Chemical Transmission and Cortical Arousal

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Because they found that cortical arousal could be elicited with cholinomimetics and blocked by atropine, Funderburk and Case10 suggested that cortical arousal might be mediated by a cholinergic mechanism. At that time, however, the role played by acetylcholine (ACh) in the CNS and the cortex was quite obscure, and there was no evidence concerning the identity of possible cholinergic pathways, or the cells these might innervate.

Although we are still very much in the dark, recent studies have given us more concrete evidence, which shows interesting correlations with problems of arousal and consciousness.

Cholinergic Mechanisms in the Cortex

Most of the recent evidence on transmitter mechanisms in the cortex indicates that ACh is not the principal transmitter concerned in fast synaptic excitation or inhibition. The main excitatory transmitter is likely to be a dicarboxylic amino acid, such as l-glutamate, or a related compound,14,15 while the main inhibitory transmitter is probably \(\gamma\)-aminobutyric acid or a close derivative.18,19 ACh, on the other hand, seems to be associated with relatively slow and prolonged excitatory effects involving only a certain proportion of cortical cells.

*ACh-Sensitive Cells. Direct micro-applications of ACh16 have shown that ACh can excite \(\frac{1}{2}\)–\(\frac{3}{4}\) of cortical neurones, on the average. The sensitive neurones are practically never found in the supragranular cell layers; they are particularly concentrated in layer V, where many can be identified as deep pyramidal cells, including corticospinal neurones.

Action of ACh. The excitation of sensitive cells is typically slow in onset and prolonged, often outlasting the application by 30–60 sec-

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Fig. 1. Two cortico-spinal neurons (cf. large and small spikes) behaving like typical ACh-sensitive units. They were found at depth of 1.1 mm. in the lateral preccuital cortex of a cat, and were identified as cortico-spinal neurons by short-latency antidromic activation from medullary pyramids (A), responding up to frequency of 300/second (B). They were spontaneously active (C), gave early responses to stimulation of contralateral forepaw (D) and afferent relay nucleus in thalamus (E), and also repetitive afterdischarges (F, G). The early and late responses were also elicited trans-callosally (H, I). Both units were excited vigorously by ACh applied from multiharrelled recording micropipette (J).

rather characteristic pattern which corresponds well with the distribution of ACh-sensitive cells. A diffuse cholinergic network appears to be present in the deeper cortical layers, where it closely invests many of the pyramidal cells (fig. 2). Some AChE-containing fibers, which form U-links around sulci and contribute to the deep network, seem to arise from cholinergic neurones lying in the deepest cortical layer. Most of the network, however, is probably made up of ascending fibers with a subcortical origin. Along the lateral and frontal aspect of the hemisphere, these fibers can be traced down to the external capsule and the deeper region of the striatum, while those in the upper and medial regions seem to come from the septum and fornix.

Lesions in the forebrain showed that the various fibers do indeed ascend to the cortex, but it was not possible to establish the exact situation of their cells of origin. The cells could be in the septum or the lenticular nucleus, or they might be further back in the mid-brain reticular formation which sends AChE-containing projections to the forebrain.

The subcortical origin of the “cholinergic” radiation to the cortex was confirmed by an ontogenetic study of the development of AChE-containing fibers. This showed very clearly that the neocortex is free of AChE throughout its early development, whereas the striatal and septal primordia contain AChE from the earliest stages onward. AChE-containing fibers from these areas grow progressively into the pallium, and invade the cortex at a relatively late stage.

Release of ACh. MacIntosh and Oborin had noticed a general correlation between release of ACh from the cortex and its electrical activity; release was much reduced as the depth of anesthesia increased. These observations were confirmed by Mitchell, while subsequently, Kanai and Szentberg found that stimulation of the midbrain reticular formation, which caused cortical electrographic arousal, also increased the liberation of ACh. More recent studies by Collier and Mitchell on unanesthetized, freely moving rabbits have revealed a striking correlation between behavior and the rate of release of ACh. According to these authors, stimulation of the lateral geniculate body increases liberation of ACh from the visual cortex by two apparently different mechanisms: the first, which is diffuse and affects both hemispheres, they ascribe to indirect activation of a reticular ascending system; the second, more specific and acting principally on the ipsilateral visual cortex, they believe is the pathway responsible for repetitive and augmenting responses.

From these various kinds of evidence, it appears that the deeper and older layers of the cortex receive a cholinergic innervation originating from the basal and relatively ancient portions of the forebrain. This innervation may be the forebrain extension of a cholinergic reticular system present throughout much of the brain-stem. Via this system of fibers, the older parts of the brain may exert some control over the cerebral cortex.

This ascending pathway could be brought into action by strong afferent volleys, through
afferent collaterals supplying the mid-brain reticular formation, and also by various descending pathways. The effect of such reticular activation would be distributed very widely over the hemispheres. On the other hand, specific thalamic nuclei may be linked selectively to certain groups of ascending cholinergic fibers, via thalamo- striatal and thalamo- septal connexions. It is thus possible that the same ascending cholinergic system accounts for generalized arousal and for more localized repetitive or augmenting responses.

Cholinergic Innervation and Its Relation to Arousal and Consciousness

Arousal. Most of the recent evidence is consistent with the original hypothesis of Funderburk and Case, and later authors. A continuous, possibly multisynaptic system of cholinergic fibers does seem to extend from the level of the mid-brain to the cortex, passing through the striatum and the septum. These fibers provide a diffuse innervation for the neurones in the deeper cortical layers. The excitability of these cells would thus depend upon the rate of release of ACh by the ascending fibers. Desynchronization of the EEG in arousal could result from a marked increase in random spontaneous activity, which would prevent the synchronized activation of cortical cells by thalamic volleys. The rhythmic thalamic activity may itself be influenced by similar ascending cholinergic pathways.
and also by the change in cortico-thalamic discharges.27

It is well known that behavioral arousal and cortical electrographic arousal can be dissociated pharmacologically 4, 10, 26; for instance, esterine can elicit cortical arousal without greatly altering the behavior of an animal. A comparable dissociation can also be induced by appropriate lesions in the brain stem.9 These observations suggest that other areas of the brain also play a vital role in the mechanism of sleep and arousal and that if cholinergic pathways are also involved,12 they may have different pharmacological properties resembling many ACh-sensitive cells in various subcortical regions.2, 3, 25

Conscious Experience. A cortical cholinergic innervation may affect the state of awareness in a very specific way. In recent experiments, Libet et al.23 examined in detail the parameters of stimulation needed to evoke conscious sensation in human subjects during direct electrical stimulation of the exposed cortex. Their most interesting finding was that single pulses were inefficient, and that even repetitive volleys had to last 0.5–1 second to be effective, except when very intense shocks were applied. On the basis of these observations, Libet 22 has suggested that cortical activity must persist for a certain minimum period, of the order of 0.5 second, before it is experienced consciously. More transient activity would remain at a subconscious level.

It is significant, therefore, that ACh is so effective in prolonging the discharges of cortical cells. One of its most characteristic actions is to enhance repetitive responses. If the cortical cells stimulated in the experiments of Libet et al.22 include ACh-sensitive cells, the release of ACh by the postulated cholinergic innervation could determine the degree of awareness of the subject.

Anesthesia. In deep anesthesia, both the spontaneous activity and the evoked discharges of ACh-sensitive cells are much reduced. Two factors may be responsible: a reduction of the sensitivity of the cells to ACh; 16 and a fall in ACh release,25, 56 causing a direct depression of the cholinergic nerve endings or diminished activity in the ascending system. The relevant cortical cells would therefore be much less readily excited by different volleys, and their responses would become much briefer. The last effect might be sufficient to eliminate consciousness.

References


DISCUSSION

Dr. Zanchetti: In support of the interesting data of Dr. Krnjević, may I recall some data by Dr. Mantegazzini and Pepeu (Mantegazzini, P., and Pepeu, G.: Increase of cortical acetylcholine induced by mid-brain hemisec- tion in the cat. J. Physiol. 173: 20, 1964). They performed unilateral transection of the midbrain in cats previously subjected to a complete transection at the midpontine level; a peculiar preparation because in the hemisphere which is ipsilateral to the hemi- transection, the EEG is mostly synchronized, as during sleep, while in the contralateral hemisphere the EEG is mostly desynchronized. So far as I can recall, they found that the content of acetylcholine was considerably higher in the synchro- nized hemisphere than in the desynchronized one. As to the point made by Dr. Krnjević that many cholinergic fibers seem to originate from the lenticular nucleus, the classic data by Nauta and Kuipers (Nauta, W. J. H. and Kuipers, H. G. J. M.: Some ascending pathways in the brain stem reticular formation. In: Reticular Formation of the Brain, London, J. and A. Churchill, Ltd., 1957, pp. 3-30) might be recalled. They observed that reticular neurons originated in the mid-brain projection to the striatum, including both divisions of the lenticular nucleus.

Dr. Krnjević: I did not mention the work of Pepeu and Mantegazzini (Science 145: 1069, 1964) because of limitations of time. They measured particularly the amount of acetylcholine in

the cortex and it was closely related to the degree of arousal. Kanai and Szerb (Nature 205: 81, 1965) have done similar experiments, and showed that when they stimulated the mid-brain reticular formation sufficiently to produce cortical electro- graphical arousal, they found a marked increase in acetylcholine release from the cortex.

Dr. Brown: The delayed response from your cholinceptive cortical neurons might be coming from the late EPSP like the one seen in sympa- thetic ganglion cells which is also blocked by atropine; this late EPSP is best brought out by repetitive stimulation rather than single stimula- tions. Have you tried this in your thalamo-cortical projection paths? If so, you might have greater success in picking up more cholinceptive neurons.

Dr. Krnjević: We did not try this when stimulating the thalamus. We tried only repetitive stimulations while stimulating further down in the mid- brain.

Dr. Brown: What sort of frequency?

Dr. Krnjević: Anywhere between 2 and 10 per second; that is the sort of frequency that brings in augmenting responses, appearing characteristically about at five a second.

Dr. Fink: Was I correct in thinking I saw cholinergic fibers on the surface of those sections of the cortex?

Dr. Krnjević: Yes, I have always been rather puzzled by these. But I gather from Professor Szentagothy that the cells in the deepest layer of
the cortex, layer 6, send collaterals to the surface which travel along layer 1. This ties in rather well with our evidence, because my impression was that the fibers which give the U-shape connections around sulci, probably originate from the same kind of cells; therefore, these same cells could give rise both to the arching U-fibers and to the ascending collaterals which end as horizontal fibers in layer 1.

Dr. Fink: When such fibers are activated is it possible to detect acetylcholine in cerebro-spinal fluid?

Dr. Krnjević: That hasn't been done systematically. This would have to be done with a completely isolated cortex, to be sure one was not getting reflex excitation by impulses passing down to the thalamus and back again.

Dr. Salmoiraghi: Have you stimulated the striatum and studied the pharmacology of this response?

Dr. Krnjević: We have tried stimulating the reticular formation but unfortunately got only rather delayed responses. These are perfectly consistent, with a direct connection to the cortex, but the long latency does not allow one to say that this must be a direct connection. It could be that, when we stimulate the striatum we get excitation back via the reticular formation, which might return via a completely different pathway. It is unfortunate that these effects are slow and therefore one cannot use the latency as a good criterion of how direct the connections are.

Dr. Salmoiraghi: Would it not be possible to transect the brain in such a way as to allow the study of these problems?

Dr. Krnjević: This would be difficult because it is such a diffuse projection over a wide area, that one would have to do a great deal of surgery to secure adequate separation. It is certainly worth looking into.