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FORMATION OF NEW CORTICORUBRAL SYNAPSES BY PARTIAL
DENERVATION AND THE PROPERTIES OF THEIR
SYNAPTIC TRANSMISSION

by

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The present thesis is mainly based on the following articles.

- I. Tsukahara N., Murakami F., and Hultborn H.:
Electrical constants of neurons of the red nucleus.
Exp. Brain Res. 23, 49-64, 1975
- II. Tsukahara N., Hultborn H., Murakami F., and Fujito Y.:
Electrophysiological study of formation of
new synapses and collateral sprouting in red nucleus
neurons after partial denervation. J. Neurophysiol.,
38, 1359-1372, 1975
- III. Murakami F., Tsukahara N., and Fujito Y.:
Analysis of unitary EPSPs mediated by the newly-formed
corticorubral synapses after lesion of the nucleus
interpositus of the cerebellum. Exp. Brain Res.,
30, 233-243, 1977
- IV. Murakami F., Tsukahara N., and Fujito Y.:
Properties of synaptic transmission of the newly-formed
corticorubral synapses after lesion of the nucleus
interpositus of the cerebellum. Exp. Brain Res.,
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Chapter I

General Introduction

It has until recently[✓] been believed that the neural networks of the central nervous system are 'rigidly' wired. However, in view of the 'plastic' behaviors of the various animals it is natural to suppose that neural circuitries which provide the structural basis of these behaviors must change their properties in response to various circumstances. It is important, therefore, to understand at the cellular level to what extent the nervous system modifies its property.

Since the introduction of intracellular recording techniques, the property of synaptic transmission and the detailed circuitry of the nervous system have been extensively investigated (Eccles, 1964; Eccles, et al., 1967). Now the experimental basis for the study of plastic changes of networks at the cellular level seems to be well founded. This experimental development has been supplemented by the theoretical advances as exemplified by the Rall's neuron models, which have been successfully applied to the analysis of the synaptic activities located on the various portion of the cell membrane (Rall, 1962;1964;1969).

These experimental and theoretical advances provide us a tool for investigating the neural plasticity at the cellular level. The purpose of the present investigation

is to obtain the answer to the general question how plastic the neuronal connections are by the experimental and theoretical analysis of the postsynaptic potentials of one of the central neurons, red nucleus neurons.

Neurons of the cat's red nucleus (RN) are excellent materials for the study of synaptic plasticity, because the synaptic organization of RN has been investigated extensively. In chapter 2, the synaptic organization of the red nucleus is briefly described. In chapter 3, the biophysical properties of the red nucleus neurons are investigated and the standard neuron model of the red nucleus neuron is proposed by applying the compartmental neuron model developed by Rall. Furthermore, the synaptic location of the inputs from the nucleus interpositus of the cerebellum and the cerebral sensorimotor cortex is determined utilizing the experimental parameters and the neuron model. In the next chapter (chapter 4), the synaptic plasticity of the cerebral input onto the red nucleus neurons is described. The main finding of this chapter is the experimental proof that the new synapses are formed in the red nucleus neurons after interruption of the other input; the fibers from the nucleus interpositus of the cerebellum. This was obtained by analysing the postsynaptic potentials produced in the red nucleus neurons by stimulating the cerebral cortex. In chapter 5 this

conclusion was confirmed by the analysis of the unitary postsynaptic potentials produced by a single corticorubral synaptic activity. In chapter 6, the properties of synaptic transmission of the newly formed corticorubral synapses were described. In the last chapter a general discussion of the present investigation will be presented. The specific introduction to each of the chapters will be presented at the beginning of each chapter.

Chapter II

Anatomy of the red nucleus

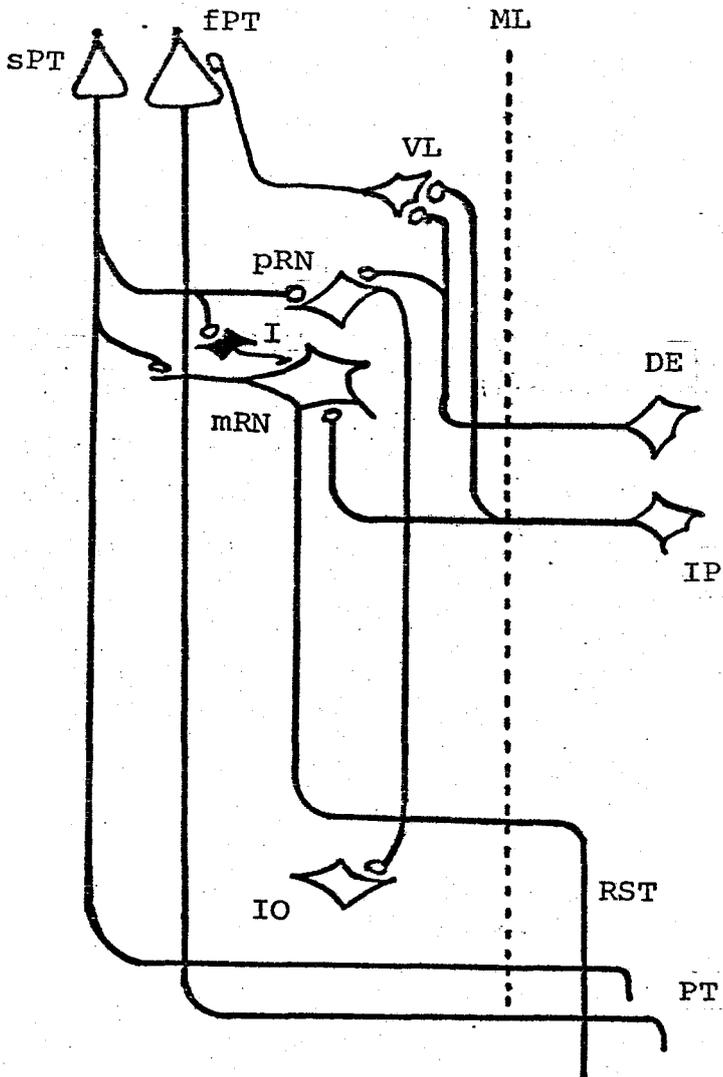
In this chapter, neuronal connections in and around the RN which have so far been revealed are briefly described. It should be noted that the circuit described here does not include all the connection but only part of the connections which is considered to be closely related with the present work.

Red nucleus of the cat is mainly divided into two parts: the rostral parvocellular part and the caudal magnocellular part. The neurons studied here belong to the latter and send axons down to the contralateral spinal cord. (The former sends axons to ipsilateral inferior olive but not to the spinal cord.)

RN neurons receive synaptic input from contralateral sensorimotor cortex (SM). This cerebral input passes through the cerebral peduncle (CP) and is arranged in a somatotopical manner. Monosynaptic EPSP evoked by stimulation of SM is followed by an inhibitory postsynaptic potential (IPSP). This is mediated by an inhibitory interneuron which is probably located within the nucleus. This interneuron is activated by the input from fast pyramidal tract neurons. The synapses from SM terminate exclusively on the distal portion of the rubral dendrite.

Contralateral interposed nucleus (IP) of the cerebellum

also makes synaptic connection with the magnocellular part of the red nucleus (Parvocellular division receives input from the dentate nucleus (DE)) by the collateral branch of the axon to the ventrolateral nucleus (VL) of the thalamus. In contrast to the cerebral input, IP axons form synapses on the somatic portion of the nucleus.



Neuronal connection around the red nucleus.

pRN (parvocellular red nucleus), mRN (magnocellular red nucleus), VL (ventrolateral nucleus), DE (dentate nucleus), IP (interposed nucleus), IO (inferior olive), SPT (slow pyramidal tract neuron), fPT (fast pyramidal neuron) I (inhibitory interneuron), RST (rubrospinal tract) PT (pyramidal tract), ML (mid line)

Chapter III

ELECTRICAL CONSTANTS OF NEURONS OF THE RED NUCLEUS

INTRODUCTION

The passive electrical properties of neurons in the mammalian central nervous system have been explored extensively by the use of intracellular recording and stimulation techniques (Coombs, Eccles and Fatt, 1955; Araki and Otani, 1955; Frank and Fuortes, 1956; Coombs, Curtis and Eccles, 1959; Spencer and Kandel, 1961; Ito and Oshima, 1965; Takahashi, 1956; Lux and Pollen, 1966; Nelson and Frank, 1967; Nelson and Lux, 1970; Lux, Schubert and Kreuzberg, 1970; Burke and Bruggencate, 1971; Barrett and Crill, 1974). Starting from a single time constant equivalent circuit, the model has been refined by taking into account the dendritic cable properties as well as the voltage and time dependent membrane conductance (Ito and Oshima, 1965; Takahashi, 1965; Nelson and Frank, 1967; Nelson and Lux, 1970).

In parallel with these experimental refinements, mathematical neuron models have been developed (Rall, 1959, 1960, 1962, 1964, 1967, 1969; Jack and Redman, 1971). Starting from the early version of the equivalent cylinder model (Rall, 1959, 1960, 1962) Rall developed the more flexible compartment model, in which dendritic trees were represented by a cascade of lumped parameter compartments (1964). With this model, it became possible to analyse the complex spatial and temporal synaptic activities generated at

the various loci on the soma-dendritic membrane of neurons. This model has been successfully applied to the synaptic excitation of motoneurons by large muscle spindle (Ia) afferents and has helped to disclose the detailed location of their synapses on the soma-dendritic membrane of motoneurons (Burke, 1967; Rall, Burke, Smith, Nelson and Frank, 1967).

Membrane voltage transients following the application of rectangular current pulses to motoneurons were found to deviate from a single exponential function, but could be expressed by a linear combination of three exponential functions having time constants of about 25, 5 and 1 msec (Ito and Oshima, 1965). It has been shown previously (Tsukahara, Toyama and Kosaka, 1967) that the large neurons of the red nucleus (RN) of the cat are free from the complication of the slow process. Furthermore, RN cells have two excitatory synaptic inputs which have been suggested to impinge on different portions of the soma-dendritic membrane (Tsukahara and Kosaka, 1968). In the present paper, passive electrical properties of the membrane of RN cells were investigated more extensively. An attempt was made to provide a quantitative electrophysiological description of the RN cell membrane based on Rall's neuron model. By the use of the compartmental model it was further attempted to account for certain properties of the excitatory postsynaptic potentials of RN cells.

METHODS

The cats were anesthetized by an intraperitoneal injection of pentobarbital sodium (35 mg/kg). Supplemental doses (10 mg) were administered as required. In some cases, the animals were immobilized by intravenous injection of gallamine triethiodide and artificially respired. The procedures of the experimental arrangement for intracellular recording from RN neurons were essentially the same as reported previously (Tsukahara et al., 1967). In order to prevent pulsation of the brain tissue, drainage of the cerebrospinal fluid at the atlanto-occipital linkage and, in some cases, pneumothorax were carried out.

Glass microelectrodes filled with 2M NaCl, 3M KCl or 2M K-citrate having an electrical resistance of 6 to 12 M Ω were used. They were inserted from the exposed surface of the hippocampus with a lateral angle of 15 degrees. As the microelectrode penetrated the brain, DC current was sometimes passed through it to prevent its blockage. The input circuit for both recording and passing current through the recording microelectrodes was similar to that described by Ito (1960). An operational amplifier (Phillbrick/Nexus 101101) was utilized in its construction. The output of the voltage and the current recording system of the input circuit was amplified by DC amplifier (Tektronix 3A3), and displayed on an oscilloscope (Tektronix, 565). The voltage output was also connected to another DC recording system in order to

monitor the resting potential. Whenever a large amount of current was passed to unblock the electrodes, the input of the amplifier was disconnected by a relay circuit to protect the operational amplifiers. Stimuli were provided by brief current pulses of 0.1 msec duration by electronic stimulator (Nihon-Koden). In order to solve the differential equations of the five-compartment model (Appendix), an analogue computer (Hitachi, ALS 2000) was used.

RESULTS

General

Neurons giving stable intracellular recordings for thirty minutes to an hour or more and with spike amplitudes of more than 60 mV were selected in this experiment. The mean amplitude of the spike potentials was 69 mV for the 25 RN cells accepted for further analysis. Many of the neurons were the same as those in which the regulation of repetitive firing in RN cells was investigated by injecting long depolarizing currents (Hultborn, Murakami and Tsukahara, to be published).

Passive Electrical Properties of RN Cells

The resistance of RN cells was found to be ohmic within the current range tested. The term 'ohmic' is used to refer to a situation in which the voltage change elicited by two current pulses is the algebraic summation of the voltages produced by current pulses delivered separately. This linear summation should occur at any time during the background

membrane potential change.

Figure 1A—D illustrate examples of the original records from which the input resistance of an RN cell was measured. From the extracellular records (lower traces of Fig. 1E—H) it can be seen that no appreciable polarization occurred. The make and break points of the intracellular voltage transients permitted an evaluation of the compensation of the electrode resistance. The filled circles of Fig. 1I illustrate the steady state voltages, partly shown in Fig. 1A—D, when plotted against current intensities. The data points were found to have a linear relation. The slope of this line gives the input resistance of $3.6 \text{ M}\Omega$. Membrane resistance was measured in this way in all 25 RN cells. The input resistance ranged from $1.1 \text{ M}\Omega$ to $4.5 \text{ M}\Omega$, the mean and S.D. being $2.5 \pm 0.9 \text{ M}\Omega$. The experimental points for the whole current range tested were easily fitted to straight line for every individual cell, thus suggesting that the input resistance did not change with increasing polarization.

In order to assess further the linearity of the voltage drop due to membrane resistance, current pulses of short duration were superimposed on the long pulse (lower traces of Fig. 2A—D). In this series, magnitudes and polarities of the short current pulses were varied. Filled circles of Fig. 2G represent the voltages of the membrane transient that resulted from changing the intensities of the short pulses that were superimposed on the long pulse. Since the voltage plot fell on the same straight line whether or not

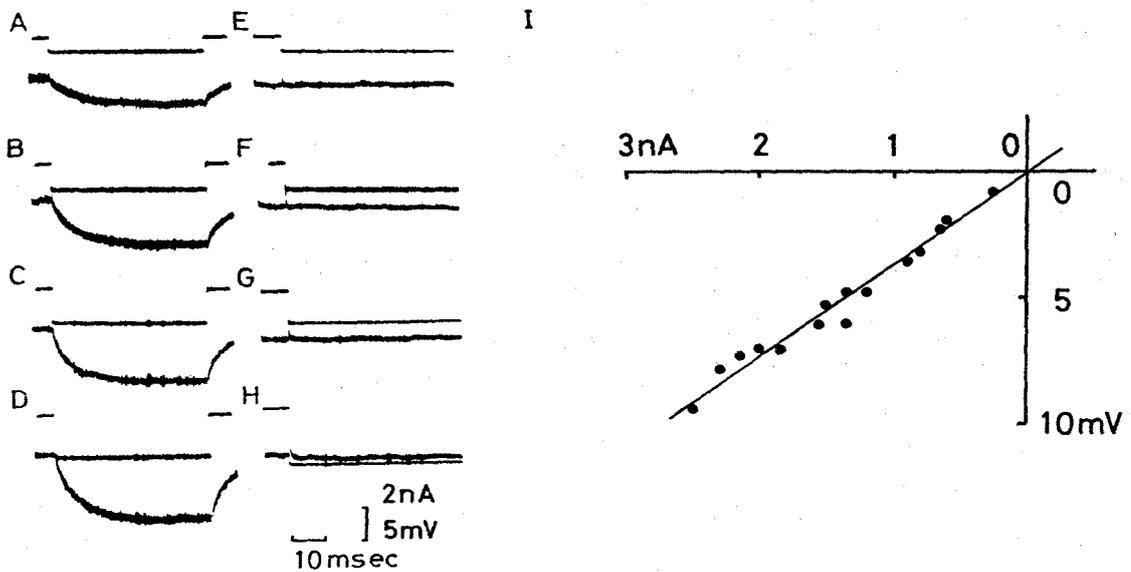


Fig. 1. Potential changes of an RN cell produced by application of current steps. A-H: intra- (A-D) and corresponding extracellular (E-H) potentials (lower traces) induced by hyperpolarizing current steps (upper traces) of increasing intensities from top to bottom. In the graph I are plotted the amplitudes of potential changes (ordinate) against the intensities of applied current (Abscissa). Voltage, current and time calibration in H are common for A-H.

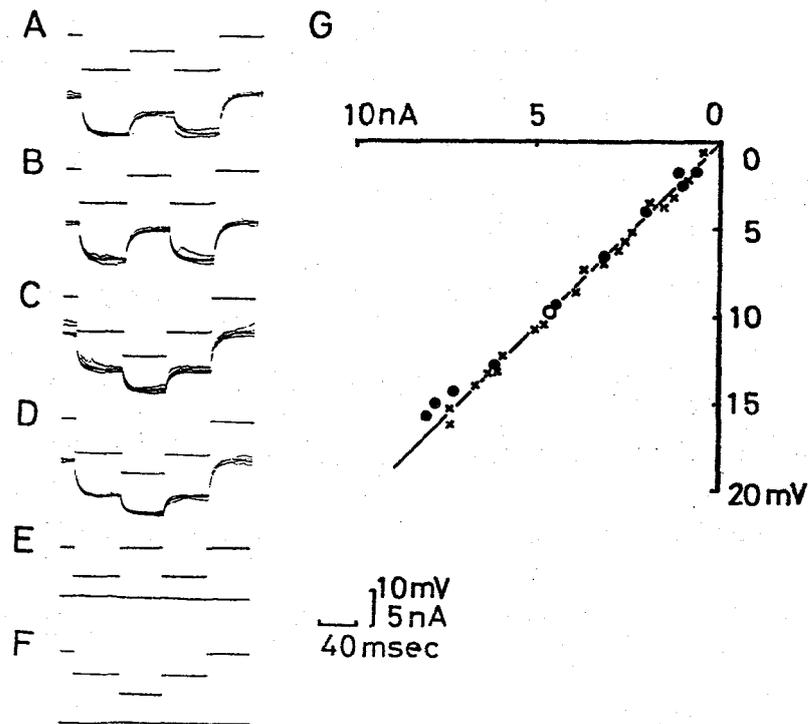


Fig.2 Potential changes of another RN cell as Fig.1 induced by the short current pulses of depolarizing (A,B) and hyperpolarizing (C,D) directions superimposed on the long current step of hyperpolarizing direction.

Corresponding current records were shown in upper traces of A-F. E-F: Extracellular records. In the graph of G are plotted the amplitudes of potential changes (ordinates) against the intensities of applied current (Abscissa). Crosses: The amplitudes of potential changes evoked by long current steps alone were measured at 40 msec after the onset of current pulses. Filled circles: The potential

changes evoked by short current pulses (shown partly in A-D) superimposed on long current step. Open circle: The potential change evoked by long current step alone of double pulse. Voltage, current and time calibration in E are common for A-F.

there was a background membrane potential change (shown by a open circle in Fig. 2G), it can be considered that the membrane resistance of RN cells is voltage independent.

In Fig. 3A—C are found records obtained by changing the time of superimposition of a short pulse on the background long current pulse. It can be seen from the plot in Fig. 3F that the membrane voltages produced by the short current pulses do summate arithmetically with those produced by the long current pulse independently of the timing. Therefore, it is evident that the membrane properties of RN cells are 'ohmic' in nature, for the current range tested. This apparent linear behaviour of the membrane of RN cells is favorable for a detailed examination of the time course of the voltage transients elicited by a current step.

Some records of membrane potential transients resulting from the application of current steps across the cell membrane of RN cells have been reported in a previous paper (Tsukahara et al., 1967). The membrane transients could be approximated by a combination of two exponential functions. In this paper, a more extensive study of the membrane transients was attempted.

Figure 4 (Two lower traces of inset) shows records of the membrane transient at two sweep speeds following the application of a rectangular hyperpolarizing current step through the microelectrode. The corresponding extracellular record taken after withdrawing the electrode is shown in the upper trace of the inset. The voltage transients thus

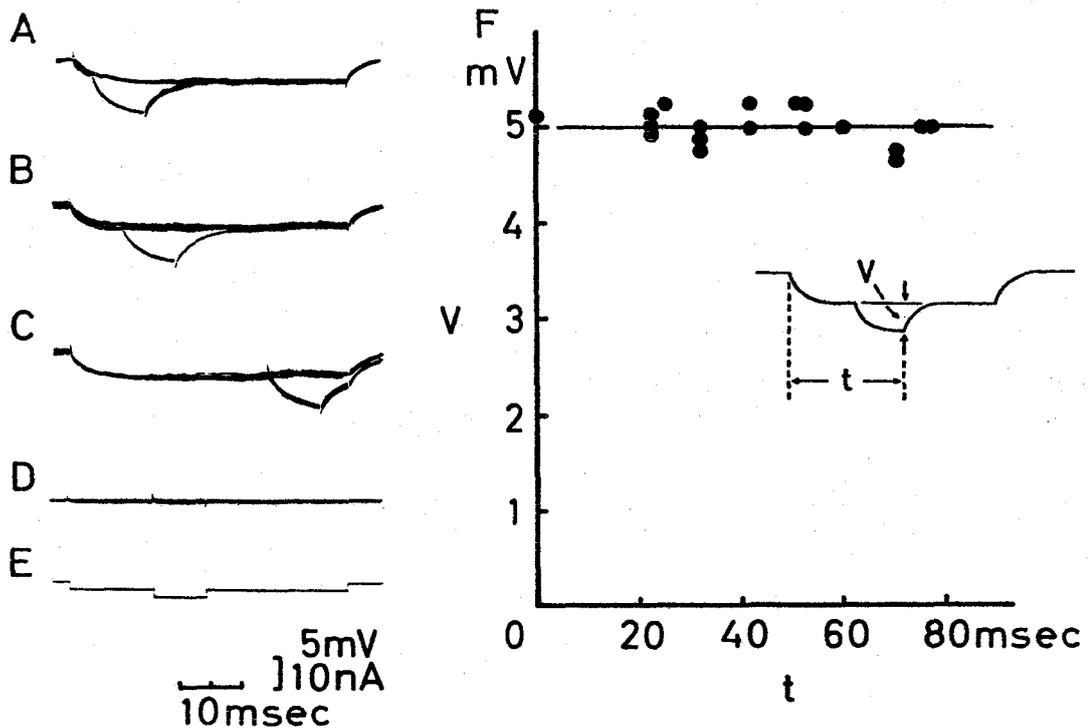


Fig.3 Membrane potential changes to short current pulses superimposed on a long current pulse. A-C: Superimposed traces of potential changes induced by long current pulses, and long pulses plus short pulses. D: Extracellular records. E: Current pulses corresponding to A-D. F: Plot of the amplitudes of potential changes (V) to short pulses (filled circles) measured at the cessation of the short pulses against the time after onset of long pulse (t). Cross: Control without background long hyperpolarization

obtained were measured at 0.2 or 0.5 msec intervals and plotted on the semilogarithmic coordinates. In agreement with the previous paper, voltage points could be fitted by a straight line except during the initial 2 msec (Fig. 4). This straight line gives the membrane time constant, $\tau_0 = 5.7$ msec. If the difference between this straight line and the deviated data points of the initial 2 msec are plotted on a semilogarithmic scale, they could be fitted by another straight line, the slope of which gives a time constant of a second exponential function, $\tau_1 = 0.7$ msec.

Figure 5 shows the frequency distribution of these two time constants of RN cells. The first and the second time constants thus obtained in 25 RN cells were 5.6 ± 1.0 msec and 0.6 ± 0.2 msec, respectively, and the ratio of their amplitudes, E_1/E_0 , was 0.18 ± 0.05 . Although the absolute values of E_1 and E_0 depend on several factors such as the initial conditions, etc. as described by Rall (Rall, 1969), the ratio E_1/E_0 does not (see Appendix).

Figure 6 is a plot of the amplitudes of these two exponential functions, E_1/E_0 , against the ratio of respective time constants, τ_1/τ_0 . Although there is some scatter of points, it is likely that there is a linear relation between these two ratios. This correlation was found to be of statistical significance ($0.01 > p > 0.001$, t-test and regression line $Y = 0.97X + 0.073$, where Y and X are E_1/E_0 and τ_1/τ_0 , respectively). The solid line in Fig. 6 represents the line drawn through the mean value of these ratios and the zero

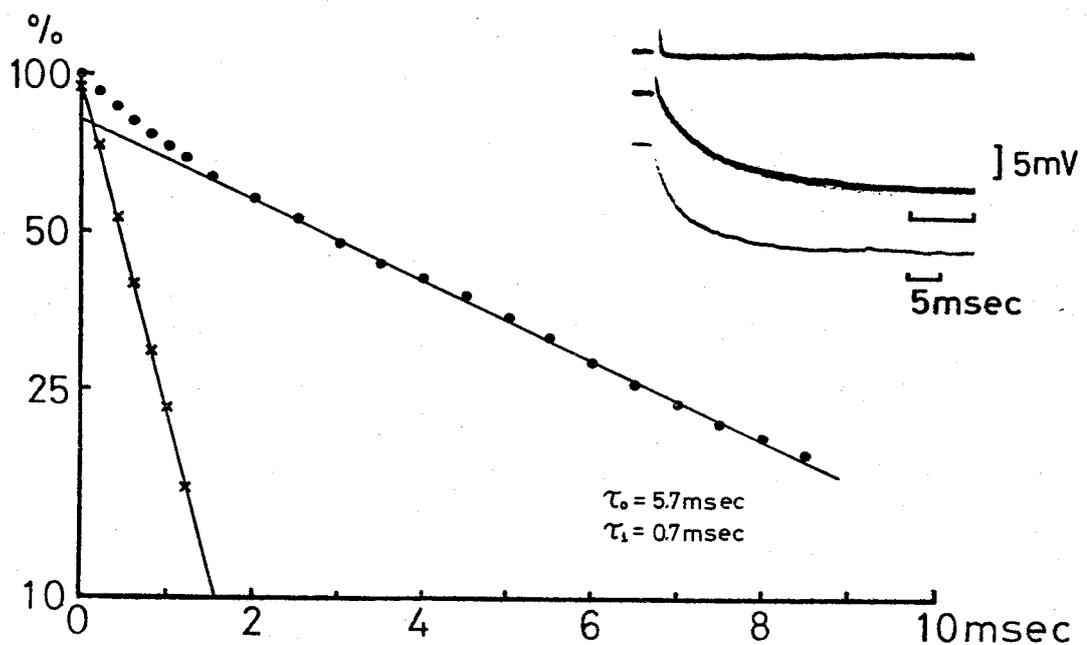


Fig.4 Semilogarithmic plot of a voltage transient at the onset of hyperpolarizing current pulse. Inset; intracellular (middle and lowermost traces, different sweep speeds) and extracellular (upper trace) voltage changes induced by a hyperpolarizing current step. The straight tail portion of the curve at times larger than 2 msec represents the first time constant, τ_0 , 5.7 msec. The difference between the experimental data (filled circles) and the extrapolated line was plotted on semilogarithmic scale (crosses). The straight line through these crosses represents the second time constant, τ_1 , of 0.7 msec.

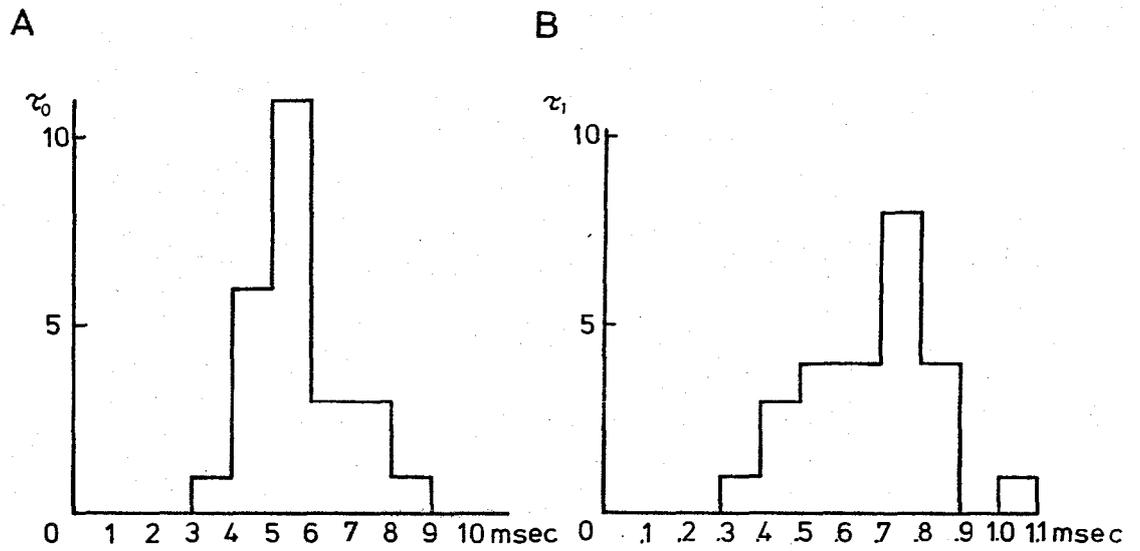


Fig.5 Frequency distribution of first and second time constants. Ordinates, number of cells. Abscissae, first time constant (A) and second time constant (B).

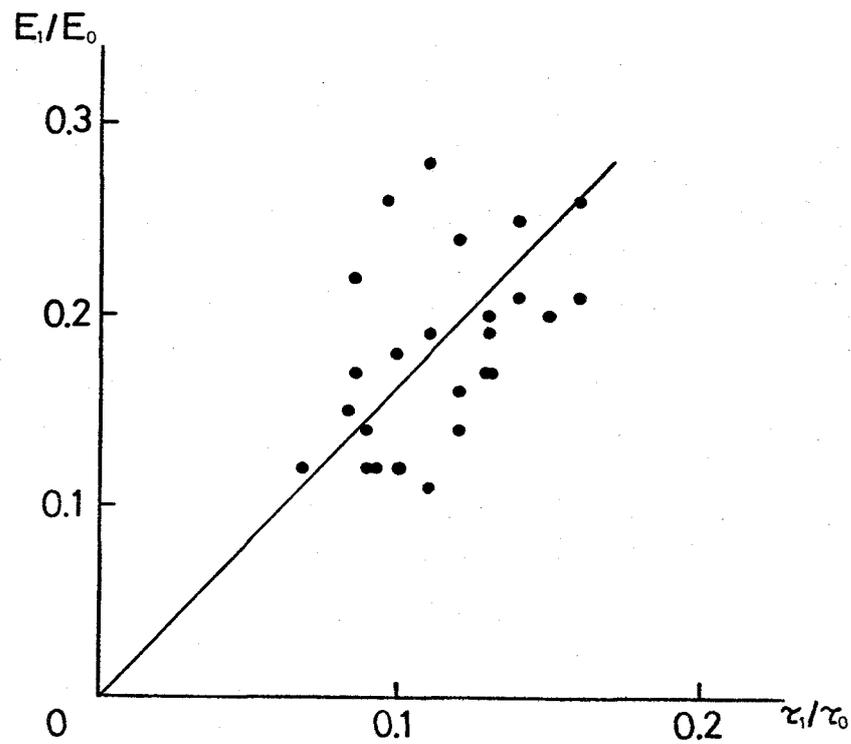


Fig.6 Linear Plot of the ratio of final values of the two exponential functions, E_1/E_0 , of the transient response against the ratio of the two time constants, τ_1/τ_0 . The line fitted for the data point is drawn by solid line. See text.

point of the coordinate, having a slope of 1.64.

In searching for the possible structural basis of this linear relation, it was found that the dendritic cable properties are especially relevant (see Discussion). By application of cable model, it was possible to obtain a linear relation between the two ratios. The experimentally obtained ratio, 1.64 ± 0.45 is not significantly different from that obtained theoretically which is 1.8 according to the five compartment model of Rall (Rall, 1964). This approximate match between experimental and theoretical results was taken to indicate that the initial deviation of the membrane transients to a current step is indeed due to the cable properties of dendrites.

By using the ratio of τ_1/τ_0 , it is possible to calculate the cable parameter or electrotonic length, L , of RN cells from the equation derived by Rall (1969)

$$L = \frac{\pi}{\sqrt{\tau_0/\tau_1} - 1} \text{ ----- (1)}$$

The mean L value of RN cells was 1.1 ± 0.15 for the 25 RN cells. Since we use the five compartment model in the next section, the increment of electrotonic length per compartment, ΔZ , relates to L by the relation, $5\Delta Z = L$. This ΔZ value was used for the later calculation. Figure 7 plots the ΔZ value thus obtained against the input resistance in the present sample. There is a negative correlation between the electrotonic length of individual RN neurons and their input resistance ($0.05 > p > 0.025$, t-test and regression line of

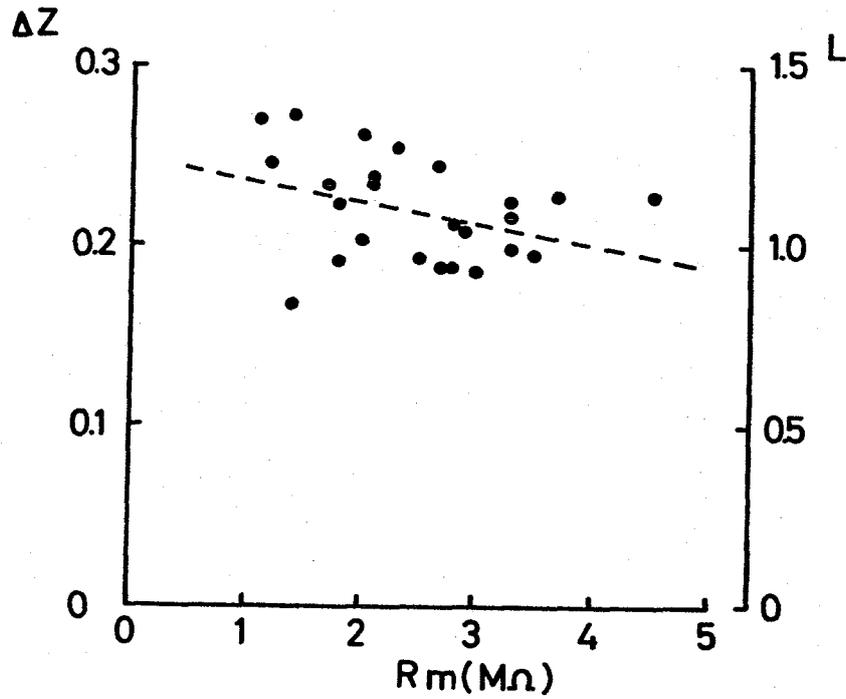


Fig.7 Plotting of the cable parameter, ΔZ , or electrotonic length, L , of RN cells against the input membrane resistance. Ordinate; ΔZ values or L values. Abscissa; input membrane resistance, R_m .

$Y = 0.25 - 0.012 X$ where Y and X are ΔZ and input resistance, respectively). This is in contrast to the findings for spinal motoneurons (Burke and Bruggencate, 1971).

Excitatory Postsynaptic Potentials (EPSPs) of RN cells

The membrane resistance of RN cells was essentially 'ohmic' (see above) and the deviation of the membrane transient from a single exponential function was interpreted as being due to their dendritic cable properties. It is known that RN cells have two excitatory synaptic inputs which have been shown to impinge on different portions of the soma-dendritic membrane (Tsukahara and Kosaka, 1968; Toyama, Tsukahara, Kosaka and Matsunami, 1970; King, Martin and Conner, 1972; Nakamura and Mizuno, 1971). It may therefore be expected that the same cable properties would affect several features of these excitatory postsynaptic potentials (EPSPs). We will now analyse their dependence on voltage displacement at the soma in relation to the dendritic cable parameter, ΔZ , obtained above.

It has been shown that EPSPs induced from cells in the cerebral cortex or their efferent fibers at the internal capsule are less sensitive to membrane potential displacement than those induced from the nucleus interpositus (IP) of the cerebellum (Tsukahara and Kosaka, 1968). By using the standard cable parameter, ΔZ , found in the RN cell material it is possible to estimate where, expressed in electrotonic distance, these two kinds of synapses are located on the soma-dendritic membrane of RN cells. This is performed by

comparing the sensitivity of these two kinds of EPSPs to membrane potential displacement and the theoretical results based on the five compartment model (see Appendix).

Figure 8A—D illustrate examples of records from which EPSP sensitivities to membrane hyperpolarization were measured. Cerebral EPSPs were induced by stimulating at the cerebral peduncle (CP). Figure 8F plots the peak amplitudes of both IP-EPSPs (crosses) and CP-EPSPs (filled circles) against applied current intensities. In both series, the points fell on straight lines, which plot these EPSPs when the amplitudes of the control EPSPs were set as 100%. Thus the ratio of the slopes of these two straight lines gives the relative sensitivity of the EPSPs.

Ten RN cells were examined in this way. In general, the relative insensitivity of the CP-EPSPs as compared to the IP-EPSPs reported by Tsukahara and Kosaka (1968) was confirmed in the present study. By using a wider range of current intensities than used previously, it was possible to compare quantitatively the sensitivities of these EPSPs to membrane hyperpolarization. The sensitivity of CP-EPSPs ranged from 29—75.9% (mean = 48%) of the sensitivity of IP-EPSPs in the ten RN cells.

Since the electrotonic length of the standard RN cell is already known and the EPSP sensitivity to membrane potential displacement is not dependent on the time course of the EPSP conductance, we can compute the theoretical sensitivities of EPSPs generated on the various loci of the

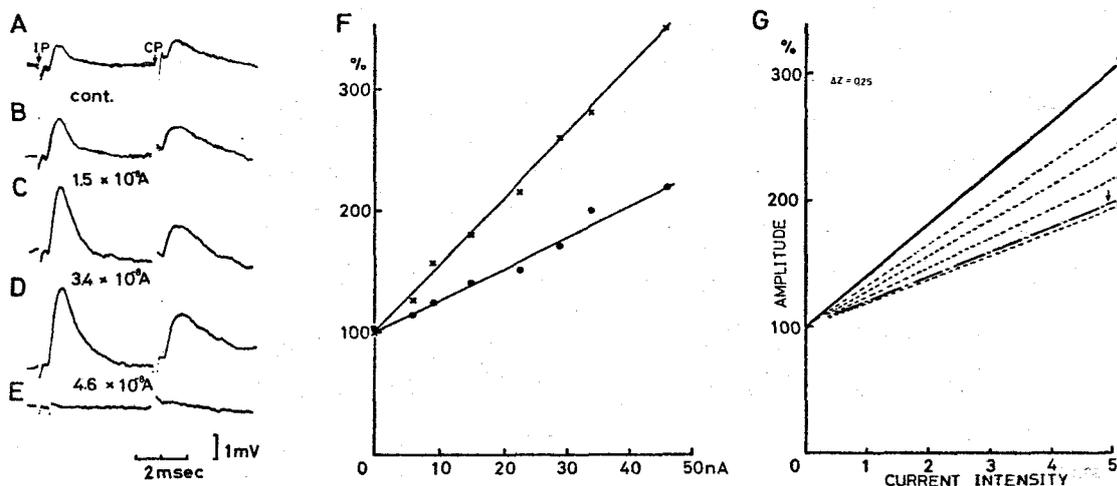


Fig. 8 Effect of membrane potential displacement on the EPSPs induced from the nucleus interpositus (IP) and the cerebral peduncle (CP). A: Control IP- and CP-EPSPs. B,C, and D: Same as in A but during passage of hyperpolarizing current of 1.5×10^{-8} A, 3.4×10^{-8} A, and 4.6×10^{-8} A respectively. F: In the graph the EPSP amplitudes (ordinate) are plotted against the respective current intensities (abscissa). Crosses for IP-EPSPs and filled circles for CP-EPSPs. G: Comparison of the experimental data with those derived theoretically by using the five compartmental model as shown in Fig. 9. The solid line represents the slope for IP-EPSP and that for computed EPSP initiated at the first compartment. The dotted lines indicate the slopes for the computed EPSPs initiated at 2, 3, ..., 5th compartments. The interrupted line (arrow) shows the slope for CP-EPSP.

soma-dendritic membrane of RN cells, by assuming a certain, but arbitrary time course for the EPSP conductance increase. We also assume the equilibrium potential of the theoretical as well as experimental EPSPs is the same independent of the site of initiation. We used the pulse-shaped conductance increase of the duration of 0.7 msec in the present calculation (Appendix).

Figure 8G illustrates the sensitivities of the computed EPSPs to membrane hyperpolarization. The peak amplitudes of the computed EPSP induced at the first compartment (labeled 1 in the inset) is plotted as a solid line against current intensities. The dotted lines represent the normalized slopes of the computed EPSPs induced at the second, third, ----, and fifth compartments (labelled 2, 3, ---. and 5 in the inset) when the amplitudes of the control EPSPs without membrane hyperpolarization were set as 100%.

In order to compare the theoretical results with the experimental ones, it was assumed that the IP-EPSP was initiated at the first compartment. The interrupted line of Fig. 8G illustrates the slope of the sensitivity of CP-EPSPs after normalization so that the theoretical and experimental slopes for the IP-EPSPs became identical. It can be seen that the slope for CP-EPSP falls between the slopes corresponding to computed EPSPs induced at compartments 4 and 5 respectively (indicated by a downwards arrow). Therefore, it is suggested that the CP synapses are located at or near compartment five. These results support the assumption that

the IP terminates at the first compartment, since the best match between experimental and computed results was obtained if IP and CP synapses were assumed to terminate at opposite ends of the chain of compartments.

DISCUSSION

In an extension of the previous study (Tsukahara et al., 1967), more extensive measurements of the electrical constants of RN cells have now been made. The average input resistance was found to be 2.5 ± 0.9 M Ω . The membrane transient to a current step could be approximated by a combination of two exponential functions having time constants of 5.6 ± 1.0 msec and 0.6 ± 0.2 msec, respectively, and the ratio of the amplitudes of these two exponential functions was 0.18 ± 0.05 .

A similar deviation from a single exponential function was found in the transient voltages of motoneurons to current step (Ito and Oshima, 1965). Ito and Oshima proposed two possible mechanisms to interpret this deviation: 1. Another time constant produced by the endoplasmic reticulum as has been shown in muscle membrane by Falk and Fatt (1964). According to the two time constant model of muscle fiber, there is a third path through a resistance and a capacitance in series, in addition to the two parallel paths through membrane capacitance and resistance of the ordinary muscle membrane. Transient response of this fiber model gives the solution of the sum of two exponential functions (equation 26, Appendix C of Falk and Fatt, 1964).

2. The initial deviation of voltage transient is due to the cable properties of the neuron. This has been extended theoretically by Rall (Rall, 1969). These two mechanisms are not mutually exclusive. However, the former emphasizes the contribution of a membrane component which has a smaller time constant than that of the ordinary membrane, with or without the geometric factor of dendrites, while the latter stresses the appearance of the initial rapid transient due to the cable properties of the geometrically complex neuron even if the electrical property of the membrane itself can be described by a single time constant.

In the present investigation, it was found that there is a linear relation between the ratio of these two time constants and the ratio of these two amplitudes. This constraining condition of the parameters of the two time constant model was found to be particularly useful for the evaluation of the two alternative mechanisms discussed above. In the former model, there would be no linear relation between E_1/E_0 versus τ_1/τ_0 (equation 26, Falk and Fatt, 1964). On the other hand, a theoretical linear relation between E_1/E_0 versus τ_1/τ_0 could be derived by solving compartment model (see Appendix). Therefore, in this study the initial deviation of the membrane transient responses of RN cells from a single exponential function was considered to be due to the cable properties of dendrites.

In order to apply the compartmental model with a straight chain of equal compartments or the equivalent cylinder model

to a real neuron, several assumptions were made. 1. The electrical properties of this neuronal membrane are linear and passive. 2. The membrane has one time constant. 3. The sum of the $3/2$ power of all branch diameters at any particular electrotonic distance remains constant. 4. The formulation used for deriving the equation (1) required the knowledge of the terminal boundary condition.

In this case, the first assumption was justified on experimental grounds. In contrast to the non-linear membrane properties found in some motoneurons (Ito and Oshima, 1965; Burke and Bruggencate, 1971; Nelson and Frank, 1967) or pyramidal tract cells (Takahashi, 1965), the purely linear behaviour of RN cells is favourable for applying the linear compartment model developed by Rall (Rall, 1964). The work by Bellman and Astrom (1970) further justifies the possibility to determine the parameters of the compartmental equation by analysing the membrane transient that is recorded at the terminal compartment and produced by the application of a current step at the same point.

As for the second and third assumptions, it was found with the compartment model as well as the equivalent cylinder model that a linear relation between E_1/E_0 versus τ_1/τ_0 could be derived theoretically (see Appendix). Experimental verification of this linear relation may be taken to suggest that the assumptions for deriving this relation i.e. assumptions 2 and 3, are justified. Finally, the terminal boundary conditions were found to be related to the slope of the linear

relation between E_1/E_0 versus τ_1/τ_0 (Sato, personal communication).

In this analysis of membrane voltage transients, only the second exponential component was considered. This was done since the experimental transient analysis showed that the third or higher components constituted only a minor portion of voltage transient. Furthermore, theoretical calculation gives a τ_2 value of 0.2 msec and a E_2/E_0 of 0.04 by using the five compartment model. This order of values would be within the range of experimental errors. Therefore, the third and higher components were neglected in the present analysis.

By applying the cable model, it is possible to define the cable characteristics of RN cells. The electrotonic length, L , of soma-dendritic membrane of RN cells was 1.1. The equivalent cylinder model has been applied to cat motoneurons by Nelson and Lux (1970) and Burke and Bruggencate (1971) to obtain the electrotonic length of the equivalent cylinder. By making assumptions of the dendritic geometry and the sealed end boundary condition of the cylinder, they obtained an L value between 1 and 2. Further, Lux, Schubert and Kreuzberg (1970) obtained an L value of motoneurons by direct matching of the morphological and electrophysiological data as 1.5. They have shown further that the $3/2$ power law indeed holds true for motoneurons. However, recent work by Barrett and Crill found that the sum of the $3/2$ power of the dendritic diameters of the reconstructed motoneurons after

Procion dye injection iontophoretically into motoneuron through the recording pipette was found to decrease with distance from the soma. They developed a computational technique to allow calculation of the passive membrane properties of motoneurons based on the reconstructed neuron geometry (Barrett and Crill, 1974).

By using the electrotonic length of RN cells, $L = 1.1$, it was estimated at what electrotonic distance from the soma the excitatory synapses of RN cells from nucleus interpositus of the cerebellum and the cerebral cortex are located on the soma-dendritic membrane of RN cells. If we assume that the equilibrium potential of both of these excitatory synapses is the same, it is possible to compare the observed sensitivities of EPSPs to membrane hyperpolarization to those derived theoretically using compartmental model. Since a change in the time course of the conductance transient does not modify the relative sensitivities of EPSPs to membrane potential change, we used a pulse-shaped conductance increase in the present analysis. Present results suggest that the cerebral synapses are located around $4 \cdot \Delta Z = 0.88 \lambda$ from the soma, where λ is the length constant of the membrane, while the excitatory synapses from the nucleus interpositus are localized at the soma. Electronmicroscopic observation of cat red nucleus by Nakamura and Mizuno (1971) has indeed shown that the excitatory synapses from nucleus interpositus end exclusively on the soma. It is relevant to note that electronmicroscopic data of King et al. (King, Martin and

Conner, 1972) have shown the purely dendritic location of corticorubral synapses in opossum red nucleus.

If the cerebral synapses are located far remotely, at the terminal dendrites, and the synapses from the nucleus interpositus at the somatic membrane, a question would arise as to the kind of synapses which are located between the soma and the remote dendrites. It has been suggested that the pyramidal inhibitory synapses are at or near the soma (Tsukahara and Fuller, 1969). This led us to consider the possibility that there are other synaptic inputs to RN cells which may terminate between the soma and terminal dendrites. There is some indication for the other synaptic inputs to RN cells although the exact location of these postulated synapses is obscure at present (Massion, 1961; Nishioka and Nakahama, 1973; Tsukahara, Hultborn and Murakami, unpublished observation).

APPENDIX

Computation on Excitatory Postsynaptic Potential and Membrane Transient Response Based on Rall's Compartment Model

By assuming that the excitatory conductance increase is localized in one compartment and that its time course is very brief, it is possible to calculate the resulting voltage change at any compartment for "EPSPs" initiated at any one of the five compartments using the five compartment model (Rall, 1964). Some properties of these computed EPSPs were

examined. For the calculations we accepted the cable parameter $\Delta Z = 0.25$ and the membrane time constant $\tau_0 = 6$ msec. By the use of an analogue computer the resulting voltage change at the first compartment (= soma) was calculated where a pulse-shaped "EPSP" conductance increase with a duration of 0.7 msec and with the same magnitude was applied to each one of the five compartments.

Figure 9A illustrates examples of these computed EPSPs initiated at the first and the third compartment for upper three and lower three traces, respectively. For these values of ΔZ , membrane time constant, and the duration of the conductance pulse, the time to peak of the computed EPSPs initiated at each compartment was 0.7, 0.9, 1.4, 2.3 and 3.2 msec, respectively for the first, second, ---, and fifth compartment EPSPs.

In order to calculate the EPSP sensitivity to membrane potential displacement, DC current was applied at the first compartment and after the steady state voltage was attained, the excitatory conductance was given at each compartment. As would be expected, the amplitudes of the computed EPSPs increased most prominently when the conductance increase was given at the first compartment. The sensitivity to membrane potential displacement decreased progressively as the site of initiation of the computed EPSPs occurred more remotely from the first compartment. Figure 9A illustrates specimen records of these computed EPSPs initiated at the first and third compartments, with membrane hyperpolarization of two

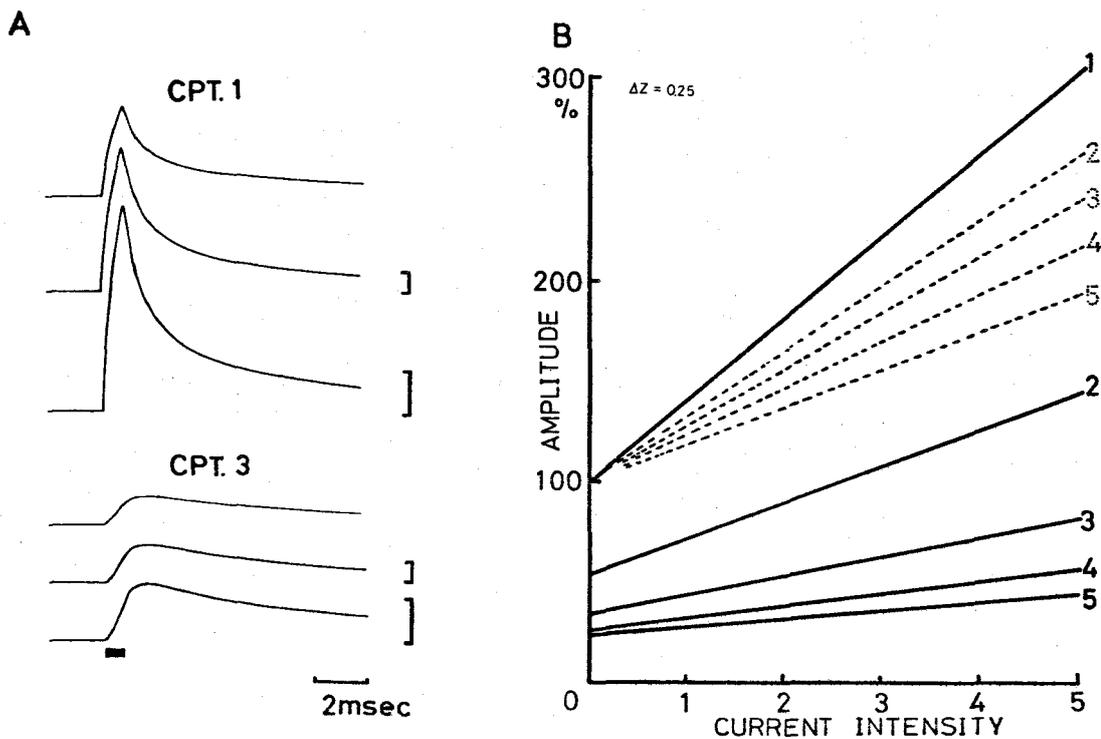


Fig.9 The computed EPSP sensitivity to membrane potential displacement. A: Computed EPSPs initiated at the first compartment (CPT. 1) and the third compartment (CPT. 3) at rest (uppermost trace) and during passage of hyperpolarizing currents as indicated by vertical bars in A using arbitrary unit from the compartment 1 (middle and lowermost traces). Horizontal bar indicates the duration of conductance increase. The graph in B shows the relation between the peak amplitudes of the computed EPSPs generated at each compartment (labelled in the figure) and applied current intensities. The dotted lines represent the normalized slopes of the computed EPSPs when their control amplitudes without current injection were set as 100 percent.

different magnitudes. Figure 9B plots the peak amplitudes of these computed EPSPs against corresponding current intensities that were used to displace the membrane potential. In order to facilitate the comparison between the computed EPSPs and those obtained experimentally, the amplitudes of the control EPSPs were adjusted to be the same. The dotted lines of Fig. 9 represent the normalized slopes of these computed EPSPs, initiated at each of these five compartments with the same control amplitudes. These slopes thus give the relative sensitivity to hyperpolarization of the EPSPs initiated in respective compartments. In the present case the slopes corresponding to the 2nd, 3rd, 4th and 5th compartments were 80, 68, 57 and 45% of the slope of the first compartment. These percentages were used in Results for comparing the computed and experimental EPSPs.

Theoretical membrane transient to current step applied to the 1st compartment was also computed by using analogue computer, but it was possible to obtain the analytical solution. The transient voltage had a functional form

$$V = \sum_{i=0}^4 E_i (1 - \exp^{-t/\tau_i}), \text{ where } i = 0, 1, \dots, 4. \text{ By observing}$$

the solution thus obtained, it was found that there is a relation between these parameters as $E_1/E_0 = 1.81 \cdot \tau_1/\tau_0$.

It was further known that this is a relation which holds true more generally in the case of n-compartment model as E_1/E_0

$$= (1 + \cos \frac{\pi}{n}) \cdot \tau_1/\tau_0, \text{ where } n \text{ denotes the number of compartments.}$$

(Sato, personal communication). Since this linear relation

is one of the important properties that this model can predict, it was used in Results for interpreting the applicability of the model to the experimental results of RN cells.

SUMMARY

1. Membrane electrical constants have been studied in neurons of the red nucleus (RN) of the cat which were identified antidromically from the spinal cord. For each cell, the input resistance was determined from the membrane potential changes to current steps and was found to be 2.5 ± 0.9 M in twenty five RN cells studied. In addition, linear summation of the membrane response induced by two current pulses was demonstrated.

2. From the membrane voltage transients to current steps, the first membrane time constant, τ_0 , and second time constant, τ_1 , were determined as 5.6 ± 1.0 msec and 0.6 ± 0.2 msec, respectively. The ratio of the amplitudes of two exponential functions, E_1/E_0 , was 0.18 ± 0.05 . A linear relation was found between the ratio of these amplitudes of exponential functions and that of the two time constants.

3. The cable parameter (electrotonic length, L) of the combined soma and dendrites of the RN neurons was estimated as 1.1 from membrane transient data using the relation developed by Rall (1969).

4. By using this parameter, an attempt was made to estimate the location of the excitatory inputs on the soma-dendritic membrane of RN cells.

Chapter IV

ELECTROPHYSIOLOGICAL STUDY OF FORMATION OF NEW
SYNAPSES AND COLLATERAL SPROUTING IN RED
NUCLEUS NEURONS AFTER PARTIAL DENERVATION

INTRODUCTION

The possibilities for regeneration of neurons in the central nervous system (CNS) of higher animals have been regarded for a long time as very poor (Clemente, 1964). In view of this, the capacity for functional compensation after brain lesions by the remaining nervous structures must be regarded as surprisingly good. The compensation of the initial dysfunction by the remaining nervous system following cerebellar lesions has been described repeatedly since the first report by Luciani in 1891 (Luciani, 1891). However, little is known about functional changes subserving this compensation and their morphological correlates at the synaptic level. As far as lesions in the peripheral nervous system are concerned, it is well established that sprouts from remaining intact motor axons are able to reinnervate denervated muscle fibers (Edds, 1953). Recent detailed morphological evidence indicates that collateral sprouting of intact axon may operate in rat CNS (Lynch, Deadwyler and Cotman, 1973; Lynch, Mosko, Parks and Cotman, 1973; Moore, Björklund and Stenevi, 1971; Raisman, 1969; Raisman and Field, 1973; Steward, Cotman and Lynch, 1974).

In order to examine possible plastic changes of synaptic

activities, corticorubral excitatory postsynaptic potentials (EPSPs) were investigated in the giant neurons in the red nucleus in adult cats after destruction of the interpositorubral connection. Physiological and histological investigations have shown that corticorubral synapses terminate at the remote dendritic portion of the cells (Brown, 1974; King, Martin and Conner, 1972; Tsukahara and Kosaka, 1968; Tsukahara, Murakami and Hultborn, 1975), while synapses from nucleus interpositus (IP) end on the soma (King, Dom, Conner and Martin, 1973; Nakamura and Mizuno, 1971; Toyama, Tsukahara, Kosaka and Matsunami, 1970). Electrophysiologically, distal dendritic synaptic input is dominated by the dendritic cable properties, which cause a much slower time course of these dendritic excitatory synaptic potentials (EPSPs) than that of somatic EPSPs (Rall, 1964; Rall, Burke, Smith, Nelson and Frank, 1967; Tsukahara and Kosaka, 1968; Tsukahara, Murakami and Hultborn, 1975). Taking advantage of this synaptic organization, it is possible to test electrophysiologically whether corticorubral fibers form new synapses on the more proximal portion of the soma-dendritic portion of RN membrane after interruption of the cerebellar nuclear input. This paper reports evidence of such a plasticity of the corticorubral synapses after destruction of the cerebellar input in a way which may be partly responsible for the compensation of the cerebellar dysfunction. Preliminary reports of the present investigation have been published (Tsukahara, Hultborn and

Murakami, 1974; Tsukahara, Hultborn, Murakami and Fujito, 1975).

METHODS

The experiments were performed on 51 adult cats. Three groups of Nembutalized cats were used; one control group of normal animals (n = 20) and test groups with IP lesions (n = 29) or lesions of the sensorimotor cortex (n = 2). In the cats with IP lesions, the right IP was destroyed electrolytically 4 days to more than 90 days before the acute experiment. Similarly, the left sensorimotor cortex was destroyed 20 days before the acute experiment. The IP nucleus was destroyed electrolytically by changing the electrode position systematically several times and passing cathodal current of 2 mA for 30 s each time. The sensorimotor cortex was destroyed by electrocoagulation. The surgical procedures and the techniques for stimulation and recording from the red nucleus neurons were the same as those employed previously (Tsukahara, Toyama and Kosaka, 1967) and will be summarized only briefly here.

Stimulation and recording

The C₁-C₂ segment of the spinal cord was exposed and bipolar acupuncture needles, insulated except at the tips, were placed on the lateral surface of the cord for antidromic activation of the rubrospinal neurons. Bipolar electrodes made of acupuncture needles, insulated except at

the tips, were inserted stereotaxically for stimulation of several sites. During acute experiments the corticorubral fibers were stimulated at three levels: within the sensorimotor cortex (SM), the rostral part of the cerebral peduncle (rostral CP: at the stereotaxic coordinates of A8.5, L6, and H-3.7), and the caudal part of the cerebral peduncle (caudal CP: at A4.5, L4.5, and H-6.1). In normal as well as in chronic cats, stimulating electrodes were inserted in the right IP and the left ventrolateral nucleus (VL) of thalamus. In normal cats, stimulation of the VL evokes monosynaptic EPSPs in RN neurons due to collateral activation of the interpositorubral projection (Toyama, Tsukahara, Kosaka and Matsunami, 1970; Toyama, Tsukahara and Udo, 1968; Tsukahara, Toyama and Kosaka, 1967). During acute experiments following lesions, VL as well as IP were stimulated to estimate physiologically the degree of destruction of IP. The subsequent histological control always verified the physiological estimation.

Glass microelectrodes, filled with 2 M NaCl, 3 M KCL, or 2 M K citrate and having an electrical resistance of 6-12 M Ω , were used. Microelectrodes filled with Procion yellow dye were used occasionally. The electrodes were inserted from the exposed surface of the left hippocampus with a lateral angle of 15°. The input circuit, for both recording and passing current through the recording microelectrodes, was similar to that described previously (Tsukahara, Toyama and Kosaka, 1967). An operational

amplifier (Phillbrick/Nexus 101101) was utilized in its construction. The output of the voltage and the current-recording system of the input circuit was amplified by a DC amplifier (Tektronix 3A3) and displayed on an oscilloscope (Tektronix, 565). The voltage output was also connected to another DC recording system in order to monitor the resting potential. Stimuli were provided by brief current pulses of 0.1 ms duration from an electronic stimulator (Nihon-Koden).

Histology

The degree of lesion of IP and the location of the stimulating electrodes were confirmed by histological examination. At the end of the experiments a 10% formalin solution was injected into the carotid artery. The sites of stimulating electrodes were coagulated by passing currents, 500 μ A for 30 s, through the electrode, and subsequently located by serial sections. Some RN neurons were identified, in addition to the antidromic activation from the spinal cord, by injecting Procion yellow dye, as shown in Fig. 1. Electrodes filled with an aqueous solution of 5% Procion yellow dye with an average electrode resistance of 50 M Ω were used. Hyperpolarizing current of 10–20 nA was passed for 5–10 min across the cell membrane for dye injection. After 1 wk, frozen sections, 100 μ m thick, were examined using a Nikon fluorescence microscope. Figure 1 illustrates a chronic cat RN cell stained with Procion yellow.

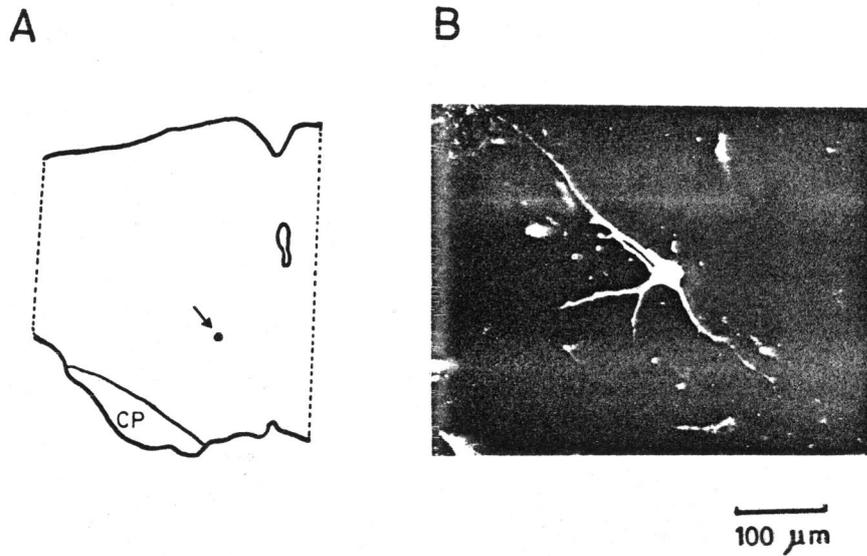


Fig.1 Staining of an RN cell of a chronic cat by Procion yellow dye. A, Drawing of a histological section of the midbrain of a chronic cat. A dot (arrow) represents the location of an RN cell stained by Procion yellow. B, Photomicrograph of a Procion yellow filled RN cell with high magnification.

Computer simulation

The theoretical shape of the EPSP in RN neurons were analyzed by Rall's compartment model using an analogue computer (Hitachi, ALS 2000), as described previously (Tsukahara, Murakami and Hultborn, 1975).

The model used consists of the straight chains of five equal compartments of conventional synaptic membrane equivalent circuit having resting (G_r) and excitatory (G_e) conductance channels with resting (E_r) and excitatory (E_e) synaptic batteries. Membrane capacity (C_m) is inserted in parallel with these conductance channels. Five such compartments of equal parameters are connected with each other by mutual conductance (G_{ij}), from j th compartment to i th compartment. The membrane potential at i th compartment is V_m . The normalized membrane potential at i th compartment, v_i , is expressed by the following ordinary differential equations which are linear and first order (equation 13 of ref 38). The i th compartment satisfies the following equation

$$\frac{dv_i}{dt} = \sum_j \mu_{ij} v_j + f_i$$

where

$$v_i = \frac{V_m - E_r}{E_e - E_r}$$

$$f_i = \frac{\epsilon_i + \chi_i}{\tau}$$

$$\mu_{ij} = G_{ij} / C_m$$

$$\mu_{ij} = - \frac{1 + \epsilon_i}{\sum_{j \neq i}^n \mu_{ij}}$$

$$\epsilon = G\epsilon_i / Gr$$

$$\tau = Cm / Gr$$

$$\chi_i = I_i / Gr \cdot (E_e - E_r)$$

In this model, somatic membrane is represented by the compartment 1 and dendritic membrane is represented by the compartments 2, 3, ..., 5. Current I_i is injected at i th compartment externally. In the case of straight chains of equal compartment as treated here, $\mu_{ij} = \tau^{-1}(\Delta Z)^{-2}$, where ΔZ is the increment of electrotonic length of the equivalent cylinder model and the total length of the cylinder is $n \cdot \Delta Z$. The membrane time constant, τ , was assumed to be the same in all compartments.

By assuming that the excitatory conductance increase is localized to one compartment and that its time course is very brief, it is possible to calculate the resulting voltage change at any compartment for EPSPs initiated at any one of the five compartments using the five-compartment model described by Rall (Rall, 1964). Some properties of these computed EPSPs were examined. For the calculations we changed the cable parameter ΔZ to various values (see Fig. 9) assuming $\tau = 6$ ms. By the use of an analogue computer, the resulting voltage change at the first compartment (-soma) was calculated where a pulse-shaped EPSP conductance increase with a duration of 0.9 ms (see DISCUSSION) was applied to each one of the five compartments. This model

was used for quantitative evaluation of the location of the newly formed components of CP EPSPs after partial denervation at the various sites of the soma-dendritic membrane of RN cells (see DISCUSSION).

RESULTS

1. Change of time course of corticorubral EPSPs after destruction of cerebellar nuclear input

The typical slow CP EPSPs evoked by stimulation of rostral CP and fast IP EPSP in a normal cat are illustrated in Fig. 2A, B. In contrast, the rostral CP EPSP shown in Fig. 2C-G, from a cat with chronic IP destruction, has a much faster rise time (time to peak) and larger amplitude than in A. The mean latency (\pm SD) of the fast-rising components of rostral CP EPSPs was 0.8 ± 0.1 ms ($n = 93$ cells) for the chronic cats, which is slightly shorter than that of the normal cats (1.0 ± 0.2 ms ($n = 100$)). By changing the stimulus intensities, the fast-rising component of the CP EPSP could be graded in amplitude, as shown in Fig. 2D-G, the minimal amplitude in this case being 1.2 mV (Fig. 2D). Frequently, there is no simple decay following the rapid rise and early summit; instead, a second peak may occur as if the slow EPSP were superimposed on the fast one (Fig. 2F). Spike potentials were usually initiated with shorter latencies and the field potentials were much larger than in normal cats (Fig. 2G).

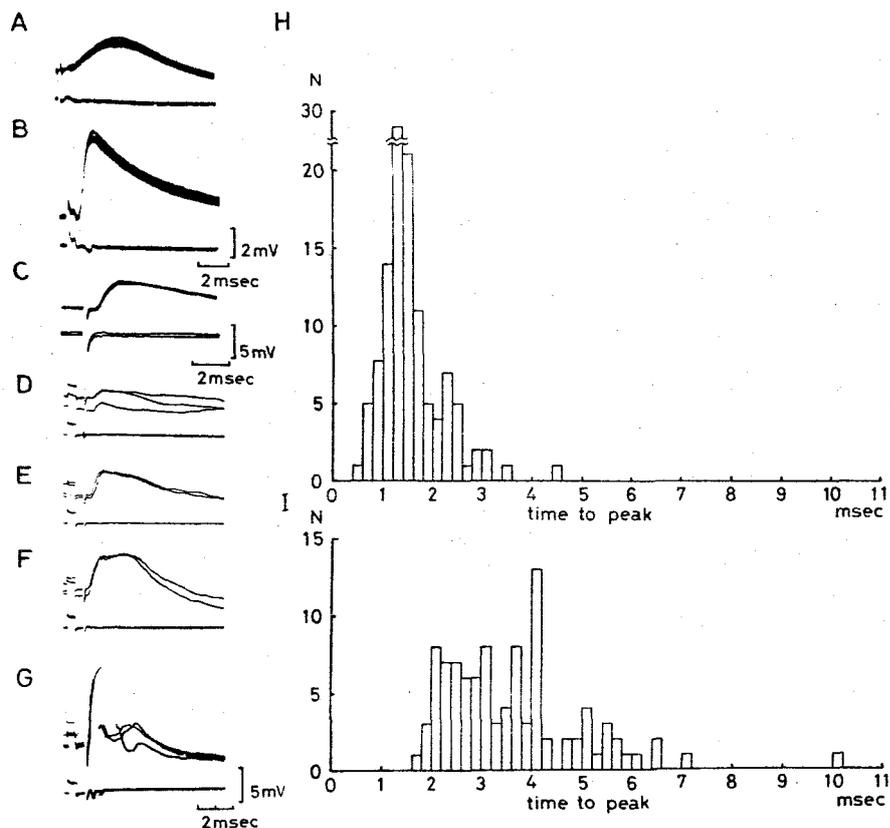


Fig.2 Rise time of the CP-EPSP after IP lesion. A-G, Upper traces are intracellular responses in RN neurons, while the lower traces show the corresponding field potentials recorded at a just extracellular position. A and B illustrate a CP-EPSP and an IP-EPSP respectively (same cell) from a normal cat. C-G show CP-EPSPs after IP destruction. C and D-G are records from different RN cells. Stimulus intensity was increased from D to G. Time and voltage calibration of B and G also apply for A and D to F respectively. H, Frequency distribution of the time-to-peak of CP-EPSPs (rostral CP-EPSPs) in chronic cats. Ordinate; number of cells. Abscissae, time-to-peak of CP-EPSPs. I, The similar histogram as in H but from normal cats.

Time-to-peak measurements of rostral CP EPSPs corrected for extracellular field potentials were made in chronic as well as normal cats. In case of dual peaks, the initial peak was used. The frequency distribution of the times to peak of the rostral CP EPSPs of 119 RN cells of chronic and 100 RN cells of normal cats are shown in Fig. 2H and I, respectively. The mean time to peak of rostral EPSPs in the chronic cats was 1.5 ± 0.6 ms ($n = 119$), which was significantly shorter ($P < 0.001$) than that of the normal cats (3.6 ± 1.4 ms ($n = 100$)). In the majority of RN cells, the times to peak of rostral CP EPSPs of chronic cats were shorter than those of normal cats. However, it must be noted that there were RN cells in which the times to peak of rostral CP EPSPs were within normal range. Since the results varied with survival time (see Fig. 7), the present sample of 119 RN cells is composed of chronic cats more than 12-days survival time after IP lesion.

The time to peak of the later peak of the rostral CP EPSPs, as shown in Fig. 2F, were measured by assuming that this component has the same latency as the initial fast-rising component. The mean time to peak of this later component is 3.0 ± 1.2 ms ($n = 28$), which is close to that of the normal cats. These results thus indicate that a new component with faster rise time, which appeared after chronic IP lesions, is simply added to the normal slow-rising corticorubral EPSPs.

Figure 3 illustrates the relation of the time to peak of the initial and second components of rostral CP EPSPs in

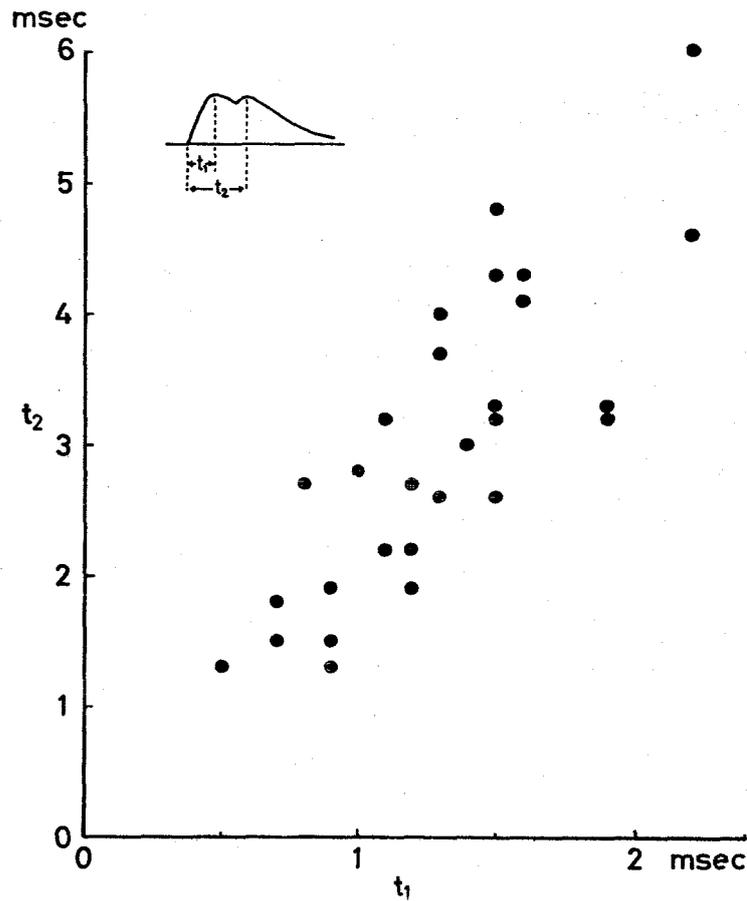


Fig.3 Relation between the time-to-peak of the initial and the second components of rostral CP-EPSPs in chronic cats. Ordinates; time-to-peak of the second component of rostral CP-EPSP (t_2) measured as in the inset diagram. Abscissae; time-to-peak of the initial components (t_1):

chronic cats. It is noted that there is a strong positive correlation between these two values. These data may be taken to indicate that the time to peak of these two components is determined largely by the cable properties of neurons (see DISCUSSION).

2. Conduction velocities of fibers responsible for fast-rising component of CP EPSPs

Similar fast-rising EPSPs, but with different latencies, could be induced by stimulation of the sensorimotor cortex (SM) and caudal CP. Figure 4A and B illustrate records of corticorubral EPSPs induced from two loci; the SM (with a distance from the RN of about 30 mm) and the caudal CP (about 2 mm from the RN). Collision experiments partly shown in Fig. 4C-E have shown that these EPSPs were generated by the corticofugal fibers originating in the sensorimotor cortex and passing down through the CP. By comparing the latencies (second arrows in Fig. 4A and B) with stimulation at these two loci, the conduction velocities of the fibers responsible for the new fast-rising components of the EPSP in the chronic cat shown in Fig. 4A, B was estimated to be 14 m/s. A similar estimation was made on 29 RN cells, and the mean conduction velocity was 21 ± 8 m/s. Figure 4F shows the frequency distribution of these conduction velocities. This value corresponds to that of the normal corticorubral fibers, 20 m/s (Tsukahara and Kosaka, 1968).

Frequently, however, a very small depolarization with

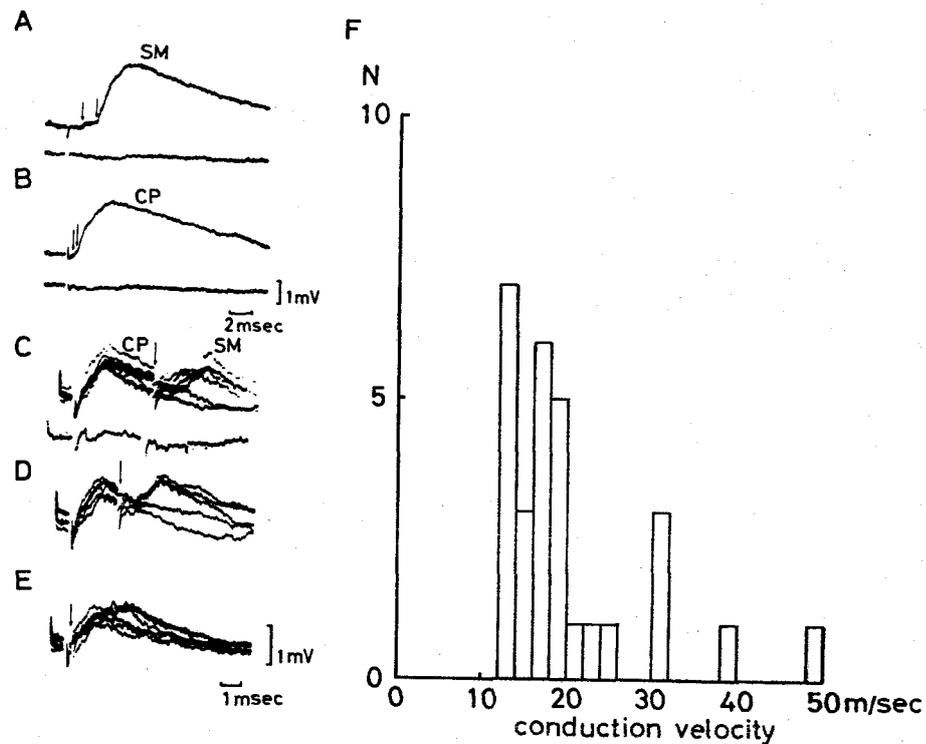


Fig.4 Conduction velocities of fibers mediating the newly appeared fast rising components of corticorubral EPSPs in RN cells. Upper traces in A and B are intracellular records of EPSPs evoked from SM(A) and CP(B). Lower traces are corresponding extracellular controls. C-E show the records of collision experiment of EPSPs induced from SM and CP. SM stimuli were applied at various time intervals after preceding CP stimuli. Downward arrows in C-E indicate the onset of SM stimuli. Lower trace of C shows the extracellular control. F, Frequency distribution of conduction velocities of fibers mediating the fast rising component of the corticorubral EPSPs. Ordinate: number of cells. Abscissae; conduction velocities.

somewhat shorter latency from the main component could also be produced by stimulation of SM (initial arrows in Fig. 4A, see also Fig. 8C and caudal CP (initial arrows in Fig. 4B). The conduction velocity of fibers responsible for this small component was 40 m/s, which is in the range of fast-conducting pyramidal tract fibers (Takahashi, 1965). In normal cats, the fast-conducting pyramidal tract fibers end in the RN region, forming synaptic contact with inhibitory interneurons (Tsukahara and Fuller, 1969; Tsukahara, Fuller and Brooks, 1968), but not with rubrospinal neurons directly. Therefore, it is suggested that after IP lesion, formation of synaptic contact onto RN neurons, as identified antidromically from the spinal cord, occurred from fast-conducting pyramidal tract fibers. However, the potential was usually very small.

3. CP EPSPs during membrane potential change

Figure 5 illustrates a CP EPSP of a chronic cat during membrane-potential displacement by injecting current through the microelectrode. With hyperpolarizing current, the CP EPSPs increased in size; and with depolarizing current they decreased; and by further increasing the depolarizing current, they reversed in sign. This result, together with the smoothly graded nature of the fast-rising component, indicates that this component is due to an EPSP and not due to a dendritic spike potential such as observed in hippocampal pyramidal cells (Spencer and Kandel, 1961) or alligator Purkinje cells (Llinás and Nicholson, 1971).

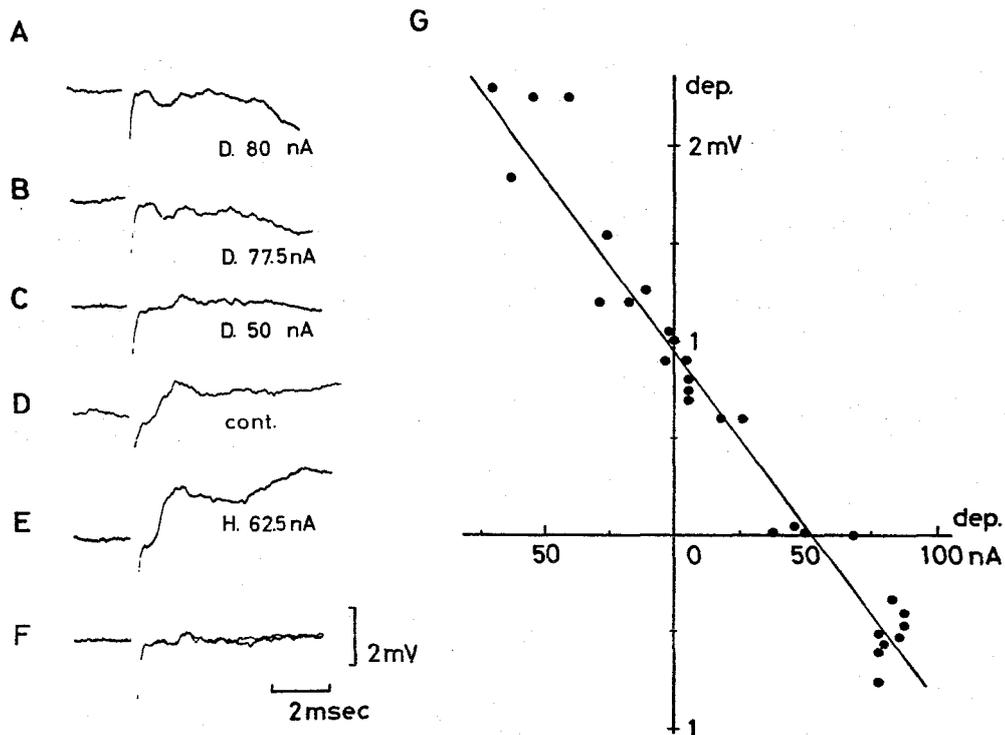


Fig.5 Effect of membrane potential change on the CP-induced EPSP. A-C, during passage of depolarizing current of 80 nA, 77.5 nA and 50 nA, respectively. D, Control. E, during passage of hyperpolarizing current of 62.5 nA. F shows the corresponding extracellular record. G, The amplitudes of the fast-rising component of CP-EPSPs (Ordinates) are plotted against the respective current intensities (Abcissae).

4. Comparison of cable properties of RN cells before and after IP lesions

The simplest interpretation of the results presented in sections 1-3 is to assume that corticorubral terminals sprout to form new synaptic contacts at a more proximal portion of RN cell membrane so as to compensate for the removal of its principal excitatory input. However, an alternative explanation is to assume that the partial denervation causes a morphological change of the RN cells, resulting in drastic changes of their cable properties.

In order to assess this possibility, the electrical cable properties of RN cells were analyzed in cats with chronic IP lesions and compared with those of a normal sample (Tsukahara, Murakami and Hultborn, 1975). Figure 6A illustrates an example of a membrane transient response of an RN cell in the preparation where IP was destroyed 16 days previously. By plotting the voltage thus obtained against time on semilogarithmic coordinates, it was found that the membrane transient response could be approximated by the sum of two exponential functions of different time constants, and final values as in normal cats. The main time constant, τ_0 , was 8.4 ms and the shorter time constant, τ_1 , was 0.7 ms in the cell of Fig. 6A. Similar measurements were made on 11 RN cells. The mean τ_0 value was 7.2 ± 1.2 ms, which is slightly larger than the τ_0 value of 5.6 ± 1.0 ms reported for normal cats by Tsukahara et al. (Tsukahara, Murakami and Hultborn, 1975). The mean value of τ_1 was 0.7 ± 0.2 ms (normal cats

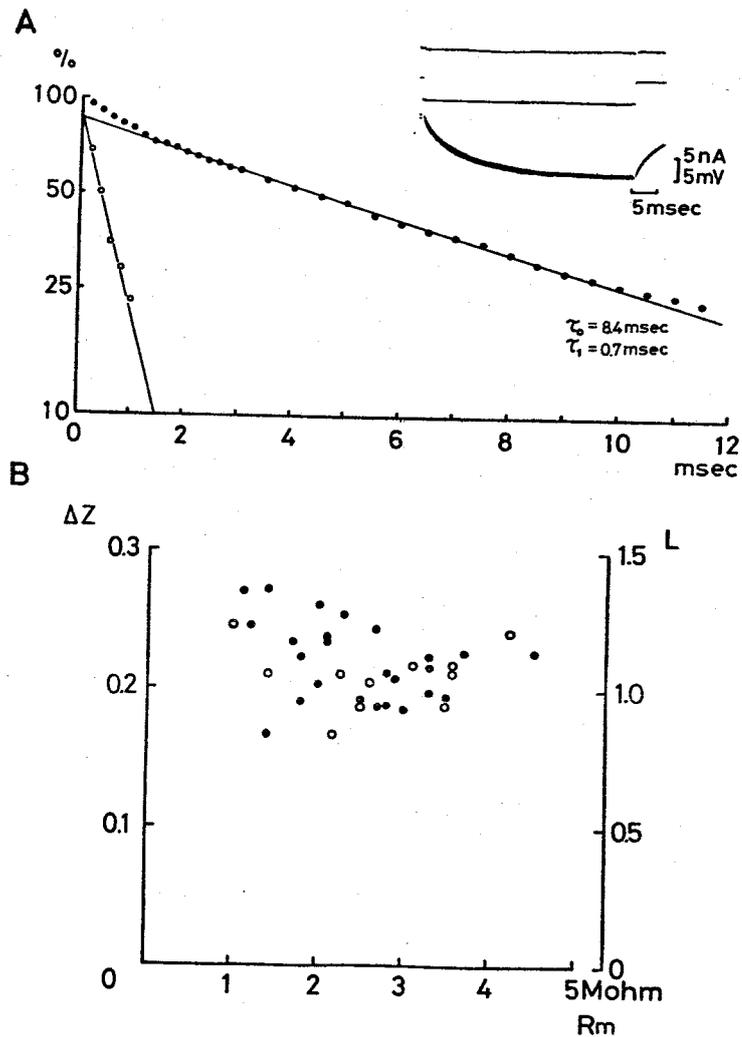


Fig.6 Dendritic cable properties and input resistance of RN cells after IP lesion. A, Inset of A shows the membrane transient responses (lowermost trace) of an RN cell induced by applying a step of current (middle trace) through microelectrode. Uppermost trace of the inset is the corresponding extracellular control. The membrane

transient response thus obtained is plotted on a semilogarithmic coordinates. The straight tail portion of the curve at times larger than 2 msec represents the first time constant, τ_0 , 8.4 msec. The difference between the experimental data (filled circles) and the extrapolated line was plotted on semilogarithmic scale (open circles). The straight line through these circles represents the second time constant, τ_1 , of 0.7 msec. B, The electrotonic length (L) or increment of the electrotonic length of five compartment model (ΔZ) of the model RN cell membrane (Ordinates) is plotted against the respective input resistance of RN cells (Abcissae). Open circles, the data of chronic IP lesion. Filled circles, the data of normal cats drawn from the previous experiment (52).

0.6 ± 0.2 ms (Tsukahara, Murakami and Hultborn, 1975)). The mean membrane resistance of RN cells was 2.7 ± 0.8 MΩ, which is not significantly different from that of normal cats (2.5 ± 0.9 MΩ) (Tsukahara, Murakami and Hultborn, 1975). From the ratio of two time constants, τ₀ and τ₁, it was possible to estimate the cable parameter, ΔZ, or electrotonic length, L, of RN cell membrane from the following equation (Burke and Bruggencate, 1971; Rall, 1969; Tsukahara, Murakami and Hultborn, 1975)

$$L = 5 \cdot \Delta Z = \frac{\pi}{\sqrt{\tau_0/\tau_1} - 1}$$

The mean ΔZ value in the 11 RN cells was 0.21 ± 0.02, which is not significantly different from that of normal cats, 0.22 ± 0.03 (Tsukahara, Murakami and Hultborn, 1975). Figure 6B summarizes the relation between ΔZ or L values and the input resistance in the present sample of RN cells. Open circles represent data from chronic cats and filled circles represent data from previous work on normal cats (Tsukahara, Murakami and Hultborn, 1975).

5. Time course of change of rise time of CP EPSPs after IP lesions

In order to know the time course of the development of supposed collateral sprouting and formation of new synapses, time to peak of CP EPSPs was investigated in experiments performed at different intervals after destruction of IP nucleus. Figure 7 illustrates the relation between the average time to peak of the corticorubral EPSPs initiated at

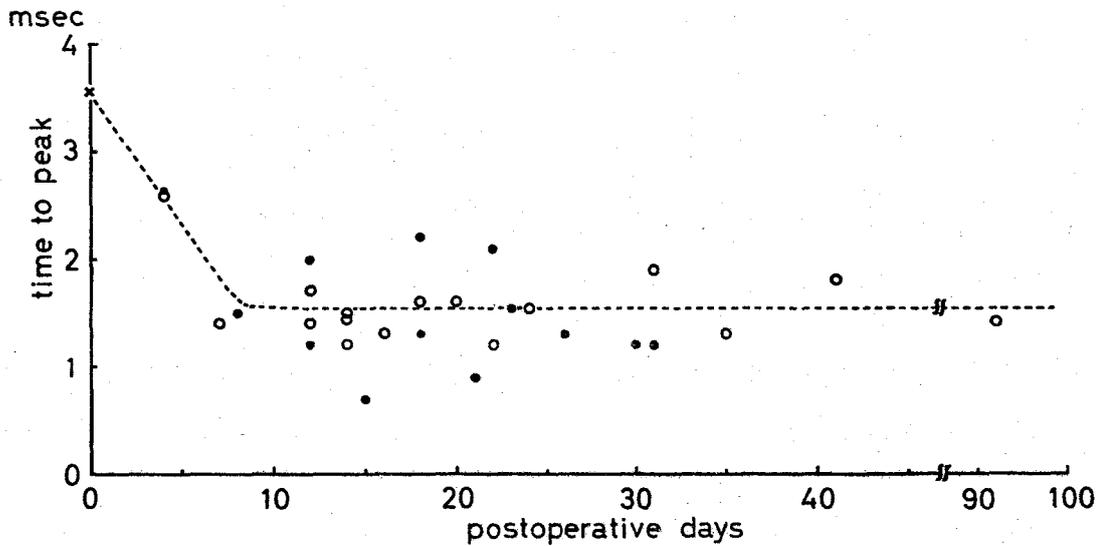


Fig.7 The time course of the change of the rise time of the CP-EPSPs after IP lesion. Ordinates, rise time of the rostral CP-EPSPs. Abscissae, the days after IP lesion. Open circles, the mean rise time of the rostral CP-EPSPs of more than six RN cells. Filled circles, the mean rise time of the CP-EPSPs of two to five RN cells. Each point represents the data from one cat. Cross indicates the mean rise time of the rostral CP-EPSPs of 100 RN cells in normal cats.

rostral CP and the numbers of days after IP lesion for each experimental animal. Four days after lesion, a significant shortening of the time to peak already occurred. After 10 days it reached the maximum and remained constant for at least about 80 days.

6. Cortical sites producing fast-rising EPSPs

According to histological (Mabuchi and Kusama, 1966; Nyberg-Hansen and Brodal, 1964; Pompeiano and Brodal, 1957; Rinvik and Walberg, 1963) and physiological investigations (Padel, Armand and Smith, 1972; Padel, Smith and Armand, 1973; Tsukahara and Kosaka, 1968), the normal corticorubro-spinal projection is arranged in a somatotopical manner; from the forelimb sensorimotor area, through the dorsomedial part of RN, to the upper spinal segment; and from the hindlimb sensorimotor area, through the ventrolateral part of RN, to the lower spinal segment. It was of interest to see whether the new fast components of the corticorubral EPSPs in RN neurons also follow this somatotopical arrangement.

Figure 8A illustrates the antidromic invasion from C₁ as well as L₁ spinal segments in an RN cell, which was thus identified as a "hindlimb" unit. Figure 8B shows the arrangement of the stimulating SM electrodes, five on the precruciate gyrus and five on the postcruciate gyrus. Stimulus pulses of constant intensity were applied between the two electrodes placed both on the pre- and postcruciate gyri. Figure 8C illustrates the cortical EPSPs obtained from

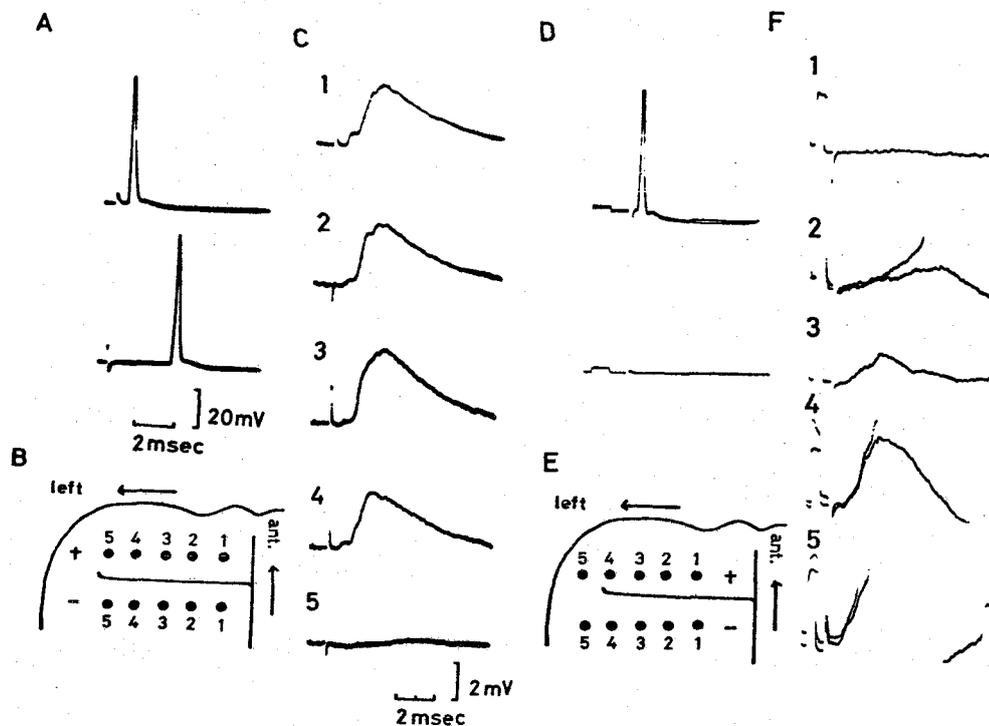


Fig.8 Cortical projective area of the corticorubral EPSPs after chronic IP lesion. A, Upper trace, antidromic spike potential of an RN cell induced from C₁ spinal segment. Lower trace, that induced from L₁ spinal segment. B, The diagram showing the position of the stimulating electrode. The overview of the left cruciate gyri. C, The SM-EPSPs induced in the same cell in A by stimulating points labelled in each trace (cathodal electrodes in postcruciate against respective anodal electrodes in precruciate points). D, Upper trace, antidromic spike potential of an RN cell induced from C₁ spinal segment. Lower trace, that failed to be induced from L₁ spinal segment. E, The position of the stimulating electrode as in B. F, EPSPs induced in the same cell as in D by stimulating points labelled in each trace similar to C. Voltage and time calibration in A and C also apply for D and F, respectively.

these five pairs of electrodes in the RN cell identified in A. The IP nucleus had been destroyed 41 days previously. It can be seen that cortical EPSPs were produced predominantly, but not exclusively, from the hindlimb region of the SM. The same degree of overlap was also observed in normal cats. Correspondingly in other RN cells, which were shown to innervate only the upper spinal segments (Fig. 8D), cortical EPSPs were produced predominantly from the forelimb region of SM (Fig. 8E, F). Therefore, it was concluded that there is still a tendency toward somatotopy.

7. Chronic cortical lesions and IP EPSPs

The results presented in sections 1-6 have all been concerned with changes in the corticorubral system after interruption of the interpositorubral projection. It would, of course, be of interest to extend the experiments to also cover conceivable changes in the interpositorubral termination after chronic cortical lesions. Preliminary evidence indicates that when the SM cortex was destroyed 20 days prior to the acute experiment, there was no appreciable change of the time course of the IP or VL EPSPs. Perhaps the interpositorubral system does not possess the same plastic properties as observed in the present experiments for the corticorubral projection.

DISCUSSION

The present investigations have shown that the time

course of the depolarization of red nucleus neurons following stimulation of the corticorubral pathway changed after chronic lesion of the nucleus interpositus of the cerebellum. The faster rising component of the potential started to develop several days after the IP lesion. Furthermore, the corticorubral depolarization increased in amplitude so prominently that it was much easier to initiate spike potentials in RN cells. This was revealed both by intracellular recording and by the increased field potentials induced in RN region by stimulation of the corticorubral fibers.

As for the nature of the new fast-rising component of the potential, it could be argued that it may not be an excitatory postsynaptic potential but rather a regenerative potential, such as dendritic action potentials (Llinás and Nicholson, 1971; Spencer and Kandel, 1961). The latter possibility was excluded for the following three reasons. First, the new fast-rising component could be smoothly graded by changing the stimulus intensity down to the amplitude of about 1 mV. Second, by changing the membrane potential either in depolarizing or in hyperpolarizing direction, the amplitude of the new component varied gradually, as would be expected for an EPSP (Eccles, 1964). By intense depolarizing current, it was even possible to reverse the potential. Third, the new component exhibited frequency potentiation by double or triple stimuli with a time course similar to that found for the normal slow corticorubral

EPSPs (Tsukahara and Kosaka, 1968; unpublished observation). Therefore, the new fast-rising component is concluded to be an EPSP.

There are several ways of interpreting the change in the time course of the corticorubral EPSPs. The most straightforward interpretation of the results is to assume sprouting from the intact corticorubral fibers and formation of new synaptic contacts at more proximal portions of the RN cell membrane. An alternative explanation would be that the partial denervation produced a morphological change (Cerf and Chacko, 1958; Jones and Thomas, 1962; Mathews and Powell, 1962) of RN cells, resulting in a drastic change of their cable properties, as has been observed in muscle membrane after denervation (Albuquerque and Thesleff, 1968). If, for example, the electrotonic distance from the soma to the distal dendrites had become shorter, the EPSP generated at the distal dendrites would be less affected by the cable properties and would appear less attenuated and with a faster rise time (Rall, 1964; Rall, Burke, Smith, Nelson and Frank, 1967). This possibility was excluded for the following two reasons. First, this cannot account for the dual peaks of the cerebral EPSPs. Second, there was no significant difference in the electrotonic length of RN cell membrane after IP lesion. There was a 10% increase of the main time constant but this should cause an increase, not a decrease, of time to peak of the CP EPSPs.

Another factor which had to be considered is the con-

ceivable development of a denervation hypersensitivity. Partial denervation is known to increase the sensitivity of postsynaptic membrane to transmitter substance, as demonstrated after complete denervation of muscle fibers (Axelsson and Thesleff, 1959; Miledi, 1960). Such a hypersensitivity could possibly be responsible for an increase of amplitude of the cortical EPSPs in RN cells, but fails to account for the decrease of rise time of the EPSPs. After this consideration of conceivable interference from changing cable properties and developing denervation hypersensitivity, it must be concluded that a new component appears after chronic IP lesion, which has never been observed in normal cats. The new corticorubral component must be generated at a more proximal portion of the somadendritic membrane of the RN cells than the original corticorubral EPSP, as judged by its faster rise time.

By assuming that the corticorubral fibers in normal cats terminate at the most distal portion of dendrites (the most distal compartment in terms of Rall's (Rall, 1964) compartment model), it is possible to estimate how this new component should be generated proximally. If the time to peak of the rostral CP EPSP (3.6 ms) of normal cats is only determined by the cable properties of dendrites ($\Delta Z = 0.22$), the model (see METHODS) predicts that the duration of the excitatory conductance increase should be 0.9 ms, when a pulse-shaped conductance increase was assumed. From Fig. 9, the expected location of the new component of cerebral EPSPs

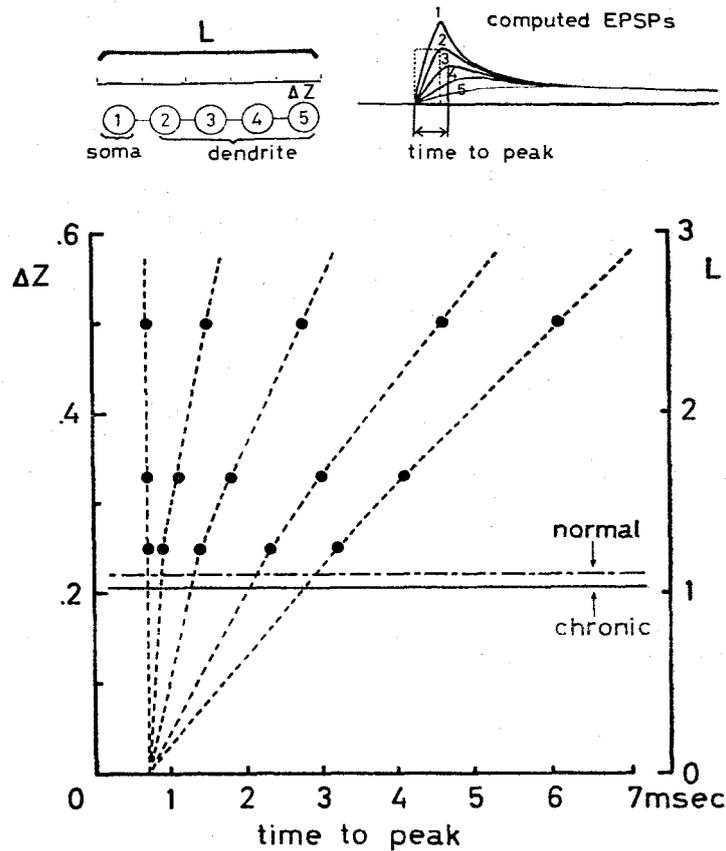


Fig.9 Estimation of the location of the fast rising component of CP-EPSP of chronic cats based on the compartment model of Rall. The inset illustrates a chain of five equal compartments having the same electrical parameters (see Methods). The pulse-shaped conductance increase of 0.9 msec duration (dotted line in the inset diagram) occurs in each one of these compartments and the resultant potential changes at the first compartment (soma) are called the "computed EPSPs". The times-to-peak of

these "computed EPSPs" are plotted against electrotonic length (L) or increment of electrotonic length (ΔZ). The computation was performed at three different values of ΔZ . The resultant times-to-peak of these "computed EPSPs" are plotted as filled circles. The normal cortico-rubral EPSP is considered to be initiated at 5th compartment (52). Dashed line shows the electrotonic length of normal cats (average of 25 RN cells). The solid line shows that of chronic cats (average of 11 RN cells).

which produced the time to peak of 1.3 ms, the peak value of the histogram of Fig. 2, must be the second compartment. Therefore, the new component is assumed to be generated at the proximal dendrites very close to the soma. This view may also account for the shortening of latencies of the CP EPSPs in chronic cats, since the latency of the distally located EPSPs tends to be estimated longer due to the delay of passive propagation along dendrites (Rall, 1964).

There are possible alternatives to the interpretation of our data other than occurrence of sprouting and formation of new synapses. One possibility would be that corticorubral fibers always make morphological synaptic contacts on the proximal as well as remote dendrites of RN cells, but that the former is physiologically inactive for one or more reasons (Cass, Sutton and Mark, 1973; Dennis and Miledi, 1974). However, this possibility seems to be refuted by ultrastructural evidence (Brown, 1974; King, Martin and Conner, 1972) that the corticorubral synapses in normal animals terminate selectively on distal dendrites.

The postulated formation of new synapses does not imply a priori an increase of the number of presynaptic fibers. Instead it may merely represent a dislocation of the synaptic contact from peripheral dendrites to more proximal portions, without any accompanying increase of presynaptic fibers. Although the question undoubtedly deserves a morphological analysis, it seems that the double peaks of the SM and CP EPSPs in cats with chronic IP lesions (see section 1 in RESULTS)

strongly favors the idea that the original dendritic projection remains intact, while newly formed collaterals terminate the synapse onto the more proximal region. This conclusion is further strengthened by the knowledge that formation of new synapses with an increase in the number of presynaptic fibers (collateral sprouting) occurs in the peripheral nervous system at the neuromuscular junction (Edds, 1953), in the sympathetic ganglia (Guth and Bernstein, 1961) as well as in some central synapses (Raisman, 1969; Raisman and Field, 1973).

The classical morphological evidence for the formation of new synaptic contacts at the light-microscope level (Goodman and Horel, 1967; Liu and Chambers, 1958; McCouch, Austin, Liu and Liu, 1958) gained considerable weight with the detailed ultrastructural studies (Raisman, 1969; Raisman and Field, 1973), as well as with the histochemical study (Moore, 1971) on the partially denervated septal nuclei of the adult rat by Raisman (Raisman, 1969), and Raisman and Field (Raisman and Field, 1973). Lynch et al. (Lynch, 1973) and Steward et al. (Steward, Cotman and Lynch, 1974) showed that field potentials in rat appear in parallel with the process of reinnervation established morphologically (Lynch, Mosko, Parks and Cotman, 1973), and their results and other (Wall and Egger, 1971) suggest that the new connections are functionally active. In this connection, it should be noted that most of the work referred to above was performed on the rat CNS, which normally continues to develop even in the adult. It may be of interest, therefore, to investigate the possibility

for collateral sprouting and formation of new synaptic contacts in species in which there is no further growth in the adult CNS (see also Guillery, 1972; Kerr, 1972; Lund and Lund, 1971).

The present results have provided strong physiological evidence for formation of new synaptic contacts with RN of adult cats after partial denervation of IP input. Recent electron microscopic work by Nakamura et al. (Nakamura, Mizuno, Konishi and Sato, 1974) has supported the present conclusion.

The recovery of function after cerebellar lesions is well known in the literature (Dow and Moruzzi, 1958). The mechanism for this recovery, however, has remained obscure. The formation of new synaptic contacts from the cerebral cortex onto the brain stem neurons, which receive both cerebellar and cerebral excitatory inputs, may provide one of the possible mechanisms for this compensation. The corticorubral synapses, for example, can compensate for the loss of the major synaptic input of RN cells from IP nucleus by occupying the more favorable location of spike initiation than the previous remote dendritic location.

SUMMARY

1. Intracellular recording was made from cat's red nucleus after chronic lesion of the nucleus interpositus (IP) of the cerebellum and the properties of the corticorubral

EPSPs were examined.

2. It was found that the time to peak of the corticorubral EPSPs induced from cerebral peduncle (CP EPSP) of chronic cats was much faster than that of normal cats. There was no simple decay following the rapid rise and early summit; instead there was another peak as if the slow normal CP EPSPs were superimposed on the fast ones.

3. By stimulating two loci, sensorimotor cortex and the cerebral peduncle, the conduction velocities of the fibers responsible for the newly appeared fast-rising component of the corticorubral EPSPs were measured. They were almost the same as those of normal corticorubral EPSPs.

4. The newly appeared fast-rising component of the CP EPSPs increased in size by membrane hyperpolarization and decreased and finally reversed in sign by membrane depolarization.

5. Electrotonic length of the red nucleus neurons, as estimated by analyzing the membrane transient response to current steps, did not change significantly after chronic IP lesion.

6. Time course of the change of the rise time of the CP EPSPs after IP lesion was investigated. At 4 days after lesion, there was already a significant shortening of the time to peak of the EPSPs. After about 10 days it reached the maximum and remained for more than 80 days.

7. There was a somatotopical organization of the projective areas of the fast-rising component of the corticorubral

EPSPs similar to that of normal cats.

8. It was concluded that the dendritic corticorubral synapses sprout to form synaptic contacts at the more proximal portion of the soma-dendritic membrane of red nucleus neurons. A quantitative estimation of the possible sites of the newly appeared synapses on the soma-dendritic membrane was made.

Chapter V

ANALYSIS OF UNITARY EPSPs MEDIATED BY THE
NEWLY-FORMED CORTICO-RUBRAL SYNAPSES AFTER LESION OF
THE NUCLEUS INTERPOSITUS OF THE CEREBELLUM

INTRODUCTION

It has until recently been considered that synaptogenesis does not take place in the central nervous system of adult mammals, although in the peripheral nervous system there is efficient regeneration (Clemente, 1964). However, Raisman (Raisman, 1969; Raisman and Field, 1973) reported electron microscopic evidence suggesting formation of new synapses in the septal nucleus of adult rat following partial denervation. A histochemical study (Moore et al., 1971) has supplied additional evidence of growth of sprouts in the septal area. Steward et al. (1974) have further provided knowledge that fibers from the contralateral entorhinal cortex give sprouts to the granule cells of dentate gyrus of adult rat following lesion of the ipsilateral entorhinal cortex.

The question needs to be raised at this stage whether these newly formed connections are physiologically effective. Attempts were made to test this problem by using indirect methods such as field potential analysis (Wall and Eggar, 1971; Lynch et al., 1973), but unfortunately there has been almost no detailed intracellular study of this problem in CNS.

Neuron of the red nucleus (RN) provides an excellent

material for testing this problem directly by using intracellular recording techniques, since quantitative electrophysiological properties of the synaptic organization and the electrical constants of the neurons have been investigated extensively (Tsukahara and Kosaka, 1968; Tsukahara et al., 1975c; Sato and Tsukahara, 1976). Indeed, it has recently been shown by intracellular recording that synapses are newly formed in RN cells after partial denervation (Tsukahara et al., 1974, 1975a,b). Cortico-rubral fibers which make synapses originally on the remote dendrites (Tsukahara and Kosaka, 1968; Tsukahara et al., 1975c; King et al., 1972), make new synapses on the proximal portion of the soma-dendritic membrane following lesion of interpositus nucleus (IP) of the cerebellum. This can be detected by the change in the time course of the cortico-rubral EPSPs; a new component with faster time to peak and larger amplitude appears after IP lesion superimposed upon the slowly rising ones.

The present paper is concerned with the properties of the unitary EPSPs generated through the newly-formed cortico-rubral synapses after chronic lesion of IP, while the subsequent paper (Murakami et al., 1977) deals with the properties of synaptic transmission.

A preliminary report on some part of the present study has been published (Murakami et al., 1976).

METHODS

Two types of preparations were used. The first type were with chronic IP destruction of the right side from twelve to one hundred and sixty one days previously. The second were cats without destruction of the IP nucleus. All animals were anesthetized with pentobarbitone sodium (35 mg/kg), immobilized by gallamine triethiodide, and artificially respired. Supplemental doses of pentobarbitone were injected intravenously as required. The body temperature of the animals was kept between 36°C and 38.5°C throughout the experiment.

The methods of recording from RN neurons and of stimulation and destruction of IP nucleus were essentially the same as those employed in our previous report (Tsukahara et al., 1975b). Intracellular recording was performed from RN neurons on the left side. Microelectrodes filled with 2M NaCl or 3M K-citrate and those with an electric resistance of 4-8 M Ω were preferred. Membrane potentials were monitored through a low gain, D.C. coupled amplifier on an oscilloscope (Tektronix 565) and amplitudes of the action potentials were monitored.

A pair of acupuncture needles insulated except at the tips was used for stimulation. Stimulating electrodes for cerebral peduncle (CP) were composed of 2-3 such acupuncture needles separated by 1.5 mm. Those for sensori-motor cortex (SM) were composed of two lines of five acupuncture needles

separated by 1.5 mm. A repetition rate of 1/sec was used for CP and SM stimulation.

The output of the voltage recording system of the preamplifier was amplified by a D.C. amplifier (Tektronix 3A3) and displayed on an oscilloscope (Tektronix 565) and also on a magnetic tape (Sony PFM-15). After the experiments the data in the tape were averaged by an averaging computer (Nihonkoden ATAC-250). 30 traces were averaged for the present analysis.

An analogue computer (Hitachi ALS-2000) was used for calculation of the theoretical EPSPs by Rall's compartmental model as previously (Tsukahara et al., 1975b, c).

RESULTS

The experimental results were based on 22 RN cells of seven normal cats and 26 RN cells from eleven cats with chronic IP lesion (referred to as 'chronic cat' below), which showed spike amplitudes of more than 50 mV ranging from 50 mV to 85 mV.

1. Time Course of Unitary EPSPs

Figure 1 illustrates examples of the unitary EPSPs evoked in RN cell by stimulating the sensorimotor cortex (SM). During SM stimulation with a fixed intensity cortico-rubral EPSPs appeared in an all or none fashion and also exhibited fluctuation of their amplitudes. The EPSP evoked in this all or none manner is considered to be due to activation of a single cortical cell or a fiber, or less likely a group of

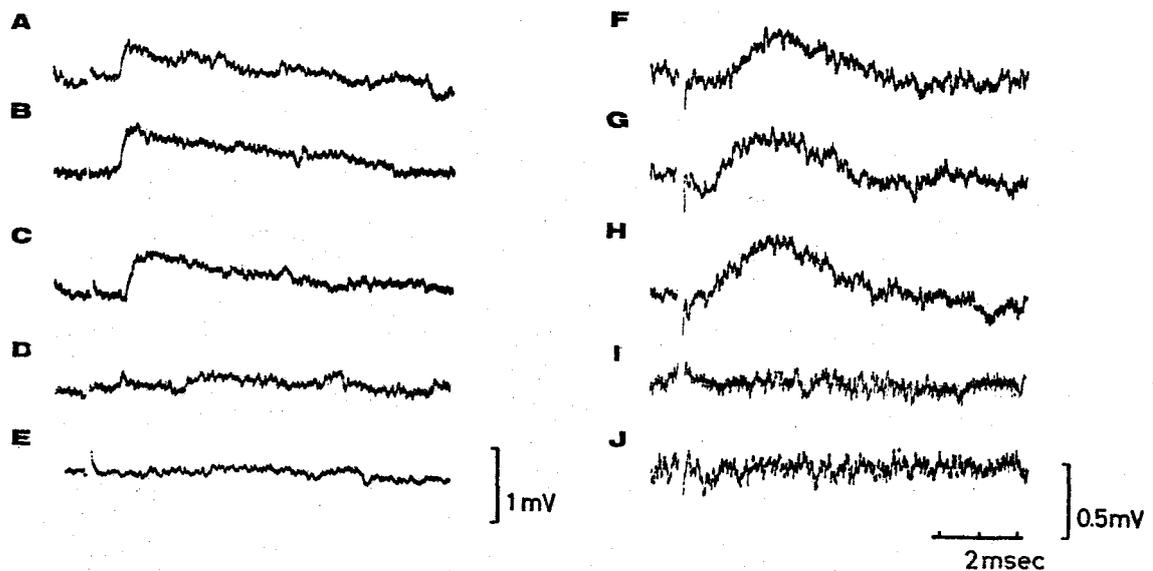


Fig. 1 Cortico-rubral unitary EPSPs. A-C: intracellular EPSPs evoked by stimulation of sensori-motor cortex at a rate of 1/sec in a cat with IP lesion 27 days before acute experiment. F-H: those in a normal cat. D: failures of responses which appeared at the same stimulus intensity as A-C. I: that in a normal cat. E, J: extracellular recordings just outside of the cell. Traces in A-D and F-H are part of consecutive records. Voltage calibration in E apply to A-D and that in J apply to F-I also. Time calibration is common for A-J.

single cortical cell or a fiber, or less likely a group of cortical cells behaving in an all or none manner with this stimulus condition which converges onto the recorded RN cell, and is referred to as an unitary EPSP. The time course of these cortico-rubral unitary EPSPs was compared in normal and chronic preparations.

Examples of cortico-rubral unitary EPSPs in a chronic cat are illustrated in Figure 1A-C whereas those in a normal cat are shown in Figure 1F-H. The traces shown in D and I are failures of responses with the same stimulus intensity as A-C or F-H, respectively and the traces shown in E and J are the extracellular recordings just outside the cell. As exemplified in this figure, the times to peak of many cortico-rubral unitary EPSPs are shorter and their amplitudes are larger in chronic cats than in normal ones. Photographs of 30-40 such consecutive responses were taken, and the time to peak, amplitude, and the latency were measured for each trace. In a few cases of chronic cats there were apparently double-peaked EPSPs. In these cases their amplitudes and their times to peak were measured for the first peak.

Time to Peak

As can be seen in the example of Figure 1, time to peak of the cortico-rubral unitary EPSPs is evidently shorter in chronic cats than in normal ones. Frequency distributions of the time to peak plotted from records of the EPSPs partly shown in Figure 1 are shown in Figure 2A and B for the

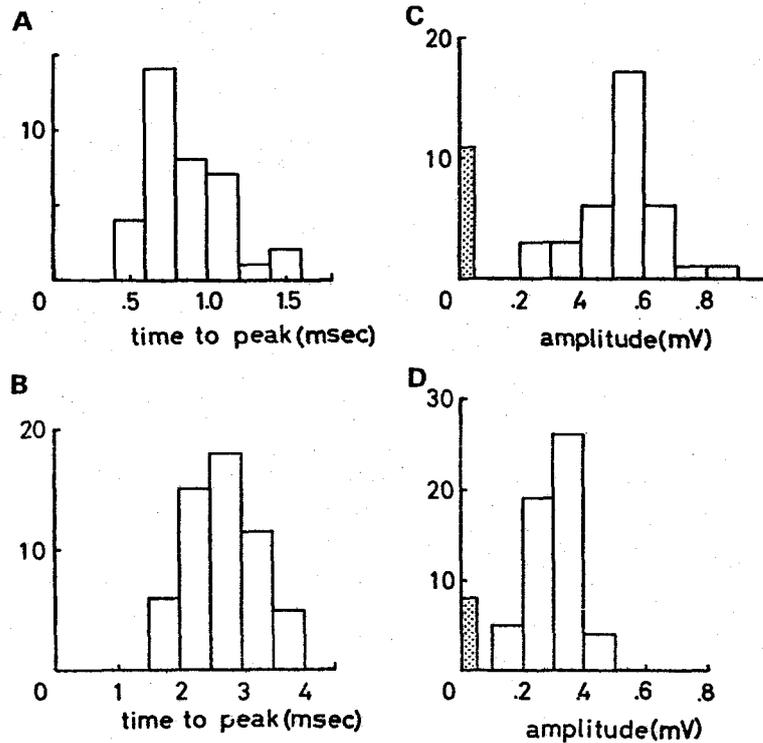


Fig. 2 Frequency distribution of the time to peak and amplitude. Time to peak and amplitude of the EPSP was measured from each of the consecutive traces of the EPSPs partly shown in Fig. 1. A: histogram of the time to peak of the unitary EPSP in a cat with IP lesion. B: that of the EPSP in a normal cat. C, D: histogram of the amplitude of the EPSP in a cat with IP lesion and normal cat, respectively. Shaded areas represent number of failures of the responses.

unitary EPSPs in a chronic and a normal cat, respectively. The average times to peak of the unitary EPSPs shown in Figure 2A B are 0.9 msec and 2.9 msec, respectively.

Amplitude

The amplitude of the unitary EPSPs is larger in chronic cats than in normal ones. The frequency distribution of their amplitudes for the EPSPs partly shown in Figure 1 is plotted in the histograms of Figure 2C and D, respectively. The distribution is unimodal and the mean amplitude of the EPSP in a chronic cat is 0.6 mV. Histogram of those of the normal preparation of Figure 1 is shown in Figure 2D. Again the distribution is unimodal, and the mean value is 0.3 mV.

In a few cases the distribution was not unimodal. These were not used for the present analysis.

Latency

It has been reported that the latency of the compound CP-EPSPs was slightly shorter in chronic cats than in normal ones. The average latency of the unitary EPSP of a chronic cat shown in Figure 1A-C is 1.4 msec and that in normal cat is 1.8 msec.

Altogether 26 unitary EPSPs recorded in cats with IP lesion and 22 unitary EPSPs in normal cats were sampled in this way and the average time to peak, amplitude and the latency of the series of 30-40 consecutive traces thus obtained are plotted in the histograms of Figure 3. It is noted that the time to peak is shorter and the amplitude is

larger in chronic cats (Fig. 3A, C) than in normal cats (Fig. 3B, D). The mean, S.D. (and the range) of time to peak and the amplitude of the EPSPs in normal cats are 2.68 ± 0.61 msec (1.25-3.90; n=22) and 0.33 ± 0.09 mV (0.23-0.41; n=22), respectively. Similarly, the mean, S.D. (and the range) of time to peak and the amplitude of the EPSPs in chronic cats are 1.36 ± 0.59 msec (0.81-2.64; n=26) and 0.48 ± 0.16 mV (0.22-0.89; n=26), respectively. The difference of the time to peak and amplitude of the EPSPs in chronic cats and those in normal ones is statistically significant ($p < 0.001$).

Likewise, latencies of the unitary EPSPs evoked by stimulation of CP or SM in chronic cats and normal ones are shown in Figure 3E and F, respectively. The latency is slightly but not significantly shorter, 1.11 ± 0.17 msec (n=15) for CP-EPSPs in the chronic cats, than in the normal, 1.16 ± 0.17 msec (n=14). Similarly for SM-EPSPs, the latency is 2.01 ± 0.58 msec (n=11) in the chronic and 2.15 ± 0.51 msec (n=8) in the normal cats.

Figure 4 shows the relation between time to peak of the unitary EPSPs and their peak amplitudes. Filled circles represent EPSPs in normal cats and open circles represent those in chronic cats. It is noted that the EPSPs in normal cats had large time to peak and small amplitude, while those in chronic cats showed a wider range of variability on the graph. Furthermore, there is a tendency for the larger unitary EPSPs to show a shorter time to peak.

A similar relation of the time to peak versus amplitude

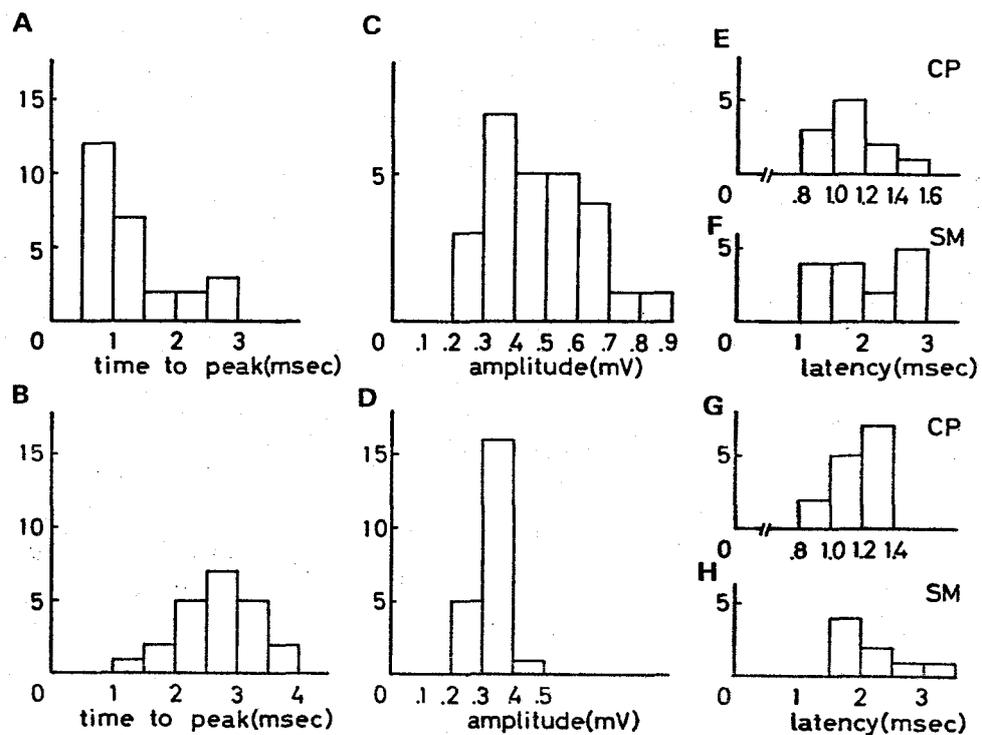


Fig. 3. Summarized histogram of the mean time to peak, amplitude and latency of the cortico-rubral unitary EPSPs.

A, B; histogram showing the relation between number of EPSPs and mean time to peak of the EPSPs. C, D: histogram showing the distribution of amplitude. E, G and F, H: histogram showing the distribution of latency by stimulation of CP and SM, respectively. A, C, E and F: for chronic cats. B, D, G and H: for normal cats.

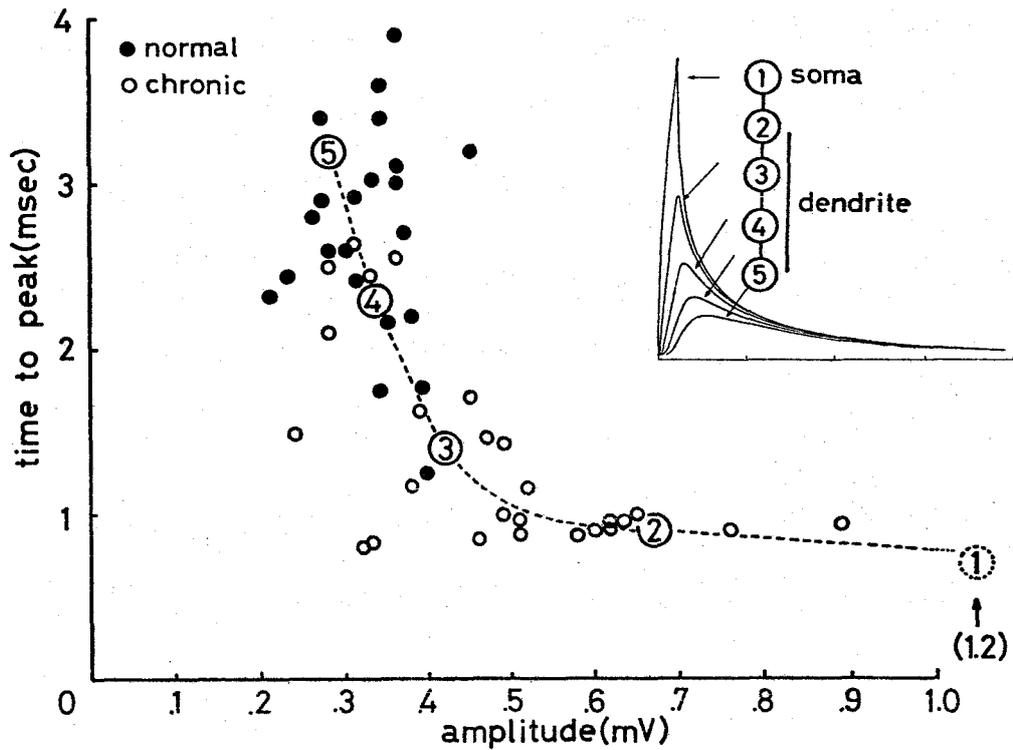


Fig. 4 Relation between time to peak and amplitude of unitary EPSPs. Open circles represent unitary EPSPs of chronic cats and filled circles represent those of normal cats. Large open circles represent those of theoretical EPSPs derived by Rall's compartmental model initiated at the each compartment of a chain of five compartment. Numbers 1 - 5 in each circle indicate corresponding compartments. The time course of the theoretical EPSPs generated in those compartments is diagrammatically shown in the inset of the figure.

was derived theoretically using Rall's compartmental model (Rall, 1964) and calculating the theoretical EPSPs generated at each compartment of the five compartment model as illustrated in the inset diagram of Figure 4. Times to peak and amplitudes of these theoretical EPSPs are plotted in the same graph as large circles labelled as 1-5. For deriving this relation the same parameters as in our previous computation (Tsukahara et al., 1975c) were used and an excitatory conductance increase of the same time course was assumed to be localized in each compartment, i.e., membrane time constant of RN cells was assumed to be 6 msec and the total electrotonic length as 1.1. Pulse-shaped excitatory conductance change of 0.9 msec duration was employed for the generation of EPSPs at each compartment. By assuming that the peak amplitude of the theoretical EPSP caused by the conductance change at the most distal compartment (compartment 5) is about $300\mu\text{V}$, it is possible to compare the theoretical results with those derived experimentally. A good agreement of the theoretical curve with the experimental points strongly supports the view that cortico-rubral synapses are newly formed in chronic cats on the dendritic trunk electrotonically close to the soma.

Presence of the EPSPs of the normal range either in the time to peak or the amplitude is also noticed in chronic cats. This suggests that there are cortical fibers which do not give any sprouts (see section 2).

In a few cases cortico-rubral unitary EPSPs with

combined fast and slow components were clearly observed. Figure 5A-E shows part of the consecutive records where an EPSP with double peak appeared in all (A-E) or none (F) fashion. Slight decrease in stimulus intensity caused complete failure of responses.

2. Fast and slowly rising cortico-rubral EPSP during membrane potential displacement.

Sometimes both fast and slowly rising EPSPs were recorded in the same RN cell when stimulating different loci of the sensori-motor cortex of chronic cats. Fig. 6 illustrates an example of a pair of averaged records of fast and slowly rising EPSP in an RN cell.

When the membrane potential was hyperpolarized (Fig. 6B and C) by injecting current through the impaling microelectrode, the amplitude of the fast rising EPSPs increased whereas the amplitude of the slowly rising EPSP remained almost constant. The relation between the amplitude of the EPSP and the amount of injected current is plotted in Figure 6D. Open circles correspond to the fast rising EPSP and filled circles relate to the slowly rising EPSPs. Crosses represent the amplitude of fast-rising EPSP when initial amplitude without injection of current was normalized to be the same as that of slowly rising EPSP.

It was necessary to average the data because of the fluctuation of the amplitude of the EPSP. A large scatter

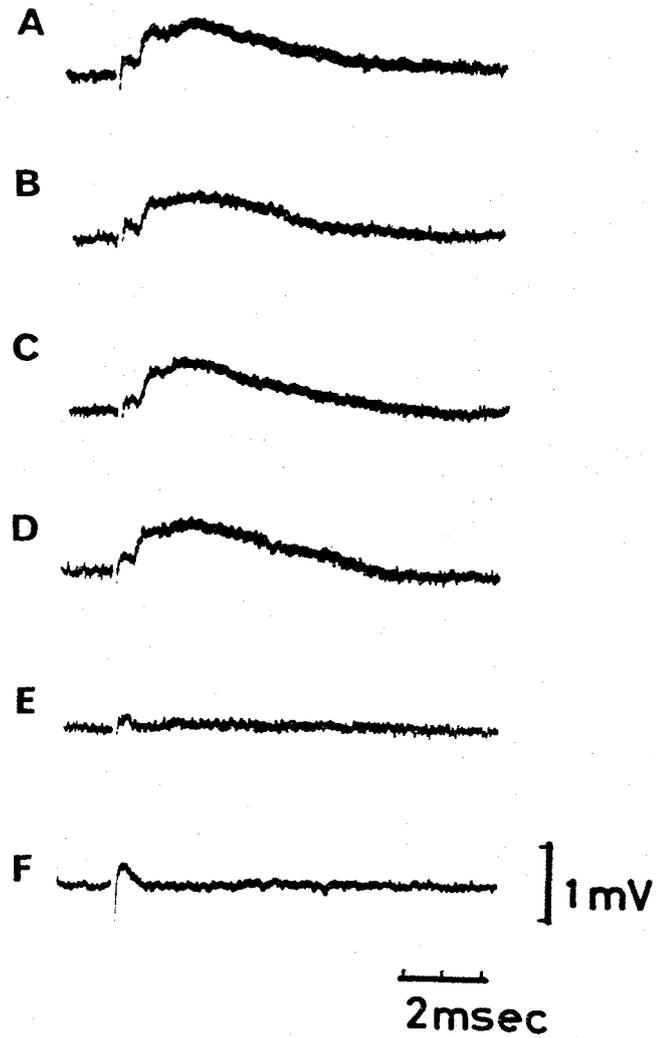


Fig. 5 Unitary EPSP with double peak A-E: part of consecutive records of cortico-rubral unitary EPSPs evoked by stimulation of cerebral peduncle in a cat with IP lesion. F: a failure of response.

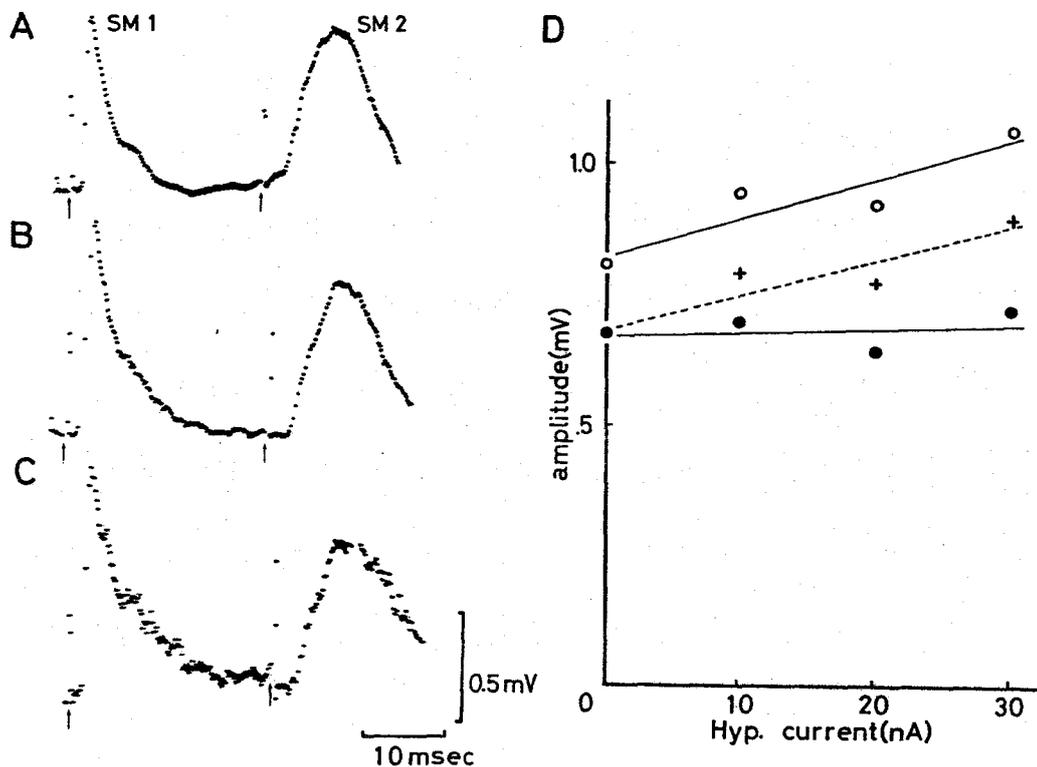


Fig. 6 Sensitivity of amplitude of the EPSPs to membrane polarization. A: fast rising (left) and slowly rising (right) corticorubral EPSP of unitary level recorded in the same cell by stimulating different points of sensorimotor cortex of a chronic cat. B: same as A but EPSPs when membrane potential was hyperpolarized by injecting current through microelectrode. Hyperpolarizing current of stronger intensity than in B was injected in C. 30 consecutive EPSPs were averaged in A-C. Upward arrows

in A-C indicate the time of onset of stimulation. D: relation between amplitude of EPSP and injected current. Open circles correspond to the fast rising EPSP and filled circles correspond to slowly rising ones in A-C. Crosses represent the percentage increase (right ordinate) of the amplitude of fast rising EPSP. The control amplitude without current injection was adjusted to be the same as that for slowly rising ones. Left ordinate; amplitude of EPSP. Abscissa; intensity of injected current. Lines were drawn by the method of least squares.

of the points in Figure 6D might be accounted for by the variation of the membrane resistance, since it took at least 30 sec to obtain one point of the graph of Figure 6D. However, it is noted that the amplitude of the fast rising EPSP increased more prominently (34%) than that of the slowly rising one (7%). Further, the coefficient of regression (obtained by the method of least squares) is significantly larger for the fast rising EPSP (dashed line) than that for the slowly rising one.

Similar difference of sensitivity of the amplitude of EPSPs to injected current was observed in two other RN cells. This can be accounted for by proximal location of the synapses responsible for the fast rising EPSPs on the soma dendritic membrane of RN cells compared to the slowly rising one and supports the view that the fast rising cortico-rubral EPSPs are newly formed at the proximal portion of the soma-dendritic membrane of RN cells (see discussion).

DISCUSSION

In an extent of a previous study (Tsukahara et al., 1975b), a more detailed analysis of the cortico-rubral EPSP after chronic lesion of the IP nucleus has now been made. By providing a detailed knowledge of the properties of the unitary EPSPs the present investigation has confirmed the previous conclusion that new synapses are formed in RN cells from corticorubral fibers after chronic lesion of IP (Tsukahara et al., 1975b). Firstly, a new group of unitary EPSPs with faster rising time course and larger amplitude than in normal cats was observed in RN cells of chronic cats. Secondly, this new group of unitary EPSPs had a time course and an amplitude quantitatively predictable by a theoretical consideration that new cortico-rubral synapses were formed at the proximal portion of the soma-dendritic membrane of RN cells. Thirdly, when the membrane potential was hyperpolarized the amplitude of the fast rising group increased whereas that of the slowly rising unitary EPSP remained almost unchanged. Taken together these results indicate that sprouting and formation of new synapses of the cortico-rubral fibers occurred at the proximal portion of the soma-dendritic membrane of RN cell.

A theoretical prediction of Rall's model which can be compared to the experimental neuronal membrane tran-

sient was investigated previously (Sato and Tsukahara, 1976). The theoretical transient of somatic membrane potential change induced by applying current step to the soma is expressed as a sum of exponential functions. The ratio of amplitudes of the fast and the second largest exponential functions of this theoretical membrane transient was found to be proportional to that of their respective time constants. This relation is useful to evaluate the applicability of the Rall's model to RN neurons. Experimental analysis of the membrane transient responses produced by injecting current step to RN cells showed that this theoretical relation holds true for RN cells (Tsukahara et al., 1975c). Furthermore, it was confirmed that there was not significant difference in membrane time constants and electrotonic length of RN cells between normal and chronic cats (Tsukahara et al., 1975b). Hence we accepted this model for calculating the theoretical unitary EPSPs and used the same parameters as used previously for normal cats. It should be noted, however, that calculation of the theoretical unitary EPSP is based on the assumptions that duration and intensity of transmitter action are the same for all synapses and the equilibrium potential of the EPSPs is also the same.

The presence of unitary EPSP with double peak which behaves in all or none manner as shown in Figure 5 indicates that there exist cortico-rubral fibers making new

synapses on the same RN cell as their original termination. A frequent observation of cortico-rubral unitary EPSP with fast time to peak and simple decay may indicate that there exist newly formed cortico-rubral synapses produced by cortico-rubral fibers running close to the RN cell without original synapses on these RN cells. In the previous report (Tsukahara et al., 1975b) it was found that fast conducting pyramidal tract fibers which normally form synaptic contact with inhibitory interneurons in RN region make new synapses on rubro-spinal neurons. However, the potential produced by the fast conducting pyramidal fibers was usually very small. It must also be considered that absence of the slow component superimposed on the fast rising ones in cortico-rubral EPSPs should be interpreted with caution, because a slight reduction of the membrane resistance diminished the electrotonic propagation of the remotely located dendritic EPSPs to the soma. Thus, the slow component of the cortico-rubral unitary EPSP of small amplitude tends to be overlooked.

In summary, the appearance of the fast rising component of cortico-rubral unitary EPSP after chronic lesion of IP is considered to be due to the formation of new synapses on the dendritic trunk electrotonically close to the soma of RN cell.

SUMMARY

1. Unitary EPSPs were recorded intracellularly from neurons of the red nucleus (RN) by stimulating the cortico-rubral fibers in normal cats as well as those with chronic lesion of interpositus nucleus (IP) of the cerebellum

2. Two groups of unitary EPSPs were recorded in cats with IP lesion. One consisted of cortico-rubral unitary EPSP with shorter time to peak and larger amplitude than those in normal cats. The other consisted of unitary EPSPs of the normal range.

3. The appearance the fast rising group of cortico-rubral EPSP caused by IP lesion was theoretically predictable based on Rall's compartmental model by assuming that new synapses were formed close to the soma of the RN cells.

4. The amplitude of the fast rising EPSPs increased more prominently by hyperpolarising the membrane potential than that of the slowly rising ones.

5. It was concluded that the fast rising group of the unitary EPSPs which appeared following IP lesion was due to the formation of new synapses on the proximal portion of the soma-dendritic membrane of RN cell.

Chapter VI

PROPERTIES OF THE SYNAPTIC TRANSMISSION OF THE NEWLY FORMED
CORTICO-RUBRAL SYNAPSES AFTER LESION OF THE NUCLEUS INTER-
POSITUS OF THE CEREBELLUM

INTRODUCTION

Modification of synaptic transmission caused by preceding stimuli has been found in neuromuscular junctions and central synapses (Eccles, 1964) as an increase of amplitude of EPSPs for a short period after a preceding stimulus, 'facilitation' or as a prolonged enhancement of the EPSPs, 'posttetanic potentiation' (PTP), after repetitive synaptic activation. The mechanisms of facilitation and PTP have been shown to be due to an increase in the probability of transmitter release from presynaptic terminals (Kuno, 1971). Extensive studies on the frog neuromuscular junctions have further shown that this is caused by influx of Ca ion into the presynaptic terminals (Katz and Miledi, 1968; for review see Kuno, 1971).

There are considerable variations in the degree of facilitation or potentiation in synapses of different presynaptic origin even in the same central neurons (Eccles, 1964; Kuno and Weakly, 1972; Phillips and Porter, 1964; Tsukahara and Kosaka, 1968; Toyama et al., 1970) in contrast with the matching of facilitation among the nerve fibers converging onto a single muscle fiber in crustacean neuromuscular junctions (Atwood and Bittner, 1971; Frank, 1968),

In the red nucleus (RN) neurons, it has been reported that cortico-rubral EPSPs show a marked facilitation

(Tsukahara and Kosaka, 1968), while interposito-rubral EPSPs exhibit neither facilitation nor depression on repetitive stimulation of the presynaptic fibers (Toyama et al., 1970). The cortico-rubral synapses normally make synapses on the distal dendrites of RN cells (Tsukahara and Kosaka, 1968; Tsukahara et al., 1975b). Following the lesions of the nucleus interpositus (IP), whose termination in RN is somatic (Toyama et al., 1970), occurrence of sprouting and formation of new cortico-rubral synapses on the proximal dendrites of RN cells have been reported previously (Tsukahara et al., 1975a).

Then a question arises whether the newly appeared cortico-rubral synapses have the same characteristics of synaptic transmission as the original cortico-rubral ones or not.

The present study attempted to obtain an answer to the above question. Synaptic transmission of the newly formed cortico-rubral synapses after IP lesion was analysed by examining facilitation of cortico-rubral unitary EPSPs in comparison with those of normal cats. Some properties of PTP were also examined both in chronic and normal cats.

A preliminary report on some part of the present investigation has been published (Murakami et al., 1976).

METHODS

The experimental procedures were essentially the same as those described previously (Murakami et al., 1977). In order to avoid complications which might be introduced by stimulating

transsynaptically the cortico-rubral neurons in the cerebral cortex, cortico-rubral fibers were stimulated at the cerebral peduncle (CP) in the present study. In the 'facilitation' experiment the stimulus intensity of CP was adjusted as small as possible so that the amplitudes of cortico-rubral EPSPs are in the range of unitary EPSPs in order to avoid contamination by polysynaptic IPSPs (Tsukahara et al., 1968) and to make the summation of EPSPs in the linear range (cf. Martin, 1955).

RESULTS

The data presented here were sampled from RN cells in cats where the interpositus nucleus was destroyed from fifteen to one hundred and sixty-one days prior to the acute experiment (referred to as chronic cats below) and those in normal cats. Data were collected from RN cells having spike amplitudes of more than 50 mV and showing stable resting potentials.

1. Facilitation of Cortico-Rubral Synaptic Transmission

When double stimuli with the same stimulus intensity were applied to cerebral peduncle (CP), the amplitude of the second EPSP was larger than that of the preceding one as shown in Figure 1A-D. This facilitation of the synaptic transmission produced by the preceding stimulus was seen for CP-EPSPs in both the chronic (Fig. 1A and B) and the normal cat (Fig. 1C and D). In all the experiments described here,

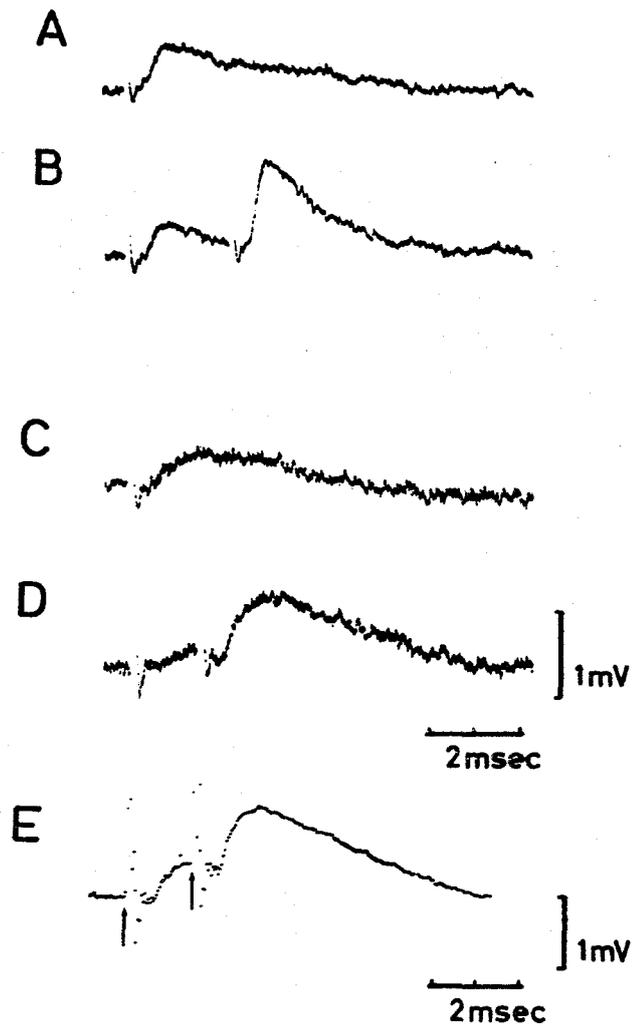


Fig. 1 Facilitation of cortico-rubral EPSPs. A, C: examples of EPSPs evoked in RN cells by stimulation of CP. B, D: same as A and C but the EPSPs in response to two successive stimuli to CP. A, B: in a chronic cat. C, D: in a normal cat. E: an averaged record of the EPSPs partly shown in D. Upward arrows in E indicate the onset of stimuli. Voltage calibration in D applies to A-C also. D.C. recording.

the interval between the paired double stimuli was fixed as large as 1 sec so that there would be no residual facilitatory effect due to the preceding double stimuli. As has been described in the previous paper (Murakami et al., 1977), there were considerable fluctuations in the amplitude of cortico-rubral EPSPs. Therefore, the responses were averaged by an averaging computer and the averaged records as exemplified in Figure 1E were used for the subsequent analysis.

The degree of facilitation was defined as the ratio of the difference between the amplitudes of the first (V_1) and the second (V_2) EPSP against the first response,

$$f_{12} = (V_2 - V_1)/V_1,$$

as illustrated in the inset diagram of Figure 2A. This is plotted in Figure 2A for an example of a normal cat against stimulus interval. As shown in the example shown in Figure 2A the facilitation attains its peak at the interval of about 3 msec and thereafter declines in two phases; it initially decreases rapidly and then slowly.

The time course of the facilitation thus obtained was measured in 14 RN cells of chronic cats and 12 RN cells of normal ones. The mean and standard deviation were then calculated and plotted in Figure 2B and C against stimulus interval for the chronic (Fig. 2B) and the normal cats (Fig. 2C), respectively.

Although the degree of facilitation for the EPSPs in chronic cats (Fig. 2B) is slightly smaller than those in normal ones (Fig. 2C), the difference is not statistically

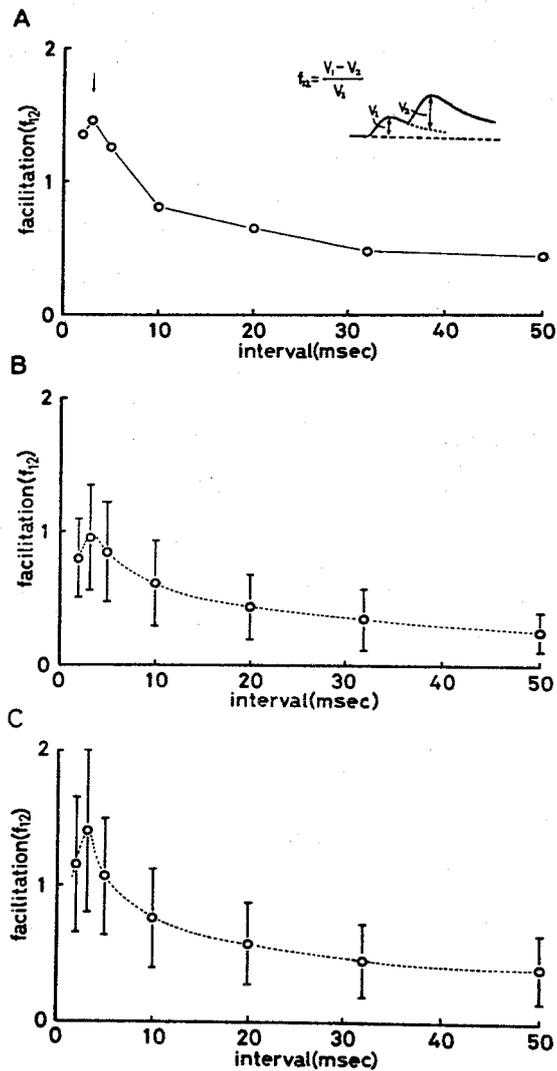


Fig. 2 Time course of facilitation of cortico-rubral EPSPs.

A: degree of facilitation of an EPSP in a normal cat measured in the manner as illustrated in the inset is plotted on a logarithmic ordinate against the interval between two stimulus pulses of CP. B: the mean time course of facilitation of 14 EPSPs in chronic cats was plotted on logarithmic ordinate against stimulus interval (open circles). The plotted points could be fitted by a straight

line except for the initial 10 msec. The slope of the straight line gives a time constant of 44 msec. The differences between the experimentally obtained values (open circles) and the extrapolated straight line (dotted line) were replotted in the same graph (filled circles). These values could be fitted by another straight line which gives the second time constant of 6 msec. C: same as B, but the mean time course of facilitation of 12 EPSPs recorded in normal cats. The time constants are 54 msec and 3 msec for the normal cats. Straight lines giving the large time constants were drawn by applying the least-square method for points between 10 msec and 50 msec and those giving the small time constants were drawn by applying the same method for points between 3 msec and 10 msec.

significant ($p > 0.05$, t-test, compared at each interval). By comparing the mean time course of facilitation in chronic and normal cats shown in Figure 2B and C, it is noticed that time course of facilitation decays approximately exponentially on the average with a comparable time course both in chronic and normal cats except for the initial 10 msec.

The amplitude of the third EPSP was still larger than the second as shown in a specimen record in Figure 3A, where three successive stimuli of the identical interval and intensity were applied to CP in a chronic cat. The degree of facilitation caused by the preceding stimuli was defined by the ratio of the difference between the EPSPs produced by the third stimulus and that by the first stimulus to CP divided by the amplitude of the first EPSP as illustrated in Figure 3B. The degree of facilitation for the third EPSP as defined above was measured in the same RN cells of chronic cats as used in Figure 2B at various intervals and the mean facilitation was plotted in Figure 3C against stimulus intervals. The larger degree of facilitation for the third EPSP than for the second may be consequence of addition of facilitation such as linear summation of facilitation (Mallart and Martin, 1967; Muir and Porter, 1973).

In the experiment shown in Figure 4, the amplitude of the first and the second cortico-rubral unitary EPSPs responding to two successive stimuli to CP were measured directly from photographic records without averaging.

A facilitation of the second fast rising cortico-rubral

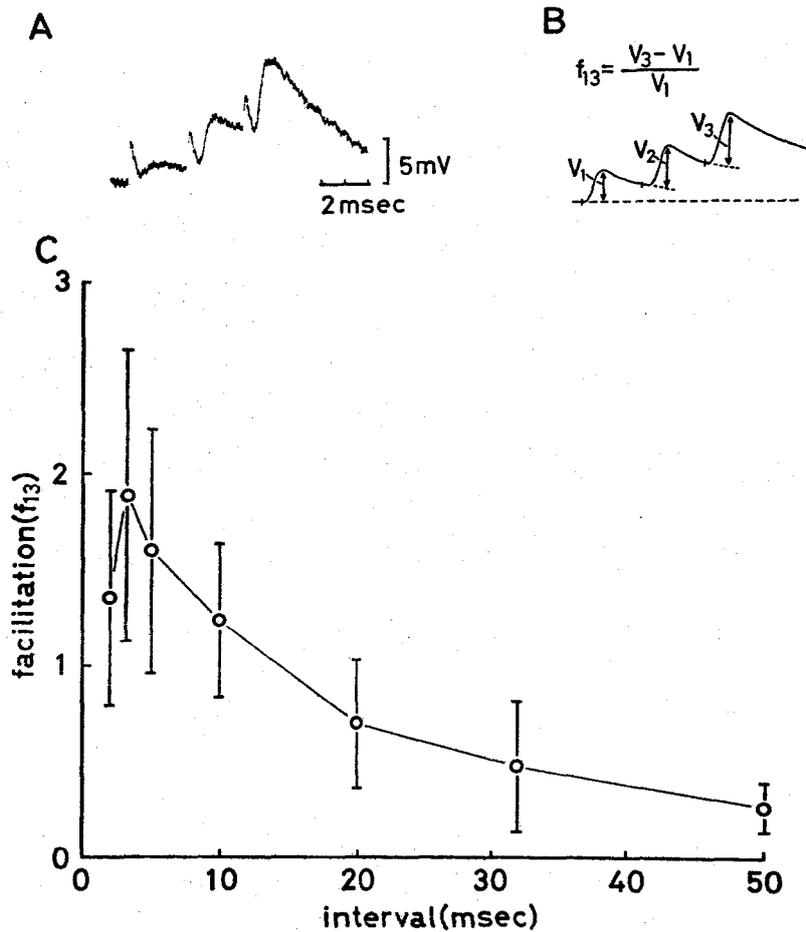


Fig. 3 Facilitation of cortico-rubral EPSPs to three successive stimuli.

A: a specimen record of cortico-rubral EPSP in response to three successive CP stimuli in a chronic cat. D.C. recording. B: the method for measuring the facilitation for the third EPSP (f_{13}).

f_{13} is defined as a difference between the peak amplitude of the third and the first corticorubral EPSP ($V_3 - V_1$),

divided by the first EPSP (V_1). C: facilitation for the third EPSP. Ordinate; mean facilitation for the third response of 12 EPSPs when triple stimuli of identical interval and strength were applied to CP of chronic cats. Abscissa; interval between stimulus pulses. Vertical bars represent standard deviation.

EPSPs was observed even when no fast rising EPSP was evoked by the first stimulus to CP (Fig. 4B, control in Fig. 4A). Further, as would be expected the amplitudes of the second EPSPs were mostly larger than the first ones (Fig. 4A and C). Figure 4D shows the relation between the amplitudes of the first cortico-rubral EPSPs (V_1) and those of the second (V_2). It is clear that V_2 is larger than V_1 ($p < 0.01$), and the amplitudes of the second EPSPs are significantly larger than V_1 ($p < 0.02$, t-test) even when $V_1 = 0$ (points on the ordinate). Figure 4D further indicates that frequency of occurrence of failures is markedly reduced for the second CP stimulus and that there was no correlation between the amplitudes of the second EPSPs and those of the first ($p > 0.1$, t-test, not including failures). Measurements of V_1 and V_2 from photographic records were performed with appreciable accuracy in 4 RN cells of chronic cats and 2 RN cells of normal ones. In all of them similar relations between V_1 and V_2 as described here were obtained.

2. Relation Between the Time Course of EPSP and the Degree of Facilitation

The peak value of the facilitation (maximum facilitation) differed from cell to cell as is noticed from large standard deviation of the data shown in Figure 2B and C. It also varies depending on the time course of the EPSP as shown in Figure 5. It was found that there is a tendency that the more rapidly rising EPSPs (Fig. 5A) have the smaller facilitation (Fig. 5B) and vice versa (Fig. 5C and D). Figure 5E

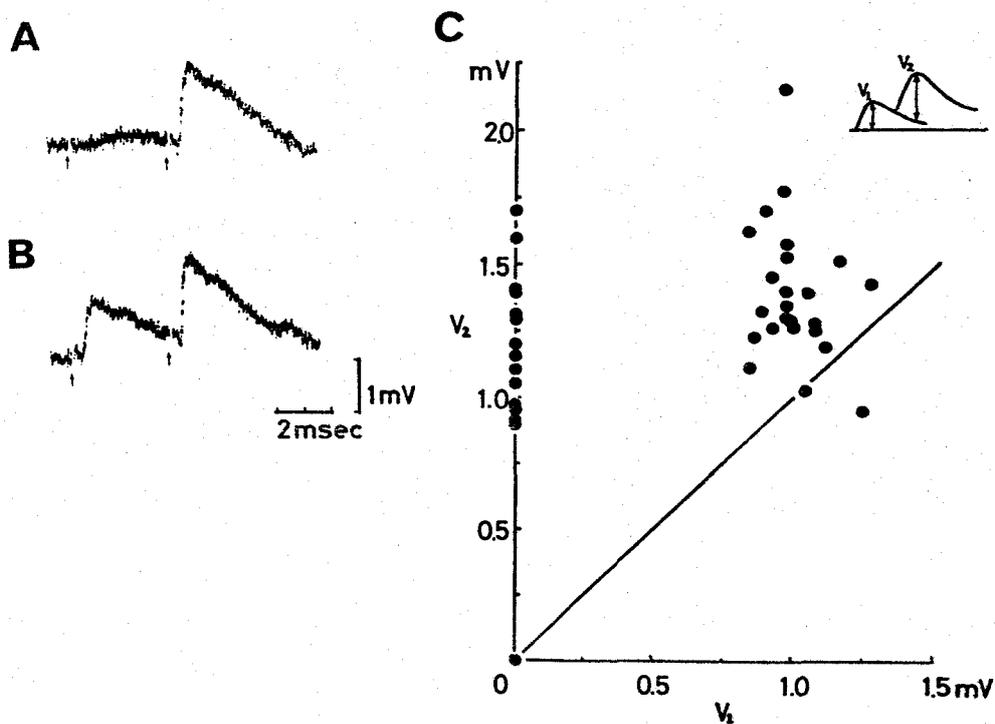


Fig. 4 A relation between the amplitude of first and the second cortico-rubral EPSPs. A: a photographic record of a cortico-rubral EPSP in response to a single CP stimulus. B: the same cortico-rubral EPSP as A in response to double CP stimuli with failure of response for the first CP stimulus. C: same as B but without failure. Upward arrows indicate the moment of CP stimuli. D: a relation between the amplitude of the first (abscissa) and the second (ordinate) cortico-rubral EPSPs. Amplitudes of the second EPSP (V_2) were measured as in the inset figure. The straight line represents the relation satisfying $V_1 = V_2$.

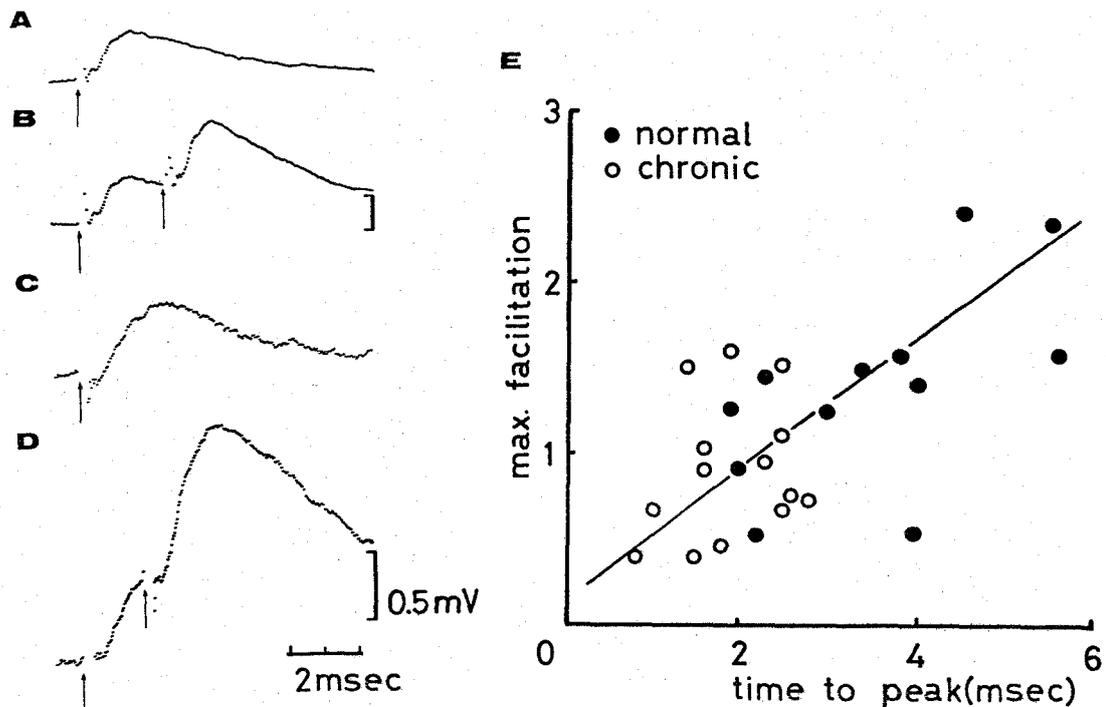


Fig. 5 A relation between the degree of facilitation and the time to peak of the EPSPs. A: a fast rising cortico-rubral EPSP. B: same as A but in response to two successive stimuli to CP. C: a slowly rising cortico-rubral EPSP. D: same as C but the EPSP in response to two successive stimuli to CP. Upward arrows indicate the moment of CP stimuli. E: the relation between the maximal degree of facilitation (ordinate) and time to peak of the EPSP (abscissa). The maximal degree of facilitation was defined by the summit value of facilitation curve as shown in Fig. 2A. Filled circles for normal cats and open circles for chronic cats. A straight line was drawn by the least-square method from the data points.

shows the relation between the maximum facilitation and the time to peak of the EPSP. The maximum facilitation was measured at the summit of the facilitation curve as shown in Figure 2A (downward arrow). The correlation between the maximum facilitation and the time to peak of the EPSPs is statistically significant ($r = 0.63$; $p < 0.001$, t-test) (cf. Porter, 1970).

3. Posttetanic Potentiation

A posttetanic potentiation of the cortico-rubral EPSPs was also found both in chronic cats and in normal ones after repetitive CP stimulation. The upper traces of Figure 6A and B show examples of cortico-rubral EPSPs in a chronic cat before tetanic stimulation of CP. Following tetanic stimulation at 200 Hz for 10 sec (represented by stippled area) the amplitudes of the EPSPs increased as shown in Figure 6C and D. Similar increase of amplitudes of the EPSPs was also seen in normal cats (control in Fig. 6E and F upper traces) after tetanic stimulation of CP as shown in Figure 6G and H (upper traces). Judging from the simultaneously recorded membrane potential change produced by the injection of a square-wave current (lower traces in Fig. 6A-H) there was no appreciable change of membrane resistance of the RN cell after tetanic stimulation.

Typical examples of the time course of the potentiation are shown in Figure 7A and B for the EPSPs in a chronic and a normal cat, respectively. Amplitudes of the EPSPs are represented by large filled circles. The maximal potentiation

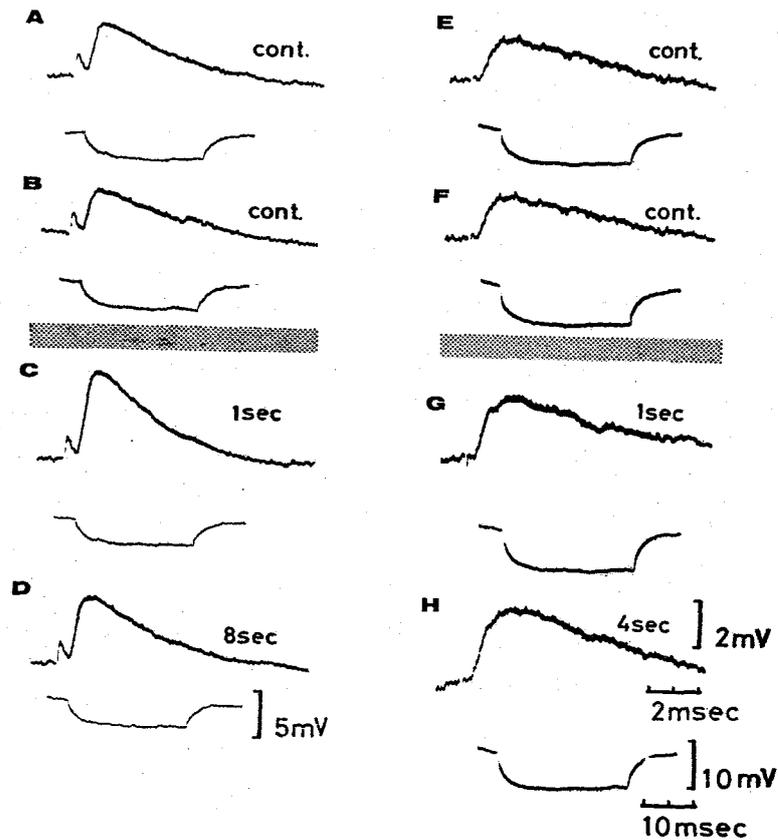


Fig. 6 Posttetanic potentiation of cortico-rubral EPSPs. A fast rising cortico-rubral EPSPs before (A, B, upper traces) and after (C, D, lower traces) tetanic stimulation of CP at 200 Hz for 10 sec in a chronic cat. E, F: same as A and B but the EPSP in a normal cat (upper traces). G, H: same as C and D but in a normal cat after tetanic stimulation of CP at 200 Hz for .20 sec (upper traces). Times after tetanic stimulation are labelled in each trace of C, D and G, H. Lower traces in A - H are the membrane potential changes produced by the passage of current pulses through microelectrode immediately after recording the EPSPs.

Stippled areas represent the period of tetanic stimulation. The voltage calibration in D applies to the lower traces from A to D and that in the lower trace of H applies to the lower traces from E to H. The voltage calibration in the upper trace of H applies to the upper traces from A to H. Time calibration of 2 msec in the upper trace of H applies to all the upper traces and that in the lower trace of 10 msec of H applies to all the lower traces. Lower traces are all D.C. recordings.

of the amplitudes of EPSPs was attained immediately after the tetanic stimulation. The potentiation declined slowly lasting for several minutes. Similar potentiation of the amplitudes of EPSPs was seen in 4 RN cells in chronic cats and 5 RN cells in normal ones. Open and filled circles in the lower part of Figure 7A and B represent membrane resistance and change in resting membrane potential, respectively. It can be seen that there is no appreciable change in the membrane resistance or in the resting membrane potential associated with the increase of the amplitudes of EPSPs. Thus the increase of the amplitudes of the EPSPs is a genuine PTP and not due to a change in the membrane potential or in the conductance of RN cells. A more prominent potentiation was observed as the frequency and/or duration of tetanic stimulation was increased. However, the degree of potentiation of fast rising cortico-rubral EPSPs in chronic cats was not appreciably different from that of the slowly rising EPSPs of normal cats.

DISCUSSION

The results in the present investigation have shown that cortico-rubral EPSPs mediated by the newly formed synapses exhibited facilitation and posttetanic potentiation as observed in the cortico-rubral EPSPs of normal cats. Furthermore, the degree of facilitation was larger for the slowly rising EPSPs than for the fast rising EPSPs; there was a correlation between the time to peak of the EPSPs and their

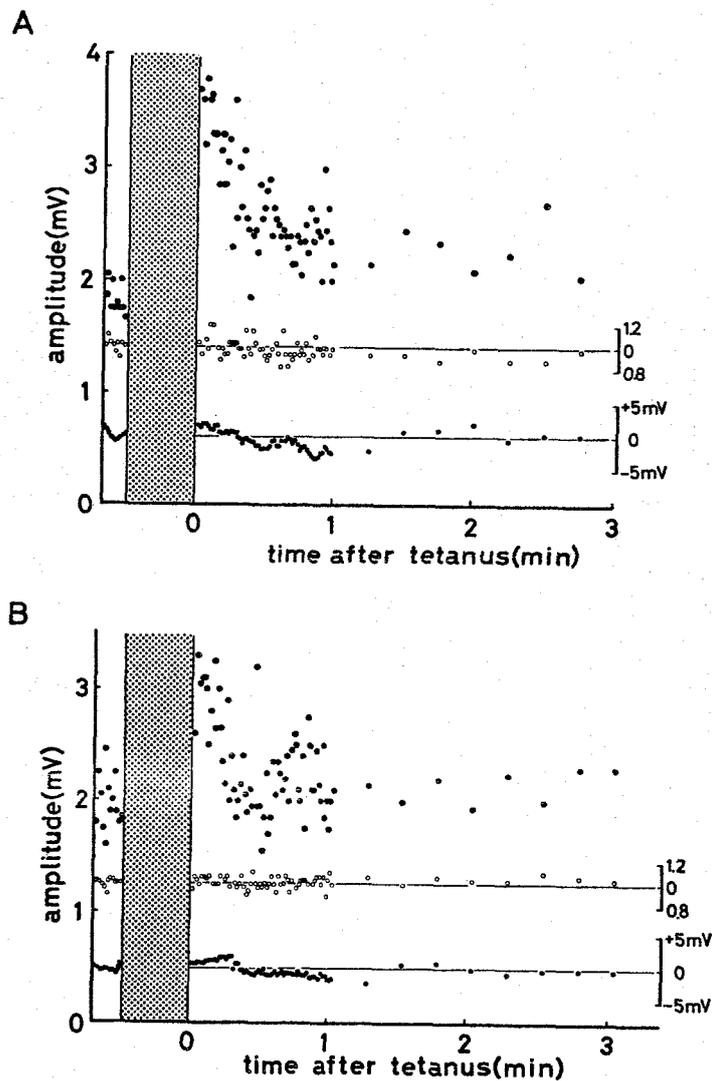


Fig. 7 Time course of posttetanic potentiation. A: the amplitude of cortico-rubral EPSPs (ordinate), membrane resistance, and resting membrane potential in a chronic cat are plotted against time before and after tetanic stimulation to CP. Tetanic stimulation to CP at 200 Hz for 30 sec is expressed as stippled column. Time 0 indicates the moment of cessation of tetanic stimulation.

Large circles; amplitudes of cortico-rubral EPSPs.
Open circles represent the membrane resistance as the
ratio of the mean value before tetanic stimulation.
Small filled circles represent the deviation of the
membrane potential from the mean value before tetanic
stimuli. B: same as A but in a normal cat.

degree of facilitation.

In agreement with the previous reports in neuromuscular junctions or peripheral and central synapses (Eccles, 1964; Kuno, 1971), the mechanism of facilitation in cortico-rubral synapses is assumed to be presynaptic in nature for the following reasons. In the first place, there was an enhancement of the second cortico-rubral EPSP even when the first stimulus to CP failed to evoke any EPSP. Secondly, the frequency of failures was much less for the second response than for the first. Thirdly, the amplitudes of the second EPSPs were not correlated with those of the first. These observations cannot be explained by any postsynaptic mechanisms as a change in sensitivity of postsynaptic membrane to the chemical transmitter (Watchel and Kandel, 1971; Bennett, 1971).

The presence of a correlation between the degree of facilitation and the time to peak of the EPSPs does not come about secondarily to IP lesion, because there is still a significant correlation ($p < 0.05$, t-test) between the degree of facilitation and the time to peak of the EPSPs in normal cats (filled circles in Fig. 5E). The correlation rather indicates that there is a positive correlation between the degree of facilitation and the location of the synapses producing the EPSPs, since the difference in the time course of the EPSPs has been fully explained by the different location of the synapses on the soma-dendritic membrane of RN neurons (Tsukahara and Kosaka, 1968; Tsukahara et al., 1975a;

Murakami et al., 1977; Rall, 1964).

Although the mechanism of facilitation for cortico-rubral EPSPs is considered to be presynaptic as discussed above, there is a possibility that the difference in the degree of facilitation in individual EPSPs might be due to some postsynaptic mechanisms which modify the amplitudes of cortico-rubral EPSPs recorded by the intrasomatic microelectrodes.

1. A non-linear property of the membrane such as the anomalous rectification of the membrane (Nelson and Frank, 1967), which produces an increase of membrane resistance for a depolarization, would modify the amplitudes of the EPSPs. It is expected that EPSPs produced at the distal dendrites are susceptible to this modification more prominently, because larger depolarization might be produced by the same synaptic current due to larger input resistance of distal dendritic branches (Rall and Rinzel, 1973). 2. A participation of some active process such as a dendritic spike (Spencer and Kandel, 1961).

Dendritic spikes may be generated by the second stimulus with a higher probability than for the first, because the second EPSP is superimposed upon the depolarization caused by the first EPSP. 3. A shortening of the dendritic electronic distance caused by the preceding depolarization; in this situation the second cortico-rubral EPSP is propagated from distal dendrites to the soma with less attenuation than for the first EPSP. This would result in an apparent increase of the EPSPs generated by the second stimulus.

With the above mentioned mechanisms which presuppose

dendritic depolarization by the preceding EPSP it could not be possible to account for the long-lasting time course of the facilitation. However, as for the facilitation at short stimulus intervals it is possible that the above mechanisms play an important role. Nevertheless, these possibilities are contradicted by the following reasons. a) The non-linear property of the membrane has not been observed in RN cells when tested by injecting rectangular current through intrasomatically penetrated microelectrode (Tsukahara et al., 1975b). b) The amplitude of cortico-rubral EPSPs was increased linearly by the hyperpolarizing current and decreased by depolarizing current either in chronic (Tsukahara et al., 1975a) or in normal cats (Tsukahara et al., 1975b). The linear change of the amplitudes of the EPSPs to injected current can hardly be expected if the current-voltage relation of the membrane is not linear. The reason against the second possibility is that there is no inflexion in the rising phase of the second cortico-rubral EPSPs judging from the photographic recordings as shown in Figure 1 and 4, unlike dendritic spikes observed in chromatolysed motoneurons (Eccles, 1964) and hippocampal pyramidal neurons (Spencer and Kandel, 1961).

The third possibility is readily excluded since a shortening of the dendritic electrotonic distance should result in a reduction of the time to peak of the second cortico-rubral EPSPs but no appreciable change in the rise time of these EPSPs was observed during the period of facilitation.

It now seems unlikely that the difference of the degree of facilitation is related to some postsynaptic mechanisms. Therefore, the alternative possibility that it is related to a property of presynaptic fibers is more likely.

There are several possible presynaptic mechanisms for the above property of the facilitation. The most attractive of these is that presynaptic terminals of RN cells receive a kind of information about the location of the postsynaptic sites. This postulated information might be utilized to control the degree of facilitation of that presynaptic terminal. Indirect support of this interpretation is the accumulative evidence in neuromuscular junctions that there is a kind of information flow from postsynaptic site to the presynaptic terminals (Kuno et al., 1974; Yip and Dennis, 1976; Brown and Irons, 1977; Grinnell et al., 1977).

The alternative explanation for the observed relation between the degree of facilitation and the synaptic location of these synapses is to assume that synapses which are not activated by a single impulse because of the blockade of impulse propagation at the branching of the presynaptic terminals are activated by two or more successive stimuli, and that the degree of this recruitment of the inactive synapses is more prominent in the more distally located cortico-rubral synapses. This would require that several conditions be met. First, it would require that there are a significant number of branches of the cortico-rubral fibers, and second that they should be blocked for some reason,

resulting in the presence of the inactive synapses. Third, it necessitates that the degree of the blockade of impulse conduction is reduced by the second or third impulse than by the first, thus producing the enhanced facilitation. However, there is nothing to indicate that these three conditions are fulfilled.

SUMMARY

1. Properties of synaptic transmission during and after repetitive activation of the newly formed cortico-rubral synapses were examined in the red nucleus neurons (RN) of cats after lesions of the nucleus interpositus of the cerebellum (chronic cats) as well as in normal ones.

2. A prominent facilitation of the amplitude of cortico-rubral unitary EPSPs was observed in both normal and chronic cats when a stimulus to the cerebral peduncle (CP) was preceded by another stimulus by 2-50 msec.

3. Time course of the facilitation shows that it attains maximum at the interval of about 3 msec and decays approximately exponentially lasting for 50 msec or more.

4. When three successive stimuli of identical intensity were applied to CP, the degree of facilitation was more prominent than that for double shock.

5. There was a positive correlation between the time to peak of the cortico-rubral EPSPs and their maximum value of facilitation.

6. The posttetanic potentiation of the cortico-rubral

EPSPs was observed after tetanic stimulation to CP in chronic and normal cats. It lasts for a few minutes in both cases.

Chapter VII

General Discussion

The present study revealed that collateral sprouting and formation of new synapses occurs in adult cats following chronic lesion of IP.

The study of collateral sprouting started more than twenty years ago, when occurrence of axonal sprouting from intact axons was found following partial denervation of the motor nerve (Edds, 1953). The anatomical evidences for collateral sprouting at the light microscopic level has been subsequently accumulated in the spinal cord (Liu and Chambers, 1958; McCouch et al., 1957) as well as in the superior cervical ganglion (Murray and Thompson, 1956; Guth and Bernstein, 1960). Further anatomical evidences for lesion-induced collateral sprouting have been presented in various parts of the CNS based on histochemical (Moore et al., 1971; Stenevi et al., 1972; West et al., 1975; Steward et al., 1974) as well as on electronmicroscopic results (Raisman, 1969; Raisman and Field, 1973; Lund and Lund, 1971; Mathews, 1976a; b). However, these histochemical and morphological studies failed to demonstrate the functional effectiveness of the synapses formed by the new collateral sprouts. There have also been some physiological works in which collateral sprouting was suggested to occur in the CNS by the analysis of the field potentials (Wall and Eggar, 1971; Lynch et al., 1973; West et al., 1975; Steward

et al., 1974). But such field potential analysis remained indirect and could not decide whether the newly appeared components are due to collateral sprouting or denervation hypersensitivity (Axelson and Thesleff, 1959; Kuffler et al., 1971) or dendritic spike (Spencer and Kandel, 1961).

The present study proved that the newly formed cortico-rubral synapses following IP lesion are indeed functional. It was found that they show properties of synaptic transmission similar to those of normal corticorubral synapses, when presynaptic fibers were activated repetitively.

Nadler (1973) reported that lesion of the entorhinal cortex of the rat eleven days after birth was followed by formation of anomalous septohippocampal projection, but that the thereby observed increase in activity of acetylcholinesterase was transient. Further, Chow (1975) found formation of ipsilateral retinal projection to lateral half of superior colliculus, which is normally innervated contralaterally, 14 - 26 days after enucleation in the rat just after birth. However, these new connections were mostly not functional when the animals were fully grown up. Also in the case of salamander functionally suppressed synapses by foreign nerve was finally eliminated morphologically (Dennis and Yip, 1978).

One may wonder whether the newly formed synapses in the RN continue to function permanently or not. The results of the present study indicate that the newly formed CR synapses are functionally effective at least for six month (see Fig. 7 of chapter IV) after lesion of IP.

It should be noted that CR unitary EPSP with double peak was observed in chronic cats and this was taken to indicate that the collateral sprouting occurred from CR fiber. This observation indicates that mother branch keeps its synaptic contact with the distal dendrites and also that these synapses maintain their functional effectiveness even after formation of new daughter branch at the proximal dendrite.

The CR unitary EPSP with double peak (Fig. 5 of chapter IV) may be the first direct evidence which showed both collateral sprouting with axon elongation and the functional effectiveness of the newly formed synapses. In the multiple synapse of the septum (Raisman, 1969) the distance between two different synaptic site is of the order of synaptic size ($\sim 5\mu\text{m}$) and this indicates that there was no axonal growth nor terminal proliferation. But in the case of RN neurons, the situation seems to be different. Since the interval between the first time to peak and the second one is about 2 msec, the electrotonic

distance between two synaptic sites is estimated to be about $4/5$ of the electrotonic length of the whole neuron, which is much larger than the synaptic size. This indicates that in the case of RN neurons axons have indeed grown new collateral branches and formed synapses.

Most of the works on collateral sprouting deal with abnormal situation, i.e., the growth of intact axons following lesion of their neighboring tissues. One may ask if the collateral sprouting as described here occur in the animal of normal situation and if it is the substrate for the plasticity of the behavior. The experiment that gives insight into this question is the one done by Tsukahara and Fujito (1976). They demonstrated that the collateral sprouting of the corticorubral synapses occur following cross-innervation of the forelimb nerves in the adult cat. They also observed behavioral recovery of the forelimb after the operation. Therefore, it is probable that axonal sprouting is the substrate for behavioral plasticity or adaptability, although other mechanisms such as postsynaptic plasticity (e.g. cf. Rutledge, 1976) should also be considered.

SUMMARY

1. The present study showed collateral sprouting and formation of new synapses in the mammalian CNS as well as their functional effectiveness which histochemical and morphological studies have failed to demonstrate.

2. The significance of the present study was discussed in view of the fate of the newly formed synapses and the original ones after IP lesion.

3. The difference between the present evidence of collateral sprouting and formation of multiple synapses was discussed.

4. The relation between axonal sprouting and the behavioral plasticity was also discussed.

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List of publications

- I. Tsukahara N., Hultborn H. and Murakami F.: Sprouting of cortico-rubral synapses in red nucleus after destruction of the interpositus of the cerebellum. *Experientia*, 30, 57-58, 1974

- II. Tsukahara N., Hultborn H., Murakami F., and Fujito Y.: Physiological evidence of collateral sprouting and formation of new synapses in the red nucleus following partial denervation. In: Golgi centennial symposium proceedings. Ed. Santini, Raven Press New York. 1975

- III. Tsukahara N., Murakami F., and Hultborn H.: Electrical constants of neurons of the red nucleus. *Exp. Brain Res.*, 23, 49-64, 1975

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- V. Murakami F., Fujito Y., and Tsukahara N.:
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- VI. Murakami F., Tsukahara N., and Fujito Y.: Analysis
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- VIII. Murakami F.: Formation of new corticorubral synapses
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Abbreviations used

CNS = central nervous system

CP = cerebral peduncle

EPSP = excitatory postsynaptic potential

IPSP = inhibitory postsynaptic potential

PTP = posttetanic potentiation

IP = interpositus nucleus

SM = sensori-motor cortex

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