1. ELECTROPHYSIOLOGICAL ANALYSIS OF SINGLE NEURONS IN THE CAT'S AMYGDALOID NUCLEAR COMPLEX

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The stimulation- and ablation studies on the amygdaloid nuclear complex have verified that its function has an important relation to the emotional, autonomic, and sexual manifestations of animals. In order to understand its functional role it is both necessary and significant to trace the electrophysiological characteristics of the said single neurons by means of microelectrode technique in parallel with anatomical as well as other physiological methods. And yet this type of reports is very few besides the pioneer work by Machne and Segundo and our previous report. The observations reported here were performed on the single neurons in the principal nuclei of the amygdala and are concerned with the reactions of the neurons caused by the stimulation of the temporal tip, midbrain- and thalamic reticular system.

The results obtained will contribute to the elucidation of the functional role of the amygdaloid nuclear complex especially when they are considered in reference to our previous report which is concerned with the regulatory influence of amygdaloid nuclei upon the hypothalamic unitary activities.

Methods

The experiments were performed on 35 curarized adult cats. The left posterior sylvian gyrus was exposed as widely as possible, the dura being incised and reflected. The microelectrode was inserted horizontally through the cortical surface covering an area of about 2-3 mm² in the rostral part of the lower portion of the ectosylvian sulcus. The principal nuclei (baso-lateral nucleus) of the amygdala are found at the depth of between 5-6 mm and 9-10 mm measured from the cortical surface, the shallower part corresponding to the lateral principal nucleus, the comparatively basal deeper part to the medial principal nucleus and the middle part to the intermediate principal nucleus. (cf. Usui).
When satisfactory records were obtained, a fine steel needle was inserted in the same manner as the microelectrode was inserted, an electrolytic lesion was made, and the tip position of the microelectrode was ascertained by histological examination.

As the microelectrode, a glass pipette electrode filled with 3-MKCl was used. The electrical resistance was 20-30 MΩ. The electrical activities picked up by means of a microelectrode, were led to D C and C R amplifier through a cathode follower preamplifier, observed with the use of 2 or 3 beams cathode-ray oscilloscope, and recorded with a long recording camera.

The stimulating electrodes were steel needles of 0.2 mm in diameter insulated with Silicon Varnish except the tips. The electrodes were placed bipolarely at 1.0 mm distance on the inferior limit of the posterior ectosylvian gyrus, and also stereotaxically inserted into the desired portions of the midbrain reticular formation, the specific and nonspecific nuclei of the thalamus.

Results

Stimulation of the temporal tip region: Since the inferior limit of the posterior ectosylvian gyrus of the cat is said to be homologous with the anterior tip region of the monkey’s temporal lobe,1320) electrical stimulation or local strychninization was given to this region. As the electrical stimulation, the square wave of 5-10 c/s or sometimes 30 c/s, about 5 V in amplitude, 1 msec in duration was used in most cases. The units thus recorded were 59, of which 49 (5 submitted to intracellular recording) were tested by electrical stimulation and 10 (3 submitted to intracellular recording) were tested by the local application of 1% strychnine solution. The reactions observed in the unitary activity of the three principal nuclei of the amygdala were roughly devided into two types, i.e. excitation and inhibition. By electrical stimulation 21 units showed the former, while 16 showed the latter and in the remaining 5 no detectable reactions were observed. There was no areal difference perceivable in the proportion of these two reactions seen in the three principal nuclei, namely, lateral, intermediate and medial.

Fig. 1 shows these phenomena. There were seen two types of excitation; One is that the spike appeared with almost constant latency in response to each shock, the number of the spikes to each shock being increased by strong stimulation (A); the other is that no direct relation was discerned between spike response and each shock, and the number of the spikes increased as a whole during prolonged stimulation (B). Inhibition occurred in the form of decrease or disappearance of the spikes by stimulation (C). In most cases no spikes appeared both during and for a while after the stimulation although weak stimulation failed to cause complete disappearance of the spikes. Strong stimulation, on the contrary, caused an increase of the number of spikes after cessation of the stimulation or during the later part of it, the phenomenon showing the reversal
Fig. 1. Effect of stimulation to the temporal tip. A shows unitary activities of lateral principal nucleus. Spikes responded to stimulation (5 c/s, 4 V, 3 msec.) with latency of about 8 msec., but not always following each shock. This unit came to respond to every shock under prolonged application of high frequency stimulation. B shows unitary activities of the medial principal nucleus. Spikes increased diffusely by stimulation (25 c/s, 4 V, 1 msec.). C shows unitary activities of lateral principal nucleus. Spikes disappeared by stimulation (34 c/s, 10 V, 0.5 msec.). Bottom line indicates period of stimulation in each figure. Calibration: 5 mV. Time mark: 10 msec. in A and B, 20 msec. in C.

from inhibition to excitation. In such cases seizure discharges were often observed in the corticograms obtained from the stimulated region.

Fig. 2 shows the intracellular records from the amygdala neurons excited by the stimulation. The depolarizing wave with characteristic form, i.e. EPSPs, was generated by each shock with constant latency of about 8 msec, and spikes were initiated at a certain level of the EPSPs which was the critical level of spike firing (A and B), though the full-sized spikes were not always generated. This explains the fact that in the extracellular record (Fig. 1 A), spikes are not always caused by each shock. Hyperpolarizing waves, i.e. IPSPs, are also discerned with the latency of 25-30 msec. regardless of whether spike potentials precede them or not (A-a, B).

We can infer, from this, that there are both excitatory and inhibitory synapses derived from the temporal tip upon the said neuron in amygdala. Although it is rather difficult to compare the two neurons shown in Fig. 2 A and B, because both recording and stimulating condition differ from each other, it may be suspected
that the neuron of A has fewer inhibitory synapses. The EPSPs were increased in their amplitude and prolonged in their time course due to temporal and spacial summation as the shocks were repeated; consequently constant firing of the spikes and then the increase of the number of spikes by each shock were observed (b. c.). One the other hand, the IPSPs decreased in their amplitude or disappeared during the frequent repetition of the stimulation (c). Then in response to further high frequency stimulation of 24 c/s, 6 V, 1 msec, the membrane potential began to keep above the spike firing level soon after the stimulation was started, high frequency spike discharges being generated.

The intracellular record of a neuron in which activities were inhibited by the stimulation is shown in Fig. 3. As soon as the stimulus was applied, hyperpolarization was distinctly observed. Both during and for a few seconds after the cessation of the stimulation there was observed no spike firing. Variance was seen in the grade of hyperpolarization according to the intensity of the stimulation. For this reason, the neuron showed yet spike firing even during the weak stimulation, though the number of spikes was decreased; on the contrary, it needed several seconds for the appearance of the spike even after the cessation of the stimulation by the strongest stimulation; of course, in such case there were observed no spike during the stimulation.

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Fig. 3. Intracellular record from the lateral principal nucleus. Stimulation (50 c/s, 14V, 1msec.) was given to the temporal tip. During stimulation, hyperpolarization was seen and no spike discharge (Upper trace). This state continued for a while after cessation of stimulation. In course of gradual repolarization, IS spikes were observed (Lower trace). Calibration: 10 mV. Time mark: 10 msec.

Also by local strychninization of the cortical surface of the posterior sylvian gylus two types of reaction, i.e. excitation and inhibition, were observed. The intracellular recording revealed depolarizing waves on which spikes were superimposed as well as fairly long lasting hyperpolarizing waves.15)

Stimulation of the midbrain reticular formation: The electrical stimulations which ranged from 1-10 V, 0.5-1.0 msec. and 100-200 c/s were applied to the ipsilateral midbrain reticular formation. Among the 41 recorded units, 20 showed the phenomenon of excitation and 15 showed that of inhibition and the remaining showed no detectable reaction. (Fig. 4 A. B.) The areal difference in the principal nuclei in connection with these phenomena was difficult to trace. Fig. 4 C shows the intracellular record of a neuron excited by the stimulation. In 15-20 msec. after the start of the stimulation depolarization of the membrane potential began to appear. The grade of depolarization gradually increased until spikes appeared about 100 msec. later, and during the stimulation approximately the same potential level was kept with the frequent appearance of spikes. Such slow depolarization of membrane potential caused by high frequency repetitive stimulation explains, it seems to us, the fact that an effective electrical stimulation of the reticular formation requires a high frequency (above 100 c/s) and the latencies of the spike responses are above 100 msec. even with such a high frequency stimulation.

The effect of the stimulation on the reticular activating system is reduced by anesthesia. In Fig. 5 is shown the effect of the intravenous injection of Nembutal upon an inhibitory reaction.

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Fig. 4. Effect of stimulation to the midbrain reticular formation. Period of stimulation is indicated by broken line in bottom of each figure. A was recorded from the lateral principal nucleus. Stimulation: 120 c/s, 2 V, 1 msec. Spike discharges increased markedly from about 140 msec. after start of stimulation. B, recorded from the medial principal nucleus. Stimulation: 200 c/s, 2 V, 1 msec. Several spikes were seen during 150 msec. after start of stimulation but thereafter hardly any spikes. C, recorded intracellularly from the intermediate principal nucleus. Stimulation: 120 c/s, 2 V, 1 msec. During stimulation the membrane potential was shifted gradually toward depolarization and then spikes were generated frequently. Rapid upward deflection immediately after start of stimulation is an artifact. Calibration: 5 mV in A and B; 30 mV in C. Time mark: 10 msec.

As a means of understanding fully the effect of the stimulation of the midbrain reticular formation on the activities of the amygdala neurons, the effect of combined stimulations both to the midbrain reticular formation and to the temporal tip were tested on the 14 units which reacted to each stimulation independently. In one of those both of the two kinds of stimulation caused excitation and in 3, both inhibition; in 4, the stimulation to the reticular formation caused excitation and that to the temporal tip caused inhibition; and in 1, vice versa.

The unit which was excited by both showed, as in Fig. 6, marked increase in the number of spikes by the combined stimulation, whereas the unit which was inhibited by both was markedly intensified in the grade of inhibition. Fig. 7
shows the case in which the stimulation of the temporal tip caused inhibition, while that of the reticular formation caused excitation. When the stimulation of the reticular formation preceded, that of the temporal tip caused, though with slight delay, complete inhibition (A) and when the stimulation of the temporal tip preceded, even with the addition of that on the reticular formation no excitation occurred (B).

With the units in which the stimulation on the temporal tip caused excitation and that of the reticular formation appeared even during the stimulation on the temporal tip. But the degree of inhibition was not so high in the case of combined stimulation as that of the stimulation of the reticular formation alone.

The results of combined stimulation above described show that, in case of both excitation as well as both inhibition, the grade of the reactions is intensified by the combined stimulation beyond the simple sum of the effect of each stimula-
Fig. 6. Effect of combined stimulation to the temporal tip and to the mid-brain reticular formation. a, b and c were recorded from one and the same neuron of the lateral principal nucleus. a: Excitation was caused by stimulation to the reticular formation (120 c/s, 5 V, 0.5 msec.). Its period is indicated by broken line. b: Excitation was caused by stimulation to the temporal tip (20 c/s, 8 V, 0.5 msec.). Its period is indicated by solid line. c: Reaction was intensified by combined stimulation. Calibration: 5 mV. Time mark: 100 msec.

Stimulation, and that, to the case in which the two kinds of stimulation gave opposite effects, the one of the stimulation to the temporal tip is more dominant and powerful, masking that of the stimulation to the reticular formation.

Stimulation on non-specific thalamic nuclei: The stimulation was of 1-10 V, 3-30 c/s, 0.1-5.0 msec in most cases. Histological examination disclosed the position of the stimulating electrode tips in the thalamic nuclei to be the midline nonspecific nuclei in 6 cases; the lateral, lateral-ventral part of the reticular nucleus in 5 cases; specific relay nuclei in 3 cases; and in 1 case the electrode was not inserted in the thalamus. The stimulation to the specific relay nuclei caused either no change or slight one, this latter being interpreted as the result due to the current spread of the stimulation to the nonspecific thalamic nuclei. For this reason the specific relay nuclei may be considered to have no relation to the activity of the single neurons of the amygdala. Of 49 recorded neuron units, 12 showed excitation, while 16 showed inhibitory reactions, and 8 showed reversal from inhibition to excitation. With reference to these phenomena no areal difference in the principal nuclei was detectable.

Fig. 8 A shows the excitatory reaction. This reaction was often obtained by the stimulation of 5-10 V, 10-20 c/s, 1 msec. By relative weak stimulation,
the excitation occurred only during the stimulation, but with the intensification of the stimulation, considerable high frequency spikes were often observed even after the cessation of the stimulation.

Fig. 8 B shows the inhibitory reaction. By the stimulation of 22 c/s, 10 V, 1 msec, this neuron was inhibited in its spike firing. There were 8 units which showed reversal from inhibitory to excitatory reaction. Fig. 9 is one example of such cases. It is true that there existed some units which had inhibitory reaction only, but if the stimulation had been tried with varied intensities more neuron units would have had such reversal of reactions.

Fig. 10 A and B were recorded intracellularly from different neurons in the intermediate principal nucleus and the stimulated portions were also different ones in the thalamic reticular system. But in both each stimulation caused depolarization, i.e. EPSPs, and hyperpolarization, i.e. IPSPs. Whereas in A, each shock induced spike, because the EPSPs were dominant, in B, on the contrary, the IPSPs were dominant; especially during the application of the first 5 shocks the grade of hyperpolarization was strengthened gradually. Accordingly, EPSPs did not seem to reach higher than the critical level of spike firing though the level could not be decided definitely because of the drift of the amplifier and
other reasons. With the repetition of the stimulation the grade of hyperpolarization tended to decrease, and a small number of spikes came to appear. From the above fact it is evident that in those neurons both excitatory and inhibitory synapses derived from nonspecific thalamic nuclei are present.

Then what should be the explanation of the phenomena of excitatory and inhibitory reactions in the extracellular records? In most cases, it may be explained that they are to be attributed to the quantitative difference of the synaptic terminals between excitatory and inhibitory synapses. The reversal of reaction may be due to the difference of summation of synaptic potentials and that of the course of fatigue between excitatory and inhibitory synapses.

Upon 14 units the effect of the combined stimulation, i.e. that on the temporal tip and the nonspecific thalamic nuclei, was tested. Both caused inhibition
Fig. 9. Reversal of reaction due to stimulation of the nonspecific thalamic nuclei. This was recorded from the medial principal nucleus. Stimulation (20 c/s, 10 V, 1 msec.) was given to the n. centralis lateralis. Inhibition of spike discharges was seen for a while after start of stimulation, then turned into excitation. Calibration: 10 mV. Time mark: 20 msec.

Fig. 10. Effect of stimulation to the nonspecific thalamic nuclei. A and B were recorded intracellularly from the intermediate principal nucleus but from different neurons.

A: Stimulation (20c/s, 10V, 1msec.) was given to the n. reticularis. Not only depolarizing waves but also hyperpolarization were seen during stimulation. The depolatization increased in latter part of stimulus application. This is the same neuron as that in Fig. 2 B. Depolarizing waves were generated also by stimulation to the temporal tip as shown in Fig. 2 B.

B: Stimulation (18 c/s, 6 V, 1 msec.) was given to the n. centrum medianum. Spontaneous discharges were extremely rare. Depolarizing waves appeared with each shock. Membrane potential shifted diffusely toward hyperpolarization during stimulation. Generation of depolarizing waves was slight at the beginning of stimulus application, increasing in latter.

respectively in 9 cases; both excitation in 3; the former caused excitation and the latter caused inhibition in 1 case; and vice versa in 1. As these neurons
responded to both the cortical and the subcortical stimulation, together with the finding of intracellular recording, that these responses are generated transsynaptically, speaks for the existence of synaptic convergence from these two regions.

More marked excitatory reaction was observed by the combined stimulation of both temporal tip and thalamic reticular nuclei in the neurons which showed the reaction by the stimulation independently applied to both regions. The same is the result on the neurons which showed inhibitory reaction. Not only the inhibitory reaction was intensified during the combined stimulation, but also there were not a few cases in which even after the cessation of the stimulation this lasted more than several seconds. In neurons which showed the opposite reactions, the results obtained by the combined stimulation were rather complicated. Fig. 11 shows the records of a neuron in which the stimulation to the temporal tip caused inhibition and that to the nonspecific thalamic nuclei, excitation. The spikes whose number increased due to the stimulation of the nonspecific thalamic nuclei completely disappeared when the stimulation of the temporal tip was
added (A). When the stimulation of the temporal tip preceded, the addition of that to the nonspecific thalamic nuclei caused hardly any spike firing (B).

Similarly in the case of combined stimulation of temporal tip and midbrain reticular formation, those results appear to show that, while the activation of non-specific thalamic nuclei gives a regulatory effect, perhaps facilitatory one in most cases, to the effect of temporal tip stimulation, the phenomena, either excitation or inhibition, due to the stimulation on the temporal tip are more dominant, masking those of the stimulation of the non-specific thalamic nuclei.

**Discussion**

The electrical stimulation and local strychninization given to the ipsilateral temporal tip caused excitatory as well as inhibitory reactions in the single neuronal activities recorded from the principal nuclei of the amygdaloid nuclear complex, the intracellular recording thereof showing the generation of EPSPs and IPSPs. This verifies the existence of a neuronal connection between the temporal tip region and the principal nuclei of amygdala, supporting the result already obtained with the evoked potentials and seizure discharges as the indicator or from strychnine neuronography.2, 7, 8, 10)

The phenomenon that EPSPs increase while IPSPs decrease due to high frequency repetitive stimulation and consequently that the membrane potential come to be kept above the critical level with successive spike discharges is also observed in the neurons of the cat's cerebral motor cortex.16, 17) This is believed to be concerned with the mechanism of epileptic seizure discharges. And we have an impression that the neurons of the amygdaloid nuclear complex tend to get in the seizure state more easily than cortical neurons from the fact that such sustained high frequency spike discharges were often generated in the neurons of the amygdala.

It is said that the midbrain reticular formation is connected with the midline- and intralaminar nuclei of the thalamus,8, 23) and that there is an afferent connection from the latter to the amygdala.9, 10, 11) That these midbrain and thalamic reticular systems serve for the arousal activation of the spontaneous rhythmic electrical activities of the neocortex is a matter of course, and the same is expected of the amygdala. It is already well known that the arousal pattern is induced in the electrical activities of the amygdala by stimulating the midbrain reticular formation.4, 21) Therefore, the excitatory and inhibitory reactions of the unitary activities in the principal nuclei of the amygdala caused by stimulation to either the midbrain or thalamic reticular systems may be regarded as corresponding to the arousal pattern of the mass electrical activities in the said region of the amygdala. Also the fact that the effect of the stimulation to the temporal tip appeared more distinct and more dominant than that to the reticular systems in the experiments with the combined stimulation seems to be an expression of
a regulatory mechanism of the reticular system over the neuronal activities of the amygdaloid nuclear complex.

Sawa\textsuperscript{13} has previously made the following view public from the results of the investigations made by him and his collaborators,\textsuperscript{2,12,18} i.e. “Amygdala is a neuronal pool as a relay station existing between the cerebral cortex particularly the temporal lobe region and the subcortical structures especially the hypothalamic region. . . . . Whereas its function is chiefly inhibitory in normal physiological conditions, it rather easily turns facilitatory . . . .” Both our previous\textsuperscript{14} and present experiments by means of microelectrode technique, we are sure, will support such a view.

**Summary**

The experimental subjects consisted of 35 curarized adult cats. With the use of superfine microelectrodes, the single neuronal activities of the principal nuclei of the amygdala were picked up and the reactions to the electrical stimulation given to the temporal tip, midbrain reticular formation, and nonspecific thalamic nuclei were observed.

By the stimulation of the temporal tip, excitatory as well as inhibitory reactions were obtained. In some cases the reversal from inhibition to excitation either during or subsequent to the stimulation was observed. It came to be clear, from the intracellular recording, that EPSPs, the spikes superimposed on them, and IPSPs were generated by each shock, and that both excitatory and inhibitory synaptic contacts are present in one and the same neuron.

With the repetition of the stimulation IPSPs decreased while EPSPs increased; and especially by high frequency stimulation, the membrane potentials were kept above the critical level, and successive spikes were generated. This was suggestive of the reversal mechanism from inhibition to excitation.

By stimulation of the midbrain reticular formation, both excitatory and inhibitory reactions were recognized. The intracellular recording showed the long lasting depolarization due to the summation of EPSPs with the increase of spike firing.

By stimulation of the nonspecific thalamic nuclei both excitatory and inhibitory reactions were observed, while that of the specific nuclei induced no response. The intracellular records revealed generation of EPSPs and IPSPs. The relation between spike responses and the postsynaptic potentials was discussed.

With reference to the same neurons, stimulations were given to the temporal tip and midbrain- and thalamic reticular systems and consequently the existence of the convergence of the synapses of projections from these regions was ascertained.

When the reactions occurring due to each stimulation of these regions are of the same kind, they were intensified by the combined stimulation; when they were
opposite, the reaction caused by the stimulation of temporal tip appeared dominant in most cases.

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References


(* written in Japanese)