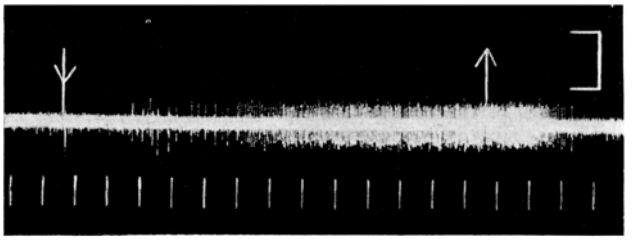
Sensitivity of Cortical Neurones to Acetylcholine

In spite of many claims for the existence of cholinergic transmission between neurones in the cerebral cortex[1,2], the direct stimulation of cortical cells by acetylcholine has never been demonstrated conclusively.

We have used compound micropipettes[3] to study the sensitivity of cortical neurones to several agents. The pipettes had 5 barrels: one, filled with 2.7 *M* NaCl, recorded the extracellular electrical activity of single units; the four others contained strong solutions of acetylcholine and other substances, which could be released in the immediate vicinity of the neurones by iontophoresis. The tips of the pipettes had a diameter of 5–10 μ and they were easily inserted into the cortex after removal of the pial layer.

We have now examined 1367 cells in various cortical areas in 13 cats anaesthetized with Dial or Chloralose. All cells were excited by *L*-glutamate; this action and any spontaneous activity were effectively blocked by γ-aminobutyric acid. Of the total, 200 cells were found to be sensitive to acetylcholine, judging by either a clear increase in the frequency of spontaneous activity, or the firing of quiescent cells. To obtain an effect, a current of 0.05–0.1 μA was passed through the acetylcholine barrel for a minimum of between 2 and 20 sec. When the acetylcholine current ceased, the heightened activity persisted for periods which varied between 5 and 60 sec. By passing a similar (or even much stronger) outward current through another barrel containing e.g. Na glutamate, it was easy to show that the observed excitation cannot be ascribed to the electrotonic effects of the flow of current.



Acetylcholine sensitive unit 1.2 mm deep in anterior part of lateral gyrus. Acetylcholine application (outward current of 0.1 μA) begun at 1st arrow and ended at 2nd arrow. Time pips are at 1 sec intervals and voltage calibration shows 0.2 mV.

Acetylcholine sensitive neurones were also excited, though to a lesser extent, by carbamylcholine, propionylcholine and succinylcholine, but not butyrylcholine. They were affected by neostigmine and edrophonium applied in a similar manner; both potentiated the action of acetylcholine, or even excited the cells. On the other hand, tubocurarine could not be shown to have any blocking action. The only effect observed in some cases was a delayed but very pronounced excitation. Nicotine had a similar exciting action, which bore no relation to the acetylcholine sensitivity. Atropine and hyoscine had a non-specific depressant effect on nearly all the cortical neurones to which they were applied (spinal cord neurones[4]). So far, only with gallamine have we been able to produce a selective block of acetylcholine.

Acetylcholine sensitive neurones tended to occur in clusters, and they very often showed spontaneous activity related to slow waves of the electrocorticogram. Although we have found a few sensitive neurones in most areas, they were distributed mainly in the primary somatosensory, visual and auditory receiving areas; the greatest concentrations were seen in the primary visual area of some of the cats.

Resumé. Sur 1367 neurones de l'écorce cérébrale du chat, examinés directement par la méthode iontophorétique, il a été possible d'en stimuler 200 par l'acétylcholine. Tous les neurones sont fortement excités par le *L*-glutamate et inhibés par l'acide γ-aminobutyrique.

K. Krnjević and J. W. Phillis

ARC Institute of Animal Physiology, Babraham (Cambridge, England), June 21, 1961.

[1] W. Feldberg, Physiol. Rev. *25*, 596 (1945).
[2] W. Feldberg, in *Metabolism of the Nervous System* (Ed. Richter, Pergamon Press, London 1957), p. 493.
[3] D. R. Curtis and R. M. Eccles, J. Physiol. *141*, 435 (1958).
[4] D. R. Curtis and J. W. Phillis, J. Physiol. *153*, 17 (1960).

New Central Stimulants

In the course of an investigation of the synthesis of new central stimulants, a series of 2-piperidyl-(1)- and morpholinyl(4)-3-substituted phenylpropionic acid esters and the corresponding propanols were synthesized. The compounds synthesized in this connection include: methyl 2-piperidyl(1)-3-phenylpropionate (CN1), 2-piperidyl(1)-3-phenylpropanol (CN2), methyl 2-morpholinyl(4)-3-phenylpropionate (CN3), 2-morpholinyl(4)-3-phenylpropanol (CN4), methyl 2-piperidyl(1)-3-(*p*-methoxyphenyl)-propionate (CN5), 2-piperidyl(1)-3-(*p*-methoxyphenyl)-propanol (CN6).

During a screening study with mice for central stimulant activity (spontaneous activity), these compounds showed the following order of decreasing potency: CN2, CN6, CN4, CN5, CN3, CN1. As CN2 was found to be most active, it was studied further for its central stimulant activity in different species of animals with the following results:

(1) 50–60 mg/kg injected intraperitoneally (i.p.) in mice produced marked increase in spontaneous activity. At 70, 80, and 100 mg/kg, the animals became extremely active. Normally docile animals could not be controlled in 12″ high cages unless a lid was put on. Clonic flexor convulsions occurred at all these dose levels. CD 50 was between 70–80 mg/kg and LD 50 around 150 mg/kg.

(2) In rats 30–40 mg/kg given i.p. caused increase in spontaneous activity within 5 min. After 100 mg/kg the response of the animals to any disturbance by auditory or visual stimuli was exaggerated. The effects seemed to disappear in 60–90 min with no signs of depression. However, at higher doses such as 150 mg/kg the animals developed flexor clonic convulsions and the surviving animals showed decreased spontaneous activity.

(3) 30–40 mg/kg given i.p. to dogs produced increased alertness and acute awareness of slightest disturbance in the surroundings. At 50 mg/kg this effect was followed by a furious struggle to get released and loss of interest in the

surroundings. In 12 min flexor clonic convulsion appeared. The effect began to decrease in 25–30 min and excitement was over in 25–30 min.

(4) In anaesthetised cats and dogs differential action towards pressor amines was noticed. The pressor effect of 5 μg adrenaline and nor-adrenaline was increased, while the effect of 2–4 mg of amphetamine and ephedrine was reduced or blocked by 20 mg/kg intravenously in dogs and 30 mg/kg in cats.

Detailed studies of compounds in these series will be communicated in due course.

Zusammenfassung. Mit 2-Piperidyl(1)-3-phenylpropanol (CN_2) wird an Mäusen, Ratten und Hunden eine deutliche zentralstimulierende Wirkung festgestellt. An anästhesierten Katzen und Hunden blockiert CN_2 die vasopressive Wirkung des Amphetamins und Ephedrins und verstärkt diejenige der Catecholamine.

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Central Drug Research Institute, Lucknow (India), March 30, 1961.

The Infundibular Recess in the Brain of *Camelus dromedarius* with Particular Reference to its Neurosecretory Pathways into the Third Ventricle

It is thought that the neurosecretion plays a part in the control of the organism's fluid balance. Since ancient times, the camel is particularly renowned for its singular water economy. It is able to drink only once a week; on the other hand, it is able to drink water of such high salinity that man, for instance, would be poisoned by it[1].

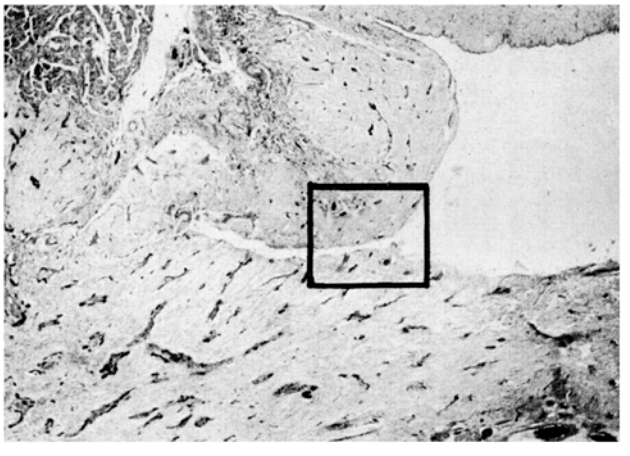

Fig. 1. Part of the floor of the infundibular recess and the bay extending caudally from it. – Haematoxylin-eosin; ×16.

The present work was undertaken in order to see if the peculiar water economy of the camel might be based on some morphological peculiarity in its hypothalamo-hypophysial system. Conditions distinct from those in other animals were found specially in the infundibular recess.

The material consisted of three brains, which were fixed in 10% formalin, embedded in paraffin, and sections were made sagittally at 5–7-micra. Staining was done with haematoxylin-eosin, Heidenhain's iron-haematoxylin, Gomori's chrome-haematoxylin and aldehyde-fuchsin.

The ventral part of the third ventricle formed a distinct infundibular recess. The posterior part of the recess was joined to a long, narrow bay running caudally, produced as a result of the forward pressure exerted on the caudal wall of the infundibulum by the pars tuberalis of the hypophysis.

The ependyma of the floor of the recess, and in the said bay, consisted of flat cells. The ependymal cell layer adjoined to a subependymal tissue with abundant quantities of hyaline mass visible in the haematoxylin-stained preparations, occurring in places in large drops. The mutual connection between the ependymal cells was broken in numerous places, where hyaline material was then seen also in the cell interstices. This made the margin of the ventricle hard to define. In certain places, hyaline material occurred also on the surface of the ependyma in the lumen of the ventricle. The hyaline material was particularly abundant around the subependymal vessels. Such

[1] Editorial, Modern Medicine *28*, 14 (1960).

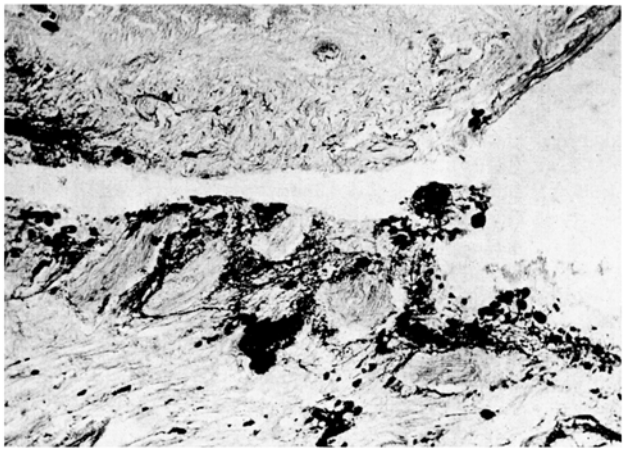

Fig. 2. The area of the rectangle in Figure 1. Neurosecretory material in great profusion in the ependymal barrier, subependymally and around the subependymal vessels. – Aldehyde-fuchsin; ×100.

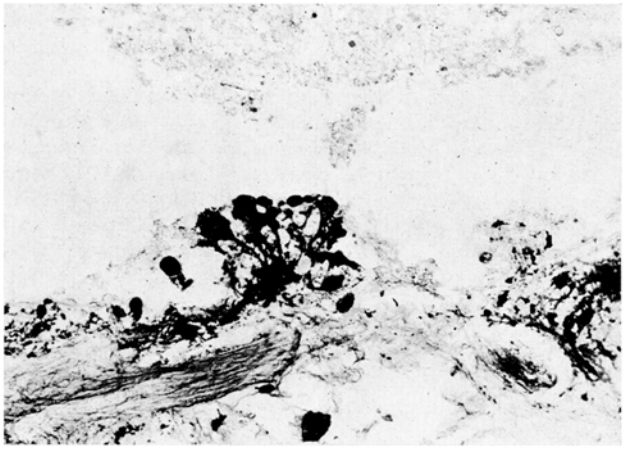

Fig. 3. Neurosecretory material around a subependymal vessel and extending as protuberances into the third ventricle. – Aldehyde-fuchsin; ×200.