MODES OF INTEGRATION IN THE CEREBELLAR CORTEX

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On the studies of cerebellar evoked potentials, many papers have been reported. These studies could be classified into two major categories, one is on the somatotopic localization obtained from their distribution\(^5\), the other concerns functional structures of the cortical components by analyzing the wave form under various circumstances\(^5\). However, the reports on the intercortical activities resulting from different peripheral nerve stimulation have been few\(^6\).

In the present study, functional organization of the cerebellar anterior lobe was studied by recording the surface evoked potentials at a fixed point on the cerebellar cortex elicited by centripetal stimulation of the peripheral nerves, to find out whether or not the phenomenon of summation or occlusion would occur, or if the cortical activity would interfere with each other in the recovery course. In this experiment the utricular (U), superficial radial nerve (R) and trigeminal nerve (T) were used as inputs and the mode of integration in the cerebellar cortex was studied from the relationship of the evoked potentials responsive to maximal or submaximal stimulation. The second positive deflection \((P_2)\) of the cerebellar evoked potentials was taken as the indicator of magnitude of excitation because \(P_2\) has been identified as the expression of synchronous firing of the Purkinje cells.

METHODS

The experiment was performed on cats (2.5–4.0 Kg) decerebrated by a precollicular transection under Nembutal anesthesia (35–40 mg/Kg). The cats were kept fully immobilized by Flaxedil and maintained under artificial respiration. For stimulation, the peripheral vestibular nerve (U) was exposed by Anderson and Gernandt's technique\(^6\). The tympanic cavity with the internal ear was opened and an electrode was attached to the nerve by means of dental cement, and paraffin oil was infused to protect the nerve from drying and to avoid spread of the current. After resecting the M. digastricus and M. mylohyoideus, the lingual nerve was exposed which runs between M.
pterygoideus internus and externus and a stimulating electrode was placed on the nerve and the incision sutured. Similarly, an electrode was attached to and buried with the superficial radial nerve.

The evoked potentials were recorded from various points of the surface of the cerebellar anterior lobe by using a monopolar elastic silver ball electrode (0.5 mm in diameter). To measure quantitatively, the potentials were made to superimpose, that is, below 50 and above 250 cps wave components of the potentials were cut off by 3db.

Amplification was provided by a differential AC-coupled preamplifier (Tektronix 122, 1 sec in time constant) leading into a DC amplifier (2A63 plug-in unit) on the 565 Tektronix oscilloscope. The stimulating pulses of 0.3 msec. were generated by the Tektronix 160 series instruments. Photographs were taken on film by a Grass Camera.

Successive single shock stimuli to a nerve was delivered at 3 sec. intervals to avoid fluctuation of the amplitude of the potentials. Great care was paid to preserve the surface of the cerebellar anterior lobe from dryness by means of coating with mineral oil.

RESULTS

1) Distribution of the evoked potentials by single shock stimulation of the ipsilateral superficial radial, trigeminal and utricular nerves at the surface of the culmen. Typical evoked potentials elicited by stimulation of the radial (R), trigeminal (T), utricular (U) nerve at the same point on the cerebellar anterior lobe surface are shown in Fig. 1. Each component of the potentials was identical in character, consisting of three deflections, i.e., the first slow positive (P₁), the second marked fast positive (P₂) and slow negative (N) deflection. Of these deflections, the P₂ deflection has been known to be susceptible to change in experimental conditions, such as administration of anesthetics or
repeated stimulation. Decrease of the amplitude was most pronounced on stimulating the utricular nerve. On the other hand, the P₂ deflection resulting from the radial and trigeminal nerve stimulation was relatively stable.

FIG. 2 shows the potentials recorded from the same point of the anterior lobe by stimulating the respective nerve. The potentials with high amplitude were recorded on the lateral part of the paravermian vein along the longitudinal axis. In the case of trigeminal nerve stimulation, this regularity of distribution of the potentials along the longitudinal line disappeared but appeared along the horizontal line of the folium. Projection area of the utricular-evoked potentials had a focus and the potentials appeared with high amplitude at a considerably localized area.

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2) Recovery curve of the evoked potentials by double shock stimulation to the same nerve. In order to observe the recovery process of the cortex following single shock stimulation, supramaximal double shock with various intervals was given to each nerve mentioned above. As illustrated in Fig. 3, the recovery
FIG. 3. Recovery curve of evoked potentials by double shock stimulation. The ordinate indicates the recovery ratio of evoked potentials and on the abscissa the interval of stimulation time in msec is represented. R+R, U+U, T+T indicate double shock stimulation to the radial, utricular and trigeminal nerve, respectively. Note that the recovery process of the three different nerves closely resembled each other., radial nerve., utricular nerve, trigeminal nerve.

process produced by stimulation of each of these nerves was nearly identical, i.e., the recovery ratio was 0% at the interval of 30 msec, 50% at 100 msec, 85% at 300 msec. respectively. However, it took one second for the influence of conditioning shock to fade away. Therefore, a smooth band as shown in Fig. 3 was depicted by enveloping the observed value of the second electrical deflections plotted on the time axis.

3) Recovery curve obtained by double shock stimulation to the different nerves. Six combinations were made for stimulation by picking two out of the three different nerves, U, T and R. Time intervals for double shock stimulation to the different combination of nerves were set and the preceding stimulation effect was evaluated by the size of the second evoked potential.

FIG. 4 indicates the recovery curve of the potentials in each group. In the case of U+T and U+R, the recovery curves showed comparatively similar processes but with regard to R+T and T+R, it seemed that nearly 100% recovery was observed by 50 msec and then decreased again. A similar process but with a different rate of recovery was also found when the relation of the evoked potentials was such that the amplitude of the preceding potentials was small in size. The recovery curve of R+U and T+U would suggest that
the curve is composed of two parts. Therefore, it is evident that the recovery curve differed in each group, but the cortical excitability shown as recovery curves fluctuated with a certain periodicity.

4) *Relationship between the negative component of the preceding potential and the recovery ratio.* As shown in Fig. 4 the recovery ratio was not determined by the modality of the nerve on which the conditioning shock was applied. But in the group of T+R, the recovery ratio seemed to be related to the amplitude of the preceding potential. Study of the relationship between the maximal amplitude of the negative wave of the preceding potential and the recovery ratio at that time showed linearity as illustrated in Fig. 5. As the amplitude of the negative wave is parallel with that of the second positive wave, it can be said that the mode of recovery process is attributed to the state of the Purkinje cell group which had reacted to the conditioning shock.
5) **The relationship among the reduction ratios of the potentials evoked by simultaneous stimulation of three different nerves.** The simultaneous stimulation designated in this section was set up so that the zero points of the second positive wave (P₂) obtained from the maximal stimulation of the individual nerves coincided at their summit. The amplitude of potentials actually obtained by maximal stimulation of two or three simultaneous different nerves was compared with the total amount of each potential height calculated arithmetically. By this comparison, the reduction ratios of simultaneous stimulation could be calculated.

A series of results obtained from the six points of the cerebellar anterior lobe are indicated in **TABLE 1.** That the reduction induced by simultaneous double shock stimulation to two nerves could be assumed as the result of occlusion, theoretical reduction ratio by simultaneous triple shock stimulation to three nerves could be mathematically calculated. Except one case in **TABLE 1,** (designated with an asterisk), estimated values well coincided with each measured value. This exception may have resulted from recording under the condition of intense potential fluctuation. Thus, as shown in **FIG. 6,** we could depict the territory of the somatotropic representation of the above three nerves by maximal stimulation. Area of a circle in **FIG. 6** indicates the relative magnitude of the potential height induced by a maximal single shock stimulation to each nerve, and the occlusion or reduction ratio is denoted by the overlapping of circles (shown by shaded area). It was found that the values of simultaneous double shock stimulation could be used to predict the value of simultaneous triple shock stimulation. Our trials will be concerned with the possibility of estimation of the reduction ratio by the size of evoked potentials elicited under natural fluctuation.

6) **Relationship between the potential height induced by the simultaneous double shock to the different nerves and its reduction ratio.** **FIG. 7** shows the relationship between relative values of the potential of higher amplitude when a single...
Table 1.

Reduction ratio of the evoked potential height responding to the simultaneous double, triple stimulation of different nerves. Expected value of simultaneous triple shock stimulation was calculated from the reduction ratio of double shock stimulation. Note that the theoretical recorded by stimulation of the radial, trigeminal and utricular nerve, except for the value with an asterisk.

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R: Radialis  U: Utricularis  T: Trigeminus

Figure 6. Convergence map on the culmen. Area of each circle R, U and T indicates the relative value of the potentials responding to a single radial, utricular and trigeminal nerve stimulation. Overlapping (hatched area) indicates the reduction ratio due to the convergence of the representations. The double hatched area indicates the overlapping common to the three representations. 1, 2, 3, 4, 5 and 6 on the triple circle indicates the locus on the anterior lobe from which the records were obtained as shown on the left hand of this diagram. Refer to the text in detail.
shock was applied to each combination of nerves and its reduction ratio resulting from the simultaneous double shock stimulation to the different nerves. Distribution of the values seemed to suggest that there was no relationship. But if we assume that the residual ratio of the potentials (i.e., 100% minus reduction %) is proportional to the potential height, the proportional constant, $K$, can be calculated in all the cases recorded at six points on the anterior lobe. A diagram of the $K$ value against the amplitude of the potentials in Fig. 8. The distribution of the values represents a rectangular hyperbola.
7) The time course of convergence resulting from stimulation of the different nerves. The time course of varying reduction ratios of the evoked potentials was studied by double shock stimulation of different nerves. The residual ratio after stimulation is shown with the ratio of the amount of cells which are estimated from individual recovery curve and that of cells actually fired, i.e.,

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\text{Residual ratio} = \frac{(F - S')}{(F - S)}
\]

F; Amplitude of the preceding evoked potentials
S; Amplitude of anticipated evoked potentials induced by test shock to the same nerve.
S'; Amplitude of the second evoked potentials obtained from double shock stimulation to the different nerves.

Fig. 9 illustrates an example of the results obtained from experiments in which a test shock with varying time intervals was employed in various combinations of nerve stimulation. In every case the ratio of convergence changed following a time course which might be understood to be due to waxing and waning fluctuation as shown by the dotted line.
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DISCUSSION

The cerebellar responses, evoked by a single shock stimulation of peripheral nerves, were first described by Dow in a decerebrated cat in 1939. Since then, distribution maps of the evoked potentials elicited by stimulation depending on the different modality of nerves have been reported. This somatotopic localization on the study of fiber connections is projected longitudinally as described by Brodal, but it has been known that the extent and arrangement of the somatotopic localization was functionally affected by various factors. Particularly, effects of anesthesia cannot be disregarded. It has been pointed out that with Nembutal anesthesia localization appears rather limited. In our experiments the distribution of the representation by single shock stimulation to the radial nerve appeared longitudinally, but in utricular nerve stimulation a relatively focal representation was observed under the same anesthetic conditions. The difference between these cases is that the evoked potentials by the utricular nerve stimulation are smaller in amplitude than that by the radial nerve stimulation. This may suggest that the extent of the area of representation is determined by the number of cerebellar cortical components which take part in the excitation. The same idea could be applied on the distribution map of representation under varying depths of anesthesia. Moreover, this dynamic concept on the localization would be supported also by appearance of otherwise unknown representation in chloralose anesthesia. From these facts, it would be assumed that the extension of the representation area is determined by dynamic factors rather than anatomical projections. Koella also investigated the evoked potentials by photic stimulation, and concluded that the extension of equipotential surface did not consist of a concentric circle. Furthermore, the morphological data are not sufficient to decide whether the transversal distribution of trigeminal representation is due to fiber projection or functional modulation.

In the second place, the mode of interference of impulses in the cerebellum through the sensory nerves by various modalities was reported by Snider and Deura by studying the evoked potentials which responded to photic and click stimuli on the cerebellar posterior lobe under Nembutal anesthesia, but relationships among representations on the anterior lobe have been rarely reported. The author, tried to study, as described above, the interference in the anterior lobe by processing interaction quantitatively, which was elicited from the radial, utricular and trigeminal nerve stimulation. Some attempts were made to establish an indicator for quantitation. There is no doubt that the Purkinje cell firing is represented in the second positive wave of the evoked potential, but there is the alternate choice of taking the potential height or the area of the second positive wave as the indicator. It seemed reasonable to measure the area as the indicator, but this was not our purpose, because
of the fact that the factors that determine the process of the descending phase of the second positive wave are not simple (Suda and Amano, 1964). On the other hand, the ascending phase of the second positive wave, as shown in Fig. 2 represents a single phenomenon, i.e., synchronous firing of the Purkinje cells. The author measured both the height and the area, and came to a conclusion that the data could be simplified when the potential height was used as the indicator.

One conclusion reached concerning cerebellar cortical activity is that the supermaximal or the submaximal double shock stimulation resulted always in occlusion and no summation of stimulation was observed. Considering the numbers of terminals which ended on the gigantic dendrites of the Purkinje cells, no subthreshold state whether excitatory or inhibitory in response to firing would be postulated. Therefore, the fact that summation could not be observed in the evoked responses to submaximal stimuli, suggests existence of a strong control mechanism over the firing of the Purkinje cells. A part of the mechanism would be attributed to the function of the basket cell considering the fiber connections and the synchronizations effect of the Purkinje cells (Suda and Amano 1964).

It is worthy to mention specially the mode of activity of the major representation in the cerebellar cortex by occlusion derived from the minor afferent system. The residual rate of the major representation was a function of the potential height with a varying proportional constant along a rectangular hyperbola. This suggests that the residual rate kept by major representation is constant in occlusion without relation to the magnitude of the input. Consequently, it is concluded that the input-output relation stated above is the basis of the stabilizing effect which is one of the major functions of the cerebellum.

The final feature of the cerebellum proposed in this paper concerns its excitability, i.e., the recovery curve of double shock stimulation to the same nerve shows a smooth long-lasting contour, but in the different combination of nerves the excitability recovers in waves, waxing and waning. It can readily be imagined that waxing and waning of occlusion would result from formation of a reverberating circuit. However, it will be a problem in the future to explain the organization of the neuronal nets.

A mode of activity of the cerebellar anterior lobe could be studied by means of quantitative processing of the evoked potentials, it would be necessary, however, to explore the extent of the electric field of the Purkinje cell firing, recorded by a surface electrode. Moreover, it will be a physiological requirement to confirm the results by recording the smaller limited area with a small tipped electrode and to investigate the relationship between the state of cortical activity and mode of manifestation in the effectors.
SUMMARY

Employing a Nembutal anesthesia, a cat was decerebrated, a quantitative study was performed on the distribution and overlapping of the evoked potentials obtained from the utricular, trigeminal and radial nerve stimulation in the cerebellar anterior lobe.

1. The waveform of evoked potentials obtained from the utricular, trigeminal nerve stimulation were the same in nature as that of radial nerve stimulation, i.e., the deflection consisted of the first slow positive, the second marked fast positive and the last slow negative deflection. The distribution of the utricular evoked potentials was localized at the ipsilateral margin of the anterior lobe but the trigeminal nerve representation spread along the folium in the cerebellar anterior lobe, contrasted with the longitudinal arrangement of the radial nerve representation.

2. The recovery process of the evoked potentials obtained by double shock stimulation of each nerve showed a smooth identical curve which recovered almost 100% by one sec, 85% by 0.3 sec. and 50% by 0.1 sec. but the second stimulation was invalid by 0.03 sec.

3. The recovery process of evoked potentials obtained by different nerve stimulation in six group of combinations showed the wavy periodical fluctuation which seemed to be due to the reverberating circuit of the cerebellum. This recovery ratio had a linear relationship to the negative components of the preceding potentials whose size was parallel to the synchronous firing of the Purkinje cells.

4. The mode of overlapping of activity due to occlusion of representations elicited by simultaneous double shock stimulation to different nerves, was different at the six points of the lateral portion of the cerebellar anterior lobe. It was demonstrated that the overlapping ratio of the simultaneous triple shocks could be anticipated from the result of overlapping in double shock stimulation.

5. It was found that the reduction ratio of the potentials, obtained from simultaneous stimulation of the different nerves, was not proportional to the amplitude of individual evoked potentials, but the ratio of the unaffected area of the major representation in double shock stimulation to the original extent of the representation, i.e., the residual ratio was a function of the potential height in a hyperbolic fashion.

6. The varying ratio of occlusion with lapse of time was followed and it was observed that the extent of the occluded area fluctuated with waxing and waning phases when activity produced from different kinds of nerve inputs interfered each other.
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REFERENCES