Interneuronal Barrages and Ripple Superposed on Inhibitory Postsynaptic Potentials

Excitatory and inhibitory mechanisms in the brain are less well known than those at lower levels of the neuraxis. However, a large number of the basic mechanisms worked out from unitary recordings in the cord have been found to apply to cerebral neurons. The wide distribution of inhibitory postsynaptic potentials (IPSPs) recorded in most brain areas upon separate inputs to any given structure has for some time suggested the existence of causal inhibitory elements similar to RENSHAW cells[1,2]. The suggestion that IPSPs in the cord were produced by a repetitive synaptic bombardment from the interneurons was originally based on the fact that the frequency of the wavelets superimposed on the IPSP was the same as that of the interneuronal barrages[1].

The significance of similar high-frequency ripple superposed on brain IPSPs has remained obscure and a matter of speculation[3,4] since no RENSHAW-like interneurons had been recorded[5]. Thalamic interneurons of RENSHAW cell characteristics have recently been demonstrated[6]. Simultaneous dual-neuron recordings have shown that independently recognizable spikes, not only ripple, do occur throughout the duration of IPSPs of identified thalamocortical relay cells[7].

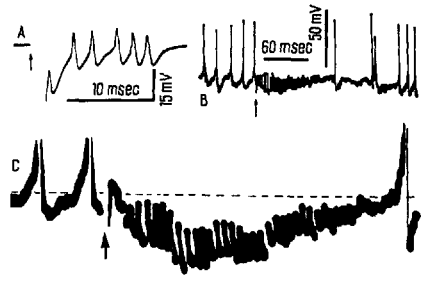
This communication (detailed study in preparation) has been extracted from another series of recordings of unitary potentials in ventrolateral thalamus upon intranuclear stimulation in anesthetized and paralyzed cats. The Figure provides direct evidence that the often-recorded ripple riding on IPSPs is due to interneuronal barrage discharges at high frequency which modulate target cells' membrane potential in a repolarizing or hyperpolarizing direction[8]. In (A) a single cathodal shock was applied to a tungsten electrode tip 1.5 mm rostral to the recording micropipette (1 μ tip; filled with 3 M-K-citrate). The stimulus evoked an interneuronal response at over 500/sec. After a sudden 50 mV negative shift of the baseline was recorded, without manipulation of the microdriver, record B emerged in the face of the oscilloscope. Single shocks applied to the same rostral electrode as in (A) produced the following concomitant events: (a) arrest of large spike lasting 100 msec; (b) post-shock hyperpolarizing potentials of equal duration; (c) barrage response of a second element, probably the same one recorded in (A) during the hyperpolarizing potential. A photographic enlargement of another hyperpolarizing potential similarly evoked 5 min after (B) is shown in (C) to demonstrate that the interneuronal barrage occupied almost the whole duration of the hyperpolarizing potential.

Recordings of extracellular unitary potentials from an intracellular electrode of 1 μ tip are unusual; the recording of an interneuronal barrage concomitantly with its target neuron spike potentials and inhibitory potential is an extremely unusual concurrence but of much value for the understanding of inhibitory postsynaptic mechanisms. These records demonstrate that an intracellular electrode may 'see' extracellular activity and record meaningful dual-neuron correlations and interdependence when high sensitivity recording is employed. They also demonstrate topological nearness between the target cells and their modulating elements. The usefulness of simultaneous recording from 2 neurons or groups of neurons rather than from individual units has been stressed by other authors[9,10]. These and other records in our experiments also establish clearly that in the thalamus interneurons are not arranged in pools separate from other cells[1,7,8].

Although no IPSP identifying transmembrane polarizing currents were used in this experiment, we feel justified, on the bases of other preparations, in assuming that the evoked hyperpolarizing potentials were true IPSPs[11,12] and that they were caused by the interneuronal discharge. The mechanism would appear to be comparable to that of recurrent collateral inhibition via RENSHAW cells in the lumbosacral segment of the cord[1,2,6-8].

The records also suggest an answer to the question of whether interneuronal discharges are briefer than their effects and hence whether the longer-lasting IPSPs would be sustained by a prolonged release of the inhibitory transmitter[12]. It can be seen that the interneuronal discharge can last as long as its effect on the target cell, the inhibitory potential. The possibility that interneurons may be triggered successively through neural chains of various complexities[10], thus ensuring relatively long barrage discharges, is suggested by the stepwise trace of the inhibitory potential (see C).

It has been assumed that the rhythmic small oscillations superposed on the inhibitory potential were usually smoothed out because they were generated by the discharge of many Renshaw cells progressively becoming out of phase beyond the summit of the IPSP; the ripple should otherwise become accentuated as the interneuronal frequency declines[1]. While there is evidence that several interneurons must contribute to the formation of an IPSP, another alternative explanation is suggested by the Figure (C) in which it can be seen that the frequency



(A) Single-shock-evoked interneuronal unitary response at about 500/sec from ventrolateral thalamus. (B) was taken shortly after 50 mV negative shift of the baseline was recorded. Single shocks arrested the large spikes for 100 msec concomitantly with hyperpolarization of cell membrane and attending interneuronal barrage at 500/sec. All three events had the same time course. (C) enlargement of another similar sequence evoked by the stimulus. Stimuli (at arrows) delivered to a locus 1.5 mm rostral to the recording micropipette. Positivity upward.

[1] J. C. ECCLES, P. FATT and K. KOKETSU, J. Physiol. *126*, 524 (1954).
[2] B. RENSHAW, J. Neurophysiol. *9*, 191 (1946).
[3] H. KITASATO, Jap. J. Physiol. *15*, 71 (1965).
[4] D. P. PURPURA and R. J. SHOFER, J. Neurophysiol. *26*, 494 (1963).
[5] H. ASANUMA and V. B. BROOKS, Archs ital. Biol. *103*, 220 (1965).
[6] P. ANDERSEN, J. C. ECCLES and T. A. SEARS, J. Physiol. *174*, 370 (1964).
[7] L. A. MARCO and T. S. BROWN, Nature *210*, 1388 (1966).
[8] L. A. MARCO and T. S. BROWN, Proc. Am. psychol. Ass. *101* (1966).
[9] R. W. GERARD, in *Handbook of Physiology* (Ed. J. FIELD; Am. Physiol. Soc., Washington, D.C. 1960), vol. 3, p. 1919.
[10] V. B. BROOKS and H. ASANUMA, Archs ital. Biol. *103*, 247 (1965).
[11] J. S. COOMBS, J. C. ECCLES and P. FATT, J. Physiol. *130*, 326 (1955).
[12] J. C. ECCLES, in *The Physiology of Synapses* (Academic Press Inc., New York, 1964).

of interneuronal discharge gradually increased rather than decreased. Concomitantly, the spike amplitude also gradually declined. It is easy to see that this interneuronal pattern of discharges can well account for the well known time course of IPSPs if it is accepted that the smaller the spike potential the smaller will be the output of inhibitory transmitter[13,14]. Indeed we have often recorded both patterns of interneuronal barrages, at gradually decreasing and at gradually increasing firing rates. Whenever the latter was obtained, spike amplitude gradually decreased to zero, probably due to refractoriness or to depolarization inactivation.

Summarizing, it appears possible to record simultaneously all primordial component events underlying the phenomenon of postsynaptic inhibition, namely target cell discharge, target cell inhibition, inhibitory potential and interneuronal barrage. The electrographic demonstration of this modulatory interdependence is indicative of underlying topological closeness of the two involved elements. The commenness of this recurrent pathway throughout the central nervous system has been emphasized from different angles by various authors[9,12,15]. The role of recurrent collateral inhibition in controlling electroencephalographic rhythmic activity[8,12,16,17] and possibly learning and total integrated behavior[15] has been adequately stressed by other authors.

Résumé. L'activité électrique des neurones du noyau thalamique ventro-latéral est étudiée ici chez des chats anesthésiés au nembutal et immobilisés avec du flaxedil au moyen d'un enregistrement intracellulaire. Nous avons observé que l'enregistrement de certains neurones montre que le potentiel nommé inhibiteur postsynaptique (IPSP) est accompagné de la décharge en barrage d'un autre neurone probablement semblable à la cellule de RENSHAW.

L. A. MARCO[18], T. S. BROWN and M. E. ROUSE[18]

Illinois State Psychiatric Institute, Chicago (Illinois 60612, USA), 3rd October 1966.

[13] J. CASTILLO and B. KATZ, J. Physiol. *124*, 586 (1954).
[14] S. HAGIWARA and I. TASAKI, J. Physiol. *143*, 114 (1958).
[15] P. M. MILNER, Psychol. Rev. *64*, 242 (1957).
[16] W. A. SPENCER and E. R. KANDEL, in *Physiologie de L'Hippocampe* (Colln int. centre national reserche sci., Paris 1962), vol. 107, p. 71.
[17] E. F. VASTOLA, J. Neurophysiol. *22*, 258 (1959).
[18] Present address: College of Physicians and Surgeons of Columbia University, Dept. of Physiology, New York, N.Y. 10032.
[19] We thank Mr. G. BILANOW for invaluable technical help.

Retinale Impulsaktivität und Elektroretinogramm unter dem Einfluss von Desipramin

Die elektrische Aktivität des retinalen Systems wird durch verschiedene zentral nervös wirksame Pharmaka verändert. So kann durch Chlorpromazin die Spontanaktivität retinaler Neurone vermindert oder ausgelöscht werden, während die Belichtungsaktivität nur wenig beeinflusst wird[1]. In der vorliegenden Untersuchungsreihe wurde Katzen Desipramin injiziert, um zu untersuchen, ob dieses Pharmakon Veränderungen von ERG, Spontan- und Belichtungsaktivität bewirkt.

In 8 mit Äther und Pentothal-Natrium narkotisierten und durch Flaxedil immobilisierten Katzen wurde der Nervus opticus intrakraniell nach der Technik von SCHUBERT[2] freigelegt; mit Platin-Iridium-Mikroelektroden wurden Impulsentladungen einzelner Axone abgeleitet. Synchron damit wurde das ERG des Auges mit einer Haftschalenelektrode aufgezeichnet (DC-Verstärkung). Die benutzten Lichtreize hatten an der Eintrittspupille eine Intensität von 70 lm/m² und dauerten 1 sec. Weitere methodische Einzelheiten finden sich bei BORNSCHEIN et al.[1] Desipramin wurde in Einzeldosen von 2–10 mg/kg bis zu Gesamtdosen zwischen 20 und 35 mg/kg i.v. injiziert. Der während der Versuche aus der Arteria carotis registrierte Blutdruck wurde durch Desipramin nur geringfügig und flüchtig beeinflusst, abgesehen von Höchstdosen, die zum Tode des Tieres führten.

Der Einfluss von Desipramin auf Spontan- und Belichtungsaktivität wurde bei insgesamt 42 retinalen Neuronen studiert. Bereits Dosen von 4 mg/kg verminderten die Spontanaktivität deutlich (Figur 1), es traten häufig Doppel- und Mehrfachentladungen (Bursts) auf. Mit zunehmender Gesamtdosis nahm die spontane Impulsaktivität der Neurone weiter ab und bestand oft nur mehr aus kurzen Bursts mit langen Zwischenpausen. Bei höheren Gesamtdosen – in einem Neuron schon bei 10 mg/kg, sonst bei 15–25 mg/kg Desipramin – setzte die Spontanaktivität vollkommen aus.

Im Gegensatz dazu wurde die phasische Reaktion der Neurone auf Lichtreize nur wenig beeinflusst; bei niedrigen Dosen wurden mitunter verstärkte Reizantworten

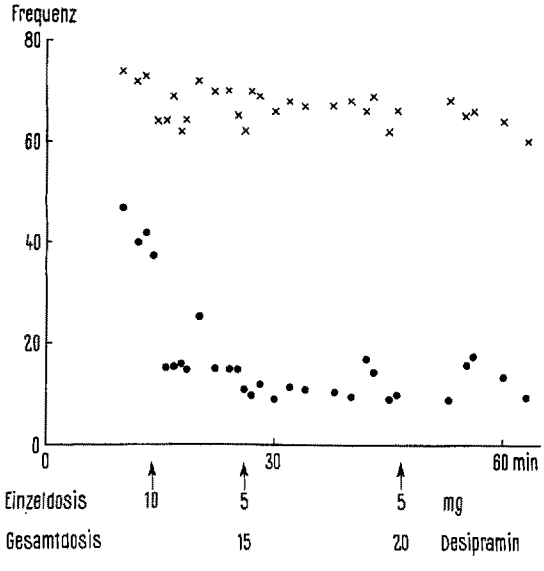


Fig. 1. Frequenz der Spontanaktivität (•) und der phasischen Reaktion eines Neurons auf Lichtreiz (×) unbeeinflusst und bei steigender Gesamtdosis von Desipramin. Katze, 1,5 kg.

[1] H. BORNSCHEIN, W.-D. HEISS, P. HEILIG und G. SANDBACH, Wien. Z. NervenHeilk., im Druck (1967).
[2] G. SCHUBERT, Albrecht v. Graefes Arch. Ophthal. *154*, 125 (1953).