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Long-Term Potentiation and Evoked Spike Responses in the Cingulate Cortex of Freely Mobile Rats

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Long-term potentiation of synaptic efficiency is regarded as a major candidate for the role of the physiological mechanism of long-term memory. However, the limited development of concepts of the cellular and subcellular characteristics of the induction of long-term potentiation in animals in conditions of free behavior does not correspond to the importance of this question. The present study was undertaken to determine whether the characteristics of potentiation in the cingulate cortex in response to stimulation of fibers of the subiculo-cingulate tract are truly long-term, i.e., develop through all known phases and last at least 24 h, in freely moving animals. In addition, the study aims included identification of the effects of application of blockers of different types of glutamate receptors on the development of long-term potentiation and identification of the characteristics of spike responses of single cingulate cortex neurons to stimulation of the subiculo-cingulate tract. Long-term potentiation, lasting more than 24 h, was obtained in freely moving adult rats not treated with GABA blockers. Injection of glutamate NMDA synapse blockers led to significant decreases in evoked cingulate cortex potentials in response to test stimulation. Activatory short-latency spike responses were characterized by a low probability of spike generation, and this increased with increases in the stimulation current. These data demonstrated that it is methodologically possible to compare, in freely moving rats, the involvement of individual neurons in the mechanisms involved in learning one or another type of adaptive behavior and the dynamics of their evoked spike activity during the formation of long-term potentiation.

KEY WORDS: cingulate cortex, long-term potentiation, spike activity, free behavior.

Long-term potentiation (LTP) of synaptic efficiency, observed as long ago as the 1970s, which is induced by tetanic stimulation of conducting pathways, especially in the hippocampus, remains the main contender for the role of the elementary physiological mechanism of long-term memory. It is also regarded as an experimental model for activity-dependent plasticity.

Potentials in monosynaptic pathways are of particular importance for identifying evoked changes in synaptic efficiency of biochemical processes, as in this situation specific synapses are known to be subject to modification; changes in responses allow the effects of added substances on these synapses to be assessed.

Study of the mechanisms of monosynaptic potentiation revealed synapses able to undergo changes in synaptic efficiency in conditions of simultaneous activation of the pre- and postsynaptic membranes, i.e., those corresponding to the theoretically predicted type of synapse whose modification might serve as the basis of long-term memory [14]. These are excitatory synapses in which the transmitter is glutamate. While the phenomenon of LTP of glutamate synapses can be demonstrated reproducibly in various parts of the hippocampus, until recently monosynaptic LTP in the cerebral cortex could only be obtained at the early stages of ontogenesis or in conditions of simultaneous blockade of inhibitory intracortical connections [7, 18]. However, recent investigations have revealed two areas of the cortex in

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which monosynaptic long-term potentiation develops without prior blockade of inhibitory circuits. These are two areas of the associative cortex to which axons of the output parts of the hippocampal formation project. This type of long-term potentiation was first observed in the prefrontal cortex in response to tetanic stimulation of the ventral hippocampus (field CA1 and the subiculum) [12, 19, 22]. Some time later, monosynaptic LTP of glutamate synapses was demonstrated in cingulate cortex neurons in response to tetanic stimulation of the subiculo-cingulate tract (SCT), which runs from the subiculum to the limbic cortex [15, 16]. However, the authors of these latter studies obtained limited data because the experiments were performed under anesthesia, and conclusions relating to the long-term nature of potentiation are made on the basis of observations of responses over periods of several hours.

The present study was performed to determine whether potentiation in the cingulate cortex in response to stimulation of fibers of the SCT met the criterion of being truly long-term, i.e., developing through all the known phases [28] and lasting at least 24 h, in free-moving animals; another aim was to identify the effects of applying blockers of various types of glutamate receptors on the development of long-term potentiation induced in these experimental conditions.

This model was used for developing concepts of the stages of memory consolidation and the neural mechanisms of behavior. The concept of "functional location," which was previously the basis of these concepts, is currently being replaced by the idea of "functional specialization" [21]. It is well known that the formation and realization of behavior depends on the involvement of groups of neurons which play significantly different roles in this process [17, 35, and others], these roles depending on the specialization of the neuron groups [1, 30]; neurons of the cingulate cortex are actively involved in forming behavior in adult animals [5, 6, 10, 26]. It is also known that potentiation of the responses of individual neurons to electrical stimulation inducing LTP of the evoked potential only occurs in some cells [3], even if these are cells of the same type lying adjacent to each other [8]; the influences of stimulating a given input on the activity of neurons is also known to depend on the characteristics of their involvement in information processing (X and Y cells of the lateral geniculate body [9]). In this connection, it is logical to suggest that comparison of the characteristics of LTP in neurons playing different roles in supporting the formation and realization of behavior and identification of the effects of LTP on the activity of these neurons in behavior (which is regarded as an extremely important but poorly developed direction of investigation [13, 23]) could facilitate the further development of concepts of the neural mechanisms of behavior. One of the most important conditions supporting the view that such comparisons can be made using cingulate cortex neurons is the observation of responses, especially short-latency responses, to stimulation of those fibers which, when tetanized, induce LTP. Thus, the aims of the present work included identification of the characteristics of the spike responses of individual cingulate cortex neurons to stimulation of the SCT.

METHODS

Studies were performed on 79 male Wistar rats weighing 250–350 g, aged eight weeks at the time of electrode implantation surgery. LTP was induced in the cingulate cortex using a protocol based on strong tetanic stimulation of the SCT, which consisted of six blocks of bipolar (100 Hz) stimulations, each of duration 600 msec. Interblock intervals were 10 sec. During surgery under pentobarbital anesthesia, animals were scalped and openings were drilled in the skull for electrode insertion and attachment of fixing screws. The bipolar stimulating electrode (two platinum-iridium wires in Teflon insulation, diameter 75 μm, cut at the tips with a cutter and glued together) was inserted into the subiculum (P = 6.7, L = 4.5, D = 2.5–3.5, Paxinos and Watson atlas [24]). The electrode for recording evoked potentials (EP) (a platinum-iridium wire, diameter 100 μm, cut with a cutter) was inserted into the posterior cingulate cortex (P = 5.5, L = 0.5, D = 1–2). The position of the stimulating electrode during surgery was changed until the maximal EP to stimulation of the SCT was reached, with a clear earliest EP component, with peaks with latent periods of about 3 msec. Electrodes were then fixed to the skull with Paladur resin.

EP were recorded during free behavior starting after a one-week post-operative recovery period. In experiments developing EP, rats received test blocks of stimuli every 15 min, blocks consisting of nine bipolar stimuli of duration 100 μsec and intensity 100–400 μA, with interstimulus intervals of 15 sec. Currents for test stimuli were determined after preliminary measurement of curves showing the relationship between EP amplitude and stimulation current; test stimulus currents were 40% of the current inducing the maximum EP. Stimulus amplitude for application of tetanic stimulation was doubled as compared with test stimuli. Signals from the implanted electrodes, after amplification, were passed to an analog-to-digital converter model M-1401 (England) and then to a Pentium-based personal computer. Computer data accumulation and processing were performed using the program Intracell. After removal of artefacts, EP from stimulus blocks were averaged and EP parameters were measured for that point in the experiment. EP parameters (leading front rise rates, amplitude of the negative component) were compared statistically in terms of the Wilcoxon criteria for small data sets and in terms of Student's *t* test for large (more than 20 observations) sets. Differences between parameters were regarded as significant at $p < 0.05$.

Spike activity from cingulate cortex neurons was recorded in another group of rats (n = 18) under pentobarbital anesthesia during surgery and then after a one-week recovery period in conditions of free behavior in the cage. Tungsten electrodes were obtained from World Precision Instruments (USA) and before insertion were coated electrolytically with gold. These electrodes were glued to the slide block of a micromanipulator attached with resin glue to the rat's skull. During surgery, the recording electrode was inserted into the upper layers of the cortex, as identified by the appearance of multineuron activity in the signal from the electrode, which after amplification was displayed on an oscilloscope. In the next four rats, this procedure was followed by smooth insertion into the cortex under anesthesia, with recording of activity evoked by stimulation of the SCT at every 50 μm of insertion depth.

After the recovery period, spike responses evoked by stimulation of the SCT were recorded in conditions of free behavior and, after amplification with a preamplifier located on the animal's head and further amplification, were passed to an M1401 analog-to-digital converter (England) and then to a computer running the Spike-2 program. Test stimulation was applied with pairs of bipolar stimuli with interstimulus intervals of 50 msec for detection of the possible effects of the presynaptic mechanisms of LTP. In most animals (n = 14), recording of multineuron activity at one site was followed by use of the micromanipulator to move the recording electrode; multineuron activity was measured at the new microelectrode position on the next or subsequent days. Data stored on computer were processed to extract spike trains from individual neurons in terms of shape and amplitude, using the Spike-2 program; the mean frequency of cell activity was measured for the ensemble being studied and peristimulus histograms of spike responses to SCT stimulation were constructed. The statistical criterion for the presence of a response was the χ^2 test, Fisher's exact test being used for small values (Epistat program). Statistically significant responses were identified by comparing specified histogram bins with the bin which in baseline conditions had the most extreme value of the same sign (the bin with the greatest value was used for activation; the bin with the smallest value was used for inhibition). Differences were regarded as significant at $p < 0.05$.

In a series of experiments based on blockade of synaptic glutamate receptors, surgery in 29 rats involved implantation of a cannula into the lateral ventricle (P = 0.8, L = 1.6, V = 4.0 according to atlas [24]) additional to the stimulating and recording electrodes. During experiments, the cannula was used to administer the following blockers: D-2-amino-5-phosphonovaleric acid (AP-5) to block N-methyl-D-aspartate (NMDA) receptors, 6-cyano-2,3,-dihydroxy-7-nitroquinoxaline (CNQX) for blockade of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, and (R,S)-α-methyl-4-carboxyphenylglycine (MCPG) for blockade of metabotropic receptors. Solutions were instilled into the lateral ventricle at a rate of 1 μl/min at concentrations which, taking the volume of the rat brain as about 2 ml, gave tissue conditions of 50 μM AP-5, 1 μM CNQX, and 500 μM MCPG. Studies of the hippocampus have demonstrated that these concentrations reliably block the corresponding receptor types, blocking respectively responses to test stimuli (CNQX) or different phases of LTP [18].

After experiments, animals were anesthetized with halothane; brains were removed and fixed for 2 h in a mixture of acetic acid, formaldehyde, and ethanol. Brains were then stored in 70% ethanol, after which the ethanol was replaced with chloroform and brains were embedded in paraffin. Sections of paraffin-embedded brains were prepared on a microtome (serial sections of thickness 20 μm) to identify electrode locations. Visually selected sections were stained with cresyl violet for accurate determination of the positions of electrode tips by microscopic examination.

RESULTS

In the first series of experiments, performed using 32 rats in free behavior, recordings were made of cingulate cortex EP produced in response to stimulation of the SCT before and after tetanic stimulation of this tract. The shape of EP produced in response to test stimulation was similar to that previously recorded in the deep layers of the cingulate cortex by Hedberg and Stanton in *in vivo* and *in vitro* experiments [15]. We observed potentiation of synaptic efficiency, which lasted more than 24 h (Fig. 1, *A*) and which can therefore be regarded as true long-term potentiation, the development of which passed through all phases known to date [28]. Increases in the steepness of the rising edge of the EP to its peak were by up to 40–50% and persisted to the following day at a level of 20–30%. This diagram shows the dynamics of the amplitude of the first component of EP, which had a latent period of about 3 msec and which repeated with a frequency of 100 Hz in conditions of tetanic stimulation; these are signs that this response was monosynaptic in nature. Thus, the long-term potentiation recorded in the cingulate cortex in these experiments was monosynaptic. Later components were more variable and potentiation of these components was seen only in some rats and also lasted for different periods of time.

The effects of intraventricular administration of blockers of different types of synaptic glutamate receptors were studied in a further 29 rats. Injections of NMDA receptor blockers led to significant ($p < 0.05$ by Wilcoxon's test) decreases in the amplitude of responses to test stimuli (about 20%) 15–30 min after application, while injection of the AMPA receptor blocker had virtually no effect on these responses (Fig. 1, *B*). The effect was identical in magnitude for the early component of EP with peak latency of 3.5 msec and for the late component with a peak latency of 7 msec.

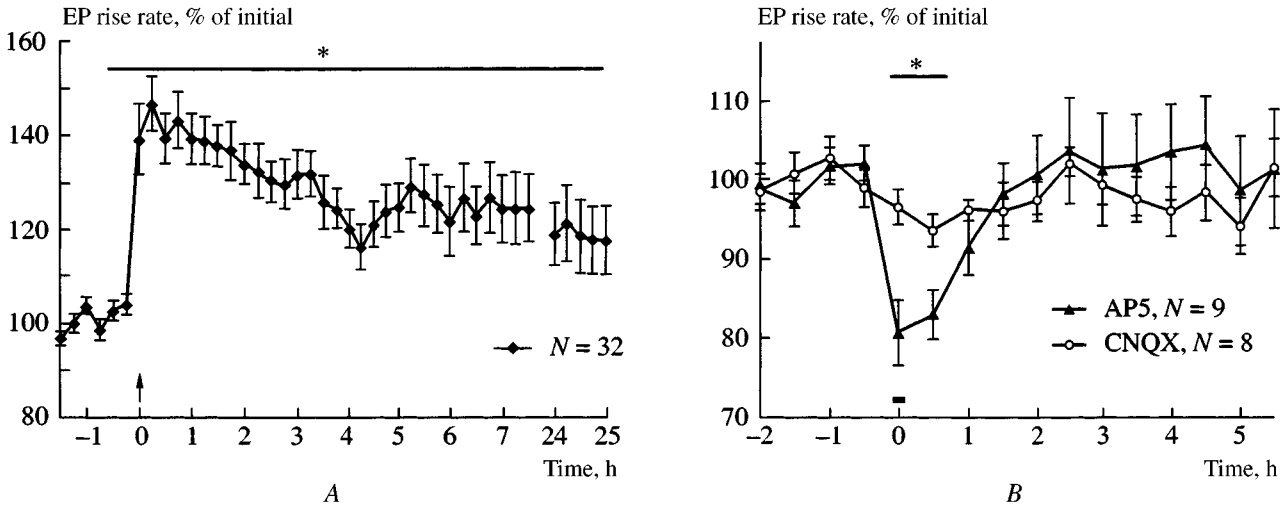

Fig. 1. Changes in evoked potentials after tetanic stimulation and application of glutamate receptor blockers. *A*) Long-term potentiation of EP in the cingulate cortex after tetanic stimulation of the subiculo-cingulate tract. The ordinate shows the EP rise rate, % of initial, and the abscissa shows time, h. The time during which post-tetanic EP was significantly (*t* test, $p < 0.05$) different from initial is noted by the line and asterisks. The moment of tetanic stimulation is marked by the arrow. The number of rats is identified as *N*. *B*) The effects of application of glutamate receptor blockers on EP produced in response to test stimuli. The interval during which the EP rise rate after application of AP-5 was significantly less than baseline and EP after CNQX is marked with a line and asterisks (Wilcoxon test, $p < 0.05$). The time during which blockers were applied is marked with the bar. Further details as in (*A*).

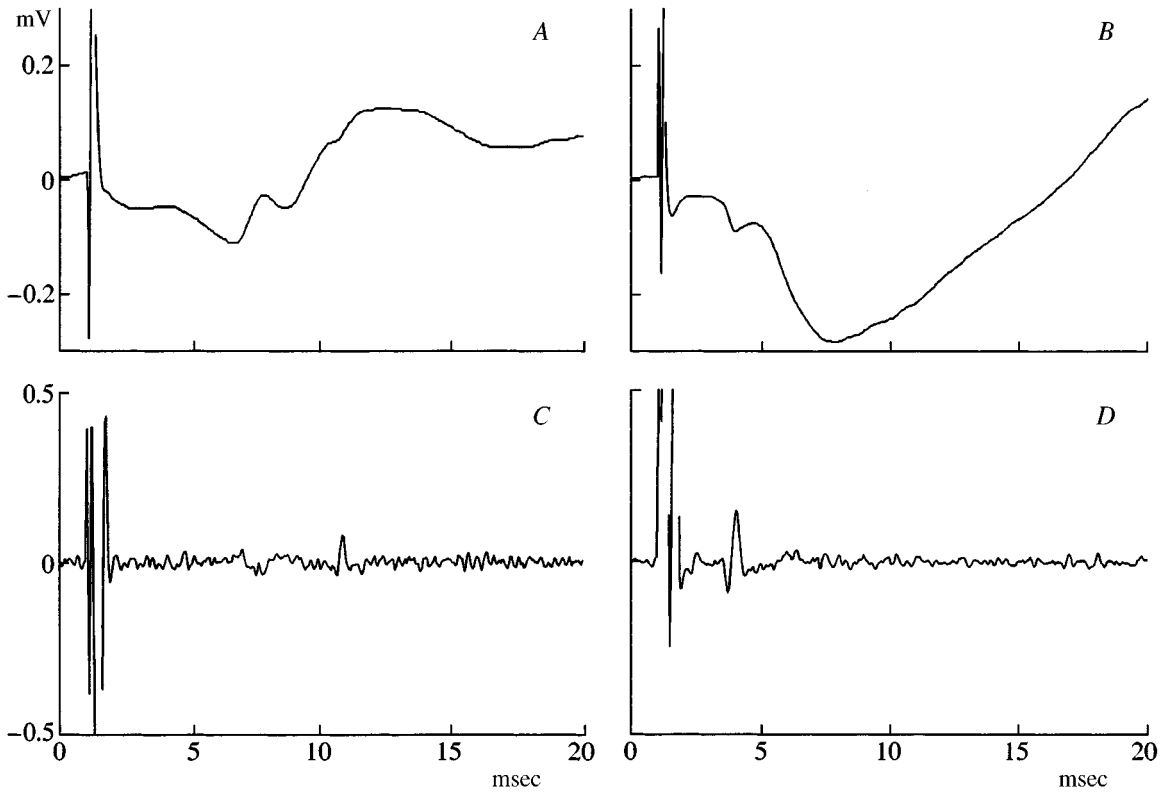

Fig. 2. Responses in the cingulate cortex to electrical stimulation of the subiculo-cingulate tract under Nembutal anesthesia. *A, C*) EP and neuronogram of responses to stimulation of the SCT with a current of 400 μA in rat No. 29; *B, D*) in rat No. 37. The moment of electrical stimulation corresponded to the 1-msec mark on the abscissa. Each curve was obtained by averaging responses to nine sequential stimuli.

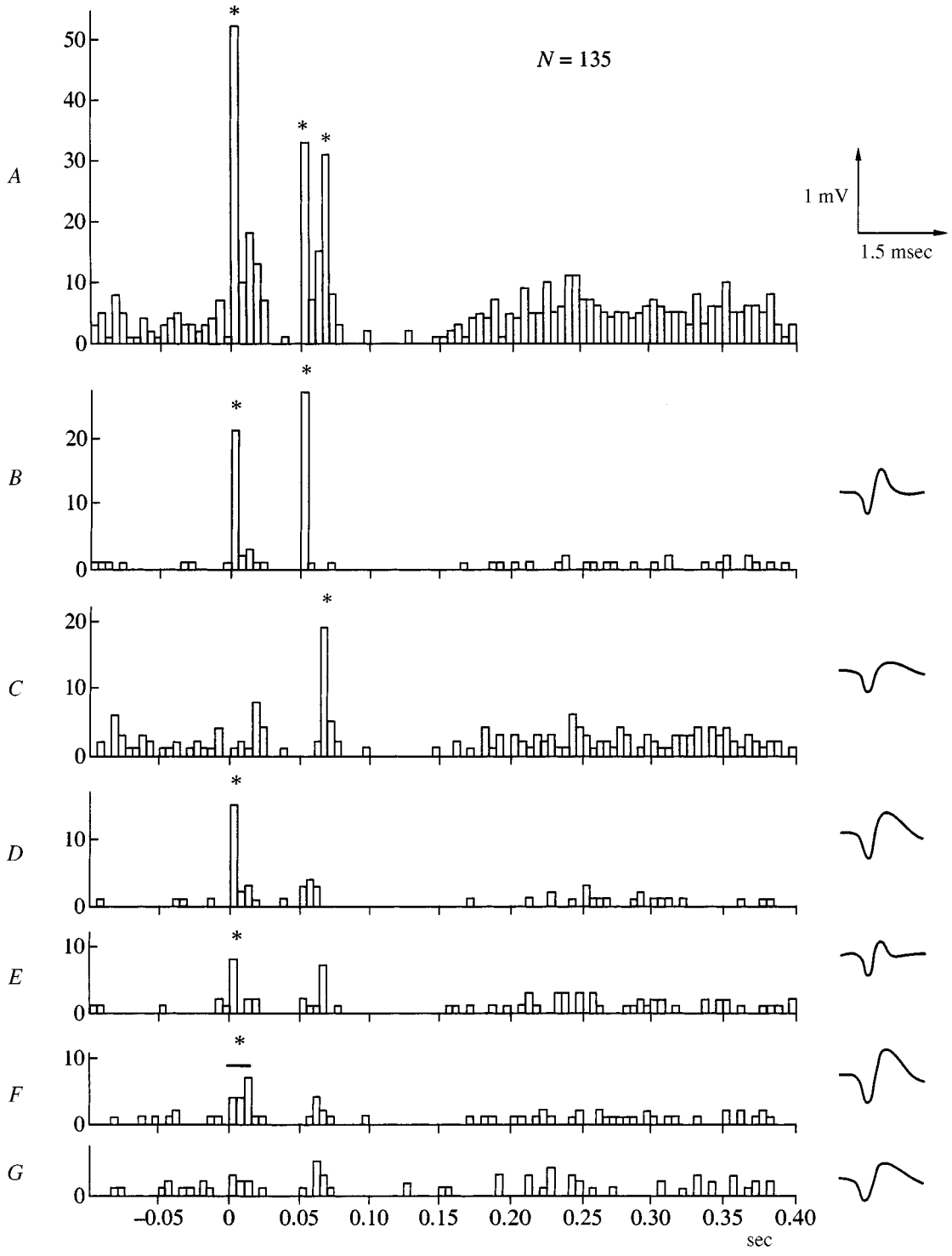

Fig. 3. Peristimulus histograms of the activity of individual neurons in response to paired stimulation of the subiculo-cingulate tract. *A*) Histogram of the spike activity of an ensemble of neurons of the cingulate cortex of rat No. 36 during stimulation of the SCT with a current of 400 μA. *B–G*) Histograms of the spike activity of individual neurons extracted from this ensemble. Plots to the right of the histograms show the averaged spike shapes for each neuron. The ordinate shows the number of spikes during the recording time; the abscissa shows time, sec. Calibration of spike shape is given to the right of the histogram showing ensemble activity. The moments of electrical stimulation correspond to the 0- and 0.05-sec points on the abscissa. Histogram bins significantly different from baseline are marked with asterisks (χ^2, $p < 0.05$). The number of stimulus pairs presented is identified as *N*.

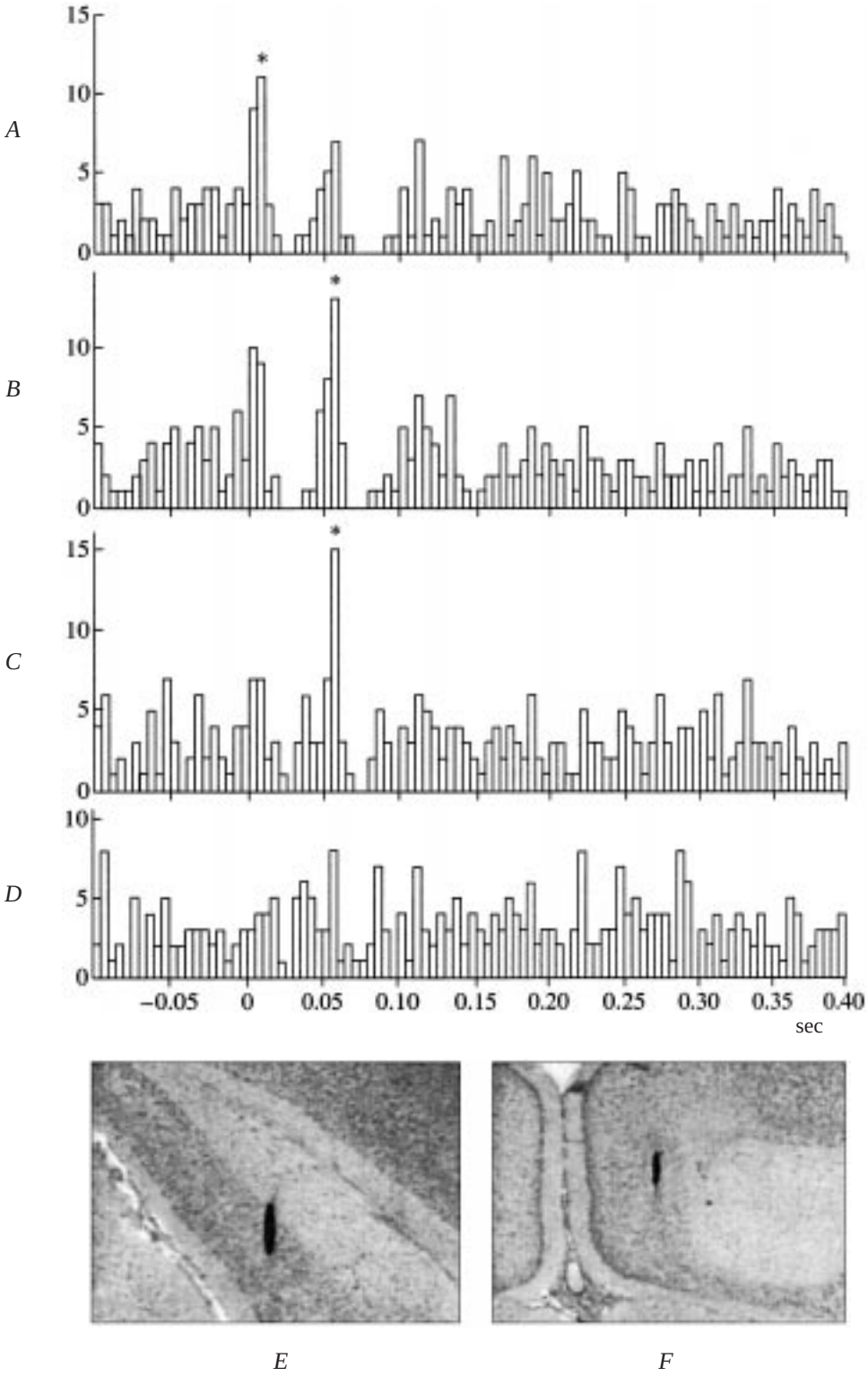

Fig. 4. Relationship between evoked spike activity of an ensemble of cingulate cortex neurons in rat No. 36 and the strength of electrical stimulation of the SCT. *A*) Histogram of activity at a current strength of 100–200 μA; *B*) histogram of activity at a current strength of 200–300 μA; *C*) histogram of activity at a current strength of 300–400 μA; *D*) histogram of activity at a current strength of 400–600 μA. The ordinate shows the number of spikes; the abscissa shows time, sec. The moments of stimulation correspond to the 0- and 0.05-sec points on the abscissa; asterisks identify bins with activity significantly greater than prestimulation activity (χ^2, $p < 0.05$). *E–F*) microphotographs of brain sections from rat No. 36 in the area of the stimulating electrode (*E*) and recording electrode (*F*). Electrode tip positions correspond to the lower boundary of the dark spot in the subiculum and deep layers of the cingulate cortex respectively. Electrode traces on the photographs are additionally contrasted.

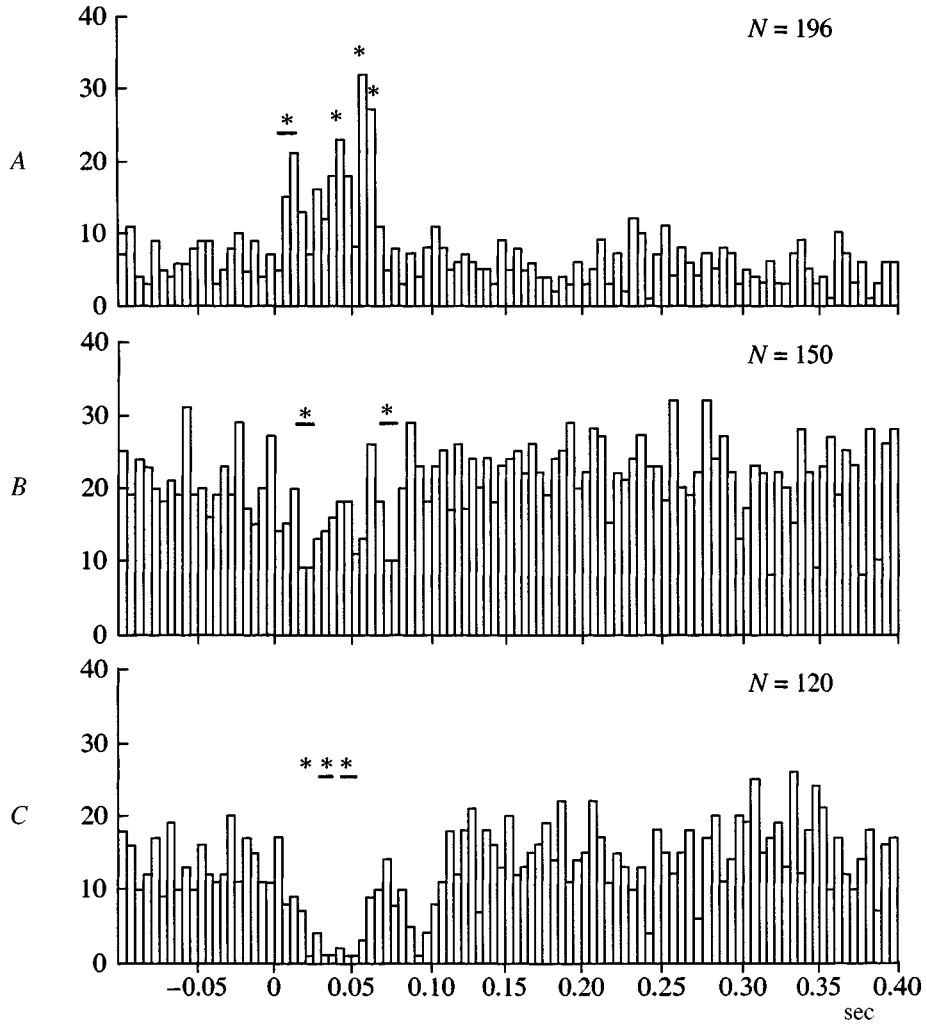

Fig. 5. Late spike responses to electrical stimulation of the SCT in the cingulate cortex. *A*) Histogram of ensemble activity in rat No. 55; *B–C*) inhibitory pauses in histograms of the spike activity of ensembles in rats No. 37 and No. 40. The ordinate shows the number of spikes; the abscissas shows time, sec. Bars and asterisks above histograms show intervals during which activity was significantly different from baseline, χ^2, $p < 0.05$. The number of stimulus pairs presented is identified as *N*.

These results are evidence for a difference in the roles of AMPA and NMDA receptors in the operation of glutamate synapses in the structure of the neocortex as compared with the hippocampus (see [11]). Considering this character of the effects on test responses, we did not study the effects of treatment with the NMDA receptor blocker on the induction of long-term potentiation. Application of the metabotropic receptor blocker MCPG had no effect on the amplitude of responses to test stimulation, while in six rats treatment with this blocker was followed 15 min later by tetanization of the subiculo-cingulate tract with parameters leading to long-term potentiation in control animals. We have previously described the result of this experiment [11], which was that application of MCPG did not block long-term potentiation.

Experiments involving recording of neuron activity in the cingulate cortex in conditions of stimulation of the SCT were performed in 18 rats. Morphological monitoring showed that the stimulating electrodes were located in the subiculum and the recording electrodes in the cingulate cortex. In four rats, neuron responses to stimulation of the SCT during surgery were recorded on the computer. Under anesthesia, arrival of the stimulating electrode into the subiculum was associated with the detection of cellular spike responses to test stimulation at 100–200 μA, with latent periods of about 3 and 10 msec (Fig. 2). After reaching a defined stimulation intensity (generally 150–200 μA), these responses were seen with a probability of 100%; Fig. 2 shows responses averaged from nine performances. Apart from the fact that spikes appeared with a probability of 100%, they were also characterized by a constant latent period, which allowed us to sum separate performances. When the stimulation amplitude was increased to a level

greater than the threshold, short-latency responses persisted at all electrical stimulation intensities used (up to 600 μA), while long-latency responses in some cases disappeared; we believe that this is associated with activation of inhibitory circuits by electrical stimulation.

After the recovery period, these and the remaining 14 rats were used for recording multineuron responses to stimulation of the SCT in free behavior. Activity was recorded from 45 neuron ensembles. The mean ensemble spike frequency was 21.6 spikes/sec. Considering the fact that 6–8 neurons could be discriminated in an ensemble, the mean discharge frequency of the cingulate cortex cells recorded was only 3 spikes/sec. Stimulation of the SCT evoked either increases or inhibition of spike activity in different neurons, or had no significant effect on spike frequency. Short-latency (with time parameters corresponding to the potentiable monosynaptic component of EP) evoked spikes were recorded in only two rats at six recording points. Figure 3, *A* shows peristimulus histograms of multineuron responses in rat No. 36 during stimulation of the SCT with pairs of bipolar impulses with a current intensity of 400 μA. Both stimuli in the pair induced spikes with a latency of about 3 msec, these falling into the first histogram bin, which means that these can be regarded as monosynaptically evoked spikes (see above). However, the probability that spikes would appear was less than 50% in total for spikes from all cells recorded simultaneously by the electrode (the Spike-2 program discriminated six different neurons in this spike activity). All individual neurons in the ensemble recorded by the electrode were activated in response to stimulation, though short-latency spikes (activity in the first histogram bin after the first stimulus was significantly greater than baseline, χ^2 criterion, $p < 0.05$) were recorded in three cells; the activity peak of the remaining cells was observed later (in the third bin, 10–15 msec). The probability that a particular cell would produce a spike response to quite strong (400 μA) stimulation varied from 3% to 27%. Plots of the relationship between the spike response in one rat and the stimulation current are shown in Fig. 4, *A–D*. These plots show that increases in stimulation current were accompanied by increases in the probability of spike generation. However, this increase was not monotonous in nature, but rather was characterized by a threshold, after which the marked spike response appeared. In addition, changes in the ratio of the amplitude of the response to the first stimulus to the amplitude of the response to the second stimulus in the pair were seen. Thus, at low stimulus intensities, the response to the second stimulus was significantly greater than the response to the first, while this ratio was inverted at high intensities. The same figure (*E–F*) shows photographs of brain sections from rat No. 36 showing the positions of electrode tips.

Seven recording points in four rats yielded long-latency (7–50 msec) evoked activatory responses. Stimulation with currents of different intensity gave maximum spike responses at mean stimulation intensities of 150–300 μA in different animals. Further increases in the stimulation current led to suppression of the spike response. An example of long-latency activation of a neuron ensemble is shown in Fig. 5, *A*.

Four rats also showed inhibitory responses to stimulation of the SCT at eight recording points. Figure 5, *B–C* shows examples of clear inhibition of long-latency changes in spike activity after stimulation of the SCT.

DISCUSSION

The observation of long-term potentiation of synapses in tracts connecting the hippocampus with structures of the neocortex is very important from the point of view of the possible role of changes in synaptic efficiency in the formation of long-term memory. The potentiation of EP demonstrated here, lasting a day, is evidence for the involvement of protein synthesis mechanisms, which can lead to long-lasting changes in cell function. In addition, particular attention should be paid to the fact that the LTP demonstrated here was recorded in conscious, freely mobile animals without blockade of inhibitory circuits. This is important, as these are the conditions in which animals are seen to learn to solve behavioral tasks. Given that the special role of neocortical structures in storing long-term memory is well recognized [31], the demonstration that LTP can be formed in these structures can be regarded as indirect support for the involvement of processes involving threshold changes in synaptic efficiency in the formation of memory.

Hedberg and Stanton [16] used blockers of different types of glutamate receptors to identify the same synapses as those studied here, though their experiments were performed *in vitro*, which does not allow the late stages of LTP to be studied, these being triggered several hours after tetanic stimulation. Unlike data obtained in [16], which showed that metabotropic receptors need to be activated for potentiation of the monosynaptic component of EP, use of blockers of these receptors in our studies had no effect on the development of LTP. This difference may be associated with the fact that different active agents were used. The present studies were based on the blocker MCPG, while Hedberg and Stanton used the less specific blocker DL-2-amino-3-phosphonopropionic acid (DL-AP3). The efficacy of MCPG as a specific blocker of the late stages of LTP has been demonstrated in the case of hippocampal neurons [20]. At the same time, data have been obtained showing the effects of MCPG on hippocampal synapses [29], which include the different effects of MCPG blockade of receptors on the course of potentiation after weak and strong tetanic stimulation of the hippocampus [34]. This difference in effects may be associated with the different roles of different types of metabotropic receptors in maintaining LTP,

these different receptor types being blocked by the blockers mentioned above; differences in tetanization conditions may also have a role.

Intraventricular administration of AMPA receptor blockers in the present study did not produce any great change in responses to test stimulation. This unexpected result may be associated with the poor diffusion of agents to the synapses under investigation or with low concentrations of AMPA receptors in SCT synapses. We did not use higher concentrations because of the risk of very severe consequences for the animals – even the intracerebral fluid concentrations used here were much greater than those needed for blockade of glutamate receptors of hippocampal synapses *in vitro*. Particular attention should be paid to the strong effects of blockade of NMDA receptors on EP produced in response to test stimulation in the present study, which surpassed the effects of application of the blocker of AMPA receptors, which are known to be responsible for up to 95% of the amplitude of EP produced in response to test stimulation in hippocampal cells [18]. Hedberg and Stanton, who tetanized in conditions of treatment with AP-5, demonstrated blockade of the induction of LTP in brain slices. Their illustrations show some decrease in the response to test stimulation after application of this blocker before tetanic stimulation. In our view, the fact of elevated sensitivity of neuronal synapses in the cingulate cortex to blockade of NMDA receptors may be associated with their special role in the formation of long-term memory. The literature includes descriptions of so-called silent synapses, characterized by the presence of only potential-dependent NMDA receptors, AMPA receptors being absent [25]. As noted by the authors of [25], these synapses are numerous at the early stages of ontogenesis, though they accumulate AMPA receptors during development. Other authors [17] have noted that processes similar to long-term potentiation play a decisive role in converting silent synapses into functional synapses during early postnatal ontogenesis in rats. It can be suggested that at the functional level, this corresponds to the process of specialization of neurons in the corresponding structures, which occurs both during the formation of behavior during early development and in adult individuals during training.

The results obtained here represent a significant step on the path to direct comparison of the course of the processes of neuron specialization during learning with changes in synaptic efficiency in the same cells during the induction of LTP. They show that in structures whose neurons become specialized during operant learning in relation to forming behavioral acts [2, 4], these neurons can, in conditions of free behavior, develop long-term potentiation in conditions of electrical stimulation of the output structure of the hippocampal formation. This suggests the possibility that the same synaptic mechanisms are involved both in processes of modification of hippocampal cells, linked in the literature with forming medium-term memory, and in rearrangements of cortical neurons, which corresponds to the formation of "pure long-term memory" [31].

From the methodological point of view, it is also very important to note that excitatory spike responses during stimulation of the subiculo-cingulate tract occur at low probability, as this virtually excludes the risk that the spikes of neighboring neurons recorded with a single chronic microelectrode will be superimposed, which allows all spikes produced in response to stimulation to be identified using standard methods of extracting spikes from a specific neuron in terms of spike shape and amplitude, for example, using the Spike-2 program.

An attempt to make a direct comparison of the spike responses of hippocampal neurons with the formation of LTP in the same part of the hippocampus showed that many neurons not only did not increase their spike activity in response to the test stimulus after tetanization, but also that decreases occurred in conditions of clear LTP as measured in terms of the usual electrophysiological measures [32]. The variability of the spike responses of an ensemble of cingulate cortex neurons to stimulation of the SCT, seen in our studies, may be associated with the morphological features of this tract and the distribution of synapses on neurons [33]. This tract is characterized by a topographical projection whereby a defined locus of the subiculum projects to a defined locus of the cingulate cortex. In combination with lateral inhibition developing in cortical structures, this may explain the large number of inhibitory responses which we recorded, as well as the fact that short-latency excitatory responses were recorded only in two rats. Considering that the synapses of this tract are located on the apical dendrites of cingulate corte pyramidal cells and, according to Hedberg and Stanton [15], have only "modulatory effects" on their activity (unlike, for example, the "leading influence" of transcallosal connections), the probability of precise coincidence of the stimulated group of fibers in the projection is very low, which explains the small number of animals in which we were able to record short-latency responses. In this sense, it is very useful to record responses on a computer during surgery for mutual positioning of the microelectrodes which, unfortunately, we commenced only in experiments on the later rats.

Summarizing the data, we note in particular: a) we recorded short-latency responses from cingulate cortex neurons to stimulation of the SCT in freely mobile rats; b) these responses were seen in a majority of individual cells extracted from the corresponding ensemble. The data obtained here demonstrate the fundamental methodological possibilities of comparing the involvement of particular individual cells in freely mobile rats in learning one or another adaptive behavior and the dynamics of their evoked spike activity during the formation of long-term potentiation. At the same time, our data also demonstrate the difficulties of performing such experiments.

CONCLUSIONS

1. Tetanic stimulation of the subiculo-cingulate tract leads to the formation of long-term potentiation in the cingulate cortex of freely moving adult rats without the use of GABA blockers.

2. The potentiable component of the evoked potential can be regarded as monosynaptic.

3. Injection of both synaptic AMPA and glutamate GABA blockers leads to significant decreases in evoked potentials to test stimulation in the cingulate cortex.

4. Stimulation of the subiculo-cingulate tract leads to recording of activatory and inhibitory spike responses with different latent periods in the population of neurons located in the cingulate cortex in freely moving rats.

5. The activatory short-latency spike responses recorded here were characterized by a low probability of spike generation, which increased with increases in the stimulation current.

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