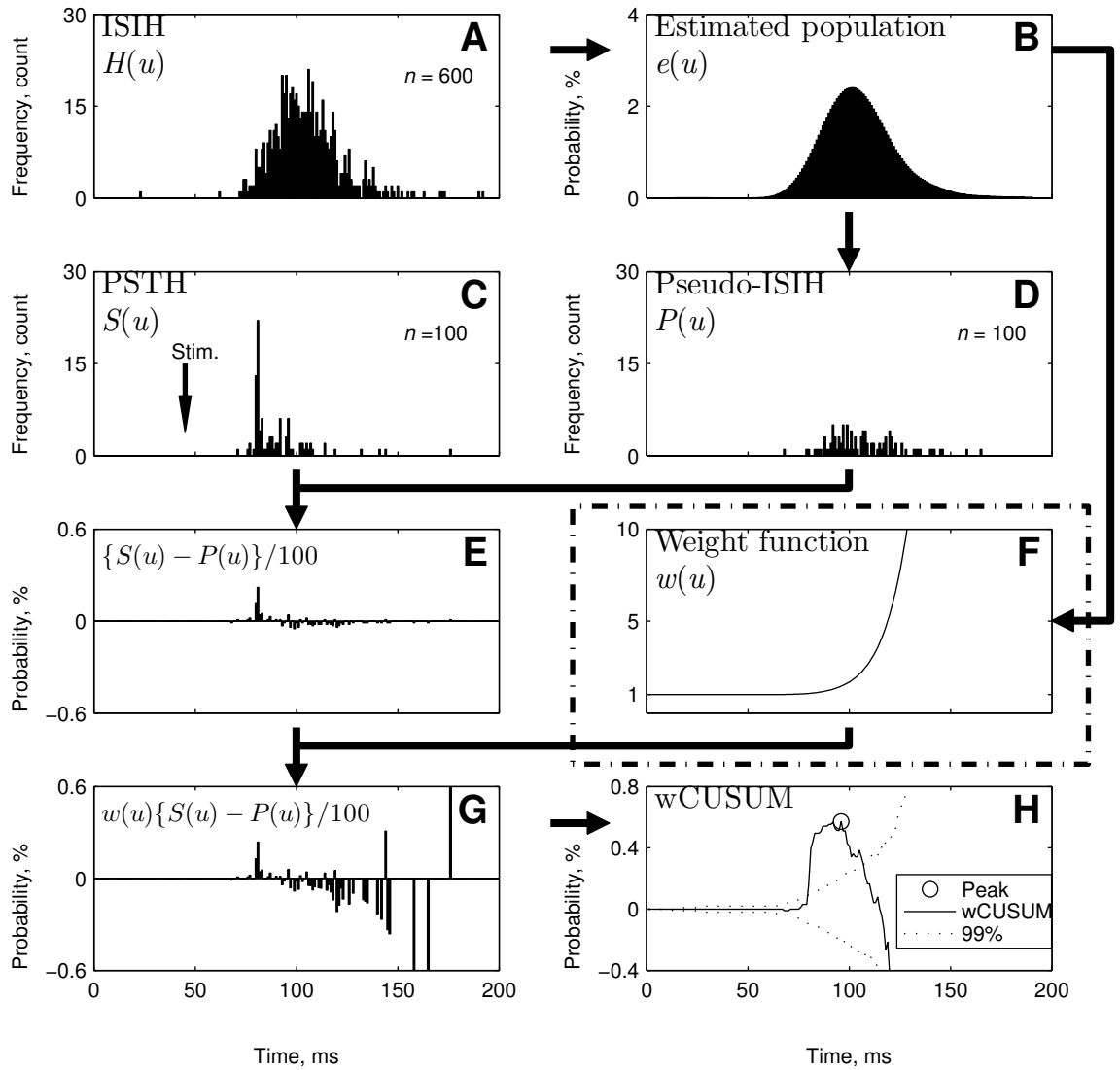

Figure 4. Scheme of the wCUSUM test. (**A-E**) These panels are the same as in Fig. 3. (**F**) Weight function derived from the estimated population. (**G**) Product of weight function and **E**. (**H**) wCUSUM curve (timewise cumulative summation of **G**; solid line). The peak value was used as a part of the estimated AHP (circle). Significance level at $p = 0.01$ is also indicated (dotted line).

We developed the wCUSUM test by modifying the CUSUM test. Our analysis is based on the fact that a discharge whose occurrence time was changed by a stimulus is expected to occur after the time point of the stimulus-induced change. To compare the PSTHs with different delay times, we have to consider the difference in their firing probability before the application time of the stimulation. In this study, we used a weight function, derived from the estimation of the background firing probability, to correct this difference.

In the wCUSUM procedure, the estimation of background firing probability at the *u*th bin (defined as $e(u)$) from an ISIH and the construction of a pseudo-ISIH using the Monte-Carlo method were conducted in the same form as the conventional method. The wCUSUM was then computed as a cumulative plot along the time course of a PSTH, $S(i)$ after subtracting a pseudo-ISIH, $P(i)$, and multiplying it with a weight function, $w(i)$, from the *i*th bin to the *u*th bin using the equation:

$$C_w(u) = \sum_{i=1}^{u} \left\{ \frac{S(i) - P(i)}{100} \times w(i) \right\} \qquad (1)$$

where: $C_w(u)$ is the wCUSUM at the *u*th bin and $w(i)$ is the weight function at the *i*th bin. $w(i)$ is given by

$$w(i) = \frac{1}{\sum_{j=i}^{200} e(j)} \qquad (2)$$

where $e(u)$ is the estimated background firing probability at *j*th and the number of 200 represents that the integration was done from the *j*th bin to the final bin.

A level of statistical significance for wCUSUM was computed by wCUSUM of two pseudo-ISIHs picked up from $e(u)$. After repeating wCUSUM procedure 100,000 times, we used the converged probability distribution of wCUSUMs to define the values of the 99th percentile of the distribution as the critical region.

The height of $C_w(u)$ represents the degree of AHP response to an electrical stimulus at a fixed interval from the previous discharge, and it is also influenced by the AHP at the interval. We hypothesized that the trend of the peaks in the wCUSUM for the delay times represents the slope of the AHP.

After the estimation of AHP in 22 sessions, we evaluated the estimated AHP by comparing its gradient with the mean firing rate of the MUP. That a high gradient of AHP causes a fast firing rate is a feature of neurons that can be generally observed in animals or computer simulations.

All analyses were carried out using custom-developed programs designed in MATLAB (Mathworks, Natick, MA, USA).

3 Results

We conducted 4–8 recording sessions per subject. The total number of triggers for the ISIHs and PSTHs ranged from 216–1200 (1200 was the limited maximum number of triggers). In 16 out of 22 sessions, the number of triggers reached the maximum (full recording). A full recording session required 5–7 min to perform. Due to accidental changes of needle position or changes in MU recruitment, recording was stopped in the middle of some sessions, but we successfully obtained a sufficient number of triggers to

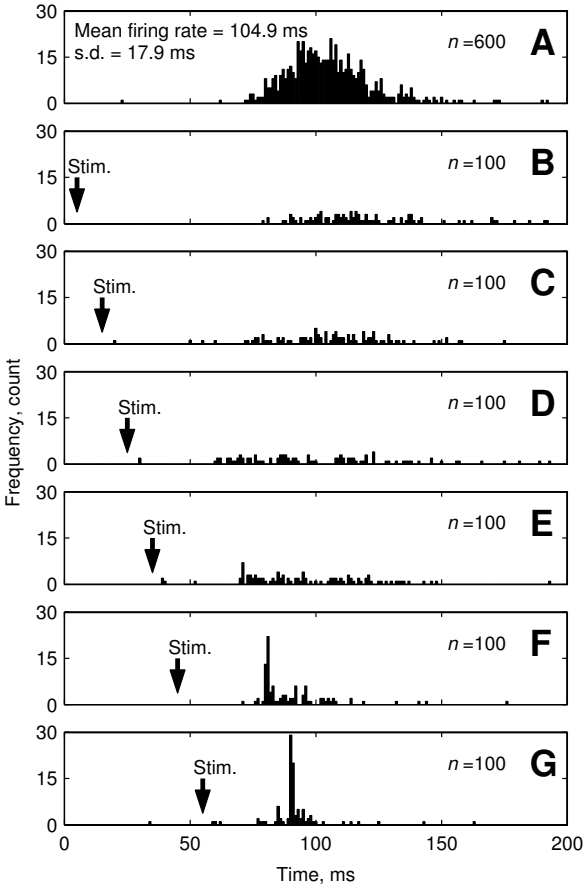

Figure 5. Delay time vs. obtained histograms. (**A**) ISIH obtained in the control situation (n = 600). (**B**–**G**) PSTHs in the test situation with an intensity of 0.90 × MT. The delay times were 5, 15, 25, 35, 45, and 55 ms, respectively.

Ergonomics of Human-System Interaction
Vol.13, No.1
February, 2011

estimate AHP in the majority of the sessions.

Changes in PSTHs with different delay times are illustrated in Fig. 5. The ISIH, which followed a Gaussian distribution, is indicated at the top of the PSTHs. An arrow in the PSTH indicates the stimulation time, and stimulation evoked sharp excitation with a latency of 30–40 ms, indicating the peak of the histogram. The peak evoked by stimulation with a delay time of 15 or 25ms was less visible than stimulation with a delay of 45 or 55 ms. Event if the peak was invisible, the CUSUM or wCUSUM tests could detect the significant effect of stimulation in the majority of cases.

Figure 6 shows the estimated AHP generated using wCUSUM. This AHP was generated from the data presented in Fig. 4 as histograms. All peak values used in the estimated AHP exceeded the significance level ($p < 0.01$). The slope of the estimated AHP was monotonically increased along with the delay time. The slope represents the AHP from 30–40 ms after the previous discharge, which was measured as a trigger, to the next discharge because a latency of 30–40 ms was required to evoke the excitation by stimulation and it is impossible to observe the response during the initial 30–40 ms.

Such a positive slope was obtained for the responses of 16 out of 22 MUs, and these data are summarized in Table 1 with the number of recorded discharges in each session. We recorded a single MU during a single session; therefore, the number of MUs can be determined from the session number. The number of discharges used to construct the ISIHs and PSTHs are also described in Table 1.

Finally, we evaluated the relationship between the mean ISI and the positive slopes when MUs generated a positive AHP ($n = 16$). The MUs were plotted in Fig. 7 and the data were analyzed using a linear least squares fit. The fit gave a correlation coefficient, r, of -0.58 ($p < 0.02$).

Table 1. Summary of recorded MU data

Subject	MU number	Number of discharges ISIH	Number of discharges PSTH	Monotonical increase
A	1	270	45	+
	2	192	32	+
	3	600	100	+
	4	600	100	+
	5	600	100	+
	6	600	100	−
	7	600	100	−
	8	600	100	+
B	9	600	100	−
	10	600	100	−
	11	600	100	+
	12	366	61	+
C	13	114	19	−
	14	600	100	−
	15	600	100	+
	16	600	100	+
	17	432	72	+
D	18	438	73	+
	19	600	100	+
	20	216	36	+
	21	108	18	+
	22	600	100	+

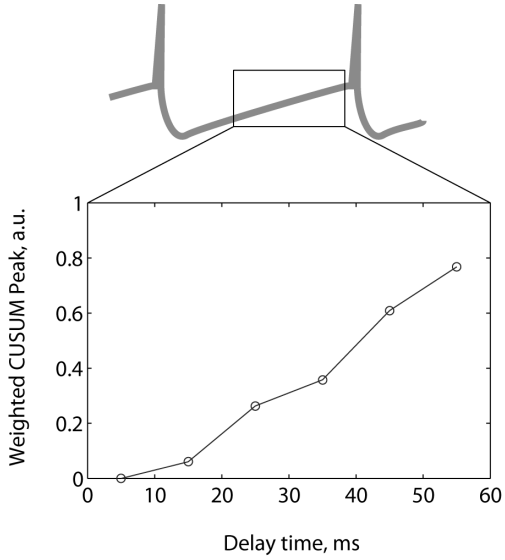

Figure 6. Estimated AHP (wCUSUM peak vs. delay time).

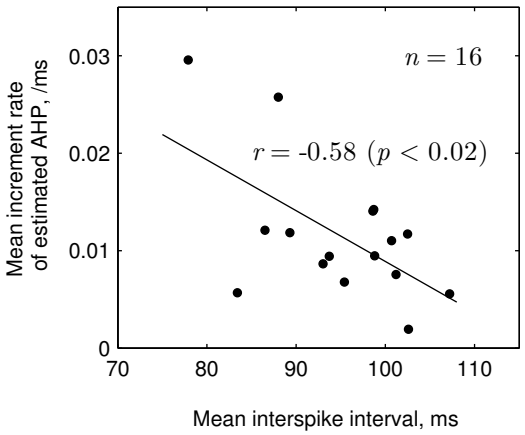

Figure 7. Mean interspike interval vs. mean increment rate (slope) of the estimated AHP. Each dot indicates single MU data ($n = 16$). The solid line is a linear least squares fit to the data.

5. Discussion

5.1. Theory and validity of the wCUSUM test

We developed a novel method, the wCUSUM test, to estimate the AHP of human alpha motoneurons. This method was based on the conventional CUSUM test. The conventional method was unsuitable for comparing PSTHs with different delay times because the delay times strongly influence the CUSUM peak values. Our wCUSUM test is based on the fact that a discharge whose occurrence time was changed by a stimulus appears after the occurrence time of the stimulus-induced change. If we try to observe the effect generating the changes of the discharge time, the discharges before the start of the effect never change their discharge time. In the comparison of PSTHs with different delay times, the number of discharges that occurred after the delay time and latency (30–40 ms) varied from PSTH to PSTH; therefore, the relationship between the CUSUM peaks and the delay time does not represent the trend of AHP. We confirmed the two following features of AHP: AHP increases with time and there is a relationship between the interspike interval and the slope of AHP.

The estimated AHP was monotonically increased in 16 of the 22 sessions. Such a positive potential slope is one of the features of AHP that receives a constant synaptic input from other neurons. During the experiment, each subject was asked to isometrically contract the TA muscle at a low force. When a subject keeps the force constant, it is assumed that the synaptic input from the cortical motor area to the alpha motoneurons is also maintained at a constant level.

Additionally, the slope of the estimated AHP was inversely correlated to the mean ISI. As indicated in Fig. 8, slower excitation of AHP resulted in a slower motoneuronal firing rate, if the synaptic input was constant. These two characteristics are natural features of neurons according to animal or simulation studies, indicating the validity of the estimated AHP.

5.2. Problems and perspectives of the wCUSUM test

The estimation of AHP in human motoneurons could reveal the underlying mechanisms of diseases with involuntary movements from the viewpoint of cell dynamics. For example, the muscles of tremor patients are periodically activated and show surface EMG bursts at a frequency of 20 Hz [8]. Conversely, single MUs that generate this 20-Hz muscular activity as a component, predominantly fire at 10 Hz [9]. This 10-Hz activity is also observed in the current study shown as a mean firing rate of 80–110 ms (see the ISIH in Fig. 5A).

In tremor patients, it is assumed that excitation by synaptic input from the cortical motor area occurs at a frequency of 20 Hz; however, this level of excitation in human motoneurons cannot be observed using direct measurements. We expect that the increase of AHP, discharging at 10 Hz at the middle time point, could be observed using our wCUSUM test.

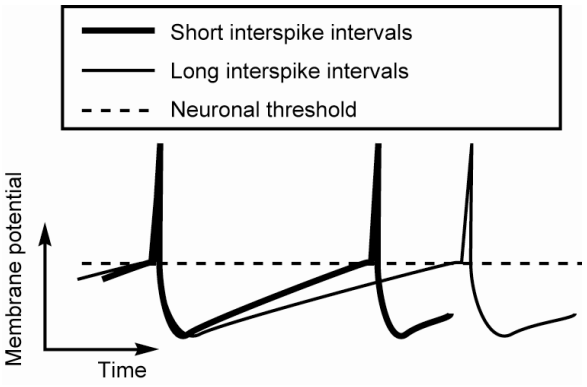

Figure 8. Spike trains with short interspike intervals and long interspike intervals.

In contrast to this expectation, there are two problems with our approach. First, the unit of the estimated AHP was arbitrary and not based on voltage. Due to this problem, a comparison between the estimated AHP and the membrane potentials acquired in animal studies is difficult. The second problem is that our method only estimates a part of motoneuronal AHP and not the whole AHP because it is impossible to observe the response during the initial 30–40 ms using PSTH. If the target motoneuron fires at 10 Hz, we could not estimate approximately 40% of the response.

We hypothesize that the incorporation of computer simulation into our estimation method is key to addressing these problems. Although computer models of membrane potential in cat or squid neurons have been established [10] [11], we will modify these models to fit human alpha motoneurons and use the model to transform the arbitrary unit into voltage and to extrapolate the unclear period of AHP. This parametric estimation method could be used to compare human alpha motoneurons in healthy subjects and tremor patients or motoneurons in animals.

In conclusion, we have developed a method, wCUSUM, to estimate membrane potential in human motoneurons using PSTHs. Using this analysis, we estimated the slope of AHP in healthy subjects and confirmed that the estimated AHP shared common features with the membrane potential of neurons.

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