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GABAergic Inhibition in the Neocortex

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Though long suspected, inhibition in the neocortex has become fully accepted as a major mechanism of control only in the last 25 years. The principal mechanism is a large increase in chloride permeability, mediated by the synaptic release of GABA from a variety of interneurons. There is still no evidence of any significant glycinergic inhibition in cortex. The functional role of cortical inhibition has been demonstrated in several ways: by global inactivation (with GABA depletion or antagonists) which causes paroxysmal activity; or by very localized release of GABA antagonists, which reveals the important role of GABAergic inhibition in determining both qualitative and quantitative aspects of receptive-field characteristics in visual and somatosensory cortex. Prolonged synaptic inhibitions are probably generated by an increase in potassium permeability. They probably account for some bicuculline resistant inhibitory actions and may be mediated either by a non-bicuculline sensitive GABA action (via GABA B receptors) or by non-GABAergic pathways.

Though early recognized as an important aspect of brain physiology by some neurophysiologists, notably those of the Russian school (Bubnoff and Heidenhain, 1881; Pavlov, 1927; Sechenov, 1863), inhibition remained widely ignored; its very existence as a cortical phenomenon was denied by most electrophysiologists for the first five or six decades of this century.

Two technical advances radically changed this situation. First, the painfully slow progress of intracellular recording from cortical cells, beginning with Phillips' investigation on pyramidal tract cells in the motor cortex (Phillips, 1956). These revealed inhibitory post-synaptic potentials (IPSP), fully comparable to the IPSPs discovered by Eccles a few years earlier in spinal motoneurons (Eccles, 1953). Though of longer duration, the neocortical IPSPs were similarly associated with a marked increase in membrane conductance (Figure 1) and were highly Cl^- sensitive, being readily reversed from a hyperpolarizing to a depolarizing potential by intracellular injections of Cl^- (Lux and Klee, 1962).

These outstanding characteristics of cortical IPSPs were not compatible

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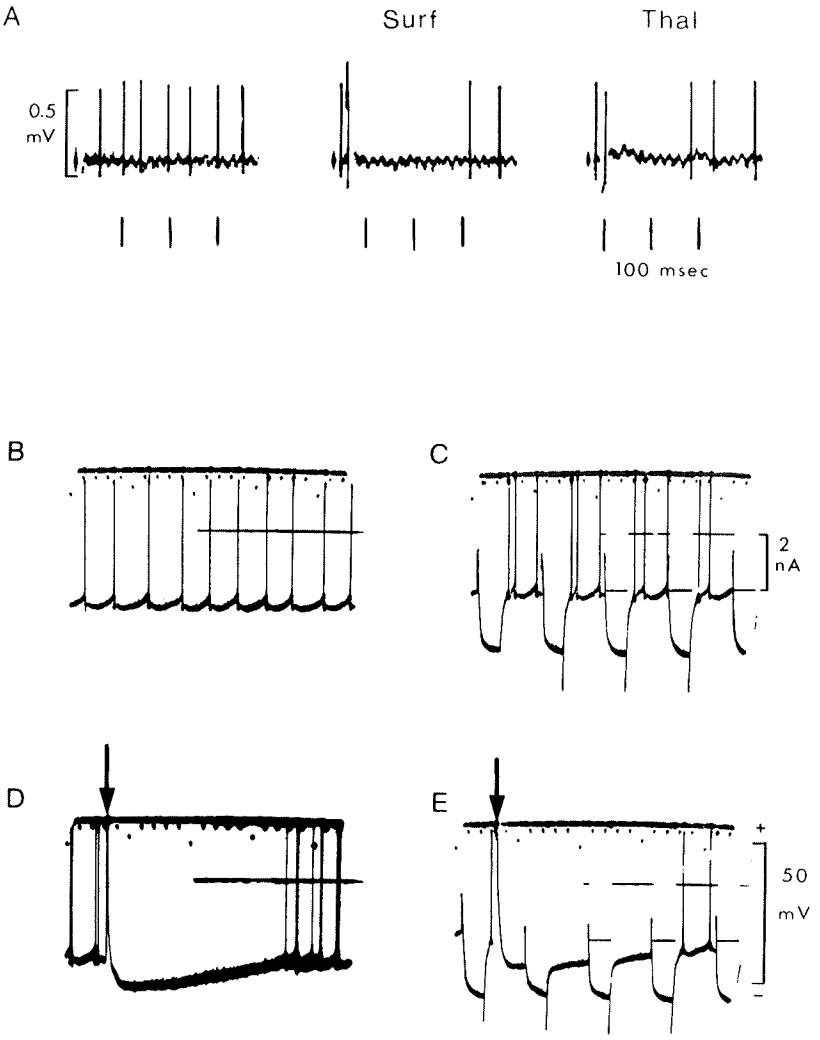

Figure 1: Manifestations of inhibition in neocortex of cat. (A) In extracellular recording, single unit in posterior sigmoid gyrus was made to discharge regularly by glutamate application to demonstrate prolonged inhibition evoked by a single electrical stimulus either to surface of cortex or to VPL nucleus of thalamus (Figure 2 from Krnjević et al., 1964). Reprinted by permission from *Nature*, London, Vol. *201*, 1294–1296. Copyright ©1964, Macmillian Magazines Limited. (B–E) Intracellular recording from another pericruciate neuron shows, (B) spontaneous discharge; (C) tests of input resistance by five identical hyperpolarizing current pulses; (D) inhibition of ongoing firing by electrical stimulus applied to cortical surface (at arrow—several traces are superimposed); and (E) corresponding large and prolonged drop in input resistance (series of identical current pulses, as in C). Resting potential initially –52 mV; time marks indicate 10 and 100 ms. (from Figure 2 in Dreifuss et al., 1969). Reprinted with permission of Springer-Verlag New York Inc., Secaucus, New Jersey.

with a variety of mechanisms previously suggested to explain *apparent* inhibitory phenomena such as refractoriness, field effects, occlusion, overexcitation, Wedensky "inhibition" and spreading depression. The only really plausible mechanism was a synaptically-mediated activation of Cl^- channels.

Synaptic inhibition nevertheless seemed speculative without some evidence that a potent inhibitory transmitter is present in the cortex. Such evidence was obtained by the use of a second major technical advance, microiontophoresis, an adaptation of the technique developed by del Castillo and Katz (1955) to study the action of acetylcholine (ACh) at the muscle end-plate. The first iontophoretic survey of cortical cells (Krnjević and Phillis, 1963) identified some prominent amino acid constituents of cortex as likely transmitters of both excitation (glutamate and aspartate) and inhibition (GABA). The iontophoretic release of glutamate also proved to be a valuable tool for demonstrating post-synaptic inhibition acting on quiescent cells—otherwise undetectable with extracellular recording. Such tests revealed surprisingly widespread inhibitory effects evoked by a variety of cortical inputs [Figure 1] (Krnjević, Randić, and Straughan, 1964).

More definitive evidence that GABA is involved in cortical inhibition required intracellular recording. Krnjević and Schwartz (1968) and Dreifuss, Kelly, and Krnjević (1969) [see Figure 2] were able to show that IPSPs and GABA produce similar changes in membrane resistance and potential, the latter having the same reversal levels over a wide range of transmembrane Cl^- gradients (see Figure 3). According to further tests (Kelly, Krnjević, Morris, and Yim, 1969) it appeared that the cortical IPSPs were mediated by purely anionic channels that were somewhat larger than those activated by glycinergic inhibition in the spinal cord (Eccles, 1964), being large enough to allow the passage of acetate and butyrate—as confirmed in recent single channel studies (Bormann, Hamill, and Sakmann, 1987).

In contrast to spinal neurons (Werman, Davidoff, and Aprison, 1968), most cortical neurons showed little or no sensitivity to glycine (Kelly and Krnjević, 1969; Krnjević and Phillis, 1963). There was minimal conductance increase, and the predominant potential change was in the depolarizing direction—which may be related to more recent findings that glycine can depress K^+ currents (Biscoe, Duchen, and Patterson, 1987) and sensitizes N-methyl D-aspartate (NMDA) receptors (Johnson and Ascher, 1987). Glycinergic inhibition was thus unlikely to be a prominent feature of cortical function; though one could not, of course, exclude the possibility that a small proportion of cortical inhibitory interneurons may release glycine (cf. Daly, 1987, this issue). No other naturally-occurring agent—including the much discussed catecholamines, serotonin, 5HT and ACh, as well as various peptides—has so far proved to be a strong, fast-acting inhibitory transmitter in the cortex. But again, one or more of these putative transmitters may well have a significant but more discrete inhibitory or modulatory function at certain sites.

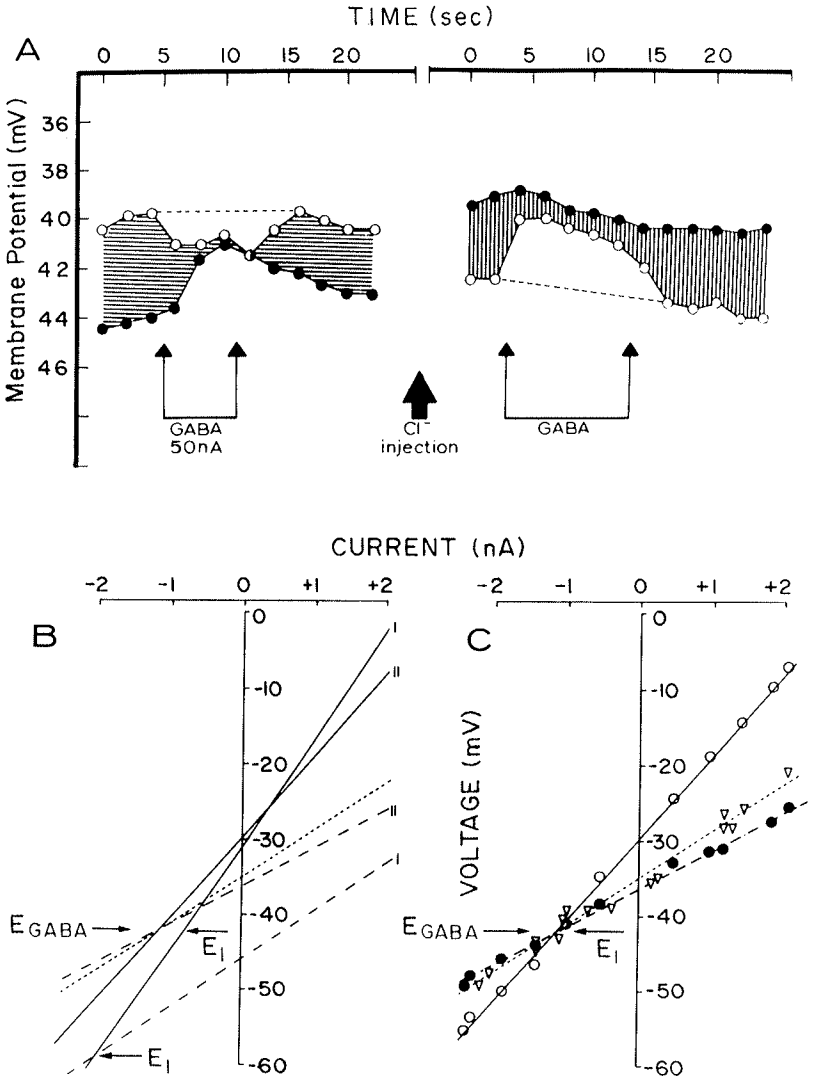

Figure 2: GABA and inhibition produce similar changes in membrane potential and resistance of cortical neurons. (A) Effect of GABA and IPSP are both reversed by intracellular injection of Cl^-. Open circles—resting potential, closed circles—potential at a peak of IPSP (Figure 3 from Krnjević and Schwartz, 1968). Reprinted with permission from Pergamon Press, Elmsford, New York, © 1968. (B) Voltage-current lines obtained before (I), during and after (II) administration of GABA to another neuron. Solid lines in (B) are for resting membrane, dashes at peak of IPSP, dots during action of GABA; reversal levels are indicated by arrows; (C) actual experimental points through which line II and GABA line were drawn (from Figure 6 in Dreifuss et al., 1969). Reprinted with permission of Springer-Verlag New York Inc., Secaucus, New Jersey.

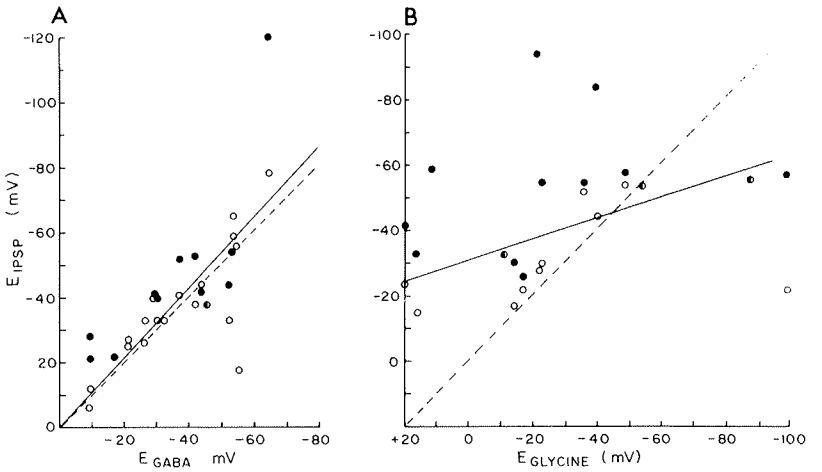

Figure 3: (A) Similarity of reversal potentials for IPSPs and for effect of GABA, shown by 18 sets of measurements on 13 different pericruciate neurons. Closed and open circles are values obtained before and after each application of GABA. Solid line indicates best fit for all the points; dashed line is the line of perfect agreement (Figure 8 from Dreifuss et al., 1969). Reprinted with permission of Springer-Verlag New York Inc., Secaucus, New Jersey. (B) Corresponding plot of reversal potentials for effect of glycine and for IPSPs show very poor agreement. Results of 14 tests in 12 neurones; open circles indicate values before application of glycine, closed circles after (from Figure 4 in Kelly and Krnjević, 1969). Reprinted with permission of Springer-Verlag New York Inc., Secaucus, New Jersey.

GABA-A and GABA-B Actions

Selective pharmacological antagonists can be very useful for the identification of the transmitter(s) operating at a given synapse. The search for a suitable antagonist of GABA led to a controversy over the usefulness of bicuculline for this purpose. This alkaloid was proposed by Curtis, Duggan, Felix, and Johnston (1970) as an effective antagonist that would distinguish between GABA and glycine receptors. Therefore it could be expected to be as useful a specific anti-inhibitory agent in the cortex as strychnine in the spinal cord.

When tested in the cortex, however, bicuculline was only a poor blocker of the typical prolonged inhibitions (Curtis and Felix, 1971; Godfraind, Krnjević, and Pumain, 1970) (see Figure 4). And yet bicuculline proved to be a highly effective tool for demonstrating local inhibitory actions evoked by sensory (visual) stimulation (e.g., Sillito, 1984; see below). This paradoxical situation may now be explained by the subsequent discovery of the existence of two varieties of GABA receptors: only one, the GABA-A type, is bicuculline-sensitive, the second, the GABA-B receptors of Bowery et al. (1980), are quite

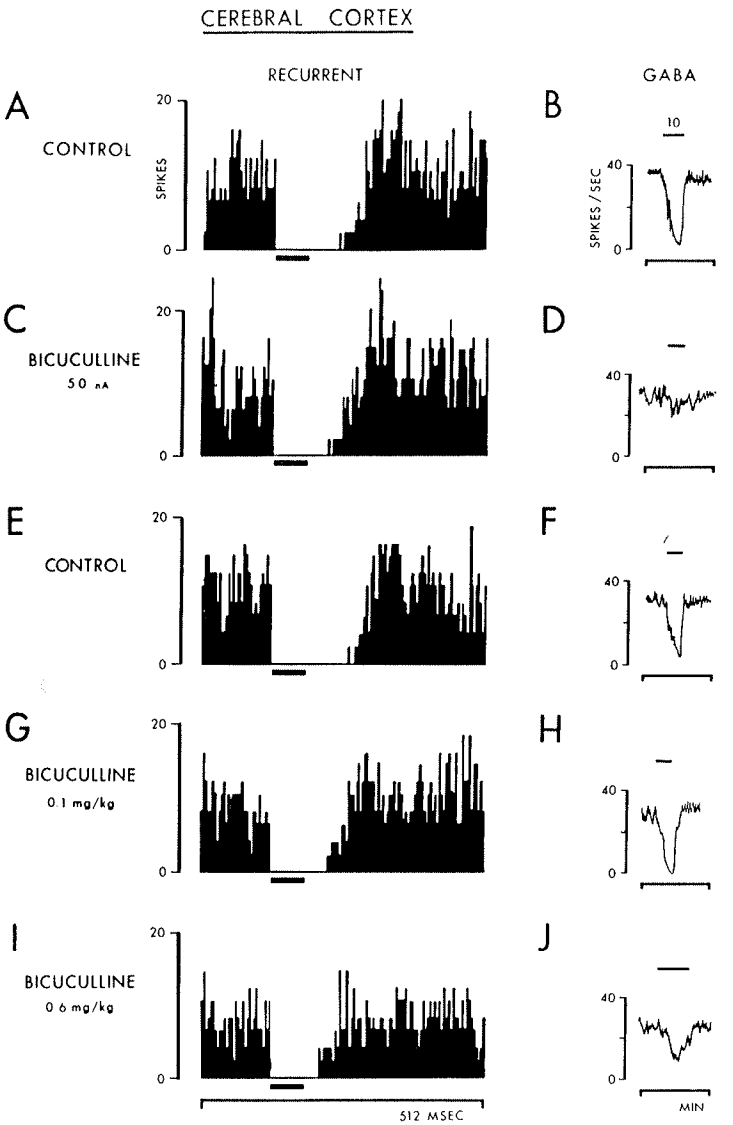

Figure 4: Effect of bicuculline on recurrent and GABA-induced inhibition of pyramidal tract neuron. Cell firing was maintained throughout with DL-homocysteate. Bicuculline was applied iontophoretically in (C) and by systemic injection in (G) and (I). Note small reduction of inhibition, in contrast with marked depression of inhibitory effect of GABA, seen especially in trace (D) and to a lesser extent in (J) [from Figure 5 in Curtis and Felix, 1971]. Reprinted with permission of Elsevier Science Publications B.V., Amsterdam, The Netherlands.

insensitive to bicuculline or picrotoxin—the other GABA-antagonist that also seemed to have little anti-inhibitory potency in the cortex (Krnjević et al., 1964).

According to a detailed investigation on hippocampal neurons (Newberry and Nicoll, 1985), the GABA-B mediated inhibition activates K^+ channels (apparently the same K^+ channels can be opened by serotonin through quite distinct but also G-protein-dependent receptors) [see also Malenka, Andrade, and Nicoll, 1987, this issue]. These GABA-B receptors—which in the hippocampus are situated mainly on dendrites—are probably responsible for the later and very prolonged bicuculline-insensitive component of IPSPs. Evidence for two distinct components of *neocortical* IPSPs, one early, relatively brief and strongly Cl^- dependent, followed by a second much longer and Cl^- independent one, though less compelling (Avoli, 1986; Connors and Malenka, 1985), is nevertheless quite suggestive (see Figure 5).

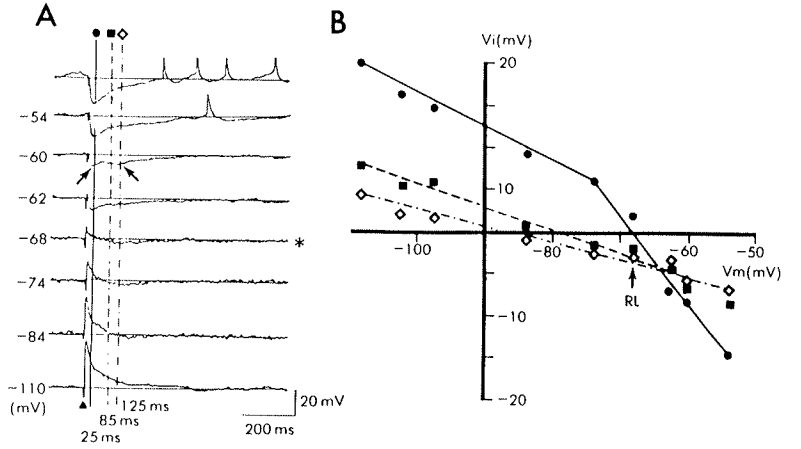

Figure 5: Double component of IPSPs in neocortical neuron (slice from rat sensory motor cortex). (A) Responses to white matter stimulation show EPSP followed by hyperpolarization, peaking at 125 ms when recording at resting potential (*). Hyperpolarization becomes larger and biphasic as neuron is depolarized (arrows at -60). (B) Plot of membrane potential during IPSP at 3 different latencies (as indicated). There were two different slopes and two different reversal levels, for early and late hyperpolarization respectively (parts of Figure 1 in Avoli, 1986). Reprinted with permission of Elsevier Science Publications B.V., Amsterdam, The Netherlands.

Role of GABAergic Inhibition in Normal ("Physiological") Cortical Function

A global role for cortical GABAergic inhibition can easily be demonstrated. Any gross interference with GABAergic inhibition, for example, by drugs which either prevent the release of GABA or block its action, has a powerful

convulsant effect. This is fully consistent with the presence of large numbers of GABAergic cells and synaptic terminals throughout the cortex (Jones, 1987, this issue). They provide the substratum for a multitude of feed-forward and feedback inhibitory pathways that operate in both a tonic and a phasic manner on practically all cortical neurons. There is now much evidence that a relative loss of GABAergic inhibitory interneurons is a very general feature of epileptic foci (Ribak, 1987, this issue; Ribak, Bradburne, and Harris, 1982). This may well be the primary defect also in human focal epilepsy, initiated by local ischemic episodes to which for some unknown reasons inhibitory interneurons appear particularly sensitive.

GABAergic inhibition plays a much more subtle role in determining the functional characteristics of the responses of cortical cells to sensory stimulation. This was first demonstrated in a remarkable series of experiments on the visual cortex by an imaginative use of bicuculline (Sillito, 1984). Both the directional and orientational selectivity of simple or complex visual neurons may be largely dictated by local inhibitory, GABAergic connections. Similarly, ocular dominance may be achieved mainly by GABAergic suppression of visual information arising from one eye (see Figure 6).

Comparable GABAergic circuits most likely operate in a similar integrative fashion in other sensory receiving areas. Recent experiments by Dykes, Landry, Metherate, and Hicks (1984) have shown that bicuculline applications in the somatosensory cortex can greatly increase the size of the peripheral receptive field of a given unit—particularly in the case of fast-adapting units (see Figure 6B)—without changing the temporal characteristics of the responses. Clearly, the fast-adapting characteristic of these units is generated by a non-bicuculline sensitive mechanism, such as increased K conductance.

Other examples of non-bicuculline-sensitive firing properties of cortical "sensory" neurons are well-known (DeBruyn and Bonds, 1986; Sillito, 1984). Since GABA-B receptors are likely to be present in the neocortex, however, it is premature to ascribe such phenomena to non-GABAergic inhibitory synaptic actions. In the absence of any useful pharmacological anti-agonist of GABA-B receptors this possibility cannot be easily excluded.

Conclusions

Though no longer a "new" transmitter, GABA continues to be the focus of numerous investigations. One reason is that GABAergic inhibition appears to be almost universally widespread throughout the CNS (and possibly even in the periphery), judging by the ubiquity of GABA-rich neurons and synapses. Moreover, as a synaptic mechanism, it is exceptionally amenable to detailed and rigorous study; the conductance changes are both large and prolonged, and therefore readily measurable; the metabolism of GABA is well established, and numerous pharmacological tools are available for the manipulation of GABA-

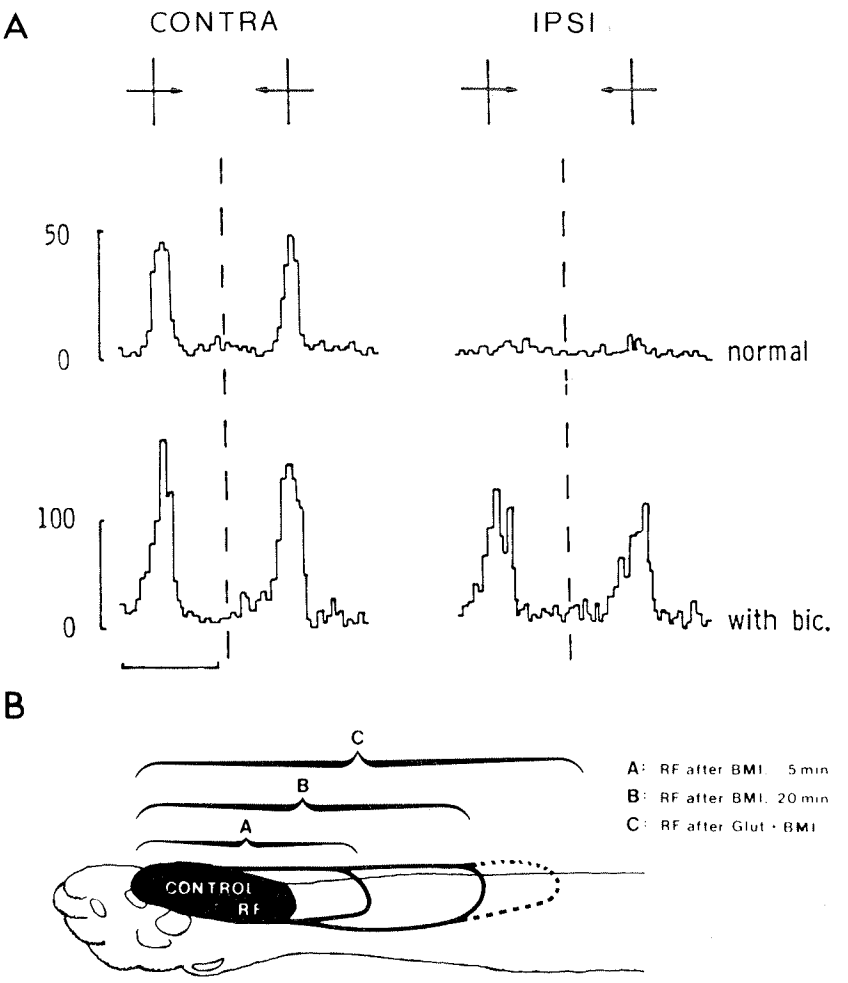

Figure 6: Cortical unit responses to sensory stimulation are determined by GABAergic inhibition. (A) Shift in ocular dominance of visual cortex complex cell during bicuculline application. Records show firing in response to optimally-oriented stimulus moving over receptive field in contralateral eye and over corresponding location in ipsilateral eye. Responses were average over 25 trials. Vertical calibration indicates counts/50-msec bin. Horizontal calibration 1 sec. Vigorous response to ipsilateral eye stimulation appears only when bicuculline was applied (from Figure 8 in Sillito, 1984). Reprinted with permission from Plenum Press, New York, New York. (B) Receptive field (RF) in somatosensory cortex (also of cat) is greatly enlarged after application of bicuculline (BMI) in cortex. Local glutamate application further increases size of field (from Figure 3 in Hicks et al., 1985). Reprinted with permission of Alan R. Liss Inc., New York, New York.

synthesis, release, uptake or action. Not the least intriguing is the multi-faceted GABA-A receptor-Cl^- channel complex, which lends itself to both

subtle and powerful modulation by so many widely used anaesthetics and other psychoactive agents, such as benzodiazepines and ethanol (DeFeudis, 1985). Our present knowledge of GABAergic inhibition and its role in cortical physiology and malfunction has probably no more than scratched the surface of an almost inexhaustible topic.

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