ELEMENTARY POSTSYNAPTIC POTENTIALS OF HIPPOCAMPAL NEURONS

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In previous investigations so-called "minimal" [6] postsynaptic potentials (PSPs), evoked by near-threshold macrostimulation [2, 3, 7] or microstimulation [1, 5] of hippocampal afferent pathways were recorded. Changes in parameters of minimal PSPs, calculated in accordance with the quantum theory of synaptic transmission [8, 11], were studied during various plastic transformations. A more accurate method of determining quantum parameters is by analysis of "elementary" PSPs [5, 6] created as a result of activation of one presynaptic element. Only one record [10] of elementary PSPs of vertebrate brain neurons (anesthetized cat) can be found in the literature, but with no quantitative data for statistical analysis.

The object of this investigation was to attempt to record elementary PSPs in the hippocampus of the unanesthetized animal and to compare their parameters with those of minimal PSPs [1-3, 5-7].

EXPERIMENTAL METHOD

Experiments were carried out on unanesthetized, unrestrained rabbits by the method described in [1, 2, 7]. At the site of projection of the dentate fascia and areas CA_3-CA_4 of the hippocampus (coordinates P4; L5.5) an electrode holder with two electrodes for extracellular recording of the "triggering" neuron (N1) and for intracellular or "quasi-intracellular" recording of the "target-neuron" (N2), was fixed to the cranial bones. The electrodes were placed at an angle relative to each other so that at a depth of 7-8 mm the distance between their tips was 0.5-1 mm. The depth of insertion was determined with a micrometer and from the character of focal potentials evoked by stimulation of the septofimbrial region (P1; L0.5; H6.3). Electrodes for recording M2 were filled with potassium citrate (resistance after sharpening 10-50 mΩ) and those for recording N1 were filled with sodium chloride (resistance 1-5 mΩ). Bridge circuits enabling the passage of a current (up to 100 nA) to increase the discharge frequency of N1 and to suppress discharges of N2 were used. Activity was recorded on a tape recorder (frequency band 0-1000 Hz) and then processed by PDP-8A (Digital, USA) computer using a combination of programs enabling: 1) triggering discharges of N1 to be distinguished from the record; 2) activity of N2 to be averaged in an assigned neighborhood of the discharge of N1; 3) segments of activity of N2 containing artefacts or discharges to be rejected; 4) the area beneath the curve within an assigned interval to be measured and converted into amplitude (E); 5) histograms of distribution of E to be plotted. Subsequent analysis on the P6060 computer (Olivetti, Italy) consisted of calculation of quantum parameters [5] and the construction of trial theoretical distributions [3]. The mean quantum composition (m), the value of the quantum (ν), and the binomial parameters (n and p) were calculated by two methods described previously [1-3, 5, 7]. Method 1 is based on subdivision of E histograms and method 2 on determination of the number of "omissions" from a histogram.

EXPERIMENTAL RESULTS

Altogether 96 N1-N2 pairs were recorded, of which 26 pairs had recordings of sufficient duration for analysis (300-800 discharges of N1) and were in a sufficiently stable state, to judge from the membrane (20-50 mV) and peak (10-40 mV) potentials of N2. Successive regions (each of 100 realizations) and the whole recorded activity of N2 were averaged. For each pair of neurons three (if the number of discharges of N1 was under 400) or more regions (if the number of discharges of N1 was greater) were distinguished.

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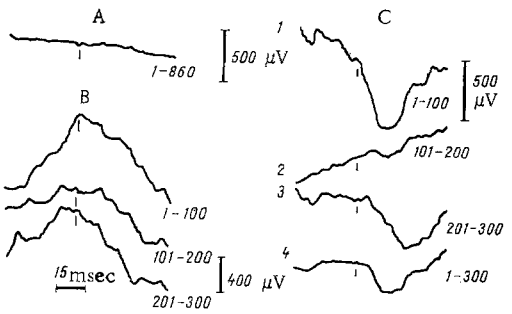

Fig. 1. Examples of averaged intracellular activity of target neurons. A) Absence of synchronized activity; B) depolarization potential commencing before discharges of "triggering" neuron; C) elementary IPSP. Here and in Figs. 2 and 3, numbers below traces are serial numbers of averaged realizations. Short vertical lines indicate time of discharge of triggering neuron.

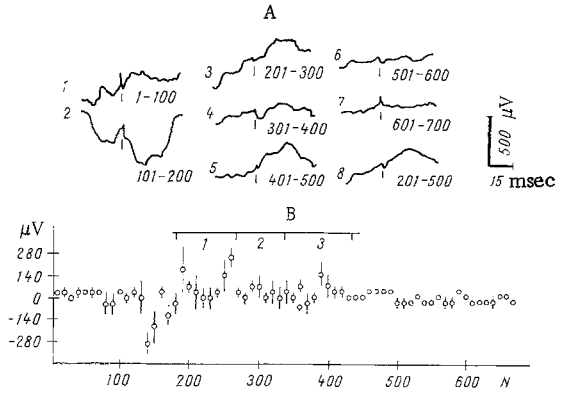

Fig. 2. Elementary EPSPs. A) Potentials of triggering neuron (1-3 and 8) and control potentials recorded after death of cell (6, 7), averaged from 100 (1-7) and 300 (8) realizations; B) dependence of amplitude of recorded activity (ordinate) on serial number of discharge of triggering neuron (abscissa). Points show mean (of 10 realizations) amplitudes calculated for time corresponding to averaged elementary EPSP. Short vertical lines show errors of means.

The criterion of correlation between N1 and N2 was the appearance of fluctuations of potential, similar in time of origin and shape to each other and to the signal obtained during averaging of total N2 activity, in all three regions (or in at least 75% of regions if they numbered more than three). According to the same criterion, no correlation was found for 18 of the 26 pairs (Fig. 1A). For five pairs, signals starting before discharges of N1 were observed (Fig. 1B). Such cases evidently reflect the presence of common inputs to the recorded neurons. Synchronized waves, starting soon after discharges of N1 and probably consisting of elementary PSPs, were discovered in the activity of three pairs. Such waves (Fig. 1C, 1-4; Fig. 2A, 1-5) had a higher amplitude than the spontaneous activity arising before discharges of N1 and the control activity recorded after death of neuron N2 (Fig. 2A, 6, 7). Hyperpolarization waves, which were regarded as elementary IPSPs, were observed in two of these three cases. Elementary IPSPs arose in two different N2 cells after discharges of the same N1. A depolarization wave, an elementary EPSP, was recorded in one experiment.

TABLE 1. Parameters of Elementary Postsynaptic Potentials

No. of experiment and of neuron	Nature of response	Number of averaged realizations	Amplitude, μV	Latent period, msec	Duration of leading edge, msec
59—3—6	IPSPs	300	250	4	10
59—3—7	IPSPs	400	250	4	14
64—1—1	EPSPs	300	150	3,5	17,5

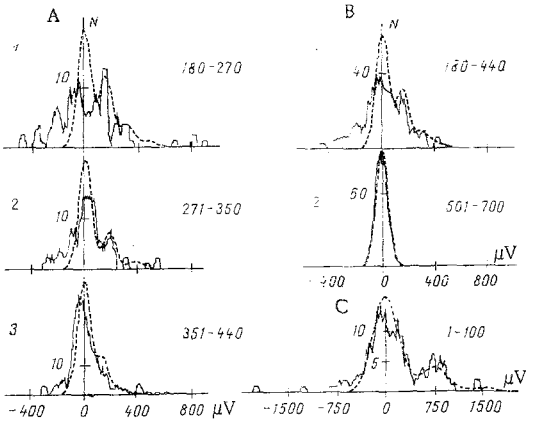

Fig. 3. Experimental (continuous lines) and theoretical (broken lines) distributions of amplitudes of recorded signals. A) Histogram of amplitudes of elementary EPSPs for different regions (1-3) of record of activity of neuron illustrated in Fig. 2; B) histograms for total region with strongest activity of same neuron (B, 1) and for control region recorded after death of neuron (B, 2); C) histograms of amplitudes of elementary IPSPs, corresponding to records in Fig. 1C. In A-C: abscissa, amplitude (in μV); ordinate, number of cases. Experimental distributions constructed by the "sliding bin" method [12]. Quantum parameters corresponding to distributions B1, A1-3, and C given in Table 2.

Data on the amplitude and temporal parameters of the elementary PSPs are given in Table 1. Since the discovery of averaged elementary polysynaptic PSPs with an amplitude higher than several microvolts seems unlikely [12], the PSPs with an amplitude of 150-250 μV (Table 1) must be regarded as monosynaptic. The relatively long latency (3.5-4 msec) matches the slow conduction velocity [4] along intrahippocampal fibers, for a distance of about 1-2 mm, and the latency of the minimal PSPs which, judging from certain features [1-3, 5-7], are monosynaptic in nature. Despite the fact that according to the criteria of selection elementary PSPs were recorded in most averaged regions, significant fluctuations of amplitudes (see Fig. 1C, 1-3, and Fig. 2A, B) and even temporary disappearance of the signal (Fig. 2A, 2) were observed. The fluctuations of amplitudes created difficulties for statistical analysis, which requires a steady state of the process [5]. Consequently, the period of most stable recording of elementary EPSPs was divided into small (about 100 realizations) "quasistationary" areas (Fig. 2B, 1-3), and the region shown in Fig. 1C, 1 was chosen for analysis of the IPSPs. Histograms drawn for such "quasistationary" regions (Fig. 3A), for the total region (Fig. 3B, 1), and for the control region (Fig. 3B, 2) obtained after death of neuron N2, are given in Fig. 3. The theoretical distributions [3, 5] were drawn from data obtained by method 1. As Fig. 3A, B, 1 and C shows, the right hand side of the histograms is well described by a distribution based on Poisson's Law. One possible cause of the difference of the left hand side of the experimental distributions from theoretical could be the appearance of relatively infrequent polysynaptic PSPs. The control histogram (see Fig. 3B, 2) is well described by the normal distribution.

TABLE 2. Quantum Analysis of Elementary Postsynaptic Potentials

Elementary post-synaptic potential	Number of measure-ments	Quantum parameters	
		ν, μV	m
EPSPs	261	165 / 79	0,54 / 0,40
EPSPs (1)	91	175 / 74	0,65 / 0,49
EPSPs (2)	80	200 / 135	0,36 / 0,28
EPSPs (3)	90	150 / 34	0,34 / 0,27
IPSPs	100	750 / 360	0,26 / 0,28

Legend. For each case numbers on top were obtained by method 1, numbers on bottom by method 2. Numbers in parenthesis indicate serial numbers of regions marked on Fig. 2B.

Only the parameters m and ν are included in Table 2, for the binomial parameters n and p were less than their own standard error. The fact that p was close to zero and that n was indeterminate is evidence of the Poisson character of the distribution. Table 2 shows that parameters of neighboring fragments of the record differ only a little (Fig. 2B, 1-3). The value of the quantum for the EPSPs (35-200 μV) lies within the limit of values obtained for spinal motoneurons [5] and hippocampal cells during microstimulation [1]. It may also be considered the value of ν was somewhat underestimated because of a decrease in resistance and membrane potential as a result of puncture of the membrane, and also on account of the background bombardment characteristic of neurons of the unanesthetized brain. These last causes could overestimate the value of ν for the elementary IPSPs (360-759 μV; Table 2). The use of a conditioning IPSP to detect minimal EPSPs [2, 6, 7] may give a rather higher value of ν than those obtained for EPSPs (Table 2) because of hyperpolarization of the cell. For all cases analyzed $m < 1$ (see Table 2), and this, together with the Poisson character of the distribution of E, corresponds to the results obtained by microstimulation [1]. During macrostimulation in most cases higher values of m were observed and the distributions of E were often approximated on the basis of the binomial rule [2, 7]. This can be explained by the fact that the procedure of selection of the "near-threshold" current (10-100 μA) facilitates activation of few but highly effective synaptic connections, whereas when elementary PSPs are recorded, such connections are difficult to find. The results suggest that hippocampal synapses are distinguished not only by low, but also by somewhat unstable effectiveness, which can change for reasons outside control. This is in agreement with the similar properties of elementary intracortical connections [6] and also with data showing a significant change in the effectiveness of hippocampal connections after tetanization [1] or low-frequency stimulation [7].

The results thus show that in about 10% of closely situated hippocampal neurons synchronized waves are found which, with respect to shape and temporal characteristics, resemble PSPs recorded during stimulation of afferent pathways. Comparison of the parameters of elementary and minimal PSPs confirms the interpretation of these parameters [1-3, 5] in terms of the quantum hypothesis, but also gives grounds for considering that during macrostimulation artificial separation of infrequently found but highly effective connections took place.

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ROLE OF MONOAMINERGIC HYPOTHALAMIC STRUCTURES IN REGULATION OF FUNCTIONS OF THE SYMPATHICO-ADRENAL SYSTEM

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Sudden cooling of the body leads to considerable activation of the sympathico-adrenal system (SAS), which is manifested as elevation of the blood adrenalin (A) and noradrenalin (NA) levels accompanied by a small decrease in the body temperature [3].

Since the body temperature is controlled in the hypothalamus through the reciprocal actions of NA and serotonin [7], the investigation described below was carried out to study the effect of pharmacologic destruction of monoaminergic terminals in the hypothalamus on basal and cold-stress-induced secretion of neurohormones by the SAS.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g in the fall and winter. The rats were divided into six groups: 1) intact; 2) control (receiving 0.5% ascorbic acid in physiological saline); 3) receiving 6-hydroxydopamine (6-HDA); 4) receiving demethylimipramine (DMI); 5) receiving DMI + 6-HDA; 6) receiving 5,6-hydroxytryptamine (HTA). The 6-HDA preparation (from "Regis Chemical"), in a dose of 200 μg in 20 μl of solvent, was injected once into the lateral ventricles of the rats (group 2). The animals of group 3 were given DMI (from "Geigy") intraperitoneally in a dose of 5 mg per rat 30 min before injection of 6-HDA into the lateral ventricles. The rats of group 4 received an injection of 5,6-HTA (from "Regis Chemical") in a dose of 75 μg in 20 μl of solvent per rat by injection into the cerebral ventricles.

After injection of 6-HDA into the cerebral ventricles in a dose of 200 μg, terminal portions of noradrenergic and dopaminergic fibers of the hypothalamus are known to be selectively destroyed [9]. Injection of DMI prevents destruction of noradrenergic fibers by 6-HDA but does not prevent injury to dopaminergic terminals [8]; after injection of 5,6-HTA into the cerebral ventricles, terminal portions of serotoninergic fibers in the hypothalamus are selectively destroyed [5].

The injection into the cerebral ventricle was given in a stereotaxic apparatus under hexobarbital anesthesia (10 mg/100 g body weight). Before the beginning of the experiment, all the rats were adapted to the chamber for 3-4 days. All the rats were kept for 1 h in a special chamber, with an air temperature of 22 ± 1 and 5 ± 1°C 7 days after injection of the preparation. The animals were then decapitated, the hypothalamus was removed, and blood was collected. Catecholaminergic structures in the hypothalamus were investigated by a fluorescence histochemical method [6]. The content of dopamine (DA) and NA in the hypothalamus and of NA and A in the blood was determined by the trihydroxyindole method [4]. Serotonin in the hypothalamus was analyzed by a fluorometric method [2]. Values obtained in rats kept at 5°C were compared with the corresponding values for rats kept at 22°C.

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