ACETYLCHOLINE-SENSITIVITY OF THALAMIC NEURONES: ITS RELATIONSHIP TO SYNAPTIC TRANSMISSION

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Thalamo-cortical relay (TCR) neurones in the ventro-basal complex (VBC) of the thalamus are excited by iontophoretically applied acetylcholine, and it has been suggested that acetylcholine may be involved in synaptic transmission in the thalamus (Andersen & Curtis, 1964a, b).

Such a conclusion would be consistent with the presence of acetylcholine (MacIntosh, 1941), choline acetyltransferase (Hebb & Silver, 1956) and acetylcholinesterase (Phillis, Tebēcis & York, 1967) in the feline thalamus. The synaptic excitation of thalamic neurones by afferent volleys in cutaneous nerves was, however, unaffected by iontophoretically or intravenously administered atropine or dihydro-β-erythroidine (DHE) which readily reduced the sensitivity of thalamic neurones to acetylcholine (Andersen & Curtis, 1964b). This finding indicates that the medical lemniscal pathway to the thalamus is non-cholinergic.

Intra-carotid administration of atropine has been shown to depress the focal potentials evoked in the ventro-lateral (VL) nucleus by brachium conjunctivum (BC) stimulation (Frigyesi & Purpura, 1966). Because the enzymatic systems for acetylcholine production and destruction are present in this pathway (Feldberg & Vogt, 1948; Hebb & Silver, 1956; Austin & Phillis, 1965; Phillis, 1965a, b), the possibility arises that this projection may be cholinergic in nature.

Cholinergic pathways from the brain stem reticular formation (RF) to the thalamus have been postulated as a result of studies on the distribution of acetylcholinesterase in normal and operated rats (Shute & Lewis, 1963). There is also pharmacological evidence for the existence of a similar cholinergic projection from the RF to the lateral geniculate nucleus (Phillis *et al.*, 1967).

The present report is concerned with the distribution of acetylcholine-sensitive cells in the feline thalamus and provides further evidence to support the postulates that cerebellar and reticular projections to neurones in the VBC of the thalamus have a cholinergic component. Pharmacological investigations into the nature of the acetylcholine-receptors on thalamic neurones will be described in a subsequent paper (McCance, Phillis, Tebēcis & Westerman, 1968). A preliminary report on this investigation has been published (McCance, Phillis & Westerman, 1966).

METHODS

Experiments were performed on forty-eight adult cats. During the first twenty-two experiments, animals were anaesthetized with pentobarbitone sodium (Sagatal, May & Baker). Subsequently a gas anaesthetic unit (Commonwealth Industrial Gases, Midget Mk. 2) was used. After induction with intravenous thiopentone sodium (Intraval, May & Baker), anaesthesia was maintained with nitrous oxide and halothane (Fluothane, I.C.I.) or methoxyflurane (Penthrane, Abbott) administered through an intra-tracheal cannula.

The head of each animal was fixed in a stereotaxic frame and the abdomen rested on an automatically controlled heating pad which maintained the temperature of the preparation at $37° \pm 0.5°$ C (Krnjević & Mitchell, 1961). After removal of the cranial vault, cortical and sub-cortical tissue of the left cerebral hemisphere overlying the thalamus was excised by suction to expose the hippocampal fornix and fimbria and the floor of the fourth ventricle between stereotaxic co-ordinates A7 to A12 and midline to L9. The rostral border of the cortical ablation was restricted to avoid excessive damage to thalamo-cortical fibres to the somato-sensory areas. The hippocampus, fornix and fimbria were left *in situ* to minimize damage to the vascular supply to dorsal thalamic nuclei.

Two glass micropipettes were inserted stereotactically at L12 to a depth of 20 mm below the surface of the brain, cut at a point 2–3 mm above the brain surface and left in position. These acted as reference points for the alignment of new electrodes and were useful markers for the orientation of blocks of tissue when brains were being prepared for histology.

Regions stimulated in the course of this investigation were the pericruciate cortex, forelimb nerves, BC and RF. Not all were employed in each experiment. Stimulation was arranged for each of these regions, respectively, as described below.

After exposure of the left somato-sensory cortex, five bipolar concentric stimulating electrodes were inserted into the pericruciate area. These were adjusted so that their tips were located about 4 mm below the cortical surface and used to stimulate the terminal portions of thalamo-cortical fibres in order to activate antidromically neurones in the VBC of the ipsilateral thalamus. The exposed cortical surface was then covered with a 2–3 mm thick layer of 4% agar in physiological saline.

Forelimb nerves were exposed and mounted on bipolar stimulating electrodes in a paraffin pool assembled from skin flaps. A bipolar concentric stimulating electrode was placed on the ipsilateral BC under direct vision after removal of the overlying cerebellum by suction. Another bipolar concentric electrode was placed in the mesencephalic reticular formation at stereotaxic co-ordinates A3, L3, D–1.

Five- and nine-barrelled electrodes were filled either by boiling in distilled water and then replacing the water with aqueous solutions of the drugs to be tested or by loading solutions of the drugs directly into the electrode and filling the tips by centrifugation (3,000 rev/min for 5 min).

Electrical activity was recorded through a 2M NaCl filled barrel of the micropipette, amplified by a negative capacitance probe (Bioelectric Instruments) and Tektronix 122 pre-amplifier and displayed on a Tektronix 565 C.R.O. The independent time base facilities incorporated in this C.R.O. made it possible to record continuously on moving film from one beam (in a stationary position) while the other trace, triggered intermittently by a Grass S8 stimulator, displayed evoked responses. The output of the pre-amplifier was also connected in parallel to a second C.R.O., two spike intensifiers (Kellet, Phillis & Veale, 1965) and an audio amplifier. The second oscilloscope was used to monitor the output pulses of one of the spike intensifiers which was connected to an electronic counter (Hewlett Packard 5214 L). This spike intensifier operated as a variable voltage gate and pulse generator, enabling the rejection of all signals below a predetermined amplitude. The counter was used with gate times of 0.1–1 sec, and its output was displayed by an ink-recorder (Texas Instruments Recti-Riter) using the analogue output coupling stage of a Hewlett Packard 562A digital recorder.

At the termination of experiments, animals were perfused with a 0.9% solution of saline followed by 10% formol saline. After further fixation, serial sections (50 μ) of the thalamus were cut on a

freezing microtome and stained with Luxol fast blue and neutral red. Photomicrographs of whole sections were taken with a Nikon 6C shadowgraph. The position of the RF stimulating electrode was also verified in serial sections.

<div align="center">RESULTS</div>

Distribution and identification of acetylcholine-excited neurones

(a) *Histological correlations.* In a survey of the distribution of acetylcholine-sensitive cells, 1,376 neurones in various thalamic nuclei were tested. These neurones were found more or less at random, being detectable either because they were discharging spontaneously or in response to stimulation of forelimb nerves, or because they were activated by L-glutamate released from the micropipette. The twenty-two cats used in this survey were anaesthetized with pentobarbitone sodium.

Sensitivity to acetylcholine was tested by passing currents of from 10 nA to 120 nA through the acetylcholine-containing barrel for periods of up to 1 min. The initial application was made with a small current, and if the cell failed to respond this was increased during subsequent applications. The location of cells tested in this manner was established by making small "acid-lesions" (McCance & Phillis, 1965) at recording sites and identifying the position of such lesions during a histological examination of 50 μ thalamic sections. An example of four electrode tracks with eighteen lesions is
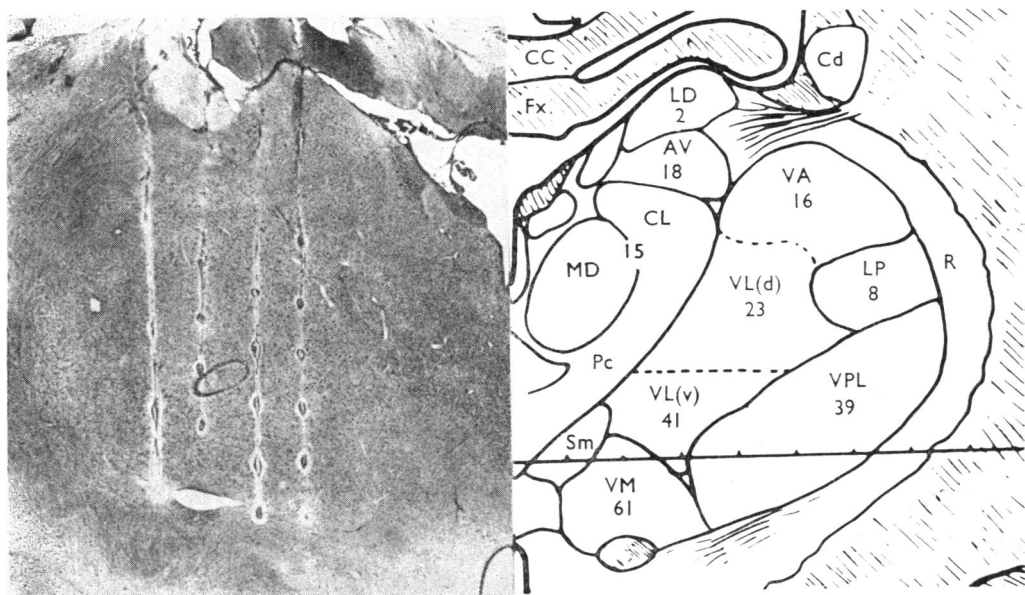

Fig. 1. Left: Photomicrograph of transverse section of thalamus at A 10.5 stained with Luxol fast blue and neutral red. Four parallel electrode tracks with numerous lesions are visible. Right: A 10.5 (diagram and abbreviations after Jasper & Ajmone-Marson (1954)). Numbers represent percentages of cells excited by acetylcholine in various thalamic nuclei. Data from N. centralis lateralis (CL) and N. medialis dorsalis (MD) and also from N. ventralis postero-medialis (VPM) and N. ventralis medialis (VM) have been combined. A broken line 1.5 mm above the Horsley-Clark zero has been used to divide the N. ventralis lateralis (VL). Percentages are based on the following total numbers of cells in each case: N. lateralis dorsalis (LD), 58 ; N. ventralis anterior (VA), 140 ; N. lateralis posterior (LP), 84 ; CL/MD, 193 ; VL (d) (above line), 287 ; VL (v) (below line), 270 ; N. ventralis posterolateralis (VPL), 170 ; VPM/VM, 135.

shown in Fig. 1. To facilitate later histological correlations, electrodes were inserted in the same transverse plane with 1 mm intervals between the tracks. After the completion of a row of insertions, the electrode was moved 1 mm anteriorly or posteriorly and another row of tracks made.

Where several neurones were found in close proximity, only one lesion was made and the position of the other neurones could be established by noting the distances over which the microelectrode tip had been advanced from the site of the lesion.

A cell was assessed as being excited by acetylcholine if it began to fire during or immediately after the application of acetylcholine. On a small proportion of the neurones tested, acetylcholine seemed to cause a subthreshold depolarization, which could be revealed when the excitability of the neurone was tested with L-glutamate. If repeated application of acetylcholine failed to excite these neurones directly, they were not included as acetylcholine-excited neurones. Diffusion of acetylcholine into the vicinity of other acetylcholine-sensitive units may have accounted for such findings. A few neurones were encountered which responded to the initial application of acetylcholine and then became unresponsive to further applications although continuing to fire when L-glutamate was applied. Although this phenomenon seems to be a result of desensitization of the receptors to acetylcholine, such cells were not included in the totals of acetylcholine-excited neurones.

The proportion of neurones fired by acetylcholine ranged between one out of the fifty-eight cells identified in the N. lateralis dorsalis (LD) and eighty-two out of one hundred and thirty-five neurones in N. ventralis postero medialis/ventralis medialis (VPM/VM). The percentages of acetylcholine-excited cells encountered in various thalamic nuclei are presented in Fig. 1. For convenience of presentation the A-P distribution of cells tested has not been taken into account in Fig. 1.

Neuronal excitation by acetylcholine occurred predominantly in the VBC, particularly in the ventral half of N. ventralis lateralis (VL), the median part of N. ventralis posterolateralis (VPL) and in VPM/VM. With the cell totals observed in each nucleus (see legend to Fig. 1) differences of approximately 10% are statistically significant.

(b) *Identification of thalamo-cortical neurones.* Thalamo-cortical neurones in the VBC can be identified by their response to an antidromic volley evoked by stimulation of the ipsilateral somato-sensory cortex. Antidromically evoked spikes were characterized by an all-or-none spike, a short fixed latency and the ability to follow repetitive stimulation at 100/sec or more.

Thirty-nine antidromically activated neurones were tested in cats anaesthetized with nitrous oxide and fluothane. The firing latencies ranged from 0.8 to 3.6 msec with most cells having latencies of 1 to 1.5 msec.

Thalamo-cortical neurones on the sensory pathways are fired repetitively by volleys from contra-lateral limb nerves (Rose & Mountcastle, 1954; Andersen, Eccles & Sears, 1964), and those on the cerebello-cerebral pathway respond monosynaptically to BC stimulation (Sakata, Ishijima & Toyoda, 1966).

Cerebral stimulation, as well as activating thalamo-cortical neurones, antidromically evokes long duration inhibitory potentials in such cells (Andersen *et al.*, 1964; Sakata *et al.*, 1966). Units which responded to cortical stimulation with a short, constant latency,

all-or-none spike but failed to respond to high-frequency stimulation, would frequently follow 100/sec antidromic stimulation if a brief pulse of L-glutamate or acetylcholine was applied just before stimulation. The poor frequency performance of such neurones, which were included as thalamo-cortical units, was attributed to cortically evoked inhibition.

Of the thirty-nine thalamo-cortical neurones tested with acetylcholine, thirty (77%) proved to be sensitive to its excitant action. Examples of two of these neurones are shown in Fig. 2. One (Fig. 2A) was relatively sensitive to the excitant action of acetylcholine, and began to fire 7 sec after the onset of application of acetylcholine by a current of 20 nA. Firing ceased 2 sec after the current applying acetylcholine had been terminated. Acetylcholine (40 nA) initiated a very rapid onset of firing (3 sec latency) and firing again ceased rapidly when the application terminated.
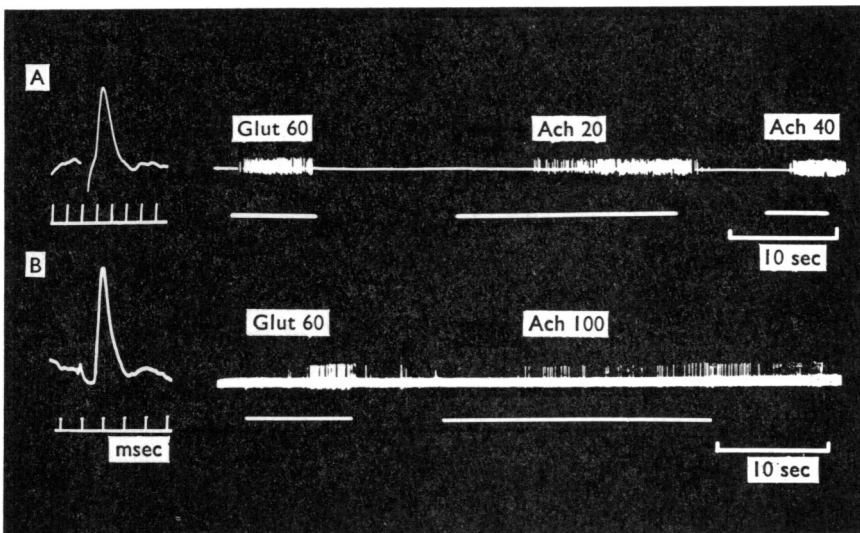

Fig. 2. A: Thalamo-cortical neurone, responding to stimulation of pericruciate cortex with a short latency antidromic spike. Acetylcholine 20 and 40 nA) had marked excitant action on this neurone. B: Another thalamo-cortical neurone, responding to cortical stimulation but relatively insensitive to acetylcholine (100 nA).

The second antidromically activated neurone (Fig. 2B) was less sensitive to acetylcholine, requiring an applying current of 100 nA before excitation occurred. In this instance, the excitation had a longer latency and the neurone continued to fire for nearly 1 min after the application of acetylcholine had ceased.

Spontaneous activity

Neurones in the ventro-basal complex of cats anaesthetized with pentobarbitone sodium were frequently characterized by rhythmic spontaneous spindle discharges. This type of activity has the same basic components as the evoked rhythmic burst discharges (Andersen & Sears, 1964), each spindle consisting of groups of spikes or " bursts "

containing several spikes at a frequency of 100–300/sec and with a group frequency of 5–12/sec. The spindles of a particular cell tended to recur at intervals of 4–8 sec. In constrast, " spindle " activity was rarely apparent in animals anaesthetized with nitrous oxide and fluothane or methoxyflurane. Administration of small doses of thiopentone sodium (2–10 mg/kg) to gas anaesthetized animals, however, would initiate the onset of spindling.

Intracellular recording from neurones in the VBC has shown that spindles result from an interaction between phased excitatory and inhibitory influences (Andersen, Brooks & Eccles, 1964; Andersen & Sears, 1964; Andersen, 1966). The results of pharmacological studies on " spindle " responses will be described later in this paper.

Nervous connections of cholinoceptive cells in the ventro-basal complex

The evidence presented has confirmed that cholinoceptive cells are present in the feline thalamus (Andersen & Curtis, 1964a, b). Such cells are unevenly distributed in the thalamus, occurring predominantly in the ventro-basal nuclei (VL, VPL & VPM). In studying the nervous pathways by which cholinoceptive cells are activated synaptically, attention has therefore been largely confined to the ventral thalamus. The types of synaptic activation studied in these experiments included responses to stimulation of limb afferent nerves, short latency and " burst " responses evoked by stimulation of the ipsilateral somato-sensory cortex, the short latency response to BC stimulation and responses to RF stimulation. The experiments described in the subsequent sections were carried out on gas anaestheized cats.

(a) *Spino-thalamic pathway.* The finding that 77% of the thalamo-cortical neurones tested were excited by acetylcholine suggested that some of the afferent, medial lemniscal pathways to the thalamus might be cholinergic, despite the earlier findings to the contrary (Andersen & Curtis, 1964b).

Stimulation of forelimb nerves evoked repetitive firing of thalamic relay neurones, most neurones responding to stimulation of several contra-lateral limb nerves. An example of an acetylcholine-excited TCR cell, which was excited synaptically by median nerve stimulation, is shown in Fig. 3. Atropine (100 nA) for 60 sec was then applied and initially depressed the responses of the cell to L-glutamate, acetylcholine and synaptic excitation. This reduction in cell excitability (Curtis & Phillis, 1960) wore off during the following 3 min and the responses to L-glutamate and median nerve stimulation returned to the control magnitude (Fig. 3B). Acetylcholine excitation, however, failed to occur even when the current through the acetylcholine barrel was increased from 40 nA to 100 nA (Fig. 3B). Responses to acetylcholine remained blocked during a further recording period of several minutes. Similar results were obtained when hyoscine was used to block acetylcholine-excitation. This substance also caused an initial reduction of 2–3 min duration in cell excitability, after which responses evoked by L-glutamate and forelimb nerve stimulation returned to pre-hyoscine levels but there was no response to acetylcholine.

Dihydro-β-erythroidine (DHE) also failed to reduce or abolish the excitation of TCR neurones by forelimb nerves. Iontophoretically applied drugs may be restricted in their actions to areas of the cell membrane adjacent to the micro-electrode tip, so it is

conceiveable that the lack of effect of atropine, hyoscine and DHE on synaptic firing induced by limb stimulation was a result of the failure of these drugs to reach active synapses even though the effects of locally applied acetylcholine had been abolished. Intravenous injections of atropine and DHE (in doses of up to 1 mg/kg) were therefore administered to several cats. Synaptic firing of thalamic neurones by volleys in limb nerves was, however, unaffected.
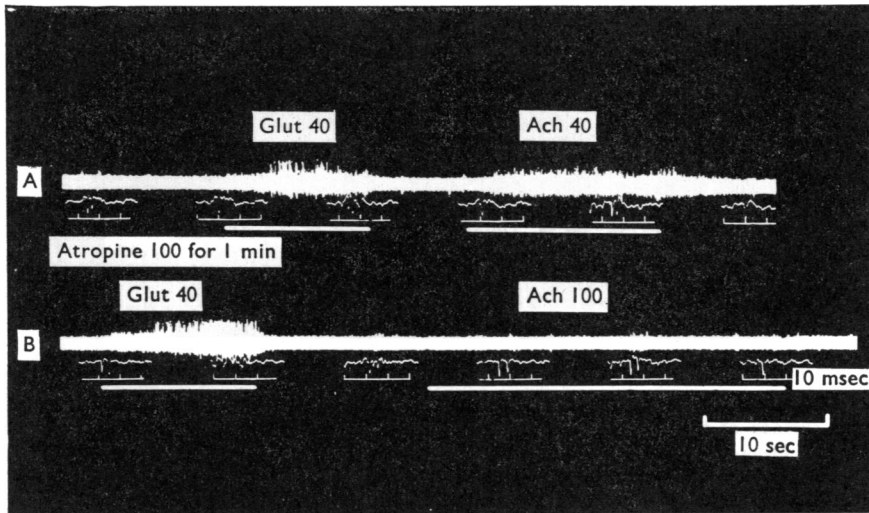

Fig. 3. A: Responses of TCR neurones to L-glutamate (40 nA), acetylcholine (40 nA), and median nerve stimulation (recording technique is described in METHODS). B: Responses of same neurone 3 min after application of atropine (100 nA for 1 min). L-glutamate and median nerve stimulation still evoked responses but acetylcholine action had been abolished.

(b) *Cortico-thalamic pathways.* Some VBC neurones were fired synaptically by stimulation of the ipsilateral somato-sensory cortex. Synaptic firing could be distinguished from antidromic responses by the fluctuations in latency and failure to follow repetitive stimulation at frequencies in excess of 10–30/sec.

Fig. 4A illustrates the responses of a neurone which responded with both anti- and orthodromically evoked spikes to cortical stimulation. The antidromic spike had a constant latency of 3.6 msec, and, if assisted by an application of acetylcholine, would follow repetitive cortical stimulation at 100/sec. The second spike had a variable latency of 7–11 msec, sometimes failed to appear (although a small prepotential was still apparent) and would not follow stimulation frequencies in excess of 10/sec. Acetylcholine (40 nA) excited the neurone. After the application of DHE (40 nA) for 90 sec, the cortically evoked synaptic responses remained unaltered, even though acetylcholine no longer excited the cell.

Cortical stimulation evoked a late burst of spikes from the neurone illustrated in Fig. 4B. This cell was situated in the VL nucleus and failed to respond to acetylcholine. Atropine (80 nA) applied for 90 sec was without effect on the synaptic response.
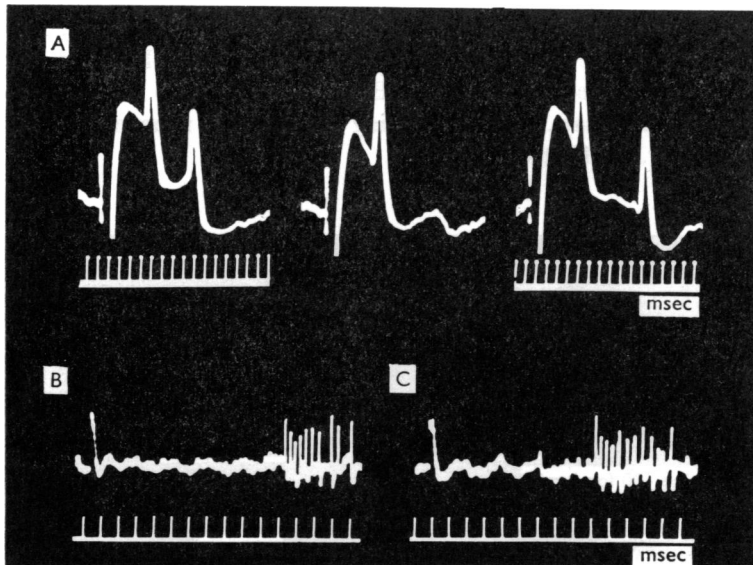
Fig. 4. A: TCR neurone which responded to stimulation of motor cortex with both orthodromically and antidromically evoked spikes. B and C: Responses of a VL neurone (depth of 4,700 μ below surface of thalamus) to cortical stimulation before and after atropine (80 nA for 90 sec). Unit was insensitive to acetylcholine.

From these results, it is clear that fibres in the medial lemniscal and cortico-thalamic pathways are unlikely to be cholinergic.

BC stimulation

Ninety-three neurones which responded monosynaptically to contra-lateral BC stimulation were encountered during these experiments. Spike latencies were of the order of 0.9 to 3 msec and the response was frequently superimposed on a negative field potential caused by the activation of adjacent neurones. With sufficiently strong stimuli the normal latency fluctuation for BC activation was very small. The neurones followed repetitive stimulation at frequencies of 10–20 sec. These properties of the BC evoked spikes in VL neurones are similar to those described by Sakata *et al.* (1966).

87% of the ninety-three BC-evoked neurones tested were found to be excited by acetylcholine. This is consistent with the findings of Frigyesi & Purpura (1966), who demonstrated that intra-carotid administration of acetylcholine or atropine has a marked effect on the magnitude of BC evoked fields in the thalamus, acetylcholine facilitating (1–12 μg doses) or depressing (in larger doses) and atropine depressing the post-synaptic components of the VL responses evoked by BC stimulation. An example of a BC evoked unit is shown in Fig. 5. This neurone failed to follow BC stimulation at 15/sec and the latency variations which occurred during repetitive stimulation are clearly visible in Fig. 5E. The neurone was relatively insensitive to acetylcholine-excitation, a current of 80 nA being necessary to initiate firing (Fig. 5F). Acetylcholine (40 nA) had a subthreshold action on this neurone, reducing the latency of the BC evoked spike and

increasing its amplitude. This action of acetylcholine was shared by noradrenaline (on those BC evoked cells that were excited by the latter) (Phillis & Tebēcis, 1967b). Repetitive stimulation at 20/sec during the period of acetylcholine application induced latency variations and spike failures, confirming that the response was still caused by a single cell spike. The increase in spike amplitude did not occur when positive current was passed through the recording barrel, eliminating the possibility that it resulted from a current-induced movement of the cell with respect to the electrode tip.
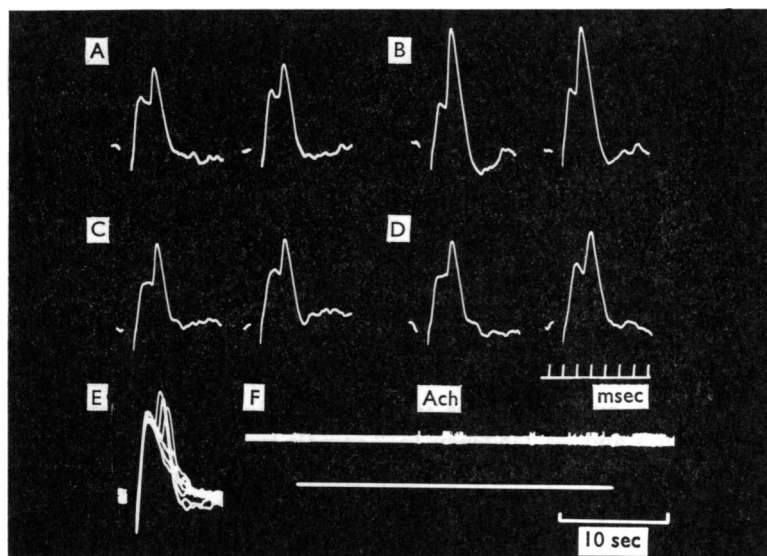

Fig. 5. Focal potentials with superimposed orthodromic spike, recorded in VL and evoked by BC stimulation. A: Control response. B: During application of acetylcholine (40 nA). C: 2 min after acetylcholine application. D: After DHE (120 nA for 60 sec). E: Response during repetitive stimulation of BC at 15/sec. F: Acetylcholine (80 nA) excited the cell.

This finding was confirmed by applying DHE (120 nA) for 60 sec. This abolished the excitant action of acetylcholine on the cell and currents of up to 100 nA failed to cause an increase in the spike amplitude. DHE, however, did not reduce the effects of BC stimulation.

Iontophoretically applied atropine, or hyoscine, also failed to block the excitant effect of BC stimulation. When administered intravenously atropine had a more pronounced action. In confirmation of the findings of Frigyesi & Purpura (1966) atropine (in doses of up to 1 mg/kg) reduced the magnitude of BC evoked focal potentials in the thalamus. Small doses of atropine (0.25 mg/kg) caused a 30–40% reduction in the amplitude of the BC fields but increasing doses had little further action.

Intravenously administered atropine blocked the BC evoked responses of some thalamic neurones, but not of others. An example of an atropine-sensitive response is illustrated in Fig. 6. This neurone was excited by acetylcholine, which also increased the amplitude of the spike (Fig. 6B). After atropine (1 mg/kg) the BC response failed, exposing the underlying atropine resistant component of the field potential (Fig. 6C).
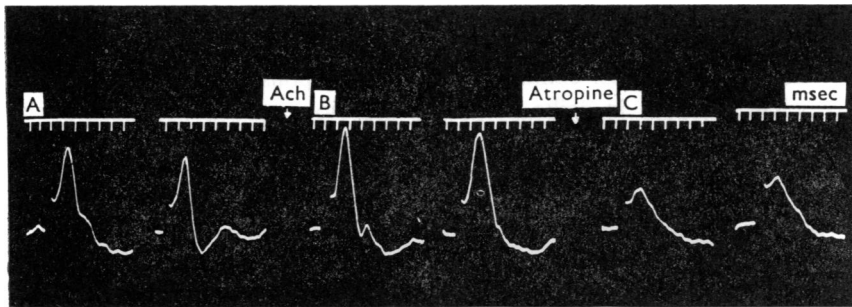
Fig. 6. A: BC evoked spike of a VL neurone. B: Application of acetylcholine (60 nA) excited neurone and increased amplitude of BC evoked spike. C: After intravenous injection of atropine (1 mg/kg) the synaptically evoked spike failed and acetylcholine-excitation was abolished.

Mesencephalic reticular formation stimulation

Stimulation in the reticular formation (RF) excites some thalamic neurones and inhibits others (Phillis & Tebēcis, 1967b). An example of a TCR neurone which responded to RF stimulation with a burst of spikes is shown in Fig. 7. This unit was excited by acetylcholine (Fig. 7C) and its tendency to " spindle-type " responses is evident in this trace. RF stimulation evoked a " burst " response which had a fairly constant latency of 8–9 msec. Although atropine (100 nA) abolished the excitant actions of acetylcholine on this cell it failed to modify the " burst " responses.
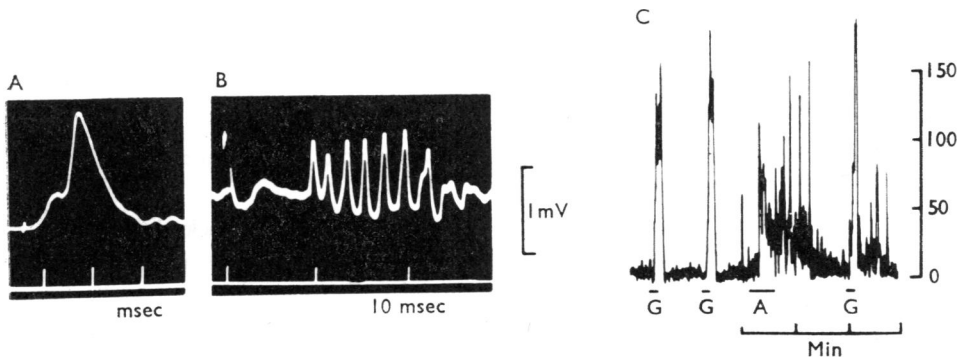

Fig. 7. A: Antidromically evoked spike of this TCR neurone. B: Orthodromic response to stimulation in mesencephalic reticular formation (RS). C: Ink-recorder trace demonstrating discharge frequencies induced by application of L-glutamate (G, 40 nA) and acetylcholine (A, 80 nA). Atropine (100 nA for 1 min) abolished acetylcholine excitation but not synaptic response to RS. In this figure as well as Figs. 9 and 10, the horizontal bars above and/or below recordings represent periods of application of drugs. Frequency of firing (spikes/sec) is indicated by calibrated vertical scale on right of recordings).

A different type of response to reticular stimulation is illustrated in Figs. 8 and 9. The neurone in Fig. 8A responded to stimulation of several forelimb nerves and the response to triceps nerve stimulation is shown. Stmulation in the RF evoked a series of spikes. This type of response had a variable latency and the interspike interval was frequently of the order of 100 msec. The cell was excited by acetylcholine.
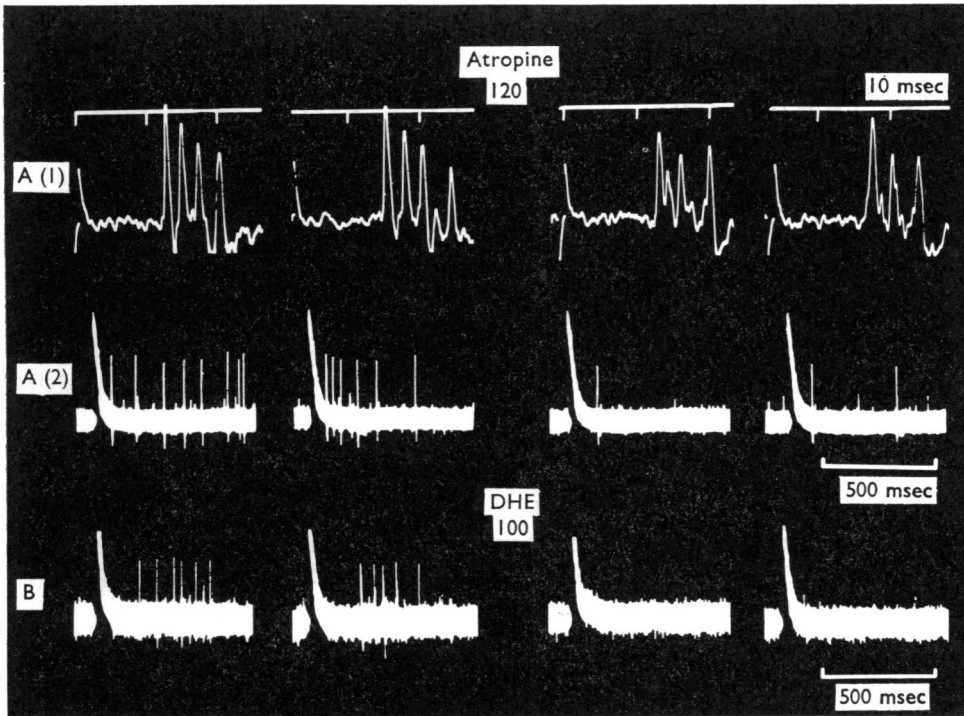

Fig. 8. A: Orthodromic responses of an acetylcholine-excited neurone evoked by triceps nerve (A(1)) and reticular formation stimulation (A(2)). After application of atropine (120 nA for 90 sec) the response to triceps stimulation was still present but response to reticular stimulation had been largely abolished. B: Response of another acetylcholine-sensitive neurone in VBC to reticular formation stimulation. DHE (100 nA for 60 sec) abolished these synaptic effects.

After the application of atropine (120 nA) for 90 sec, acetylcholine sensitivity was abolished and the response to reticular stimulation blocked, while responses evoked by triceps nerve stimulation remained unaltered.

Another cell, which was excited by reticular formation stimulation with a similar long latency, slow response, is shown in Fig. 8B. Application of DHE (100 nA) for 60 sec abolished both acetylcholine sensitivity and the response to reticular stimulation. The RF evoked responses that have just been described are likely to be a result of excitation of neuronal circuits in the brain stem, and excitation of this nature could be most readily demonstrated when the RF was stimulated repetitively at frequencies of 5–15/sec. Excitation of thalamic neurones was then evident either as a direct increase in the cell firing frequency (Fig. 9) or as an enhancement of the response to L-glutamate.
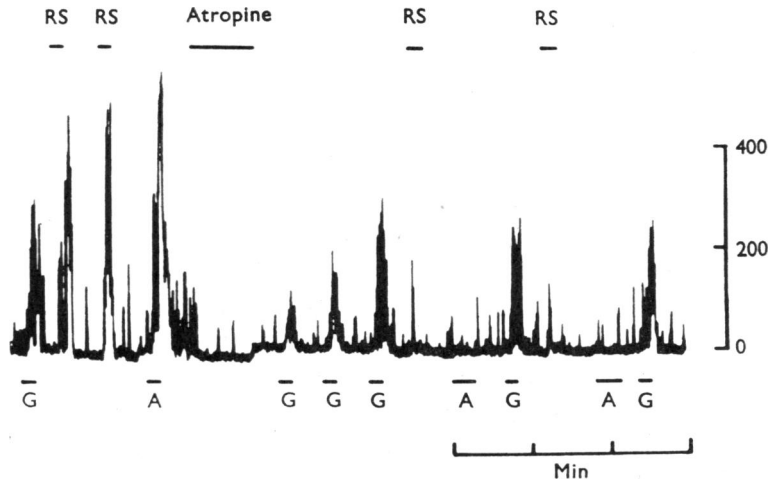
Fig. 9. Ink-recorder trace of discharge frequency of a VL neurone. Reticular formation stimulation (RS) (5/sec for 10 sec) L-glutamate (G, 40 nA) and acetylcholine (A, 40 nA) excited cell. After atropine (60 nA for 60 sec) the effects of RS and acetylcholine were abolished, but L-glutamate excitation was not.

The VL neurone in Fig. 9 was excited by L-glutamate and acetylcholine. RF stimulation (5/sec for 10 sec, 4 V) initiated cell firing at a frequency of more than 400/sec. The cell also responded monosynaptically to BC stimulation and its tendency to spontaneous bursts of firing is also evident in the record.

Atropine (60 nA) for 60 sec abolished the excitation by acetylcholine and reticular formation stimulation while L-glutamate, BC evoked and spontaneous firing remained unaltered.
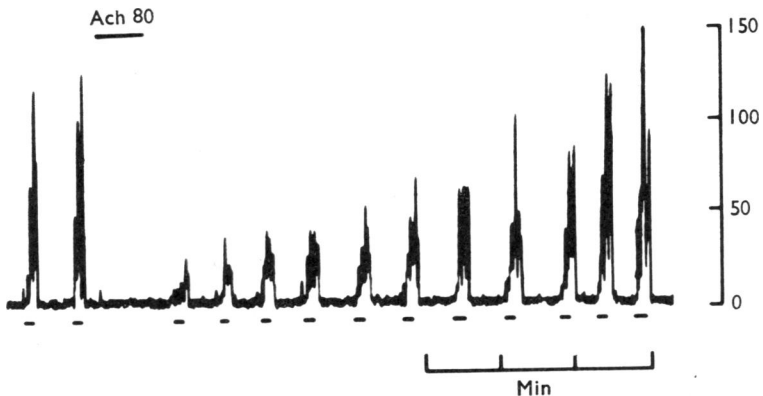

Fig. 10. Acetylcholine (80 nA) depression of response of a dorsal thalamic neurone to L-glutamate (40 nA) pulses.

Acetylcholine depression

Acetylcholine depression of thalamic neurones was of two types. An initial depression of spontaneous firing of many acetylcholine-excited thalamic neurones after the onset of application of acetylcholine has been described by Andersen & Curtis (1964a, b). This finding has been confirmed in the current series of experiments.

A different type of depression was occasionally observed when acetylcholine was tested on cells in the dorsal thalamic nuclei. An example of this type of depression is shown in Fig. 10. This cell was not excited by acetylcholine even though tested repeatedly. Acetylcholine (80 nA) had a profound depressant action on this cell and recovery took several minutes. Such responses were encountered so infrequently that an extensive pharmacological analysis was not attempted.

DISCUSSION

Results described in this paper confirm the presence of acetylcholine-excited neurones in the feline thalamus (Andersen & Curtis, 1964a, b).

Studies on the distribution of such cells in cats anaesthetized with pentobarbitone sodium have shown that they occur predominantly in the ventro-basal thalamus, although some acetylcholine-responsive cells were found in the other nuclei. Although the excitant action of acetylcholine on thalamic neurones is depressed by barbiturates (Phillis & Tebēcis, 1967a), this effect can be mitigated by repeating applications and by the use of large currents (McCance *et al.*, 1968). Both these measures were employed as seemed warranted during these experiments. Irrespective of any influence of barbiturate on the overall level of acetylcholine sensitivity, the internuclear comparisons are meaningful because the experiments were carried out under comparable conditions.

Histochemical studies on the distribution of acetylcholinesterase in the thalamus have been carried out on several cats. Although it was possible to demonstrate an increased density of staining in the VBC of some of these (see Phillis *et al.*, 1967), this was not the most usual finding. The sections presented in Plate 1 represent three A–P levels in the thalamus of a cat (A–P 12, 10 and 7). Dense staining for acetylcholinesterase can be seen in the caudate nucleus, lateral geniculate nucleus, pulvinar nucleus, habenular nucleus, centralis medialis and centrum medianum nuclei, cerebral peduncle and internal capsule. The VBC, however, has failed to stain more densely than the more dorsal thalamic nuclei.

Studies on synaptic activation of VBC neurones by volley from limb nerves have confirmed an earlier report that afferent fibres in the medial lemniscal pathway are non-cholinergic (Andersen & Curtis, 1964b). Monosynaptic and polysynaptic pathways from the cerebral cortex to the thalamus are also unlikely to be cholinergic. The results obtained with BC and RF stimulation were of special interest, because in association with the results described in other reports, they provide convincing evidence for the presence of cholinergic pathways to the thalamus.

Plate 1. Transverse sections through cat diencephalon, stained to demonstrate localization of AChE (Phillis et al., 1967). Sections represent stereotaxic AP levels, A12, A10 and A7.

Experiments on the distribution of choline acetyltransferase in the cerebellar penduncles of the dog (Feldberg & Vogt, 1948), have shown that this enzyme is present in appreciable quantities in the BC and prompted a suggestion that cholinergic efferents from the cerebellar deep nuclei were present in this pathway. Choline acetyltransferase is also present in the feline cerebellar peduncles (Hebb & Silver, 1956). The feline BC contains acetylcholinesterase (Austin & Phillis, 1965) and histochemical studies on cats in which the BC pathway had been transected several days before death have revealed that some of the efferent fibres from the cerebellar deep nuclei stain distinctly for acetylcholinesterase (Phillis, 1965a, b). The relative paucity of these stained fibres in the transected peduncle suggests that only a limited proportion of the cerebellar efferents are cholinergic.

Such a suggestion would explain the results that have been described in this paper. Although acetylcholine excited 87% of the BC evoked thalamic neurones on which it was tested, atropine only partially abolished BC evoked fields and both atropine and DHE were relatively ineffective in blocking BC evoked synaptic firing. The presence of a limited proportion of cholinergic fibres in the BC, projecting to most of the BC evoked neurones in VL, but generally dominated in action by non-cholinergic BC fibres would account for these results.

Unless the magnitude of the BC evoked EPSP was just above threshold, a block of transmission at the minority of cholinergic synapses would not cause a failure in impulse transmission.

An alternative explanation is that cholinergic fibres from other areas of the brain maintain VBC neurones in a state of subthreshold depolarization, which facilitates the responses to BC stimulation. Block of this facilitatory effect would reduce the effect of BC stimulation. This explanation is rendered unlikely by the finding that atropine and DHE do not reduce the excitation induced by stimulation of limb nerves or the cerebral cortex.

Acetylcholine caused an increase in the amplitude and duration of action potentials of BC evoked neurones in the VL which was associated with an increase in the magnitude of similarly evoked focal potentials. An increase in amplitude and duration is probably a result of facilitation of invasion of the action potential into the dendritic processes of the neurone and suggests that acetylcholine receptors may be present on dendrites.

The results with reticular formation stimulation lend support to the suggestion that cholinergic fibres project from this area to the thalamus (Shute & Lewis, 1963). A similar pathway from the RF to the lateral geniculate nucleus has been described (Phillis *et al.*, 1967). Those responses to RF simulation which were blocked by atropine or DHE invariably had a long latency and excitation could be demonstrated most effectively by stimulating repetitively.

Acetylcholine had inhibitory actions on some neurones in the thalamus. The significance of these findings has yet to be evaluated but the possibility that it may be related to the inhibition of thalamic neurones by RF stimulation should not be overlooked. It has recently been shown that acetylcholine depresses many neurones in the cerebral cortex and that this action as well as nervous inhibition of cortical neurones by RF or pyramidal tract stimulation can be blocked by atropine or hyoscine (Phillis & York, 1967). These

findings suggest that acetylcholine acts as an inhibitory transmitter in the cerebral cortex and a similar phenomenon may occur on some neurones in the thalamus. Depressant actions of acetylcholine on spinal cord inter-neurones which were considered to be unrelated to synaptic processes have been described (Curtis, Ryall & Watkins, 1966). It is clear that only further examination of the actions of acetylcholine will establish beyond doubt whether such actions are or are not related to the role of acetylcholine as a synaptic transmitter.

SUMMARY

1. The distribution of acetylcholine-sensitive cells in the feline thalamus has been studied by a combined pharmacological/histological technique.

2. Acetylcholine-excited cells were found in all areas of the thalamus tested; the highest proportions being located in the ventro-basal complex.

3. A high percentage of thalamo-cortical relay neurones and neurones responding monosynaptically to brachium conjunctivum stimulation were excited by acetylcholine.

4. Synaptic responses evoked by stimulation of limb nerves and the cerebral cortex were unaffected by acetylcholine-antagonists. Responses evoked by brachium conjunctivum and mesencephalic reticular formation stimulation were reduced by atropine and dihydro-β-erythroidine.

5. It is concluded that acetylcholine is likely to be an excitatory transmitter in the thalamus.

6. Some cells were depressed by acetylcholine.

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