Acetylcholine Facilitation, Atropine Block of Synaptic Excitation of Cortical Neurons

Abstract. *Single neurons in cat visual cortex were driven by afferent stimuli to determine the effects of drugs liberated from multibarreled micropipettes on synaptic transmission. Acetylcholine increased, and atropine decreased, the number of neuron discharges fired in response to stimulation of the mesencephalic reticular formation without necessarily affecting responses to other stimuli or affecting spontaneous firing.*

While there is much evidence to suggest that acetylcholine (ACh) acts as a synaptic transmitter in various parts of the central nervous system, central cholinergic transmission has been established only for the synapse between axon collaterals of spinal motoneurons and Renshaw cells (*1*). This was accomplished with multibarreled microelectrodes which afford expulsion of drug ions from several barrels in the vicinity of a single neuron whose electrical activity is concomitantly recorded through another barrel of the compound electrode. Application of this technique to neurons in cerebral cortex (*2*) and other parts of brain (*3*) has shown that ACh alters the spontaneous firing rate in a substantial portion of neurons, but it is unclear whether this effect is due to drug action upon synaptic sites or upon nonsynaptic structures.

In order to study the effect of ACh upon transmission of synaptic impulses in cerebral cortex more specifically, I applied ACh and atropine to neurons while they were driven by stimulation of different afferent pathways. Because general anesthesia alters neuronal ACh susceptibility (*4*), spontaneous firing, and synaptic responses, the 21 animals used for this investigation were prepared by transection of the upper cervical cord under brief inhalation anesthesia; their condition was maintained, under thorough local anesthesia, by artificial respiration and by monitoring heart beat, blood pressure, expired CO_2, temperature, and occipital electrocorticogram, the latter indicating both the cortical condition near the microelectrode recording site and the level of alertness of the preparation. Stimulating electrodes were inserted stereotactically into the lateral, ventral part of the mesencephalic reticular formation (MRF), into the optic radiation, and into the contralateral homologous cortex. Discharges of neurons in the visual cortex were recorded through a potassium citrate–filled central recording pipette whose tip protruded up to 60 μ beyond the orifices of six to ten surrounding drug barrels which ended at a common level. At this level, the outer diameter measured 3 to 10 μ, each orifice having an inner diameter of about 1 μ (*5*). At least one barrel was filled with sodium chloride solution and was used to distinguish electrical effects of the retaining and expelling electrophoretic currents which ranged between 10^{-9} and 10^{-7} amp.

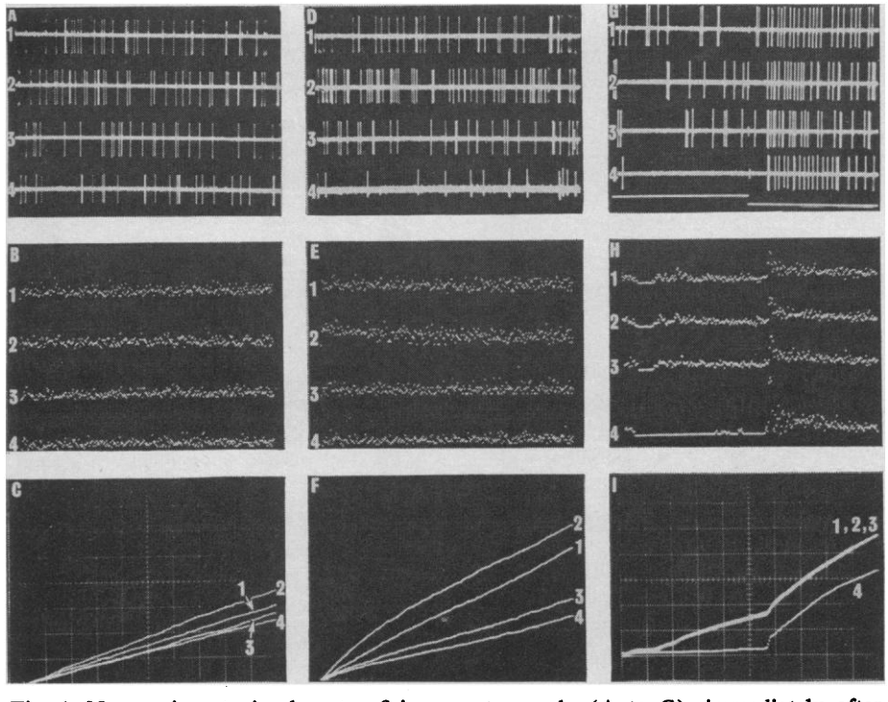

Fig. 1. Neuron in cat visual cortex firing spontaneously (A to C), immediately after 30 stimuli at 300 per second to the mesencephalic reticular formation (D to F), and during intermittent retinal illumination [(G to I) the upward deflection of bottom trace in (G) indicates bright period]; part 1, before drug application; part 2, during acetylcholine electrophoresis with 1×10^{-7} amp; part 3, during atropine electrophoresis with 5×10^{-8} amp; part 4, after intravenous injection of atropine (0.5 mg/kg). (A, D, and G), Playback of original data from magnetic tape; (B, E, and H), histograms of neuron discharges, each histogram representing 64 seconds of firing accumulated in 256 addresses of Fabri-Tek 1060 Instrument Computer; sweep length is 2 seconds for (A to F), 1 second for (G to I), gain is 16 counts per graticule division for (B) and (E), 32 counts per division for (H). (C, F, and I), Integals of histograms at a gain of 256 counts per division. The ordinate of each point represents the total number of counts accumulated to the abscissa of that point. For instance, spontaneous firing before drug application (C, part 1) reaches 3×256 discharges for 64 seconds which indicates a mean firing rate of 12 discharges per second.

Figure 1 was obtained from one neuron firing spontaneously (A to C) and in response to brief tetanic stimulation of the MRF (D to F) and to intermittent retinal illumination (G to I). Because neuronal firing in unanesthetized animals fluctuates considerably, discharges were recorded for prolonged periods. Their distribution was plotted in the form of histograms of firing after the stimulus (E and H) and of histograms of spontaneous firing obtained under otherwise identical conditions (B). Comparison of histograms was facilitated by computation of integrals (C, F, and I), the ordinate of each point on these curves representing the total number of counts accumulated to the abscissa of that point. The spontaneous firing rate of this neuron (A to C, part 1) was mildly increased during electrophoretic application of ACh (A to C, part 2) and mildly decreased during electrophoretic application of atropine (A to C, part 3). A further mild decrease of firing resulted

from the intravenous injection of atropine (0.5 mg/kg) (A to C, part 4). This procedure was used in the last neuron examined in a given cat and only after completion of electrophoretic drug tests. Reticular stimulation before drug application raised the mean neuronal firing rate during the 2 seconds following each stimulus (D to F, part 1) to one and a half times the spontaneous firing rate (A to C, part 1). Acetylcholine increased the poststimulus firing, especially in the first 600 msec after application of the stimulus (D to F, part 2). Atropine electrophoresis reduced poststimulus firing (D to F, part 3) and especially abolished the early increase in firing after reticular stimulation. Atropine applied intravenously reduced the firing after reticular stimulation (D to F, part 4) to the level that was obtained during spontaneous firing after intravenous injection of atropine (A to C, part 4). In other words, systemic administration of atropine completely abolished the activating effect of reticular stimulation. In contrast, local applications of ACh and atropine had no effect upon transmission of the light response in this neuron (G to I, parts 1 to 3). However, injection of atropine intravenously diminished firing during the bright phase of the light stimulus (G to I, part 4).

Acetylcholine facilitation and atropine depression of reticular excitatory action were observed in several neurons, many of them not showing a similar drug effect while firing in response to intermittent retinal illumination, to electrical shocks to the optic radiation, to transcallosal afferents, or while firing spontaneously. Some neurons that did show drug effects under these circumstances also showed, when tested, facilitation of the drug-susceptible activities by a conditioning stimulus to the MRF. In a few neurons, the response to electrical stimulation of the visual path was altered both by reticular stimulation and by drug application, but neither manipulation had an effect upon the flash response. In these instances, showing drug effects upon firing not induced by reticular stimulation, it can be assumed that drugs acted upon excitatory reticular input which then modified neuronal firing under the other conditions. The same mechanism could apply in cases in which reticular stimulation and drugs altered the spontaneous firing rate. Since the contribution of reticular input to the spontaneous firing rate differs from neuron to neuron and varies in a given neuron with time, level of alertness, and so forth, this mechanism would explain why testing of the spontaneous firing rate, especially in animals under general anesthesia, is not likely to reveal the nature of ACh action.

Electrophoretic application of atropine occasionally has a local anesthetic effect on single neurons (*6*). This action was seen in some instances and lasted rarely longer than a few minutes after the end of electrophoresis. It could be distinguished by the fact that it reduced all types of neuronal activity indiscriminately and was not limited to neurons reacting to ACh.

The differential effect of ACh and atropine upon transmission of reticular excitatory impulses, although not excluding the possibility of drug action upon presynaptic terminals of fiber connections from the MRF, is in agreement with the hypothesis that ACh mediates synaptic transmission of reticular excitation to cortical neurons. This hypothesis is supported by evidence accumulated with different methods (*7*). The difference in the effects of electrophoretic and intravenous application of atropine suggests that the latter reaches receptors which are presumably located at a distant link in a chain of afferent neurons and not available to the former.

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25-Hydroxycholecalciferol: Direct Effect on Calcium Transport

Abstract. *The perfused small intestine from a vitamin D deficient rat exhibits about one-half the calcium transport of the intestine from a rat given vitamin D. These levels of calcium transport can be maintained for at least 4 hours. Addition of 2.5 micrograms of 25-hydroxycholecalciferol added to the vitamin D deficient intestine via the arterial blood perfusate induces a rise in calcium transport to +D levels within 2 hours. In contrast, 250 micrograms of vitamin D_3 given in the same manner has no effect on the calcium transport level over a 4-hour period. These data provide strong evidence that 25-hydroxycholecalciferol represents the metabolically active form of vitamin D_3.*

Since the identifications of vitamins D_2 and D_3 by Windaus *et al.* (*1*, *2*) and Askew *et al.* (*3*), it has been assumed that the unaltered vitamin D itself carries out the vitamin's physiologic functions. In 1966 Lund and DeLuca established the existence of a highly biologically active vitamin D metabolite in rats (*4*). This metabolite has recently been isolated and identified as 25-hydroxycholecalciferol (25-HCC) (*5*, *6*) and has been chemically synthesized (*7*). When injected into the jugular vein of a vitamin D deficient rat, 25-HCC is approximately 1.4 times more active than vitamin D in curing rickets (*6*). More importantly, 25-HCC exhibits its in vivo activity in stimulating bone mobilization and intestinal calcium transport more rapidly than an equivalent dose of vitamin D_3 (*8*). This time lag suggests that vitamin D_3 must be converted to 25-HCC before its biological activity is manifested. Studies of early vitamin D metabolism implicated the liver in this conversion (*9*) and recently, by the use of hepatectomized rats, it has been demonstrated that the liver is the major if not the only site of 25-HCC production (*10*). In addition, a liver enzyme system has been obtained which catalyzes the conversion of vitamin D_3 to 25-HCC (*11*).

Direct evidence is now available in