NON-STATIONARY NOISE ANALYSIS AND SYNAPTIC TRANSMISSION

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Abstract

Non-stationary noise analysis is one of the most significant methods to estimate single channel conductance of receptor channels that mediate postsynaptic currents at the synapses in the central nervous system. In the present article, the principle of the peak-scaled fluctuation analysis is described in the context of synaptic transmission between neurons. Furthermore, this fluctuation analysis is applied to glutamate receptor channel in order to investigate the properties of AMPA receptor channels which underlay long-term change in synaptic strength, which is thought to be substrate of memory and learning in the brain, observed in cerebellar Purkinje cell. Whole cell patch clamp technique was used to measure the synaptic currents from Purkinje cell by parallel fiber activation. The decay phases of the postsynaptic currents were approximated by a single exponential function. The mean current and variance relationship was fitted with the parabolic curves to estimate the single channel conductance. It was suggested that no change in single channel conductance was observed before and during LTD.

Kew words: non-stationary noise analysis, glutamate receptor, single channel, Purkinje cell

1. Introduction to non-stationary fluctuation analysis

One of the most interesting and important topics in neurobiology is how the input to information in the nervous system is processed through the transfer of electrical signals from one neuron to another. In order to gain insight into this process, the elementary mechanisms of the transaction of electrical signals should be elucidated, which would then make it possible to treat the basic principle of process of information in nervous systems more systematically and logically. Fortunately, recent technical developments in neurophysiological fields, particularly single channel current recording technique, enables us to assess directly the basic principles of electrical signals from the single neuron level up to the network of neurons (Sakmann and Neher 1995; Hamill et al., 1981).

The single channel currents, which are thought to be the basic element in many nervous areas, underlie the electrical phenomena in biological cells, particularly in neurons. These currents are experimentally recorded as rectangular signals having picoampere-order amplitude and millisecond-order duration and originating from the currents which pass through pores of channel protein due to open-close gating properties. The properties of these pulse-like electrical signals are characterized mainly by three parameters, i.e., amplitudes of single channel current, open time and shut (close) time (Colquhoun and Sakmann 1985; Gibb et al. 1990). The characteristics of the macroscopic electrical signals of the cells would be described by the quantitative properties ensemble of these three principle parameters, which are directly recorded by the single channel patch clamp technique. Although this technique has numerous advantages for obtaining direct channel properties, an insufficient space of the synaptic cleft makes it difficult to bring a recording pipette close to the postsynaptic area, and the low signal-to-noise ratio of the single channel current also prevents us from separating it from the background noise. These resulted in the impossibility of obtaining single channel currents directly. Moreover, the synaptic non-NMDA channel currents during the EPSC have not been recorded directly because of their low conductance and relatively fast kinetics.

Variance analysis was subsequently developed by Robinson et al. in order to overcome these difficulties and to obtain the properties of the receptor channels in the postsynaptic membrane, which mediate synaptic transmission upon activation by neurotransmitters released from the presynaptic terminal (Sigworth 1980; Robinson et al., 1991). However their method can only be applied to simple synapses having a single release site of stable release probability and a constant number of receptors which are exposed to transmitters in each trial. The present non stationary noise analysis was developed in

order to analyze more complicated synapses which have multiple release sites and a variable number of receptors exposed to transmitters, which consequently makes it applicable to actual physiological synapses and useful in elucidating the study of plasticity of synaptic functions (Silver et al., 1992; Traynelis et al., 1993; Silver et al., 1996;).

Recently many experimental results concerning synaptic plasticity, such as LTD and LTP, from various central nervous systems have been reported. These plastic changes in synaptic efficacy are thought to originate from the transmitter release mechanisms of the presynaptic terminal and/or change in the characteristics of receptor channels in the postsynaptic membrane. Application of the present non-stationary noise analysis to the synaptic plasticity experiments for analysis may provide the information to elucidate the properties of receptor channels in the membrane of the postsynaptic site during LTP and LTD, and consequently cast light on the basic processes of the synaptic plasticity. It has been suggested that LTD of Purkinje cells in rat cerebellar cortex is due to the long-term desensitization of the AMPA receptor channel in the postsynaptic membrane of Purkinje cells via some modification, such as phosphorylation, to the channel protein from inside the cell and is not due to the transmitter release mechanisms from the presynaptic terminal. Therefore, the possible mechanisms of LTD in cerebellar Purkinje cells could be elucidated with respect to a possible change in the properties of the receptor channel, by applying the present non stationary noise analysis to synaptic currents recorded from Purkinje cells, in order to estimate the single channel current through an AMPA receptor channel in the postsynaptic membrane before and during LTD (Kojima and Ileva, 1997; Kojima et al., 1997). Comparing these two estimated values provides insight into the mechanisms at the level of the receptor channel.

2. Principle of non-stationary fluctuation analysis

2.1 Conventional non-stationary fluctuation analysis

Non-stationary noise analysis was first developed by Sigwarth in order to estimate the single channel conductance of the sodium current recorded from the node of Ranvier of the frog sciatic nerve under voltage clamp condition (Sigwarth, 1980). Then, it was further adopted by Robinson to obtain single channel conductance of the receptor channels in the postsynaptic membrane, by applying it to the synaptic currents recorded by patch clamping the cells which are in contact with presynaptic terminals. The transmitters released from the presynaptic terminal to the synaptic cleft bind to the receptor channels in the postsynaptic membrane, then activate these channels. With this activation, synaptic current would be recorded as a

waveform having two phases, the fast rising phase and the slow decay phase, corresponding to activation and to deactivation and desensitization of the channel, respectively. The decay phase of the synaptic current involves random noise caused by open-close gating properties of the channels in the postsynaptic membrane, and the variance around the mean current waveform was calculated in order to obtain the single channel current. Robinson's method is, however, limited to only simple synapses and is not applicable to more complicated ones. Thus the present non-stationary noise analysis was extended to various types of synapses, as in actual physiological preparations, by introducing some basic modifications that enable their analysis.

Since the actual biological system involves many uncertain and complicated factors, the details of the present non-stationary noise analysis were developed under several assumptions in order to simplify the actual biological system and to simplify the mathematical treatment. These assumptions are as follows,

(1) The channels in the postsynaptic membrane are homogeneous channels.

(2) These channels do not interact with each other.

(3) The open-close gating of the channel follows the Markov stochastic process. Under these hypotheses, for the synapse having a single synaptic site, upon stimulation of the presynaptic fiber, the same number of channels is always exposed to transmitters released from a single release site, as shown in Fig. 1. Total number of EPSCs, n, was obtained experimentally and each EPSC was defined as EPSCj where $j = 1, 2, \cdots, n$. Moreover, the value of EPSC at time t was $y(t)$. From the ensemble of nEPSCs, the mean EPSC, i.e., I has the value $I(t) = 1/n \sum y_j(t)$ at time t, where $y_j(t)$ is the value of each EPSCj at time t. Using these calculated values, S is expressed as

$$S = \sum(y_j(t) - k_j I(t))^2, \qquad (1)$$

where an appropriate value of k_j is chosen in order to minimize S by a prpper method such as one-dimensional downhill simplex minimization. This k_j is used as a scaling factor to define another current,

$$I_{ej}(t) = k_j I(t) . \qquad (2)$$

This $I_{ej}(t)$ is obtained by multiplying k_j with the mean current and a new current whose variance is minimum relative to the mean current. Robinson's method yields estimates of single channel conductance through the calculation of the variance as the difference between these two currents, i.e. EPSCj and $I_{ej}(t)$. Furthermore, this calculation was evaluated on a micro-scale by introducing the processes of channel kinetics; that is, the opening of a channel is probabilistic, so that after the initiation of an EPSC, each channel has a certain

probability of being in the conducting state, $P(t)$. The expected current can therefore be written as $NiP(t)$, where N is the number of functional channels at the postsynaptic site, i.e., the number of channels from which open channels can be stochastically chosen, and i is the single channel current amplitude. The variation in peak amplitudes of recorded EPSCs is evidently due to the variation of $P(t)$ (presumably due to varying transmitter release), but is effectively without variation in its normalized time course. The time course of the averaged EPSC is given by $I(t) = NiP(t)$, where $P(t)$ is defined as the time course of open probability averaged across a collection of a data set of EPSCs. Therefore, the scaled open probability of a particular EPSC in a data set is obtained as $P_j(t) = k_j P(t)$, so that the time course of the expected value of current for that scaled EPSC obtained by the least squares method is given by

$$k_j I(t) = k_j NiP(t) = Nik_j P(t) = NiP_j(t). \quad (3)$$

Then, set

$$I_{ej}(t) = k_j I(t) = NiP_j(t), \quad (4)$$

and the relationship between $I_{ej}(t)$ and variance $\sigma_j^2(t)$ is calculated using [Hille p321 ref]

$$\sigma_j^2(t) = NP_j(t)\left[1 - P_j(t)\right]i^2 + \sigma_B^2 \quad (5)$$

With both this relation and

$$I_{ej}(t) = NiP_j(t),$$

$P_j(t)$ can be eliminated. Thus the relationship $I_{ej}(t)$ and its variance is given as

$$\sigma_j^2(t) = i\, I_{ej}(t) - \frac{\left(I_{ej}(t)\right)^2}{N} + \sigma_B^2 \quad (6)$$

This is the relationship between averaged value of EPSC and the variance around it, where σ_B^2 is the variance of background noise. This formula has a parabolic function regarding $\sigma_j^2(t)$ and $I_{ej}(t)$ which could be fitted with a parabolic curve having two parameters corresponding to the single channel current (i) and the number of channels (N) in the postsynptic membrane. From the calculated single channel current, the single channel conductance (γ) is given by

$$\gamma = i/(E - E_R) \quad (7)$$

where E and E_R are the membrane potential and the reversal potential of the channel in the postsynaptic membrane, respectively.

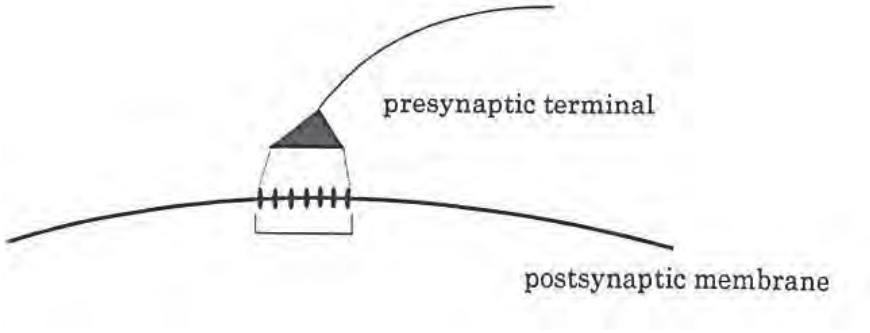
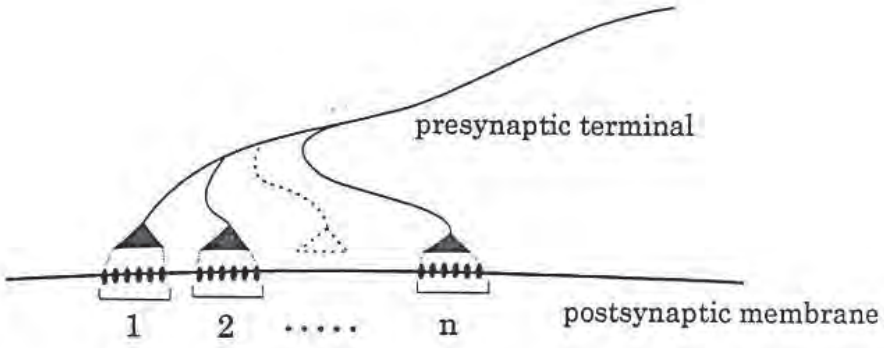


Fig. 1 A Single synaptic contact

Schematic representation of synapse having a single synaptic site is shown in the figure. When a single presynaptic fiber (for example fiber of motor neuron in the spinal cord) is activated, the same number of receptor channels are always exposed to the neurotransmitter molecules that are released from single release site.

A



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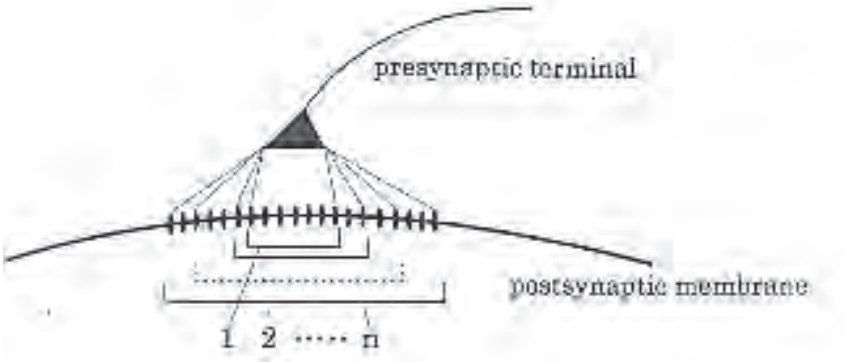


Fig. 2 Multiple synaptic contacts (A)

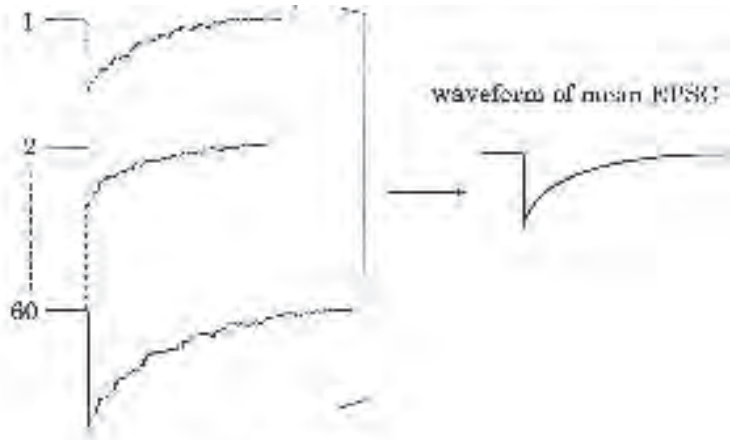
A. *The synapse having multiples release sites having is shown. The number of channels exposed to the transmitters released from each synaptic site are different each other.*

B. The synapse having a single release site but upon the stimulation of presynaptic fiber, the number of channels in the postsynaptic membrane exposed to transmitters varies from trial to trial , even if they are released from a single release site.

2.2 Present non-stationary fluctuation analysis

As previously shown, Robinson's method could be applied to a simple synapse having a single release site and a constant number of receptors exposed to transmitters. To evaluate this method in order to apply actual more complicated synaptic structures having multiple release sites, as shown in Fig. 2 A, the numbers of channels activated by transmitters released from each synaptic site are different from each other. Moreover, the present variance analysis was extended to such cases as when the number of channels in the postsynapic membrane exposed to transmitters varies from trial to trial, even if they are released from a single release site (Fig. 2 B). Therefore, the present method could be more useful method to study the mechanisms of synaptic transmission in actual physiological preparations, compared with the variance analysis described previously. In order to use variance analysis for estimating the conductance of the underlying channels which mediate the synaptic transmission, the current fluctuation associated with stochastic channel gating properties must be isolated from those that result from other factors. This can be accomplished by scaling the mean EPSC waveform to individual EPSCs and then subtracting this scaled waveform from the EPSC. Robinson's analysis employes a procedure that uses a least-squares-fitting method for scaling the mean waveform to the individual EPSCs, in order to isolate the current fluctuations due to channel gating. However, they clearly demonstrate that the minimization of the least squares difference between EPSC and the mean waveform inevitably leads to underestimation of the variance for EPSCs. Thus the underestimation in the variance of most EPSCs can lead to a considerable underestimation of single channel conductance. We therefore adopted an alternative scaling method in which the peak of the mean waveform is scaled directly to the peak amplitude of each EPSC. This approach is directly amenable to a formal theoretical treatment

A



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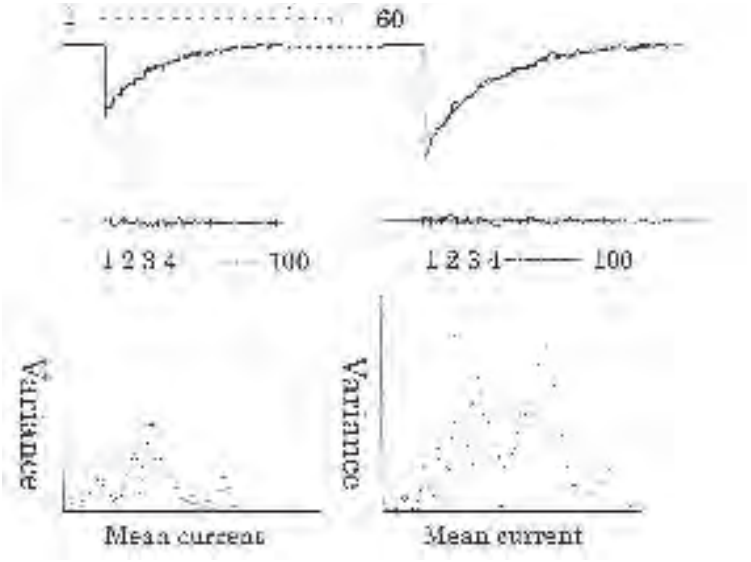


Fig. 3 Analysis process of the present variance analysis method

A. 60 EPSCs were recorded from the soma of a neuron under voltage clamp condition. The mean waveform having a single exponential decay phase was obtained.

B. The variance was calculated by subtracting the mean EPSC waveform, which was scaled up to the peak of each EPSC, from an individual EPSCs. The decy phase of EPSC was fitted with a single exponential function having time constant τ and the current-variance relationship for the decay of EPSCs was calculated by dividing the decay phase into 100 sections on the basis of an equal fractional reduction in amplitudes.

As previously described, the mean EPSC is given by $I(t) = 1/\mathrm{n}\sum y_j(t)$. When the

number of channels exposed to transmitters, (N_j) varies depending on each EPSC due to the conditions explained above and shown in Fig. 2, the current value at time t of each EPSC is given by

$$\mathrm{I}_j(t) = N_j i P_j(t) \quad (8)$$

$P_j(t)$ is the probability of a channel being open at time t.

The scaling factor k_j is defined as

$$k_j = N_j i P_j(t)/NP(t) \quad (9)$$

and the peak-scaled EPSC is given as

$$I_{ej}(t) = k_j I(t) = k_j NP_j(t)i = N_j P_j(t)i \quad (10)$$

where $NP(t) = 1/\mathrm{n}\sum N_j P_j(t)$ and according to Hille and Sigwarth,

$$\sigma_j^2(t) = N_j P_j(t)\left[1 - P_j(t)\right]i^2 + \sigma_B^2 \quad (11)$$

The relationship between $I_{ej}(t)$ and $\sigma_j^2(t)$ is then

$$\sigma_j^2(t) = iI_{ej}(t) - \frac{\left[I_{ej}(t)\right]^2}{N_j} + \sigma_B^2 \quad (12)$$

In order to describe the present analysis separately from Robinson's, the following expression is used:

$$\sigma_{\mathrm{p-s}}^2(t) = iI_{ej}(t) - \frac{\left[I_{ej}(t)\right]^2}{N_p} + \sigma_B^2 \quad (13)$$

where $\sigma_{\mathrm{p-s}}^2(t)$ is a peak-scaled variance and N_p is the number of channels exposed to transmitters. The peak value of each EPSC is given by $N_j P_j(t_p)i$, i.e., the number of channels in the open state at the peak of EPSC multiplied by the single channel current. If the probability of the channel being in the open state is high, $P_j(t_p)$ is nearly equal to 1 and then $N_j P_j(t_p)$ is almost equal to the number of channels exposed to transmitters at the postsynaptic site. The present method is different from Robinson's with regard to the scaling of the mean waveform of EPSC up to the peak of each EPSC, and has various advantages. If different conductance levels contribute to the current, then the conductance value obtained with this technique will correspond to a weighted average of all the conductance levels present. Furthermore, the conductance estimate obtained using this method is relatively insensitive to series resistance/cell capacitance filtering and asynchronous or sustained transmitter release.

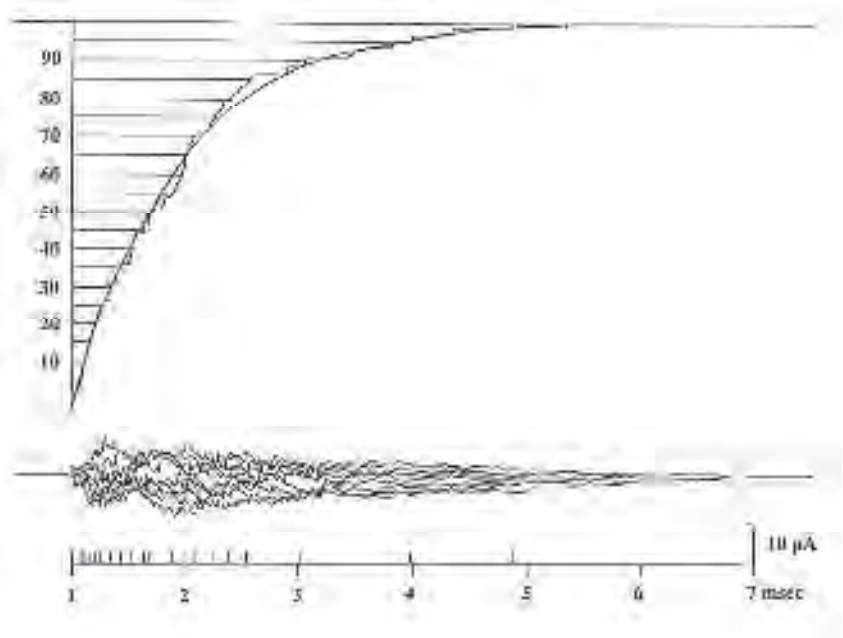
The diagram of the present variance analysis method is presented in a step-by-step fashion in Fig. 3 in order to make it clear to understand the analytical procedure. Upon stimulation of presynaptic fibers, around 60 EPSCs were recorded from the soma of a neuron under the voltage clamp condition. With these 60 EPSCs, the mean waveform having a single exponential decay phase was obtained, as shown in Fig. 3 A. The variances were calculated by subtracting the mean EPSC waveform, which was scaled up to the peak

of each EPSC, from an individual EPSCs (Fig. 3 B). The mean waveform of EPSC was aligned to each EPSC at the point where the slope of the rising phase was steepest. The decay phase of EPSC was fitted with a single exponential function having time constant τ and the current-variance relationship for the decay of EPSCs was calculated by dividing the decay phase (10×τ decay) into 100 sections on the basis of an equal fractional reduction in amplitude. The mean current for each section was calculated, as well as the sum of squares difference between the measured and peak-scaled mean waveform at each data point. For some analyses, the variance of each section of a given EPSC was normalized to the event amplitude. No significant difference was found in the ratio of mean conductances determined from the absolute and normalized variances, which is consistent with no variation in the channel conductance with changing EPSC amplitude (figure is not shown). The baseline variance was measured from the post-event current and subtracted from the variance associated with each section. The single channel current was calculated using the theoretical relationship for the peak-scaled variance that is shown here in order to investigate the properties of receptor channels, especially single channel conductance that mediates the postsynaptic currents at the synapses.

3. Estimate of single channel conductance of computer generated EPSCs

The present variance analysis was applied to computer-simulated EPSCs involving channel kinetic processes in the decay phase to estimate single channel conductance. The computer-generated EPSCs, with various amplitudes, were due to the variation in the amount of transmitters released from the synaptic terminal and /or the number of functional channels in the membrane of the postsynaptic site, as well as to random open close-gating properties of channels (Fig. 4 A). From these computer generated miniature EPSCs, the averaged waveform of EPSC was found to have a single-exponential decay time course. The mean waveform was then scaled up to the peak value of each EPSC by aligning the steepest points of the rising phase. Variance was measured by subtracting the mean waveform from individual EPSCs and dividing the decay phase (10×τ decay) into 100 sections on the basis of equal fractional reduction in amplitude. The base-line noise was calculated from post-event base currents and subtracted from each variance. Every 10 sections, from 1 to 100, are labeled at the left of the rising phase of EPSC in the figure

A



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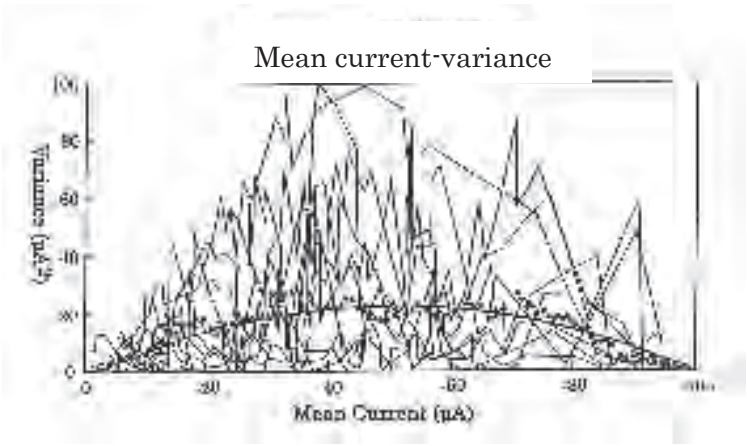

Fig. 4 Processes of non-stationary noise analysis

A. *The average waveform of EPSC having a single-exponential decay time course. Variance was measured by subtracting the mean waveform from individual EPSCs and dividing the decay phase into 100 sections on the basis of equal fractional reduction in amplitude. Every 10 sections, from 1 to 10, is labeled at the left of the rising phase of EPSC.*

B. *The relationship between the mean current and variance of 20 mEPSCs. The data points were approximated by a parabolic function.*

20 mEPSCs were analyzed with this protocol and the relationship between the mean current and variance were plotted for each mEPSC. These relationships were also

averaged with regard to the mean current and variance, and the averaged mean current and variance of 20 mEPSCs shown in Fig. 4 B. In this figure, thin lines and thick dots represent the relationships between the mean currents and variances of 20 mEPSCs and the averaged of these 20 thin lines, respectively. The thick dots were fitted with the parabolic function having the form of equation (13), when $y = \sigma^2_{\mathrm{p-s}}(t)$, $x = I_{ej}(t)$, and b = σ^2_B, the next equation is obtained.

$$y = -(1/N_p)x[x - (N_p)i] + \mathrm{b} \qquad (14)$$

The thick dots were fitted with this parabolic function, whose form is $y = f(x)$, by the least squares method, and from the fitted curve, represented by the thick line, the single channel current was found to be 0.924 pA. Fig. 4 B can produce the relationship between normalized variance and mean currents using the same data (the figure is not shown). No significant difference is found in the ratio of mean conductances determined from the absolute and normalized variances, which is consistent with no variation in channel conductance with changing EPSC amplitude.

4. Application of non-stationary flucutation analysis to synaptic plasticity (LTD)

The present non-stationary noise analysis was applied to the synapses in several regions of the central nervous system in order to elucidate the mechanisms of long-term synaptic plasticity. Here, it is shown, as an example, to one of the most significant synaptic plasticities in the central nervous system, i.e., long-term depression (LTD) at the synapse between parallel fibers and Purkinje cells in the cerebellar cortex (Ito et al., 1982).

Since Katz's formalization of the quantal theory of synaptic transmission, a central problem of the physiology of the synapse has been to identify the morphological and molecular correlates of the three key variables that characterize quantal neurotransmitter release which have been already mentioned previously in the case of NMJ; the number of release sites (N), the probability of a quantal release (p), and the size of the quantal response (q). At the frog NMJ, N and p are both large under natural conditions which means that at the physiological concentrations of Ca^{2+} and Mg^{2+} are involved [35]. The squid giant synapse (SGS) has been also studied intensively as the presynaptic terminal is exceptionally large and it is possible to insert several electrodes directly into this presynaptic terminal and simultaneously monitor the responses from the postsynaptic neuron and voltage of the presynaptic terminal. At this synapse (SGS), N and p are both very large to release sufficient neurotransmitter for the quick movement of fins to escape from sudden dangers. Furthermore, many types of synapses in the mammalian brain are

also investigated such as calyx of Held, climbing fiber-Purkinje cell synapse, are similar to the frog NMJ which have high$-N$ and $-p$ synapses. On the other hand, many synapses receive a single synaptic contact from other cells, such as CA3 Schaffer collateral and CA1 pyramidal cells [49, 50, 51] in the hippocampus and granule cells parallel fiber -Purkinje cells in the cerebellum.

To make it easy to understand the synaptic function with regard to its regulation and plasticity, the cerebellar Purkinje cell, with which other cerebellar neurons make synaptic contact, is focused on. Fig. 5 and Fig. 6 show the anatomical organization of the cerebellar cortex and its neural circuit, respectively. Fig. 6 shows the typical three neurons (Purkinje cell, granule cell and Golgi cell) of all five neurons (other two; satellite cell and basket cell). The Purkinje cell receives two major excitatory inputs from climbing fiber, and granule cell of which axon is parallel fiber. It is reported that the climbing fiber-Purkinje cell synapse is strong enough to generate action potentials (complex spike) of which firing frequency is a few Hz and that are generated by Ca^{2+} and Na^{+} influx, whereas parallel fibers-Purkinje cell synapse causes small but more frequent synaptic response (50~60 Hz in average) and also induces synaptic plasticity including long-term depression (LTD), one of the significant synaptic plasticity observed in cerebellum. This LTD is suggested to be mediated by AMPA receptors channels but not by MNDA receptor channels and to play a significant role in motor learning conducted by cerebellum.

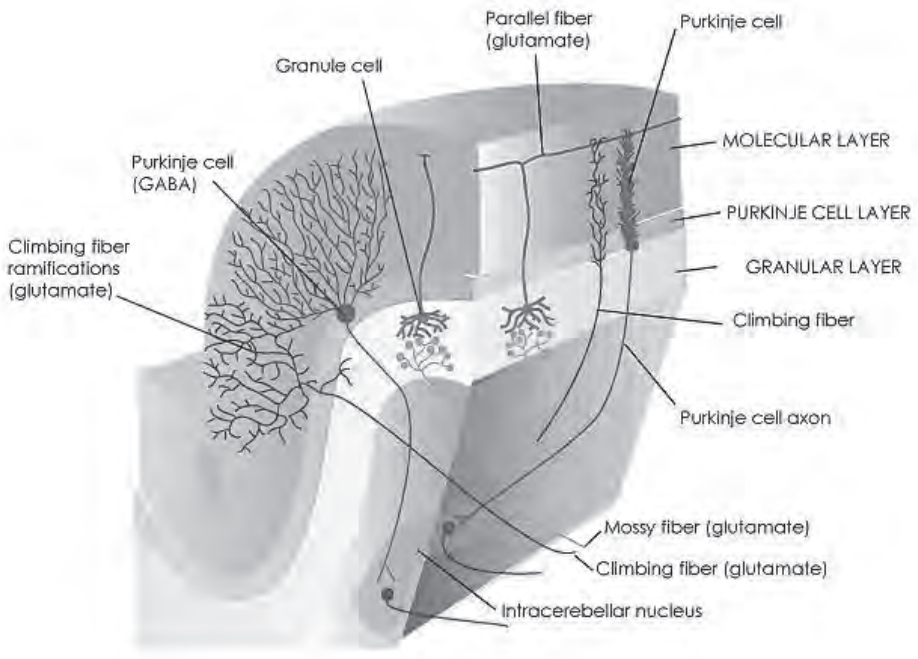

Fig. 5 Anatomical structure of the cerebellum cortex

Cerebellar cortex is composed of three layers, from the surface of the cortex, molecular layer, Purkinje cell layer and granular layer. In the molecular layer, the dendritic trees of Purkinje cells are extended and the parallel fibers have synaptic contact with the dendrite of Purkinje cell. The satellite cells are involved in this layer. Purkinje cell layer is the ensemble of the somata of Purkinje cells from where the axons of the cells extend into the intracerebellar nucleus. In granular layer there are many granule cells with which mossy fibers have synaptic contact.

In the cerebellum, information is generally conveyed continuously by the action potentials propagating along the parallel fibers contacting to Purkinje cell. On the other hand, the occasional strong input through climbing fiber to Purkinje cell generates a strong prolonged Ca^{2+} depolarization (complex spike) in the dendrite. The Ca^{2+} entered into the cell induces the biochemical reactions that produce second messengers to change the state of cell and, in some cases, to generate slow synaptic potentials. This input by climbing fiber to Purkinje cell modifies the synaptic efficacy between parallel fiber-Purkinje cells such as LTD.

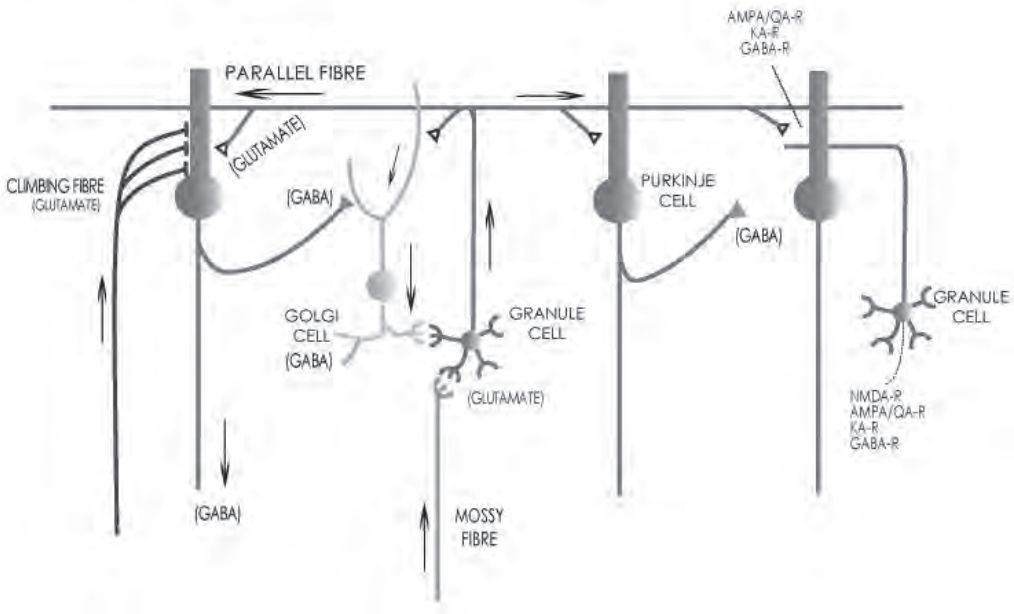

Fig. 6 Neural circuit of cerebellum cortex

The structure of the cerebellar cortex composed of three layers from bottom to top: granular cell layer, Purkinje cell layer and molecular layer where the dendrites of Purkinje cells extend toward the surface of cortex. A Purkinje cell receives two excitatory inputs; one is the axon of granule cell in the granular cell layer and another is climbing fiber from inferior olive. The sole output of cerebellar cortex is the axon of Purkinje cell that contacts to neuron of deep cerebellar nuclei. This synapse is mediated by

GABA receptor and inhibitory synapse in their nature. Other significant neurons in the cerebellar cortex are Golgi cell, satellite cell and basket cell; all of them are inhibitory interneurons. One Purkinje cell receives more than 10,000 synaptic contacts from parallel fibers of which soma are granule cells and one climbing fiber where multiple presynaptic boutons are attached to the soma and proximal dendrite of Purkinje cell. Information flows continuously from Mossy fiber to Purkinje cell via granule cell toward cerebellar nuclei and occasionally the information conveyed from climbing fiber generates the complex spikes at the Purkinje cell.

In Figure 7 and 8 schematically represent the two of the functional hypothesis of cerebellar cortex that have been already proposed on the basis of available physiological and morphological knowledge together with theoretical investigations. David Marr (1945~1980) in University of Cambridge postulated a theoretical model that predicted the change in synaptic efficacy between parallel fiber-Purkinje cell (long-term potentiation) and indicated that the function of cerebellar cortex is equivalent to that of a simple perceptron of which function is the leaning machine. Fig. 7 shows the schematic representation of the perceptron model composed of three cell layers (mossy fiber, granule cell layer, Purkinje cell layer) of cerebellar cortex. This hypothesis was later reevaluated and corrected that the synaptic plasticity is not long-term potentiation but long-term depression by James S. Albus (1935~2011) in MIT in the USA [55]. Finally, long-term change in synaptic efficacy was confirmed in the rabbit cerebellum by Masao Ito's group by *in vivo* electrophysiological experiment and at present it is called Marr-Albus-Ito theory of cerebellum.

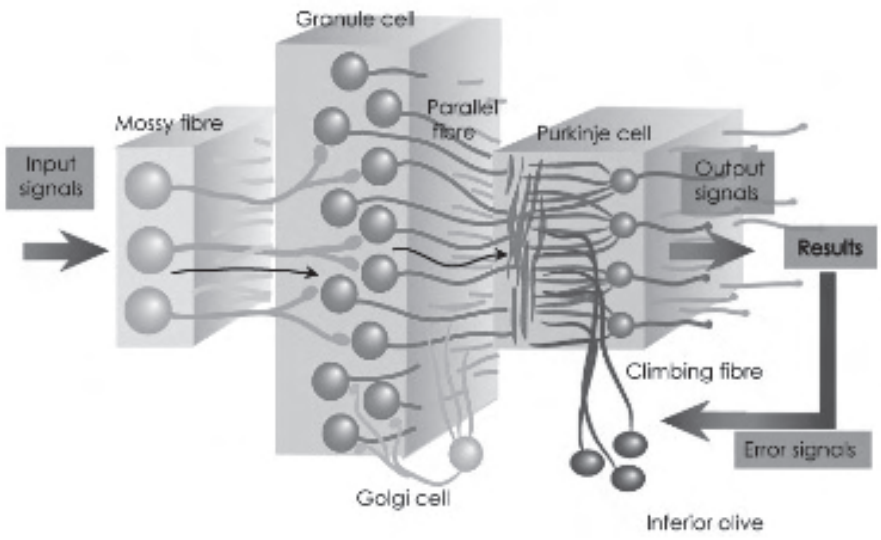

Fig. 7 Perceptron model of cerebellar cortex

The simple perceptron model composed of three layers; mossy fiber layer, granule cell layer and Purkinje cell layer. The synaptic efficacy between granule cell (parallel fiber) and the dendrite of Purkinje cell are modified according to the synaptic input from the neuron in the inferior olive of which axon is climbing fiber. A pattern that should be leant by the cortex is presented to granule cell layer vis mossy fiber and the output pattern from the perceptron is given from the signals of Purkinje cell axon. If the output pattern is not correct, the error signal is conveyed by climbing fiber in order to modify the synaptic strength between parallel fiber and Purkinje cell dendrite. The model was first proposed by David Marr then modified by Albus, finally confirmed experimentally by Ito. [modified from M. Ito, In. Physiology of Neuron, Iwanami press (1972)]

The present LTD is shown to be one of the most significant synaptic functions performed by cerebellum and not only its physiological investigations but also the molecular biological mechanisms have been studied since its discovery in 1982. One of the most significant functional models postulated by Ito who conducted the elaborated experiments is Vestibulo–Ocular-Reflex (VOR) in flocculus (Fig. 8) of cerebellar cortex.

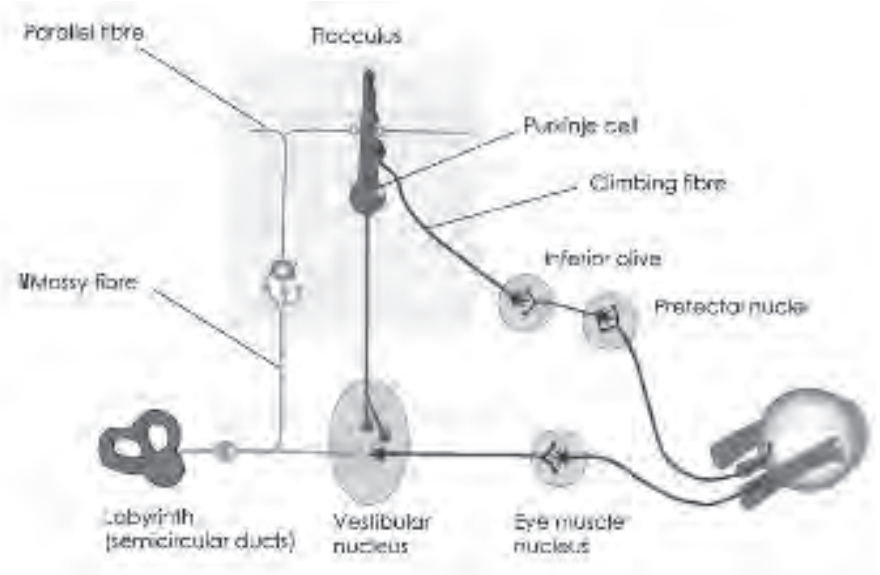

Fig. 8 Neural circuit of vestibule-ocular reflex (VOR)

The neural connection of vestibule-ocular-reflex (VOR) in which the eye movement is controlled with the motoneurones innervating eye muscles in order to coincident with the head movement (horizontal rotation) that is detected by labyrinth in the ear. The signals detecting head position from the labyrinth are sent to the vestibular nucleus in which these signals are transmitted to a set of neurons controlling eye muscles, at the same time, these signals are bypassed to Purkinje cell through parallel fiber. The synaptic strength between parallel fiber and Purkinje cell is modified by the occasional

climbing fiber inputs that correspond to error signals or instructions from retina. The instruction signals change the synaptic strength and induce plasticity (long-term depression; LTP) that reduce the inhibition of Purkinje cell output to vestibular nucleus resulting to change the signals from labyrinth to the motoneurones. Then the eye movement is changed to coincident with the head movement by the modified signals to muscle motoneurones. The neural circuit is thought to be located in the flocculus in cerebellum. [modified from M. Ito, In. Physiology of Neuron, Iwanami press (1972)]

The cerebellar cortex is an unique anatomical structure composed of only five neurons with two excitatory inputs (Mossy fiber and Climbing fiber) and one inhibitory output (Purkinje cell axon to deep cerebellar nuclei). The neural circuit by these five neurons is conserved at any sagittal section of the whole cerebellar cortex and thus varieties of functional models have been proposed by not only physiologist but also theoretical neuroscientist who tried to investigate its function theoretically. However, the detailed functional meaning remains elusive in spite of their intensive efforts.

The LTD of EPSCs by parallel-fibers stimulation was recorded from the somata of Purkinje cells using the whole cell patch clamp technique by conjunctive stimulation of parallel and climbing fibers (Konnerth et al., 1992). The sustained reduction of the amplitudes of EPSCs was thought to be due to possible long-term desensitization or other processes of AMPA receptor channels (one type of glutamate receptor channel) in the postsynaptic site of Purkinje cells by some modification of the channel protein, such as phosphorylation from inside of the cell triggered mainly by an elevation of the internal Ca^{2+} ion concentration (Linden et al., 1995). A presynaptic effect observed in long-term potentiation (LTP) in other region of the central nervous system, for example, a change in the probability and the amount of transmitter molecules released from presynaptic terminal, was not suggested to be involved in the LTD in the cerebellar parallel fiber-Purkinje cell synapse (Hirano, 1991; Ito et al., 1982). Therefore, the reduction in amplitudes of EPSCs caused by alternation of the AMPA receptor channel could be explained mainly by three possible hypotheses as elementary mechanisms of LTD, i.e.,

(1) A reduction of single channel conductance of the APMA receptor channel in the postsynaptic membrane of Purkinje cell,

(2) A change in the kinetics of AMPA receptor channels such as reduction in mean open time, burst length and open probability of channel,

(3) A change in the number of functional receptor channels in the membrane of the postsynaptic site.

In order to test hypothesis No.1, the present non-stationary noise analysis (n-SNA) was applied to estimate the single

channel conductances which mediate synaptic transmission between a parallel fiber and a Purkinje cell, and to compare the estimated single channel conductances before and during LTD. With this comparison, one can guess a possible contribution of the reduction of the AMPA receptor single channel conductance to LTD. This method has been already applied to the study of the mechanisms of long-term potentiation (LTP) in hippocampus to estimate the single channel conductances before LTP and during LTP

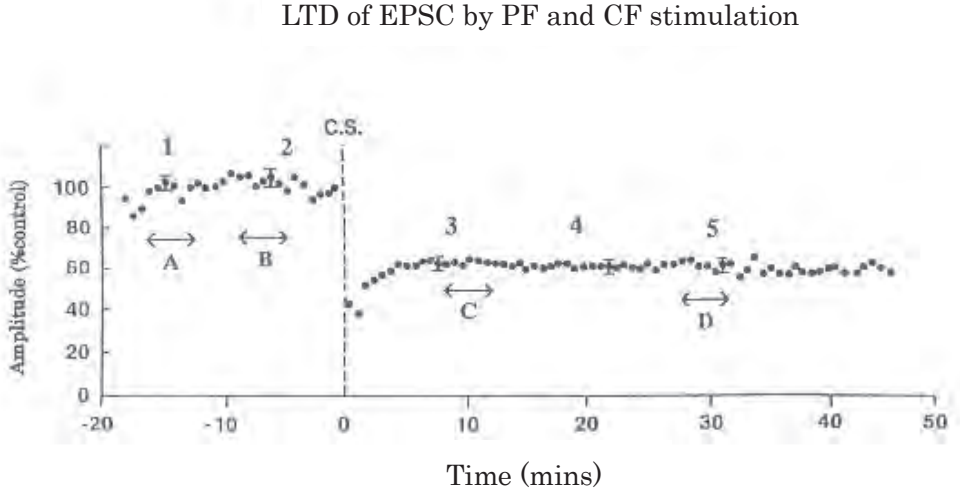

Fig. 9 An example of LTD

A typical example of LTD induced by conjunctive stimulation of the parallel and climbing fibers for 5 mins at a frequency of 1 Hz (300 pulses). The amplitudes of EPSCs decreased to around 60 % of those of control EPSCs. The time course of EPSCs picked at the points indicated (1, 2, 3, 4, 5) were compared. The present variance analysis applied to the periods indicated in the figure which correspond to before (A, B) and during (C, D) LTD. No significant difference between the value of single channel conductances was identified before and during LTD. The values of single channel conductances during A, B, C, and D are 25.73, 23. 82, 22.93, and 23.81, respectively.

The EPSCs were recorded from somata of Purkinje cells identified using infrared DIC video microscopy by patch clamping the cell in a 200-μm-thick slice preparation of Wistar rat (10 -13 days old) which was continuously perfused by normal Ringer's solution containing 20 μM bicuculine for inhibition of miniature inhibitory synaptic currents (Edwards et al., 1989; Kojima et al. 1998). Synaptic currents were filtered 10 kHz using 8-pole Bessel type-filter, and then sampled at 40 kHz using an A-D converter. In Fig. 9, an example of LTD induced by conjunctive stimulation of parallel and climbing fibers for 5mins at a frequency of 1 Hz (300 pulses) shows that

the amplitudes of EPSCs decreased to 60 % of those of control EPSCs (65.5 + 10.8 % [n = 10]). Each EPSC recorded before and after the C. S. was fitted with double exponential functions (rising phase and decay phase, respectively) by the least squares fitting method (Llano et al., 1991). On comparing the time constants of these exponential functions before and during LTD, no change in the time course of the EPSCs was observed following LTD (Fig. 10 A and Fig. 10 B), suggesting that the contribution of a change in the kinetics of the AMPA receptor channel to the reduction of EPSC amplitudes is small (Jonas and Spruston, 1994; Edmonds et al., 1995).

A B

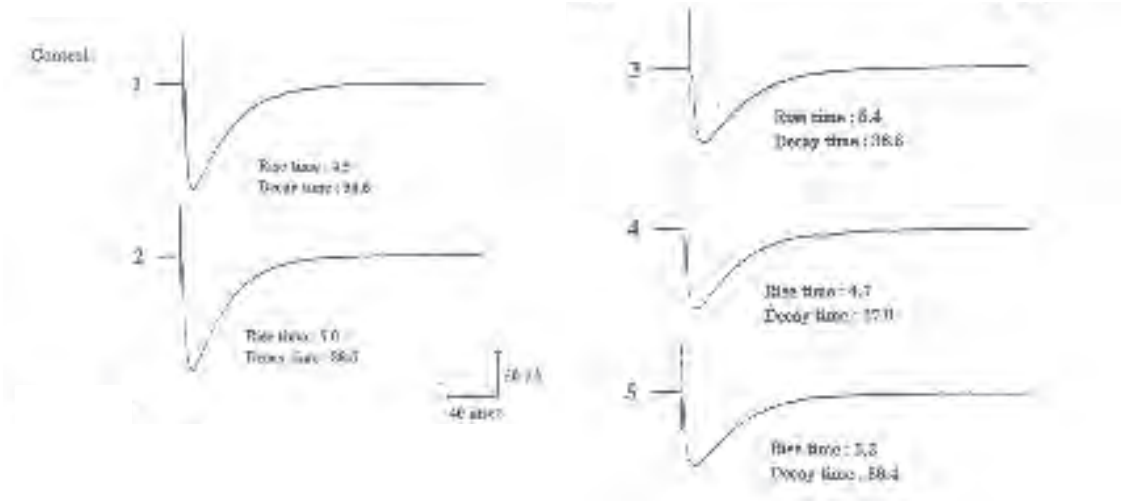

Fig. 10 A, B. EPSC wave forms of LTD

A. Time course of each EPSCs picked up a the points shown in the Fig. 54 corresponding to the periodsbefore LTD. EPSC1 and EPSC2 were approximated with two expenential functions having time constants of 4.8, 34.5 msec and 5.0, 36.5 msec, respectively.

B. EPSC3, EPSC4 and EPSC5 were picked up during LTD and of which time constants were 5.4, 36.8 msec, 4.7, 37.0 msec and 5.3, 36.4 msec, respectively when they were fitted with two exponential functions, whereas the amplitudes of EPSCs were reduced to 60 % of those of in A. No meaning ful change in time course of EPSCs was observed before and during LTD.

The single channel conductances estimated by applying n-SNA to the decay phases of EPSCs corresponding to the periods indicated in Fig. 9 were A: 25.73, B: 23.82, C: 22.93, and D: 23.81 pS. These values were obtained using the relationship between mean currents and variances calculated for 60 EPSCs by subtracting the peak-scaled mean wave form from each EPSC. The periods A and B were calculated from the EPSCs taken before C.S., and C and D were calculated from those obtained during LTD. On comparing the values of these two groups (A, B and C, D), there is

no difference in the estimated single channel conductances of AMPA receptor channels in the postsynaptic membrane. Therefore the present results suggest that the reduction of single channel conductances during LTD may not occur and the decrease of EPSC amplitude of s could be caused the two other possibilities, i.e., : (2) a change in the kinetics of the AMPA receptor channel and (3) a decrease in the number of AMPA receptor channels in the postsynaptic membrane.

5. Change in kinetics of AMPA receptor channel during LTD

One of the two possibilities, No.3, has already been examined briefly in the comparison of the time course of EPSCs before and during LTD. The time course of EPSCs was fitted by two exponential functions, a single exponential one for the rising phase with the time constant 2.21 + 0.18 msec (SD, n = 10), and another single exponential one for the decay phase with a longer time constant of 10.68 + 0.08 msec (SD, n = 10). The waveform of EPSCs could still be fitted with two exponential functions independent of the membrane potential. The rising phase of EPSC has a strong relationship with the transmitter binding to the receptor and the activation of the channels. Although several complicated factors are involved in determining the decay phase of the synaptic current, the previous results partly suggested that the desensitization and deactivation of AMPA receptor channels are not affected during LTD (Edmonds et al., 1995). Moreover, detailed analysis should be carried out by constructing the simulated EPSC based on the kinetics model of AMPA receptor channels, which was obtained from the experiment of single channel current recording.

6. The problem for using non-stationary flucutation analysis

It is suggested in the present article that the present non-stationary noise analysis provides a useful tool for estimating the single channel conductance which underlies synaptic transmission mediated by synaptic currents of the central nervous system. Since it is impossible to record the single channel current due to the narrow space of the synaptic cleft into which the recording pipette cannot reach, there have been a number of attempts to determine indirectly the biophysical properties of the synaptic channels by means of sustained or rapid application of agonist onto somatic membrane patches that contain receptor channels. The present non-stationary noise analysis provides an estimate of the single channel conductance that mediates fast synaptic transmission. Although this method is insufficient to completely determine the properties of receptor channels in synaptic transmission, it allows the estimation the single channel conductance, and consequently the characterization of the change in the

properties of synaptic transmission during the formation and development of synapses. Moreover, it sheds light on the basic mechanisms of receptor channels which are relevant to synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), the basic processes which are thought to be partly due to the properties of transmitter release from presynaptic terminals, and alternatively due to modification of receptor channels in the postsynaptic membrane. In particular, long-term depression in Purkinje cells of the cerebellar cortex was examined in order to elucidate the basic mechanisms of glutamate receptor channels in the postsynaptic membrane, since the results of the previous experiments suggest that transmitter release mechanisms from the presynaptic terminals are not involved and that long-term desensitization of glutamate receptor channels causes the reduction of EPSCs during LTD. The results of the application of non-stationary noise analysis to cerebellar LTD indicate that no change in the estimated single channel conductance which mediates the synaptic transmission between the parallel fibers to Purkinje cells occurs between before conjunctive stimulation and during LTD.

Since there is a possibility of the filtering effect to the electrical signals by dendritic trees and soma of Purkinje cell and, thus the high frequency components of these electrical signals recorded at soma could be lost from the original signals. As the result, the values of the estimated single channel conductances calculated from EPSCs by patch clamping somata of cells were lower than those of single channel conductances which should be obtained just under the synaptic site.

In order to assess the possible filtering effect of the dendritic trees and soma, the rise times and peak values of the EPSCs were plotted and the correlation between these two values was examined. Fig. 11 shows that no meaningful relationship between these two values exists, so that the filtering effect of the cell was negligible and that the waveform of EPSCs recorded at the soma was neither attenuated in amplitude nor considerably influenced.

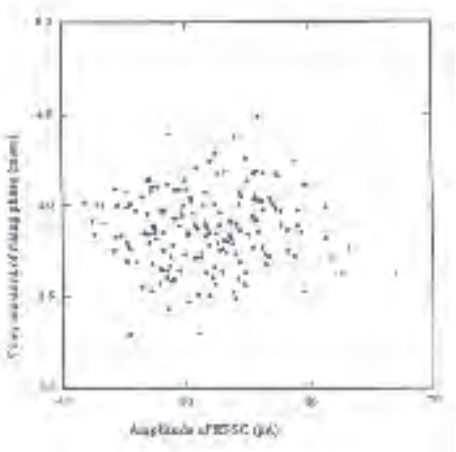

Fig. 11 Filtering effect of dendritic trees of Purkinje cell

The rise times and peak values of each EPSC, which were measured from the soma of a Purkinje cell upon parallel fiber stimulation under voltage clamp recording condition, were plotted. No meaningful relationship between these two values was observed, so that the filtering effect of dendritic trees of Purkinje cell is thought to be small.

As previously shown concerning the waveform of EPSCs before and after conjunctive stimulation comparison of these two types of EPSCs indicated that no change in the kinetics occurs during LTD, under the assumption that the influence of filtering properties of the cell are negligible. This suggestion ruled out another possibility underlying LTD, i.e., the change in the kinetics of glutamate receptor channels during LTD could not cause the reduction of the amplitudes of EPSCs by conjunctive stimulation. Even if the estimated single channel conductances were attenuated by filtering properties of cell, the reduction in these values would occur in the same fashion before and during LTD, and the possibility of a change in the single channel conductances during LTD could be detected by comparing these filtered single channel conductances. Furthermore, the single channel conductance was estimated using the cell-attached patch clamp recording technique in order to evaluate the influence of the filtering properties of the cell to the values of single channel conductance estimated by applying non-stationary noise analysis. Fig. 12 shows an example of single channel currents recorded from the Purkinje cell soma by applying a pipette containing 20 μM AMPA to elicit the current through AMPA receptor channels in the membrane of the Purkinje cell soma. The value of 22.5 pS of single channel conductance of AMPA receptor channel in the membrane of Purkinje cell was calculated from I-V relationship. Although the exact combinations of subunit pairs which make up assembled functional AMPA receptor channels in the soma membrane and dendrite were unknown, Hausser and Roth recently showed, in an electrophysiological experiment, that the properties of the AMPA receptor channel in the soma membrane and dendrite of Purkinje cells were identical (Martin et al. 1993; Hausser and Roth, 1997). Thus it is concluded that the value of single channel conductance of AMPA receptor channels in the membrane of dendrite is around 20 pS. The value of single channel conductance obtained directly from the soma, which may be similar to those obtained from dendrite, using the cell-attached patch clamp technique was the same as those estimated by variance analysis under the assumption that the AMPA receptor channel in the soma and dendrite would be the same. It is suggested that the influence of the filtering

effect of the cell to the electrical signals could be negligible.

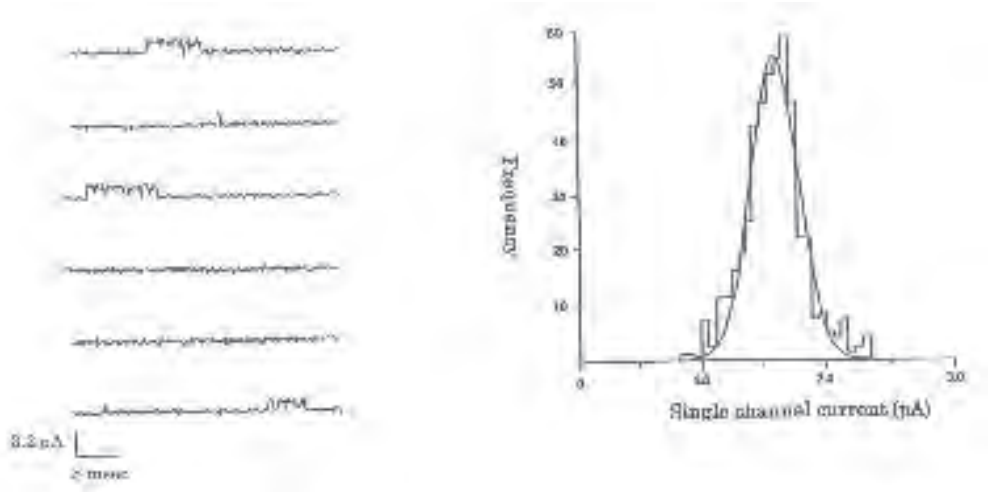

Fig. 12 Single channel currents of AMPA receptor

An example of single channel currents recorded from Purkinje cell soma by using cell-attached patch clamp configuration. Currents opened singly or in burst with a typical duration of 10 msec and amplitude of a few pA at a holding potential −80 mV inside the recording pipette. The amplitude histogram of the currents having 1.6 pA peak value that is fitted by a single Gaussian distribution.

The results of the present non-stationary noise analysis to synaptic plasticity in cerebellum suggested that the basic mechanisms underlying LTD of Purkinje cells was the reduction of the number of functional AMPA receptor channels in the postsynaptic membrane by some kind of modification of channels from inside the cell, triggered by the elevation of the concentration of internal calcium ions. The basic mechanisms of LTP should be examined using the present non-stationary variance analysis to evaluate the elementary processes of receptor channels in the membrane of the cell which partly cause the increase of the amplitudes of EPSCs during LTP such as LTD. The present non-stationary noise analysis is a useful tool for studying the basic processes of synaptic plasticity which is thought to be the basic substrates of learning and memory.

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