Thus it is clear that the intensification of phrenic nerve discharges in paralyzed animals depends on the disappearance of impulses from the lung stretch receptors which inhibit the respiratory centre in the normal state.
These experiments suggest the following conclusion. In patients with respiratory paralysis, lung compliance decreases and therefore artificial distension of the lungs with normal tidal volume cannot inhibit the respiratory centre, in contrast to the normal state. Therefore, although blood gas tension is normal, the patients need additional distension of the lungs, additional artificial ventilation. As a result of hyperventilation, $PaCO_2$ decreases and the respiratory centre adapts to hypocapnia, and this in turn creates a need for further hyperventilation. However, the primary reason for the need of hyperventilation in patients with respiratory paralysis is the insufficiency of the Hering-Breuer inflation reflex. – Of course, the results obtained in cats do not entirely justify this conclusion in human beings. Nevertheless it seems to be valid.

1 J.E. Affeldt, in: Handbook of Physiology, sect. 3, vol. 2, p. 1509. Washington 1965.
2 J.H. Auchincloss, in: Handbook of Physiology, sect. 3, vol. 2, p. 1553. Washington 1965.

Hyperventilation and inhibitory synapses

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Summary. Injection of a subconvulsive dose of strychnine (which blocked the inhibitory synapses) increases respiratory muscle activity evoked by stimulation of the sciatic nerve as well as by inhalation of hypercapnic gas mixture. Thus the inhibitory synapses prevent an excessive hyperventilation.

Strychnine is one of the stimulants of the respiratory system. Strychnine blocks the inhibitory synapses[1]. But, as was shown, strychnine does not influence the vagal inhibitory Hering-Breuer reflex[2,3]. Thus it may be supposed that the mechanism of strychnine action on the respiratory system may depend on its anti-inhibitory influence on the motoneurons innervating the respiratory muscles.
To analyse this problem, we investigated the influence of a single subconvulsive dose of strychnine-nitrate (0.07 mg/kg i.v.) (which affects respiration insignificantly): a) on the respiratory reflexes caused by electrical stimulation of the central part of the divided sciatic nerve, and b) on the respiratory muscle activity caused by inhalation of hypercapnic gas mixture.

A pair of stimulating platinum electrodes were placed on the central cut end of the sciatic nerve, and another pair of recording electrodes placed on the sternal part of the diaphragm. For stimulation of the nerve repetitive rectangular pulses, currents of 0.5 msec duration and 10/sec frequency were used. 7% carbon dioxide in oxygen was used. The experiments were performed on 12 cats anaesthetized by urethan (1.5–2.0 g/kg i.v.).
Results and discussion. Before the injection of strychnine, the frequency of respiration was 24.5 ± 1.4 per min. The threshold of the respiratory reflex (the increase of frequency and sometimes the intensity of the diaphragmatic discharges) was 20–30 V. When the stimulation of the nerve was discontinued, increase of diaphragmatic activity

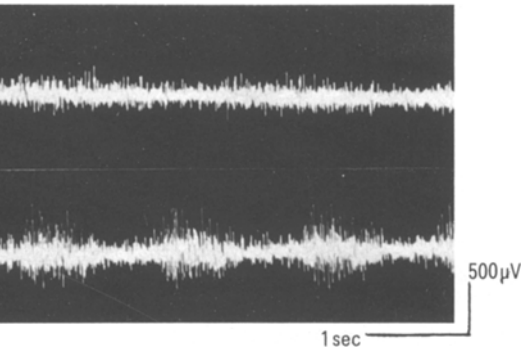

Fig. 1. Before injection of strychnine. Top record: EMG of the diaphragm before inhalation of hypercapnic mixture. Bottom record: inhalation of hypercapnic mixture increases the frequency and intensity of respiratory discharges.

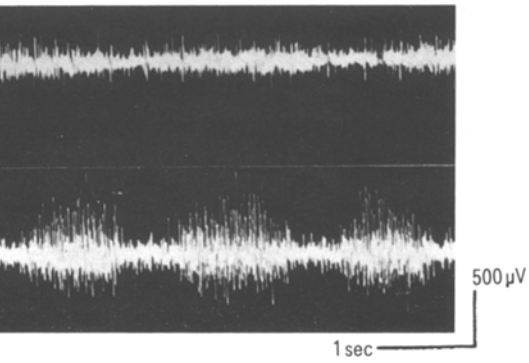

Fig. 2. After injection of strychnine. Top record: EMG of the diaphragm before inhalation of hypercapnic mixture. Bottom record: inhalation of hypercapnic mixture increases the frequency and intensity of respiratory discharges more than in the normal state (figure 1).

ceased. The subconvulsive dose of strychnine causes a small increase of the frequency (27.5±2.1 per min) and sometimes of the intensity of the diaphragmatic discharges. After the injection of the subconvulsive dose of strychnine, the threshold of the respiratory reflex was 4 or 5 times less. The respiratory frequency increases till 36±1.7 per min and persists after the cessation of nerve stimulation for about 15–60 sec. The effect of strychnine lasts 15–20 min. Inhalation of hypercapnic mixture as usual evokes an increase of the intensity and frequency of the respiratory discharges (figure 1). After injection of strychnine, inhalation of the same gas mixture increases the respiratory discharges more intensively and activity of new motor units appears (figure 2). Thus the inhibitory synapses play an important role in preventing an excessive hyperventilation. From the times of Sherrington[4], it has been known that each stimulus exerts simultaneously an excitatory and an inhibitory effect: the latter restricts excessive reflexes. Probably the stimuli influencing respiration obey the same rule.

1 J.G. Eccles, The physiology of synapses. Springer, Berlin 1964.
2 R.C. Creed and D.H. Hertz, J. Physiol., London *78*, 85 (1933).
3 S.I. Frankstein and Z.N. Sergeeva, Exp. Neurol. *19*, 232 (1967).
4 C.S. Sherrington, in: The Integrative Action of the Nervous System. Constable, London 1966.

Staining of isolated rabbit neurons and neuroglial clumps

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Summary. Isolated rabbit neurons stained just as intensely as neuroglial clumps did with Mallory's phosphotungstic acid haematoxylin, Weil and Davenport's, Marsland, Glees and Erikson's and with Gallyas's silver stain.

Isolated neurons and groups of neuroglial cells around them (neuroglial clumps) have been the subject of many studies[2–4]. During an examination of the histology of neurons and neuroglial clumps[5], it was noticed that some of the neurons were stained by the 4 techniques mentioned below[6–9], which are generally described as stains for neuroglia or their processes[10–16]. Therefore, it was decided to investigate the specificity of these stains by seeing if they would also react with neurons.

Neurons and also the adjacent neuroglial clumps (as control samples for the staining) were isolated from the cranial nerve nuclei by hand dissection using stainless steel wires by the techniques of Hydén[17]. They were placed in sucrose solution (0.25 M) in cavity slides to which they were made to adhere to the floor of the cavity. In this series of experiments, groups of at least 150 single neurons or neuroglial clumps were isolated for each staining procedure; about 15 neurons or neuroglial clumps were placed on each slide. The sucrose solution was drawn off with a Pasteur pipette, and the tissues were fixed in 10% formol saline, dehydrated with 50%, 80% and 100% ethanol (3 times), cleared with xylol and embedded in paraffin wax. The neurons or neuroglial clumps were then rehydrated and prepared with the following staining systems: Mallory's phosphotungstic acid haematoxylin[6]; Weil and Davenport's silver stain[7]; Marsland, Glees and Erikson's silver

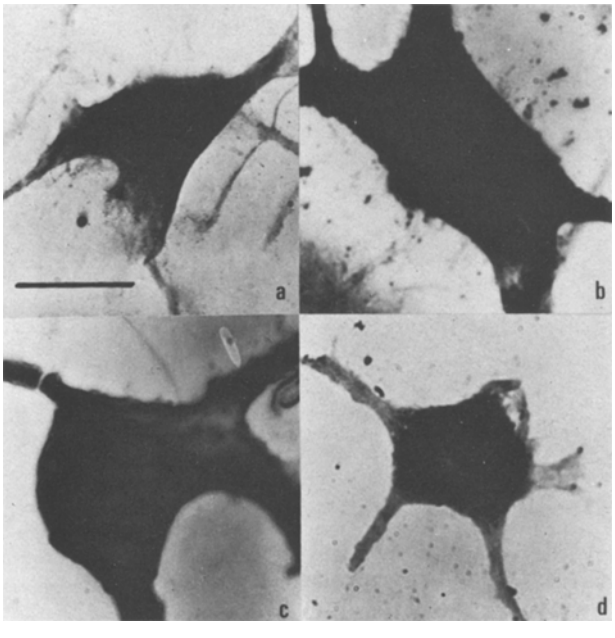

Fig. 1. Isolated rabbit neurons stained with the following systems, a: Mallory's, b: Weil and Davenport's, c: Marsland, Glees and Erikson's, d: Gallyas's. The bars in both figures are 20 µm.

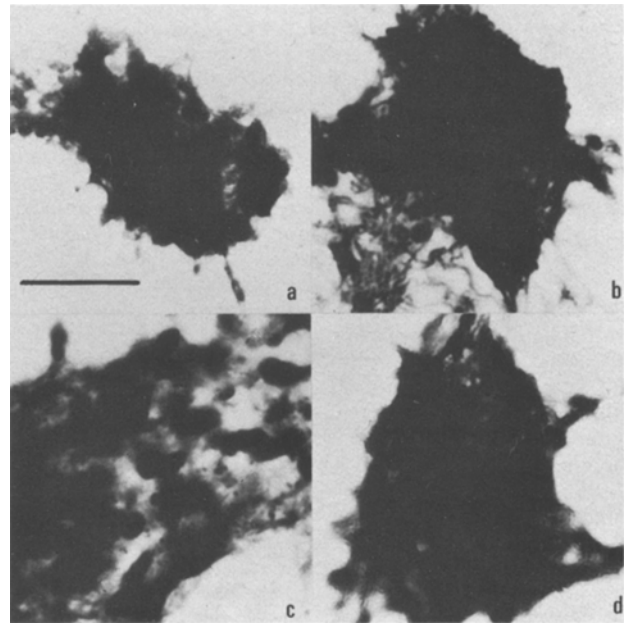

Fig. 2. Isolated neuroglial clumps, stained as were the neurons in figure 1, a–d.