Studies of Neurons of Rat's Sensory Cortex.* (28450)

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Much has been reported concerning intracellularly recorded potentials from units in the somatosensory area of the cat cortex(1, 2,3). The rat is widely used in neurophysiological research. Possibly because of the small size of the animal and of its cortical neurons(4,5), however, few intracellular recordings have been made from cortical cells in this species. Hillman and McIlwain(6) did succeed in recording the resting potentials of cells in isolated cortical tissues of rats. In this paper are described briefly cellular potentials obtained from the sensory area of the rat cortex *in situ*. Since the rat is peculiarly sensitive to curare the action of this drug on cortical units was also studied.

Methods. Rats ranging between 270 to 400 g of body weight were anesthetized by intraperitoneal injection of Thiopental Sodium (Pentothal, Abbott, 4 mg/100 g body weight). In many instances additional doses (0.5-1.0 mg/100 g) were given during the surgical procedure, but after this the rat generally remained quiet and well anesthetized throughout the experiment, no additional drug being required. Thiopental Sodium was considered to be better suited for this experiment than Pentobarbital Sodium (Nembutal; 4.5 mg/100 g) or Nembutal followed by Barbitone Sodium (3% solution; 0.6 cc/100 g) which have often been used for studying evoked cortical potentials in the rat(7). In Thiopental Sodium anesthetized rats, cortical neurons were more active and more "spontaneously" firing cells were observed than when the other anesthetics were employed. Chloralose (7 mg/100 g) was also tested, but in this case Flaxedil (Gallamine triethyliodide; 0.5-0.8 mg/100 g) and artificial respiration had to be used to keep the animal quiet. The latter procedure often caused more movement of the brain than occurred normally in spite of pneumothorax. Moreover, the repeated administrations of Flaxedil necessary to keep the animal sufficiently paralyzed to permit satisfactory artificial respiration proved to be somewhat troublesome in rats.

Stabilization of the head was accomplished by ear canal and teeth fixation. Sometimes the rat's body was also suspended by means of metal clamps attached to the hip joints. Cranial bones were removed with very small rongeurs. Great care was taken to keep the dura completely intact during this procedure. Exposure of the cortex was made as extensive as possible without damaging the saggital and transverse sinuses. Bilateral exposure reduced movement of the brain tissue. Finally, the dura was opened under mineral oil with the aid of a dissecting microscope. The animal was kept warm by a heated table top and the lamp used to illuminate its head.

Peripheral stimulations were performed through bipolar needle electrodes inserted into the skin of a contralateral forepaw. This proved to be a more natural stimulus than that resulting from direct electrical excitation of a nerve. Thalamic stimulation was carried out through a fine L-shaped tungsten electrode(8) inserted in the thalamus. This focal stimulating electrode was only a few millimeters distant from the recording surfaces and the microelectrode leads. The other pole of the stimulating electrode was attached to the skin of the head region or to another part of the body. Because a suitable stereotaxic instrument was not available at the time of these experiments, the position of stimulating electrode placement in thalamus was determined with relation to the skull and cortical surface. This was judged empirically with reference to the stereotaxic maps prepared for rats (D. W. Taylor, personal communication). Histological checks at the end of the experiments proved that this method was generally satisfactory.

In a few experiments transcallosal stimu-

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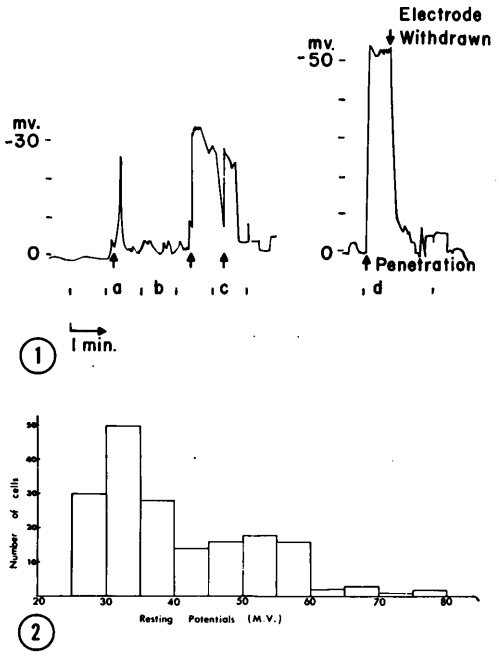


FIG. 1. Resting membrane potentials of neurons of rat sensory cortex recorded subsequent to microelectrode penetrations (indicated by arrows). Pen recording D.C. amplifiers used. a. Penetration in which membrane potential was not sustained. b. Extracellular recording showing some oscillation of state under electrode (see Fig. 3e). c. Successful penetration. There was a gradual reduction of membrane potential accompanied by cellular discharge (seen on oscilloscope tracing). The second penetration evoked no such discharges. d. Successful penetration followed by cellular discharges for a few seconds. The cell then remained quiescent. No response was evoked by thalamic stimulation.

FIG. 2. Distribution of resting membrane potentials recorded from rat sensory cortical neurons.

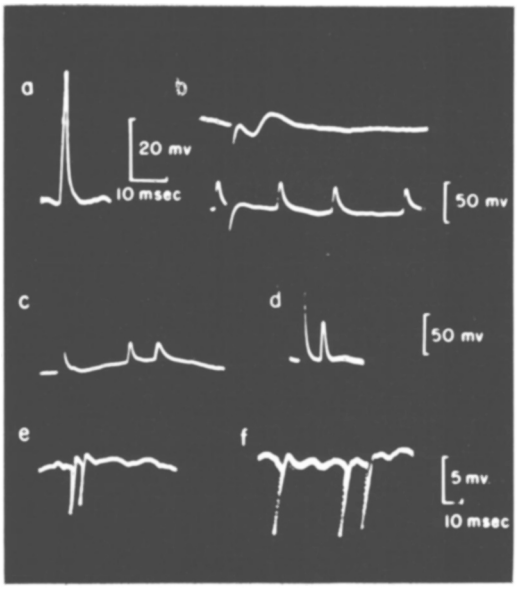
lation as well as direct cortical stimulation was performed using bipolar electrodes. The surface evoked response was recorded with fine platinum monopolar electrode. Microelectrodes used for recording intracellular potentials were conventional 4 M-NaCl filled glass capillaries having a resistance of 15-20 MΩ. The indifferent electrode used for both recordings was attached to the frontal bone. Leading from microelectrodes was through a cathode follower and DC amplifier to one beam of a double beam oscilloscope and also to a loudspeaker. The grid current was of the order of 1×10^{-13} A. The other beam of the oscilloscope recorded surface evoked responses led through a condenser-coupled amplifier. DC potentials from cells were also fed into a pen recorder.

It was customary to explore first the cortex by a surface electrode to find the spot which gave the maximum response to the stimulus employed. A microelectrode then was inserted into the brain very close to this spot by means of a micromanipulator.

Results. Despite the small size of cortical neurons of the rat [average of 20-30 μ with a maximum diameter of 33 μ *in situ*, according to Sugita(4,5)] resting membrane potentials were recorded from a number of cells. Fig. 1 shows examples of pen-recorded resting potentials of neurons and Fig. 2 gives the distribution of potentials recorded from the 180 cells which were successfully penetrated. They ranged from 25 to 60 mv. A few cells had resting potentials over 60 mv and the microelectrode tip could be maintained intracellularly for a number of minutes but in many instances it was impossible to evoke a response from these cells by stimuli normally employed in these experiments.

Fig. 3, a and b, show spike potentials recorded intracellularly from the units in the sensorimotor areas of the rat cortex. Though stimuli were applied through the thalamus, these cells were discharging "spontaneously." It was easier to find cells behaving thus than to locate them through responses evoked by any stimulus tried, *i.e.*, peripheral, thalamic, transcallosal or direct cortical activation. This difficulty in locating units showing evoked activity may have been due to the fact that very few units were excited by the stimuli used even though they were super maximal with respect to the surface evoked response. Fig. 3, c and d, show intracellularly recorded action potentials evoked by thalamic stimulation. Peripheral stimuli, however, did not succeed in evoking responses from these cells, though peripherally evoked slow potentials were easily recorded extracellularly at various depths in the cortex by means of slightly larger electrodes (1-5 MΩ resistance). A similar result was reported in studies of the striate cortex of the cat by Tasaki, *et al.*(9).

Fig. 3, e and f, show action potentials



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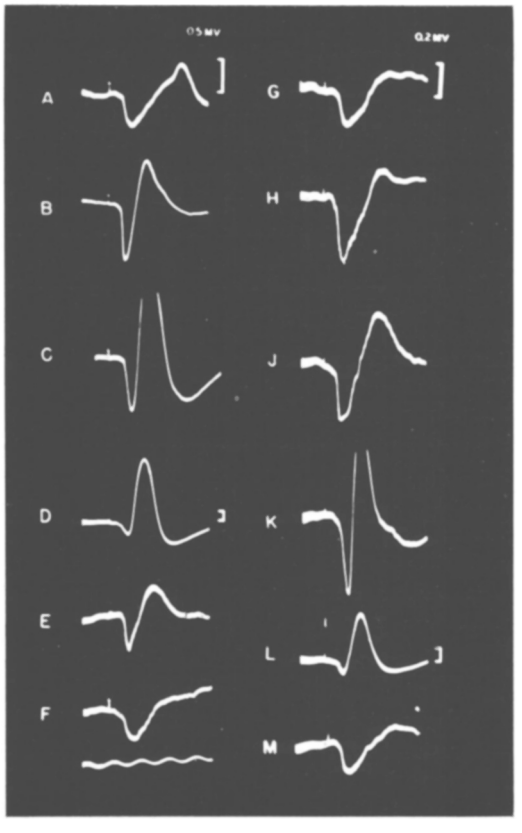


recorded extracellularly from "spontaneously" firing cells. Many such units were found at a depth of between 150 to 1400 μ and particularly at approximately 1000 μ. The number of active cells seemed to vary with the depth of anesthesia. Since histological evidence(4,10) suggests that all the cortical layers except layer I are situated within this depth, activity must be regarded as being present in every cellular layer. Layer V which is at a depth of 900-1300 μ contains large neurons and this may account for the easier detection of active cells at this depth from the surface.

d-Tubocurarine is known to produce very significant changes in cortical evoked potentials when administered locally(11,12) and the rat is much more sensitive to this drug than the cat. When 1:10,000 or 1:20,000 d-Tubocurarine chloride-Ringer's solution (approximately 10^{-4} M) was locally applied to the rat cortex for 3 to 5 minutes by forming a pool above the cortex or by placing a small disc of filter paper soaked with this solution on the sensory area, marked increases in size and changes in shape of evoked potentials were observed. d-Tubocurarine augmented both positive and negative components of the surface recorded evoked potential but the change in the negative potential was particularly great (Fig. 4).

FIG. 3. Potentials recorded from sensory cortex of rats. a. Intracellularly recorded action potential of spontaneously firing cell. b. Same as a. Thalamic stimulation was applied but no evoked response was observed. Top tracing is surface evoked potential. c & d. Intracellular potentials evoked by thalamic stimulation. e & f. Extracellularly recorded potential of spontaneously firing cells.

FIG. 4. Changes produced by d-Tubocurarine in rat cortical evoked potentials. Stimuli were applied to right forepaw and potentials were recorded from left sensory area. After control (A) was taken, 1:10,000 curare-Ringer's solution was applied over cortex for 5 min. The solution was then replaced by mineral oil after the cortical surface was washed several times with Ringer's solution. Records were taken immediately thereafter (B), 2 min after (C); 10 min after (D); 23 min (E) and 1½ hr later (F). From G to L, the effect of curare was tested by applying a disc of filter paper (2 mm in diameter) soaked in 1:10,000 d-Tubocurarine-Ringer's solution to the cortical surface near recording electrode. G—control; H—5 min; J—11 min; K—15 min and L—18 min after curare application. M was taken 30 min after filter paper was removed and cortex was washed.

TABLE I. Effect of d-Tubocurarine on the Resting Membrane Potentials of Rat Sensory Neurons. C–control, Tbc–during Tubocurarine application. Each set of recordings is from a different animal. See text.

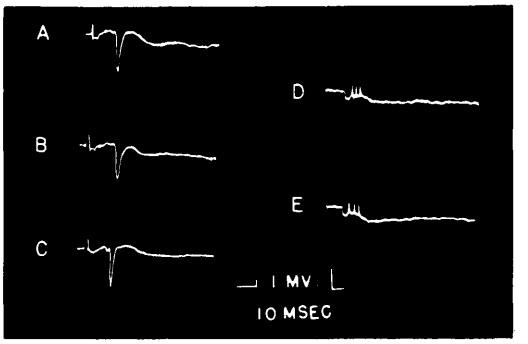
	Resting potentials (m.v.)									
	C	Tbc	C	Tbc	C	Tbc	C	Tbc	C	Tbc
	25	32	25	28	34	34	51	39	31	27
	25	25	32	50	35	25	39	40	25	50
	34	32	38	47	43	39	52	25	25	30
	52	27	46	26	35	32	28	28	27	25
	51	50	35	45	39	51	32	45	26	30
	43	30		39	36	25	36	38	33	
				28		46		28		
Avg	39	33	35	38	37	36	40	35	28	32

The effect of d-Tubocurarine on individual cell activity was also determined by intracellular and extracellular recordings. Because of the difficulty of keeping an electrode in a single cell for the long periods of time required to observe changes produced by this drug, an attempt was made to estimate such changes by intracellular recording from a number of cells before, during and after application of d-Tubocurarine to the cortical surface. In a single animal, 5-8 successful cell penetrations before, during and after drug administration usually were all that could be obtained in the 5 animals studied. The resting membrane potentials thus recorded did not show any significant change, though the successful penetration of single cells in the same animal were not numerous enough to establish this conclusion in a fully satisfactory fashion (Table I). Single cell discharges evoked by thalamic or direct cortical stimulation, recorded extracellularly, also were not affected by d-Tubocurarine even though surface evoked responses were markedly modified (Fig. 5).

The only significant change observed in cell activity following application of curare to the cortical surface was that some normally silent cells began to discharge continuously without application of any external stimulus (Fig. 6). These cells ceased to fire after the drug was removed. This phenomenon was not observed in all units studied, however.

Thus, the augmentation in evoked potentials caused by curare might be explained by an activation of a greater number of neurons. Certainly curare produced a greater degree of spontaneous firing. Study of individual units, however, gave no explanation of the mechanisms of drug arousal. Additional work must be done before the mechanism of action of curare can be explained.

Discussion. The magnitude of the membrane potentials of rat cortical neurons was found to be 25-60 mv, which is slightly lower than that recorded by Hillman and McIlwain(6) from units in isolated tissues. A few cells having high resting potentials (over 60 mv) did not respond to any stimulus applied. Phillips(13) reported the same oc-



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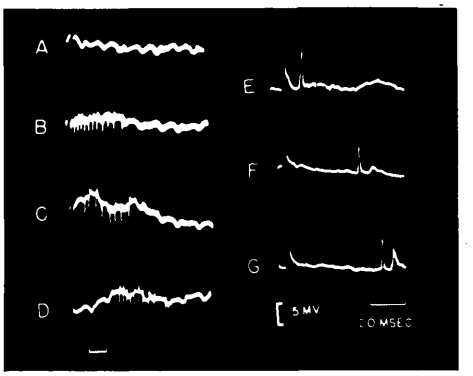

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FIG. 5. Cellular response of rat's cortex to thalamic stimulation before and after curare application. A, C and D are controls; B and E during curare application.

FIG. 6. Spontaneous firing of sensory neurons caused by d-Tubocurarine chloride locally applied to rat's cortex. A—control. B, C and D show that grouped firing occurred during curare application. E to G are from another experiment. Though stimuli were applied to the thalamus, the cell remained silent until drug was administered. The cell then showed spontaneous firing (E, F and G), but no evoked response was seen.

currence in cat motor cortex and Li(3) suggested such cells are glia cells. The amplitudes of the action potentials recorded from rat cortical cells were usually not more than 50 mv which is considerably smaller than those recorded by Li(3) from spontaneously firing neurons of the cat's somatosensory cortex. These amplitudes varied from 62-120 mv but in his earlier work much smaller values were reported, *i.e.*, 1-87 mv(2). Li attributed the variations he observed to effects of injury to the surface membranes. It is possible that the potentials recorded intracellularly are somewhat subnormal due to penetration damage to these very small cells; nevertheless the technique appears to be adequate for studies of drug actions on the rat cortex.

This work confirmed the observed high sensitivity of the rat cortex to locally applied drugs such as curare, atropine(14) and to schizophrenic serum(7). Surface recorded potentials were augmented markedly by curare. The negative component of surface potential evoked by peripheral afferent stimulation was increased most significantly. This result is similar to those observed in cats (12) but the concentration of d-tubocurarine solution applied in the latter was 10 times greater than that which was used for rats. Intracellular recording, however, merely indicated arousal of previously quiescent cells. It can be assumed that this appearance of activity was due to some change in cellular state which sensitized the cells to background activity. The drug-induced facilitation might have been due to some action on terminals of the dendritic tree which affected cellular stability. Changes in cellular states do take place even spontaneously since arousal has often been observed to occur in the cortical and other central neurons of the cat(13). The facilitory action of curare and the effects of this drug on the evoked cortical potentials require further study.

Summary. Resting and action potentials were recorded intracellularly from neurons of rat sensory cortex. Resting membrane potentials in most of the neurons were found to be 25-60 mv but a few cells had potentials of 60-80 mv. Action potentials of spontaneously firing cortical neuron as well as those evoked by thalamic stimulation were not more than 50 mv in amplitude. d-Tubocurarine chloride applied locally to the cortical surface did not modify evoked discharges of single cortical neuron nor resting membrane potentials significantly, though there was a marked change in evoked response recorded simultaneously from the surface or by a larger microelectrode from deeper layers of the cortex. d-Tubocurarine sometimes induced neurons to fire "spontaneously" but only during its application to the cortex.

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1. Buser, P., Fessard, A., *Microphysiologie comparée des Éléments Excitables, Colloq. Internat. C.N.R.S.*, Paris, 1955, No. 67, p333.

2. Li, C. L., *J. Physiol.*, 1955, v130, 96.

3. ———, *J. Neurophysiol.*, 1959, v22, 436.

4. Sugita, N., *J. Comp. Neurol.*, 1918, v29, 119.

5. ———, *ibid.*, 1918, v29, 241.

6. Hillman, H. H., McIlwain, H., *J. Physiol.*, 1961, v157, 263.

7. German, G. A., *ibid.*, 1962, v160, 10p.

8. Hubel, D. H., *Science*, 1957, v125, 549.

9. Tasaki, I., Polley, E. M., Orrego, F., *J. Neurophysiol.*, 1954, v17, 454.

10. Sugita, N., *J. Comp. Neurol.*, 1917, v28, 511.

11. Chang, H. T., *J. Neurophysiol.*, 1953, v16, 221.

12. Feldberg, W., Malcolm, J. L., Sherwcod, S. L., *J. Physiol.*, 1956, v132, 130.

13. Phillips, C. G., *Quart. J. Exp. Physiol.*, 1955, v41, 58.

14. Gundu Rao, H. V., Ph.D. Dissertation, Aberdeen University, 1963.

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