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Electrophysiological Study of Presumed Interneurons in the Lateral Nucleus of the Amygdaloid Complex

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ABSTRACT. The response properties of presumed interneurons were analyzed by stimulation of posterior sylvian gyrus (PSG) and ventral amygdalofugal tract (VAF). The presumed interneurons showed a tendency to discharge repetitively in response to shocks applied to these structures. The present study indicates that inhibitory interneurons could be activated monosynaptically to PSG and VAF stimulation. It is concluded that they mediate inhibition in the lateral nucleus (LN) of the amygdaloid complex and regulate the activity of projection cells.

Key words: interneuron, monosynaptic response, inhibition, projection cell, short latency.

In recent years diverse anatomical [1,2] and biochemical [3,4] evidence has been accumulated, which indicates the presence of inhibitory interneurons that mediate fast inhibitory post-synaptic potentials (IPSPs) of principal cells. They form large numbers of synapses in the soma, proximal dendrites and axon hyloc of the projection neurons. However, the corresponding electrophysiological data on these neurons are still very scanty. In my study the aim has been to investigate extra and intracellularly the response properties of electrophysiologically identified interneurons in the LN of the amygdaloid complex.

7 adult cats were used. The acute experiments were performed under Nembutal anesthesia (the initial dose of 35 mg/kg i.p. and an additional dose of 5-10 mg/kg i.v. every h). After tracheotomy and vein cannulation, the animals were fixed in stereotaxic apparatus. After craniotomy, coaxial stimulating electrodes were placed in the PSG. Another pair of stimulating electrodes was inserted stereotaxically into the VAF. Square pulses of 0.1ms duration and 8-30V. intensity were employed. Stimulation was applied with single and 2-4 shocks, delayed by different time intervals. Glass micropipettes, with tip diameter less than 1.0 μm and filled with 3M KCl solution were used for extra and intracellular recordings. Micro-electrodes were inserted into the LN with the use of micromanipulator and were connected through an amplifier. Membrane potentials were displayed on an oscilloscope, from which photographic records could be made. Most recordings from the presumed interneurons were made in the LN from A9 to A11. To ensure recording stability the cisterna was drained, the cat suspended and a bilateral pneumothorax performed. Blood pressure and body temperature were maintained within the physiological range. The location of stimulating microelectrodes was histologically checked.

The response properties of the presumed inhibitory interneurons are as follows: 1. The interneurons are not activated antidromically by VAF stimulation. 2. They have a tendency to discharge repetitively following stimulation. 3. They tend to discharge spontaneously. 4. They are activated synaptically to VAF and PSG stimulation. 5. The number of discharges of presumed interneurons is temporarily facilitated by volleys from the peripheral stimuli. 6. The spike onset latencies of the interneurons are shorter than those of the fast initial IPSP in the principal cells. Neurons which did not satisfy these criteria were discarded. When a unit discharge was encountered in the LN I first checked whether the unit satisfies these
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criteria. During the course of downward tracking the recording electrode from the dorsal surface of the LN I occasionally encountered single units that satisfy the criteria for inhibitory interneurons. Among the 42 inhibitory interneurons stable and long-lasting impalements were performed only in 11 cells. 5 interneurons received convergent synaptic inputs from the PSG and VAF.

In extracellular recordings the response consisted of a short excitation. Each latency value was calculated as the mean of the last 12 responses at suprathreshold stimulation. 12 spikes had latencies below 10 ms. 16 - between 12-20 ms and the other values were longer than 20 ms. Response latencies to VAF stimulation were usually less than the average latencies to PSG stimulation.

Fig. 1 shows the latency histograms to PSG (A) and VAF (B) stimulation elicited orthodromic discharges.

The number of discharges elicited by a single shock of suprathreshold intensity in presumed interneurons varied from cell to cell and was typically 2-6 (Fig. 2. A.B.C.). The discharge lasted from 10 to 50 ms to PSG stimulation and 6-35 ms after VAF shock. Cortically evoked single or repetitive spike responses appearing in LN cells at 3-6 ms were undoubtedly not transmitted through multisynaptic corticofugal circuits, but rather through oligosynaptic and direct pathways. Fig. 2D-I shows the traces from one neuron of this population. This unit responded with bursts of impulses with latency of 4.5 ms to single PSG suprathreshold stimulation (D), but when the stimulus intensity was low (70-90 μA) it responded with one single action potential to a single stimulus (E). The latency of response was remarkably stable (The range of fluctuation did not exceed 0.3ms. Fig. 1F) and did not collide with the spontaneous potential (G). These results indicate that this unit was activated orthodromically and transsynaptically. The nature of response, either monosynaptic or polysynaptic, was determined by applying double pulses at short intervals. The following criterion was used: firing activity able to follow both pulses given at 4 ms pulse interval and failing to respond to the second pulse with the interpulse interval decreased to 2.5 ms was considered to be monosynaptic, whereas firing activity already failing at interpulse intervals of 10-20 ms was labeled polysynaptic. Examples of this test are demonstrated in Fig. 1 H and E. The analysis of responses at different stimulation frequencies indicates that the response properties of 5 units satisfied the criteria for monosynaptic activation.

The attempts to record intracellularly from the presumed interneurons were often fruitless and I have an impression that the present inhibitory interneurons were relatively small in size. However, I occasionally succeeded in recording from them. The average resting potential of the interneurons was 62 ±5.6 mV. This value is significantly less than that in projection cells. In four of the impaled cells to PSG and VAF stimulation only EPSPs were present, in other 5 cells the characteristic sequence of EPSP-IPSP was described. The amplitude of the hyperpolarizing component was consistently smaller than in the projection cells. The EPSPs evoked with latent periods of 3.5-8.5 ms (mean 6.05±24 S.D.) had the amplitudes of 0.22-0.65 mV (mean 0.437±0.21 mV. S.D.). Fig. 3 shows the intracellular recordings from the presumed interneurons. Fig. 4. A. B. shows the response of neuron to graded electrical stimulation. Initial stimuli of 100 μA evoke the EPSP (A). With increase in stimulus intensity the latency of the EPSP became shorter and its duration and amplitude increased (B). In 2 units the stimulation elicited a depolarization-hyperpolarization sequence, followed by rebound discharges. Fig. 3C illustrates the response of another interneuron. The sequence of synaptic potentials elicited by the stimulation was quite differ-

Fig. 1. Latency histograms of LN neurons, activated synaptically by PSG (A) and VAF (B) stimulation

Fig. 2. Extracellular recordings from candidate interneurons in the LN. A-C responses to suprathreshold stimuli. Response of neuron from the short latency group to PSG shocks of a suprathreshold (D) and slightly subthreshold (E) intensity. F. Five superimposed sweeps illustrate negligible fluctuation of the latency. G. The stimuli were applied 2 ms. after the occurrence of orthodromic spike. No collision occurs. H. Response of the unit to application of 2 shocks at 200 Hz. Note one response to every two shocks. I. Test response is blocked at 2.5 ms. time interval. Time calibration 4 ms. For D-I oscillograms 2 ms. Voltage calibration: 0.2 mV. Upward deflection is positive.
ent from that of the principal cells. The shock elicited an initial EPSP with a superimposed spike, followed by a relatively brief and small hyperpolarization. The small hyperpolarizing potential was followed by a later EPSP, which gave rise to bursts of 3-5 spikes. The EPSPs were followed by hyperpolarization of about 2-5 mV, which lasted for about 9 ms.

Thus, the present experiments show that the inhibitory influence of the PSG on amygdaloid neurons is transmitted by interneurons. Cortical stimulation evoked short latency EPSPs in the interneurons. These EPSPs are monosynaptic in nature. My results are in agreement with ultrastructural findings [5], indicating that axons of cortical units establish asymmetric synaptic contacts with the interneurons of the LN. The small phase of hyperpolarization after depolarization is similar in its influence to the other interneurons. The recordings indicate that immunoreactive inhibitory interneurons of the basolateral part of the amygdaloid complex receive a smaller proportion of symmetric presumable inhibitory synapses than the projection cells. When the shortest latencies of interneuronal discharges were compared with those of the fast IPSPs of the principal cells, it was established that the shortest latencies of the presumed interneurons preceded the shortest latencies of the IPSPs by 1.0 ms for cortical shocks and by 0.7ms for the VAF shock. The time differences of the onset latencies between those of the interneuronal discharges and those of the IPSPs were thus in the range of monosynaptic delay. The shortest latency of antidromic responses of the principal cells preceded by 0.8 ms the shortest latency of the orthodromic activation of the principal cells. These results support the hypothesis that inhibitory interneurons are activated through the axon collaterals.

The presumed inhibitory interneurons showed a strong tendency to discharge repetitively to single shock. The inhibitory interneurons, showing repetitive discharges, have been reported in the spinal cord [6], hippocampus [7], the cerebellum [8], the thalamus [9]. They seem to have the following morphological properties: 1. They do not project axons out of the LN; 2. The size of their somata may be small in comparison with the pyramidal cells; 3. Their somata are located close to the somata of the principal cells. The most probable candidates of inhibitory interneurons are the group of sparsely spiny neurons that have locally ramifying axons. In the present study opposite response profiles were established in the interneurons and projection cells. This fact indicates that inhibitory interneurons are involved in controlling the excitability of amygdaloid projection cells.

Fig. 3. Intracellular recordings from presumed interneurons. A.B. Responses to graded stimulation. A. Threshold stimulation elicits EPSP, which becomes larger by increasing the stimulus intensity (B). C. Recording from monosynaptically activated presumed interneuron. Time calibration 10 ms. For C oscillogram 40 ms. Voltage calibration 0.2 mV.
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REFERENCES


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