Spine title : The Habenulo-Interpeduncular Pathway

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THE HABENULO-INTERPEDUNCULAR PATHWAY

Electrophysiological studies with extracellular macro- and microelectrodes in 58 cats revealed spontaneous rhythmic oscillations in the interpeduncular nucleus, ranging from 3 - 4 Hz to 9 - 10 Hz under different anaesthetics. There was little obvious correlation between these waves and the spontaneous firing patterns of interpeduncular units. Possible origins of the waves are discussed. Stimulation of the habenula evoked in the nucleus brief positive-negative waves followed by a slow negative, and then a slow positive wave. The majority of interpeduncular units excited by habenular stimulation initially fired during the brief early waves. The unit discharge was either (i) a constant latency spike, which followed repetitive stimulation at 100 Hz or more, or (ii) at relatively variable latency, a single spike or a short burst of 2 - 6 spikes at 500 - 1000 Hz. Some units showed, after the habenular stimulus, sequences of initial excitation followed by inhibition, while other units showed pure inhibition. Over 80% of units either excited or inhibited by habenular stimulation were excited by acetylcholine applied microiontophoretically; this excitation was potentiated by neostigmine and blocked by atropine.

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LA VOIE HABENULO-INTERPEDONCULAIRE

Au cours d'une étude électrophysiologique conduite sur 58 chats à l'aide de micro-électrodes, il a été mis en évidence dans le noyau interpédonculaire des oscillations spontanées rythmiques dont la fréquence varie de 3 ou 4 Hz à 9 ou 10 Hz selon le type d'anesthésie. La corrélation entre ces activités globales et les modalités de décharges spontanées des neurones du noyau interpédonculaire est faible et incertaine. Les origines possibles de l'activité ainsi décrite sont discutées. La stimulation de l'habénula évoque dans le noyau interpédonculaire une réponse qui se compose d'une onde rapide positive-négative suivie d'une onde lente négative et enfin lente et positive. La décharge initiale de la majorité des neurones interpédonculaires, en réponse à la stimulation de l'habénula, est synchrone des ondes rapides précoces. La réponse cellulaire est: soit un spike de latence constante pouvant suivre une stimulation répétitive à 100 Hz ou plus, soit, à une latence variable, un spike unique ou un train de 2 à 6 spikes à 500-1000 Hz. Quelques cellules sont excitées puis inhibées par la stimulation habénulaire et d'autres purement inhibées. Plus de 80% des cellules influencées d'une manière ou d'une autre par la stimulation habénulaire sont excitées par des injections micro-iontophorétiques d'acetylcholine; cette excitation est potentiée par la néostigmine et bloquée par l'atropine.

THE HABENULO - INTERPEDUNCULAR PATHWAY

by

Norma Lake, B.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Department of Physiology

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INTRODUCTION

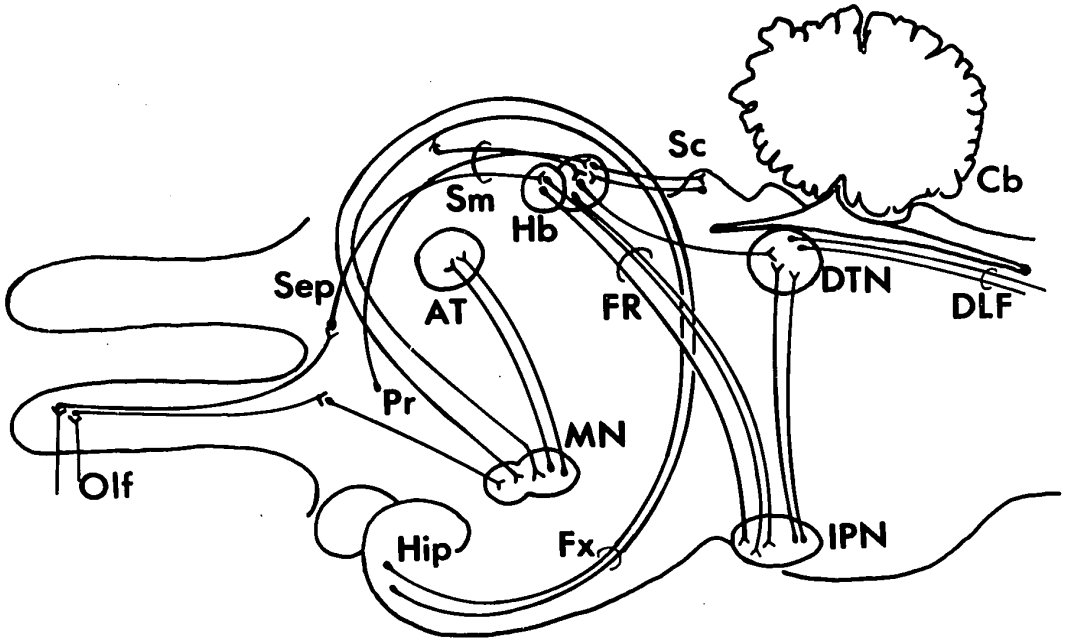
Historical

The first studies of the habenulo-interpeduncular pathway were anatomical. The habenular nuclei, discovered in 1824 by Serres (according to Cajal, 1911), are small paired structures on either side of the third ventricle above the thalamus (in the epithalamic region) anterior to the pineal gland (see Figure 1). The nuclei on either side give rise to a mainly unmyelinated fibre bundle, the fasciculus retroflexus, which travels to the mesencephalon and terminates in the interpeduncular nucleus (IPN). The IPN was discovered by Forel (1872) in the rabbit, and described in detail in 1877. Forel's description was confirmed by Von Gudden's (1881) studies on the rabbit, and Ganser's (1882) studies on the mole.

According to Cajal (1911), it was Meynert who first established the relationship of the fasciculus retroflexus with the habenula; but Van Gehuchten (1893) demonstrated in teleosts that this tract originated from habenular cells and terminated in the IPN. Both nuclear groups and the fasciculus retroflexus are a constant feature of vertebrate brains, but they are much larger in some lower species than in higher ones.

Figure 1 Connections of the habenula and interpeduncular nucleus (after Huber & Crosby, 1928)

AT	anterior thalamic nuclei	IPN	interpeduncular nucleus
Cb	cerebellum	MN	mammillary nuclei
DLF	dorsal longitudinal fasciculus	OLF	olfactory bulb
DTN	dorsal tegmental nucleus	Pr	preoptic area
FR	fasciculus retroflexus	Sc	superior colliculus
Fx	fornix	Sep	septum
Hb	habenula (medial & lateral)	Sm	stria medullaris
Hip	hippocampus		


Sc
Cb
Sm
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AT
FR
DTN
DLF
Pr
MN
Olf
Hip
Fx
IPN

Comparative anatomy

The diencephalon and the mesencephalon show parallel development in the course of phylogeny, and this is illustrated by the habenulo-interpeduncular system. In general the ventral midbrain nuclei serve as relays in the efferent paths from cortical centres, the striatum, some regions of the diencephalon, the tectum and the cerebellum, to centres in the brainstem and spinal cord. Thus the development of the higher centres and their efferent paths contributes greatly to midbrain complexity.

One factor which appears to determine the differentiation and prominence of the habenulo-interpeduncular system is the development of olfactory function. According to Edinger (1899) the size of the habenula is proportional to the size of the olfactory bulb and the olfactory cortex; for example, the habenular nuclei are small in man and in animals with a poor sense of smell.

In the lowest vertebrates studied, the cyclostome myxinoids and petromyzonts, the habenular mass appears macroscopically as one body, but microscopically one can distinguish a right and a left nucleus. The nuclei are asymmetric, the right being larger, with more afferents from the telencephalon and giving rise to a larger fasciculus retroflexus. Although most of the midbrain is still in the primitive undifferentiated state, with cell bodies lying mainly at the site of their embryonic origin in the periventricular gray, the interpeduncular neurons have migrated towards their usual ventral position "under the neurobiotactic influence of the retroflex bundle" (Ariens Kappers et al, 1936). In myxinoids the fasciculus retroflexus

has both fine and coarse fibres which form myriads of fine coils within the IPN before terminating among its bipolar spindle-shaped cells (Jansen, 1930).

The IPN of plagiostomes is larger than in petromyzonts but occupies the same basal position. The direction of the asymmetry of the habenular nuclei is opposite to that in petromyzonts; even the more advanced sharks and rays have a larger left habenula and a thicker and more heavily medullated left fasciculus retroflexus (Ariens Kappers et al, 1936).

The brain of ganoid and teleost fishes is more sharply differentiated than in plagiostomes, but the olfactory forebrain is not well developed and the habenulae are smaller. It is notable that the habenular nuclei are symmetrical as in the other remaining vertebrate series (Ariens Kappers et al, 1936).

Amphibians and reptiles have well-developed habenulae with dorsal and ventral components. A remarkable feature of the fasciculus retroflexus, especially well documented in Necturus and salamanders by Herrick (1933, 1948), is its terminations in peculiar flattened spiral endings which travel along the length of the IPN, possibly influencing almost all cells (see Figure 2). These spiral endings typify the fasciculus retroflexus but are in a much more simplified form in Mammals (Von Gudden 1881; Ganser 1882; Cajal 1911).

Most authors (e.g. Huber 1929; Craigie 1931) have recognized in the avian brain, medial and lateral habenular nuclei, smaller in size than the reptilean homologues, in correspondence with the reduction of the olfactory system. The closely packed neurons of the medial nucleus

Figure 2 Terminal paths of fasciculus retroflexus fibres in the interpeduncular nucleus.

Upper, (from Herrick, 1948) Horizontal section through decussation and spiral endings of fasciculus retroflexus of salamander. Golgi method X 50. f.retr. = fasciculus retroflexus; nuc.inp.n. = interpeduncular nucleus neuropil

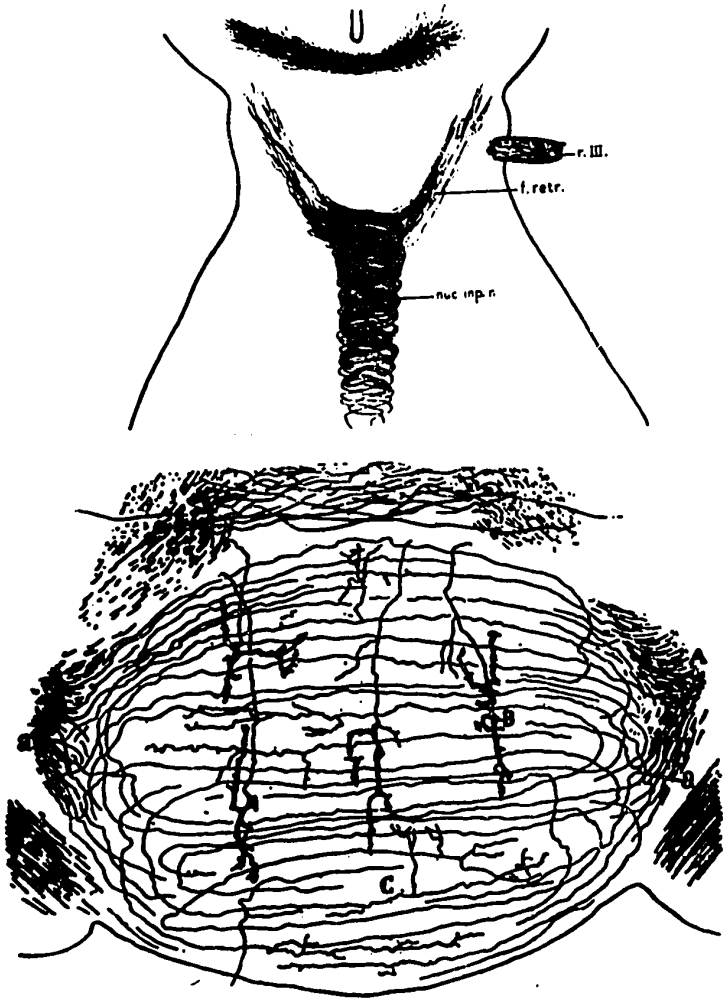
Lower, (from Cajal, 1911) Frontal section through interpeduncular nucleus of 4 day-old mouse. Golgi method.

A- f.retroflexus entering nucleus

B- cell of the nucleus seen in section

C- terminal arbor of a fibre

a- branching of fibre


U
r. III.
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C

give rise to a medial bundle, and the scattered cells of the lateral nucleus to a lateral bundle, both ofiwhich eventually terminate in the IPN.

The habenulo-interpeduncular system is present in all mammals studied, (Ariens Kappers et al 1936; and others) and is particularly large in rodents, such as rats, which have a well developed sense of smell.

Neuroanatomical circuits: the limbic system

In vertebrate evolution the habenulo-interpeduncular system and areas connected with the olfactory bulb show coordinated development, which suggests stable neural groupings or circuitry. Early anatomists classified these structures and interconnections under the term 'rhinencephalon' or the 'smell brain'. The French surgeon Paul Broca in 1878 described the rhinencephalic structures which formed a border (limbus) around the junction between the forebrain and the diencephalon as the 'grand lobe limbique' - implying functional in addition to spatial relationships. These limbic structures are the septal area, the cingulate gyrus, the entorhinal cortex, the hippocampus, and the amygdala. The more recent term 'limbic system' (cf. Maclean, 1955) also includes some reciprocal connections of these structures with other parts of the brain, such as the anterior thalamic nuclei and the mammilary bodies of the hypothalamus. The latest expansion of the limbic system is that proposed by Nauta (1958), mainly on the basis of anatomical studies: thus a certain region of the midbrain is considered 'limbic' because the projections of limbic structures,

e.g. septum, hippocampus, converge and terminate in this particular region, and in addition, tracts originating in this area ascend to more rostral limbic nuclei.

In addition to anatomical connections limbic structures have in common other features. Stimulation of various limbic areas in anaesthetized animals gives rise to a wide range of somatomotor and autonomic responses. Unanaesthetized animals with chronically implanted electrodes in limbic structures react to stimulation with the attention or arousal response. From more restricted areas than those which yield the attention response are obtained flight or defence reactions to limbic stimulation. The behavioral reaction is mild if the stimulus intensity is low; by increasing the stimulus strength and duration a more complex and complete behavior pattern is produced. McCleary and Moore (1965) suggest that "the amount of electrical stimulation thus seems to mimic the intensity of emotional arousal in the normal occurrence of these instinctive response patterns". In human patients, conscious during brain surgery, stimulation of some limbic areas gives rise to vague feelings of fear. Surgical damage to parts of the limbic system yields changes in general "emotional behavior" (temperament, etc.) of operated animals, and deficits in complex learning tasks and memory. These symptoms also occur in man with brain damage to limbic areas, or in certain types of epilepsy. Briefly then, limbic structures form an anatomical and physiological system that is apparently involved in emotional aspects of behavior, and memory.

Histology: afferents to, and efferents from the habenula

In mammals the habenula is a terminal nucleus for many fibres of diverse origin (Cajal, 1911; Marburg, 1944; Cragg, 1961a; Mitchell, 1963; Akagi & Powell, 1968; Yamadori, 1969) (see Figure 1). In the cat Nauta (1958) demonstrated by the Nauta-Gygax technique that there are two major projections to the habenula: one originates in the supracommissural part of the septal region and, as a branch of the fornix, joins the stria medullaris to terminate in the medial habenula; the other arises from the lateral preoptic region, joins the stria medullaris and terminates partly in the lateral habenula, partly in the lateral ventral tegmental area of the brain stem.

Nauta's work also shows that the compact fasciculus retroflexus arises in both the medial and the lateral habenula and terminates in the IPN, while a more diffuse habenulo-tegmental tract originates only in the lateral habenula. This diffuse pathway rejoins the compact bundle after a course through the centre median nucleus and the mesencephalic reticular formation (Burgi & Bucher, 1955; Nauta, 1958; Wakefield, 1968). It gives off fibres to the parafascicular and centre median nuclei, and to a large central and lateral tegmental region of the upper midbrain, but unlike the compact bundle it by-passes the IPN laterally and curves dorsally, giving off fibres to the tegmental nucleus centralis superior, and the fountain nucleus of Sheehan, in the caudal part of the central gray. Nauta suggests that both these habenular-midbrain pathways represent "indirect projections from the limbic system, in turn relayed to the mesencephalon" (Nauta, 1958), and he groups them with the medial forebrain bundle and the mamillo-tegmental tract as limbic-midbrain projections.

Cajal (1911) described the fibres entering the fasciculus retroflexus from the lateral habenula as being thicker than those from the medial nucleus, but he did not indicate if both groups terminate in the IPN. According to Lewis & Shute (1967) cholinesterase-staining fibres from both the lateral and medial habenula travel in the fasciculus and terminate in the IPN. A group of fibres which do not stain for cholinesterase are present in the dorsal part of the fasciculus retroflexus, but appear to leave the bundle before reaching the IPN. Lewis & Shute suggest that these fibres project to the midbrain tegmental nuclei (cf. Nauta, 1958).

In addition to the fasciculus retroflexus, projections from the medial habenula terminate in the superior colliculus, and those from the lateral nucleus terminate in the inferior colliculus (Akagi & Powell, 1968). Nauta (1958) and Mitchell (1963) also describe reciprocal connections between the lateral habenula and the preoptic region, via the stria medullaris and diagonal band, and between the medial habenula and the medial posterior septal region. There are also reciprocal habenulo-thalamic tracts (Huber & Crosby, 1928; Marburg, 1944) and habenulo-hypothalamic tracts (Haymaker et al, 1969).

Fine structure of the habenula

According to Golgi studies on the cat (Cajal, 1911; Wakefield, 1968) the habenula has two distinct divisions, the medial and the lateral nuclei, which differ in cell size, dendritic ramifications, and cell density (see Figure 3). The small round cells of the medial

nucleus are about 10μ in diameter (Milhaud & Pappas,1966a), with multiple branching dendrites, of the pattern described by Moliner (1962) for cells in primary or secondary sensory relay centres characterized by afferents of homogeneous origin. The medial nucleus does appear to have a homogeneous input only from the septal area. Cajal describes the cells as being grouped in islands, separated by dendritic arborizations which he calls habenular glomeruli in analogy with the olfactory bulb glomeruli. The axons of these cells, after a tortuous course through the nucleus to its ventral border, form the compact fasciculus retroflexus which has a diameter of 440 to 710 microns (Akagi & Powell, 1968).

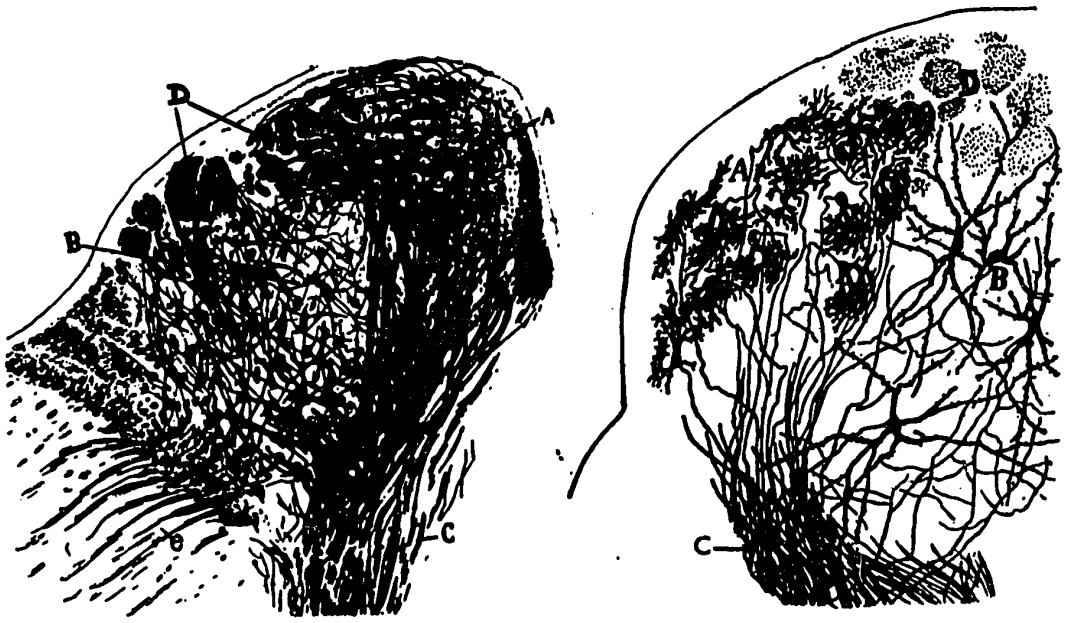
The cells of the lateral nucleus are larger, 20-26μ in diameter (Cajal, 1911), and are not as densely packed as those of the medial nucleus, being separated by myelinated fibre bundles of the stria medullaris and groups of small interneurons with very short axons (Cajal 1911). The large cells are fusiform or triangular, with radiating dendrites. Moliner (1962) has related this dendritic pattern to an input of heterogeneous origin or to the presence of widely spaced afferents. Afferents to the lateral habenula come from the lateral preoptic area (Nauta, 1958), the thalamus (Huber & Crosby, 1928), the tectum (Huber & Crosby, 1928), and the IPN (Guillery, 1959; Massopust & Thompson, 1962), constituting an input of widely spaced and heterogeneous origin. Some of the axons of the lateral nucleus cells join the compact fasciculus retroflexus, but many more are involved in the diffuse habenulo-tegmental bundle (Akagi & Powell, 1968).

Figure 3 Fine structure of the habenula nucleus (from Cajal, 1911)

Left, Frontal section of the habenula in the guinea pig Weigert-Pal and carmin method

Right, Frontal section of the habenula in the dog. Golgi method

A medial nucleus
B lateral nucleus
C origin of the fasciculus retroflexus
D fibres of the stria medullaris
e origin of the diffuse habenulo-tegmental tract


D
A
B
C
D
A
B
B
C

Using the electron microscope Milhaud and Pappas (1966 a & b) have observed habenular dendritic spines, clear and dense-core presynaptic vesicles, and dense granular postsynaptic bodies, usually in spines. They emphasize the widespread distribution of these postsynaptic bodies, and suggest some association with the habenula's high monoamine oxidase content. Wakefield (1968) described somatic spines in Golgi preparations.

Fine structure of the IPN

Cajal (1911) gives one of the most complete descriptions of the IPN from studies on the mouse, rabbit, dog and cat. Using a silver technique he demonstrated two layers of cells, forming a superficial zone and a deep zone (see Figure 4). Cajal clearly states that the superficial zone is the anterior part of the nucleus, while the deep zone is posterior, or more caudal. This terminology has been confused in numerous later studies which refer to inner and outer zones, which do not imply the same regions as Cajal's deep and superficial zones (cf. Brown, 1943; Taber, 1961).

The superficial zone (Cajal) contains small to medium-sized multipolar neurons -- ovoid, fusiform, or triangular -- with thick spined dendrites which extend more or less parallel to the surface of the brain and show marked varicosities and many fine branches (see Figure 4: a,b). The axons of these cells often follow a very tortuous course among the processes of the deeper zone cells, and give off a thick recurrent collateral before ultimately passing into the overlying tegmental gray.

The deep zone has smaller star-shaped cells with multi-branched processes radiating out in all directions from the cell soma, and a larger type of cell similar to that found in the superficial zone. Each axon of the larger cells gives off a recurrent collateral before entering the tegmental region, probably joining the axons of cells of the superficial zone to form the pedunculo-tegmental tract, which travels to the tegmental nuclei of Gudden. According to Cajal, the axons of the smaller cells of the deep zone divide into numerous arborizations in regions near their cell bodies.

The principal inputs to the IPN are the bilateral fibre bundles of the fasciculus retroflexus which approach the nucleus in a rostral-caudal path, and become transversal after entering the nucleus. In the cat the fibres pass through the nucleus to the opposite side, then curve back to the side of entry along a slightly more caudal transverse plane, and turn once more to terminate on the side opposite to entry (see Figure 2). Collaterals are given off at right angles from the main fibre tract, particularly during the latter part of its course through the nucleus. Some fibres bifurcate upon entering the nucleus and the branches follow a 'figure-of-eight' path like the single fibres, but are distributed in different planes. Herrick (1948) has described the far more complex spiral endings in Amphibia (see Figure 2).

In lower vertebrates and in some mammals, other than the cat, collaterals from the mamillo-tegmental tract terminate in the IPN (Haymaker et al 1969). Lewis & Shute (1967) described

Figure 4 Fine structure of the interpeduncular nucleus (from Cajal, 1911)

Sagittal section of the interpeduncular nucleus in 8 day-old rabbit Golgi method

a,b large cells of the superficial zone
c,d small cells of the deep zone (star-shaped, Golgi type II)
g fasciculus retroflexus fibres entering the nucleus

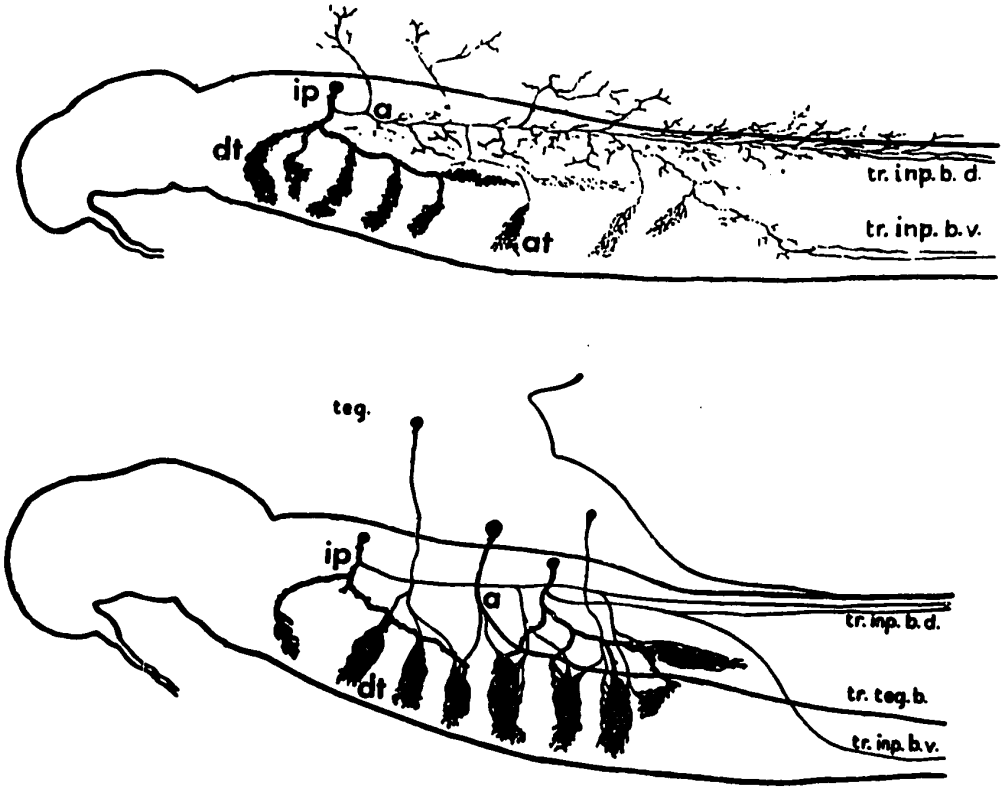

a
a
g
b
b
d
c
c
a

Figure 5 Components of interpeduncular glomeruli (from Herrick, 1948)

Upper, Median sagittal section in salamander X 40 showing neuron of interpeduncular nucleus contribution to glomeruli

Lower, Median sagittal section in salamander X 45 showing composition of glomeruli

a	axon	teg	tegmental neuron
at	tufted axonic terminals	tr teg b	tegmento-bulbar tract
dt	tufted dendritic terminals	tr inp bd	interpedunculo-bulbaris
ip	neuron of interpeduncular nucleus	tr inp bv	tracts dorsal and ventral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cholinesterase-containing fibres of the dorsal and ventral tegmental nuclei of Gudden projecting to the IPN, but this conflicts with the report of Cowan et al (1964).

The IPN glomeruli

According to Cajal (1911), Forel's description in 1872 of the rabbit IPN spoke of tiny cells, interspersed with granular areas which resembled the glomeruli of the olfactory bulb. The most complete account of these glomeruli, and indeed, of all features of the nucleus, is given in Herrick's (1948) description of the tiger salamander brain. According to this, the interpeduncular ependyma branches freely throughout the nucleus and has tufted dendrite-like endings in the glomeruli. "This elaboration of the ependymal fabric within specialized synaptic fields suggests that the ependyma has some part to play in metabolism at the synaptic junctions". (Herrick 1948). The glomeruli, in addition to the capillary net and the ependymal structure contain tufted terminals of dendrites, mostly from IPN cells, and tufted axonic terminals from IPN neurons, cells of the overlying tegmentum, or collaterals of the tractus tegmento-bulbaris, interwoven with the dendrites (see Figure 5). Many axons from these sources do not enter glomeruli but arborize in the surrounding neuropil. Intrinsic glomeruli (with an axonic component mainly from the IPN cells), tend to be oriented horizontally, while extrinsic glomeruli lie vertically and are penetrated by the spiral endings of the fasciculus retroflexus situated ventrally in the nucleus.

The spiral fibres are in synaptic connection with the dendritic component of the glomeruli. "The glomeruli provide apparatus for nonspecific summation and reinforcement" (Herrick 1948).

Histochemistry

i. Succinic dehydrogenase (SDH) and Cytochrome oxidase (CYO)

SDH and CYO are important enzymes in oxidative phosphorylation and are associated closely with mitochondria, the site of respiration. The activity of these enzymes is a good index of the metabolic role played by various nuclei. Because of their close association in the Krebs citric acid cycle, the distribution of SDH and CYO is very similar. Their activity is very conspicuous in gray matter, especially neuropil, and almost absent in white matter, corresponding to the density of mitochondria. Mitochondria are concentrated at synaptic junctions (Scharrer, 1945; Palay, 1956). There appears to be a relationship not only between SDH/CYO activity and histometabolic processes, but also a close correlation to the vascular supply of a particular area (Friede, 1959).

Studies in the monkey by Manocha and Bourne (1966a) show that the IPN has strong SDH and CYO activity in both the closely aggregated small neurons and in the ventral neuropil. There is moderate SDH and CYO activity in the dorsal part of the nucleus which has a looser neuropil. These observations have been confirmed by Friede (1959, 1961) in the cat and guinea pig. In the guinea pig Friede demonstrated strong staining in the habenula also. Shimizu and Nagaaki (1957) showed a strong SDH activity in the IPN, intermediate

in the lateral habenula, and less in the medial habenula in mice, rats, guinea pigs, and rabbits.

ii. Monoamine oxidase (MAO) and lactic dehydrogenase (LDH)

MAO is involved in the inactivation of adrenaline and noradrenaline, while lactic dehydrogenase is necessary for anerobic glycolysis. Manocha and Bourne (1966b) described in the monkey very strong MAO activity in the caudal region of the IPN "with the result that the neurons, their processes, and the neuropil cannot be made out very clearly". There are patches of moderate and strong MAO activity in the dorsal neuropil, and the small dorsal neurons. The ventral neuropil is uniformly positive and the dorsal part fainter, in preparations which assay LDH, while the cell bodies of the small and medium cells reveal intense activity. Shimizu and others (1959) showed strong MAO activity in guinea pig habenula and IPN, while the rat had much MAO in the IPN but only moderate to slight amounts in the habenula. In general they found that white matter gave a weak or negative reaction; in contrast, the fasciculus retroflexus stained strongly.

The strong SDH, MAO, CYO, and LDH activity present in the IPN suggests an active metabolic role of this nucleus (Manocha & Bourne, 1966 b). Most nuclei with strong MAO activity generally show weak SDH activity and vice versa (Shimizu and others 1959). It appears that the IPN "shows not only aerobic metabolism being rich in mitochondria and linked with Krebs citric acid cycle, but may also take an active part in the detoxication of certain amines and

inactivation of adrenaline and noradrenaline. On the other hand, substantial activity of lactic dehydrogenase points towards an ability of this nucleus to carry out anerobic glycolysis" (Manocha & Bourne 1966 b). As anerobic glycolysis is an unlikely activity for the brain, perhaps the LDH may be performing another function at this site.

iii. Glutamate decarboxylase (GAD) and GABA-transaminase (GABA-T)

A recent study in the rat by Knyihar and Csillik (1970) has utilized the technique developed by Ostrowski and Barnard (1961) to detect, by autoradiography, labelled enzyme inhibitors and hence to infer the site of enzymes involved in GABA metabolism. GAD catalyzes the synthesis of GABA from glutamic acid, whereas GABA-T catalyzes the breakdown of GABA. GAD and GABA have similar distributions in central nervous tissue (Albers & Brady, 1959; Roberts & Eidelberg, 1960). The inhibitor used was thiosemicarbazide, which inhibits Vitamin B_6 dependent enzymes (Adey et al 1960; Killam et al 1960; Terzuolo et al 1960). The method is thus nonspecific, but GAD and GABA-T are among the most susceptible to inhibition (Watkins 1968). Relevant here is Knyihar and Csillik's finding (1970) that thiosemicarbazide was accumulated heavily in the medial habenular nucleus. The fine structural details show its location mainly in the neuropil and perhaps also in glial elements.

iv. Monoamines

By means of sensitive methods for detecting fluorescence it has been possible to demonstrate the presence of catecholamines,

noradrenaline (NA) and dopamine (DA), and also 5-hydroxytryptamine (5-HT) in cell bodies and nerve fibres of the central nervous system (Carlsson et al, 1962; Dahlstrom & Fuxe, 1964 a & b; Fuxe, 1965). The main concentration of fluorescent cell bodies is in the brain stem, with largest numbers situated in the mesencephalon (Dahlstrom & Fuxe, 1964). Although surrounded by high numbers of such cells the IPN does not show any cell body fluorescence (Dahlstrom & Fuxe, 1964), though it does contain a small number of fine NA terminals and fewer 5-HT terminals (Fuxe 1965). The habenula also has fine NA and 5-HT terminals, slightly more numerous in the medial than in the lateral nucleus (Fuxe, 1965).

v. Choline acetylase (ChA), Acetylcholinesterase (AChE), and Cholinesterase (ChE)

ChA is involved in the synthesis and AChE in the hydrolysis of acetylcholine. The presence of these enzymes in neural tissue is considered suggestive evidence for cholinergic pathways (Lewis et al, 1967).

Lewis et al (1967) observed that the ChA activity of the IPN is one of the highest in the mammalian brain; it is, for example, about ten times that found in the caudate nucleus. There is also very intense AChE staining present in IPN neurons and terminals, particularly around the periphery of the nucleus. The nucleus also contains a little ChE, mainly at the posterior end, at the site of entry of fibres derived from the dorsal and deep tegmental nuclei (Lewis & Shute, 1967).

Lewis and Shute (1967) describe a "continuous chain of cholinesterase-containing neurons.... from the dorsal and deep tegmental nuclei, via the dorsal and median nuclei of the raphe, which also contain AChE and ChE, to the interpeduncular nucleus" whose "neurons appear to connect with those of the ventral tegmental area". This pathway, though not confirmed in normal or degenerating material (Cowan et al 1964), forms a cholinergic link between the limbic hippocampal-fornix-habenula projections (Lewis & Shute 1967) and the ascending cholinergic reticular system (Shute & Lewis 1967). The habenular nuclei and the fasciculus retroflexus also stain strongly for ChE and AChE (Krnjevic & Silver 1966; Lewis et al, 1967).

Lewis and Shute (1967) suggest that the non-cholinergic fibres seen in the dorsal part of the fasciculus retroflexus leave the main bundle and project to the ventral tegmental area (Nauta 1958). By placing lesions in the retroflex bundle and observing accumulation of AChE staining on one side and its absence from the other, Lewis and Shute demonstrated that the polarity of the cholinesterase-staining fibres is from the habenula to the IPN, and that these fibres arise from both the lateral and the medial habenula.

Physiology and function

The habenulo-interpeduncular pathway is a constituent of larger brain systems as revealed by comparative evolutionary, anatomical, and histochemical data presented above. Consideration will now be given to physiological and behavioral studies which involve these systems, in an attempt to disclose possible functions for the habenulo-interpeduncular

system, but keeping in mind McCleary's (1965) cautionary statement that "the number of detailed findings from such physiological studies far exceeds the present ability of scientists to relate them meaningfully to behavior."

Olfactory functions

A survey of the literature shows that olfactory functions have been attributed to parts of the brain, often limbic structures, which may be only loosely, if at all, related to the sense of smell. Upon closer examination disparities become evident. For example, the 'rhinencephalic' structures, the cingulate gyrus and hippocampus are present in the dolfin and porpoise where smell is absent (Edinger 1899; Brodal 1947). In man the olfactory bulbs are small compared to macrosmatic animals, but the hippocampus and cingulate cortex attain their greatest development (Brodal 1947). Similarly the habenulopeduncular system is well represented in all vertebrates regardless of their sense of smell; a case in point is the well-differentiated fasciculus retroflexus and associated nuclei of birds (Ariens Kappers et al 1936). Brodal (1947) has discussed this point thoroughly as concerns the hippocampus, and suggests (1963) that the anatomical rhinencephalon (smell brain) should include only elements of the brain with direct olfactory connections. Earlier, on the basis of researches in comparative anatomy, Herrick (1933) suggested that the olfactory cortex was not concerned solely with the sense of smell, but through its extensive and diversified connections with the neopallium

served as a nonspecific activator for other cortical activities. He suggested that by facilitation or inhibition the olfactory cortex could influence non-olfactory sensory motor patterns, memory processes, and "the internal apparatus of general bodily attitude, disposition, and affective tone" (Herrick, 1933). He also proposed that the nonspecific facilitatory or suppressive action of the olfactory cortex might be extended into the sub-cortical field, and that in the interpeduncular complex the dominant part played by olfaction is probably of this nonspecific nature (Herrick, 1948).

Riss et al (1969) have studied and reconsidered the evolution of the olfactory and limbic connections of the lamprey, frog, and rabbit, that is from ancient fish to mammal. Their work clearly points out the homologies in the three species. However their thesis is contrary to the usual supposed evolution of the limbic system from olfactory structures; they propose that the olfactory system, reponsive to the external milieu, developed from the limbic system, responsive to the internal milieu. They assert that the nervus terminalis is a limbic structure, and appears before the olfactory nerve, though in higher vertebrates the two are apposed. However, it has not clearly been shown that the nervus terminalis responds to the chemical composition of the blood (internal milieu), or that it is indeed limbic. Their final conclusion parallels that of Herrick (1933): olfactory system activity, in the context of external stimulation, is one of the factors which facilitate or suppress limbic activity, thus modifying the reactions of the organism. (Presumably this is true of all sensations.)

The "Papez circuit"

Herrick (1933) did not specify a locus for "the internal apparatus of bodily disposition", though he did postulate a modulating influence, and thereby implicated cortical and subcortical 'olfactory' structures and projections. Papez (1937) in view of the connections between the hippocampus, fornix, mamillary bodies, anterior thalamic nuclei, and cingulate gyrus, and the fact that lesions involving this circuit caused changes in personality and affective behavior, proposed that these structures were responsible for the central experience of emotion, and that their efferent outflow led to corresponding emotional behavior. Later workers have generally confirmed his hypothesis in man and animals, and have enlarged his original circuit to include other limbic structures (Klüver & Bucy, 1939; Spiegel et al, 1940; Bailey & Davis, 1942; Gastaut, 1953; and others). Temporal lobe epilepsies, or septal lesions, for example, are manifested by symptoms of emotional disorder, apparently owing to disruption of limbic circuits.

Stimulation and ablation studies: autonomic effects

Electrical stimulation and ablations of limbic structures have been shown to stimulate or suppress other, usually autonomic, physiological processes, such as gastrointestinal secretions and motility (Bailey & Sweet, 1940; Smith, 1945; Anand & Brobeck, 1952), blood pressure and respiration (Kabat, 1936; MacLean et al, 1960; Malmo, 1961), pituitary (corticotrophic, gonadotrophic, and thyrotrophic) secretions (Critchlow, 1958; Endröczi & Lissak, 1960; Mess, 1964; Kovacs et al, 1966), and water and electrolyte regulations (Donovick et al,1968;

Grace, 1968; Lubar, 1968). Anatomical studies show that the IPN may be a relay for impulses from limbic areas and from hypothalamic regions of the midbrain (Huber & Crosby, 1928; Ariens Kappers et al, 1936; Herrick, 1948; Nauta, 1958), and so could be involved in many of these circuits. The efferent path from the IPN, first described by Ganser (1882) is the pedunculo-tegmental tract which terminates in the ventral and dorsal tegmental nuclei. The discharge path from the dorsal tegmental nucleus is the dorsal longitudinal fasciculus of Schütz which carries impulses to the motor nuclei of the fifth and seventh cranial nerves and probably to the general visceral efferent centres of the bulb (Ariens Kappers et al 1936).

Psychosomatic disease

MacLean (1949) envisaged limbic structures as forming a visceral-emotional brain, rather than a rhinencephalon. The experiments mentioned above support this formulation. He noted the close association between emotion and autonomic functions, as had Bard (1928) and Cannon (1929), and suggested that this was perhaps due to some localization of the two within the same or related structures. In this way he progressed towards a plausible theory for psychosomatic diseases such as peptic ulcers, asthma or ulcerative colitis, in which abnormal "emotional states are etiologically related" to systemic lesions, presumably mediated by the autonomic nervous system. In harmony with this, Krnjevic (1966) commented that the histochemical finding of abundant adrenergic and cholinergic fibres

in the limbic system indicates that limbic fibres may be central components of the sympathetic and parasympathetic divisions of the autonomic nervous system, which would explain close emotional-autonomic behavioral links.

An example of these close links involves the habenulo-interpeduncular pathway (Cragg 1959, 1961b). In the cat or rabbit, electrical stimulation of the habenula, IPN, or dorsal tegmental nuclei causes panting, cutaneous vasodilation, and relaxation of somatic muscle (reduced knee jerk). Prolonged stimulation results in heat loss sufficient to lower the rectal temperature. Stimulation of axons which project to the habenula via the stria medullaris, such as the diagonal band or preoptic area, elicits both potentials in the habenula and panting, but the panting response is not abolished by section of the stria medullaris. Local heating in the diagonal band or preoptic region causes panting which is also not abolished by cutting the stria medullaris. The sectioning spares another efferent path from the preoptic region, the medial forebrain bundle. Light restraint of unanesthetized rabbits also produces the syndrome of panting and cutaneous vasodilation attributed to emotional factors (Grant 1950; Cragg 1961b). Cragg found that complete bilateral section of the stria medullaris or habenulo-interpeduncular tract abolishes panting during restraint, and concluded that the habenula and associated nuclei and tracts form an "efferent path affecting respiration which appears to be influenced by emotional factors rather than body temperature".

In Cragg's hypothetical pathway impulses from the dorsal tegmental nuclei may reach bulbar respiratory neurons via the nucleus prepositus hypoglossi and its projection to the medial medullary reticular formation (Pitts et al, 1939).

Limbic structures and somatic movements

Limbic structures also influence somatic movement (e.g. Kaada, 1954) which Nauta (1958) believes are mediated by the limbic midbrain area. Kaada (1960) has shown that stimulation of parts of the cingulate gyrus and amygdala have primarily a facilitatory effect on motor responses, while a region in the subcallosal and septal area is a motor inhibitory zone. Lesions within either of these areas produce a deficit in fear-motivated behavior; deficits in active responses appear with damage to the facilitatory area, and deficits in passive avoidance with damage in the inhibitory area (McCleary, 1961). An active avoidance situation is one in which the animal must cross a barrier or press a lever, for example, in order to avoid being punished (usually by an electric shock). In the passive avoidance situation the animal must refrain from some activity such as walking or drinking for a period of time, in order to avoid punishment. Animals with stria medullaris or habenular lesions show a deficiency in passive avoidance and enhancement of active avoidance (Neilson & McIver, 1966; Van Hoesen et al, 1969). The changes in behavior have been interpreted as evidence for an inability to suppress motor activity, and Van Hoesen's group reports that rats after habenular lesions are "extremely active... exploring and continually investigating in all test situations ... and

in the home cage," though rats with septal lesions were immobile for long periods.

Obstinate progression

Bailey and Davis in 1942 reported a syndrome of 'obstinate progression' in cats with lesions mainly in the IPN region. Upon recovery from the anesthetic the animal would progress forward, turning aside for no obstacle, thus harming itself. "If he is on a table top he will walk directly ahead beyond the end and fall sprawling to the floor." The obstinate progression continued until the animal died, usually after three days. Mettler and Mettler (1941) reported a similar syndrome of obstinate progression after lesions of the head of the caudate, and in 1945 Mettler described a direct pathway from the globus pallidus to the IPN region in primates, which he suggested was inhibitory to motor movements. Nauta and Mehler (1966) described degeneration in the lateral habenula of the monkey following pallidal lesions. Although such a direct path has not been confirmed in other species, Gahm and Sutin (1968) report an inhibitory influence on the ventral tegmental area by pallidal stimulation in the cat. Herrick (1948) suggested that the IPN may also be an inhibitory motor centre and that the obstinate progression is a release phenomenon (disinhibition). The dorsal tegmental nuclei receive efferents from the IPN (Ganser 1882) and lesions here and in its efferent pathway, the dorsal longitudinal fasciculus (DLF), in cats produce very inactive animals that lie "inert" and "silent" (Bailey & Davis 1942). Herrick (1948) postulated that the DLF acts

as a nonspecific facilitating influence upon lower motor centres. IPN destruction thus removes inhibition of the DLF and causes obstinate progression, whereas DLF lesions remove tonic motor facilitation and so result in general flaccidity. Kabat (1936) found that stimulation of DLF fibres induced an increase in rate and amplitude of respiration (facilitation), while stimulation of the habenula or IPN brought about shallower and slower breathing (suppression). The last effect, although in agreement with Herrick's scheme, is contrary to the findings of Cragg mentioned above (Cragg, 1961). Thompson and Rich (1961), using a more refined technique, (the lesions of Bailey & Davis (1942) were very large), found that lesions confined to the IPN initially produced a syndrome of obstinate progression in rats, but after three weeks the animals showed no remaining behavioral disorders. They have confirmed these findings in cats and monkeys (Thompson & Myers, 1971).

Quinine aversion

Donovick et al (1970) also propose that the IPN is inhibitory on the basis of studies of quinine aversion in rats. Rats with lesions in the post-supracommissural septum or habenula rejected drinking water containing quinine in a lower concentration than that accepted by controls, but lesions of the IPN decreased sensitivity to quinine. Donovick theorizes that ingestion of a bitter substance such as quinine, is stopped by inhibitory impulses from the IPN. As the animal becomes thirstier and the drinking 'drive' increases, the inhibitory outflow from the hypothalamus (Raisman, 1969) into the septum is reduced, thus altering septal activity. This change in septal activity is

reflected in the initiation of hippocampal theta activity (Routtenberg, 1968) as a component of the orienting response. Increased output from the posterior supracommissural septum drives the habenula, which via the fasciculus retroflexus inhibits the IPN and therefore disinhibits the efferent components of drive. The authors expect that the latter effect is mediated through IPN connections with the DLF, but they did not record from any of these brain sites, or report relevant work of others. I am unaware of any evidence in the literature to the effect that the septum "drives the habenula" or that the habenula "inhibits the IPN" via the fasciculus retroflexus.

Electrophysiological studies

The literature lacks electrophysiological studies of the habenulo-interpeduncular pathway. Sutin and co-workers (Trembly & Sutin, 1962; Wells & Sutin, 1963; Gahm & Sutin, 1968) have studied with macroelectrodes the electrical activity of the ventral tegmental area of Tsai (VTA) adjacent to the IPN. They have described a spontaneous bilaterally-synchronous, oscillating slow wave activity in the VTA. Similar activity, phase-related to the recorded from the VTA, is seen in the lateral habenular, entopeduncular and subthalamic nuclei. The authors have proposed a circuit which mediates this activity, part of which closely follows the habenulo-interpeduncular tract. As reviewed in an earlier section, Nauta (1958) has described a habenulo-tegmental tract which originates in the lateral habenula and terminates in the VTA, but not in the IPN. Presumably this forms part of the pathway of Sutin et al.

Intracranial stimulation: reward and punishment systems

Electrical stimulation of certain regions of the brain has a similar effect on an animal's behavior as external reward (positive reinforcement) or punishment (negative reinforcement), perhaps because the same neural systems are involved in both cases (Olds & Milner, 1954; Delgado et al, 1954; McCleary & Moore, 1965). In some of these experiments the animal can stimulate parts of his own brain through intracranial electrodes. Sites at which negative reinforcement is obtained are mainly in the brainstem-thalamic reticular system, whereas sites of positive reinforcement are mostly located in the limbic system, closely following the course of the median forebrain bundle (Olds & Olds, 1963). The neocortex is neutral. The work published so far indicates that the various areas from which self-stimulation (positive reinforcement) can be elicited form a system, since lesions in one area often influence responses elicited from another area, but no clear picture has emerged of the overall organization of this system (Asdourian et al,1966; Boyd & Gardner , 1967). "In particular, it seems not unlikely that a pathway through the stria medullaris and habenulo-interpeduncular tracts is involved in septal self-stimulation." (Boyd & Celso 1970). Initially it was reported that very high rates of self-stimulation occurred with electrodes in the IPN (Olds 1958), but further experimentation has led to the conclusion that the high rates were due to activation of adjacent fibres of the medial forebrain bundle (J. Olds, 1970 personal communication).

Learning and memory

The appropriate use of reinforcement can promote the learning of associations between stimuli and can condition animals to behave in a predictable way. Neural correlates of such conditioning are of interest because they presumably underlie the observed behavior. Olds et al (1969) studied anticipatory behavior of single units in rats with chronically implanted electrodes. The animals were trained to remain motionless for a certain time in order to receive food. Once the animals were trained, the firing rates of cerebral units were examined during this waiting period. Units were found in the IPN, the dorsal reticular formation and the thalamus which reliably showed an increase in firing rate prior to reinforcement. Kamikawa (1964) also showed conditioning in single thalamic and habenular units. In this case the conditioned stimulus was a light flash, while the unconditioned stimulus was a shock to the foot. The habenular unit initially showed no change of firing rate in response to the light flash, but the shock decreased the rate. After 50 to 75 pairings of the flash and the shock the unit responded to the flash with a cessation of firing. The previously neutral flash appeared to have acquired the negative properties of the shock via some learning process. Psychologists often claim such experimental evidence is the neural substrate of learning, but the evidence in no way reveals a mechanism for the change of unit activity.

Lewis and Shute (1967) suggest that cholinergic neurons receiving efferents from the hippocampus (their groups 2a and 2c) may be involved in the putative role of the hippocampus in memory

(Penfield & Milner 1958; Thompson et al 1961, 1964; Flexner et al 1964; Nielson et al 1965). A cholinergic element is suggested by the fact that atropine induces in man impairment of memory, loss of attention, drowsiness, and a decrease in spontaneous speech (Ostfield et al 1960). These symptoms also appear to indicate a partial loss of consciousness.

Consciousness, sleep, and cholinergic pathways

A fundamental psychological assumption is that some degree of consciousness or awareness is essential for most adaptive behavioral events (McCleary & Moore, 1965). The ascending reticular system forms a polysynaptic path through the core of the brainstem, receives direct projections and collateral fibres from the specific sensory systems, and makes synaptic connections with cells in the diencephalon which project diffusely on the cerebral cortex (French, 1960 and others). It also receives a large afferent input from the cortex and other forebrain structures. Moruzzi and Magoun (1949) first showed that stimulation of the midbrain reticular formation in the sleeping cat produced both behavioral and EEG evidence of wakefulness, indistinguishable from the effects of arousing the cat by a sudden sound. This effect is not due to stimulation of specific sensory systems travelling through the brainstem, but rather to direct stimulation of the reticular formation. The histochemical studies of Shute and Lewis (1967) have demonstrated that the reticular formation has cholinesterase-containing neurones which may be cholinergic. They have also shown (Lewis & Shute 1967) that a major output from the (limbic) hippocampus through links with the ascending cholinergic reticular system, such as the habenulo-

interpeduncular pathway, may possibly reach the lateral cortex of the cerebral hemispheres, where it could influence cortical electrical activity as in arousal. Green and Arduini (1954) noted that changes in hippocampal theta rhythm preceded neocortical EEG changes during behavioral transitions; Lewis and Shute (1967) interpret this as evidence for limbic influences on arousal mediated by their cholinergic pathways.

There has been other evidence for the role of limbic pathways in mechanisms of wakefulness and sleep, from experiments utilizing ablations and stimulation, either electrical or with acetylcholine. Nauta's (1958) description of limbic midbrain pathways mentioned diffuse projections of the lateral portion of the medial forebrain bundle in regions occupied by the ascending reticular activating system. He suggested that experimental or inflammatory lesions in or immediately behind the caudal hypothalamus lead to drowsy or sleep-like states (Von Economo 1918; Ranson 1939; Nauta 1946; Collins 1954; and others) by interferring with phasic descending excitatory discharge from the hypothalamus into the reticular formation.

Jouvet (1962) implicated the ascending limb of Nauta's (1958) limbic midbrain circuit in the electrical patterns occurring in deep sleep. Jouvet found that lesions limited to the midbrain region receiving limbic projections would suppress both the neocortical desynchronization and the hippocampal theta rhythm of deep sleep, leaving unaffected the associated fall in postural tone, the rapid eye movements, and other behavioral signs. These observations are

supported by the results of stimulation experiments in the cat (Jouvet 1962) and the rabbit (Faure et al 1962). The latter authors particularly emphasize that episodes of deep sleep are precipitated by stimulation along pontomesencephalic projections to the limbic system. Hernandez-Peon et al (1963) have also implicated the limbic midbrain circuit in the induction of sleep. They report that intracerebral implants of crystals of acetylcholine, with or without eserine, and carbachol elicited light and deep sleep from a pathway extending from the upper medial preoptic area into the medial pontine tegmentum along the medial forebrain bundle, the IPN, and the dorsal and ventral tegmental nuclei. These authors, unlike Jouvet, maintain that descending rather than ascending connections from forebrain to midbrain are involved in the induction of sleep. In contrast to both groups, Carli et al (1965) report that in cats interruption at different levels of either the ascending or descending limb of the limbic midbrain circuit does not prevent any of the electrographic aspects of deep sleep, showing these connections to be non-essential for deep sleep. It is unclear why these various authors disagree, but there is broad scope for varying interpretation of EEG recordings and behavior in animals, especially in those with extensive traumatic lesions. However the Carli studies seem to have been done carefully and thoroughly, and are perhaps less equivocal than those of Jouvet or Hernandez-Peon.

Summary

Studies of comparative anatomy show that the habenulo-interpeduncular complex is well-represented in all vertebrates, from the most primitive cyclostomes to primates such as man. This constancy implies that its function must be of some general importance. Neuroanatomical studies have established the membership of the habenulo-interpeduncular complex in the limbic system, linked strongly with the septum, hippocampus and preoptic area. Histochemical techniques have revealed that many limbic structures and interconnecting fibre tracts, including the fasciculus retroflexus, stain for cholinesterase, suggesting that these pathways may be cholinergic.

A survey of limbic system physiology has revealed its involvement in autonomic activities, somatic movements, reinforcement, learning and memory, emotional aspects of behavior, and arousal. The evidence for the importance of the limbic midbrain area, and its possibly cholinergic pathways, in mechanisms of sleep, is in dispute. Gloor (1960) has emphasized that though limbic structures are clearly able to influence parts of the brain involved with visceral adjustments and body movements, they appear not to contain cells that normally initiate such responses. (Lesions in the limbic system do not cause obvious motor or autonomic deficits, as might be expected on the basis of results of limbic stimulation.). Rather, through inhibition and facilitation of these autonomic and motor systems, limbic structures may select reponses appropriate to the

immediate needs. Physiological experiments, however, do not suggest under which psychological circumstances this response selection is active.

Selection of responses by limbic structures is also implied by studies (reviewed above) which show that stimulation at certain sites in the brain mimics the effects of externally presented reward (positive reinforcement) or punishment (negative reinforcement), perhaps because the same neural substrates are involved. Sites at which positive reinforcement is obtained lie mainly within the limbic system, especially along the path of the medial forebrain bundle. Reward tends to increase, and punishment to decrease, the probability of recurrence of the behavior it follows. In this way the activity of limbic structures appears to facilitate the connections between stimuli and responses (learning and memory), and to select responses, but the neural mechanisms of reinforcement are not understood. The fact that the rewarding effects of stimulation are not abolished by large and varied lesions throughout the limbic system and midbrain indicates that there is massive redundancy of these pathways, and possibly also a plasticity which provides a basis for reorganization (c.f. Valenstein, 1968).

The precise role of the limbic system in emotional aspects of behavior, and in arousal, is not clear. Disruption of limbic circuits leads to emotional disorders: changes in temperament, insight, etc., but the effects are complex, and cannot be simply explained.

The hippocampal theta rhythm appears to be an index of arousal or wakefulness, and several authors (Green & Arduini, 1954; Lewis & Shute, 1967) have suggested that changes in hippocampal waves precede and perhaps influence neocortical arousal levels. The role of the limbic system in arousal may be important because of the connections of the hippocampus on the one hand with limbic structures mediating reinforcement, emotional aspects of behavior, and homeostatic mechanisms, and, on the other hand with structures of the ascending reticular activating system (the cholinergic ventral tegmental pathway of Shute & Lewis, 1967). These connections might provide a basis for changes in arousal level due to emotion, learned associations, or physiological needs.

There have been few psychological or physiological investigations specifically involving manipulations of the habenulo-interpeduncular (Hb-IPN) complex. None of these experiments provides clear evidence for the precise relation between Hb-IPN activity and the observed behavior. In view of the many interconnections between limbic structures the effects reported may be secondary, or only very loosely related to the stimulation or lesion of the Hb-IPN complex. For example, experimental manipulations may interfere with hippocampal, septal, or hypothalamic outflows relayed through the habenula, rather than with some function wholly localized in the Hb-IPN complex. The data do not allow definite conclusions. On the other hand, in view of the many connections between limbic

structures, deficits after Hb-IPN lesions may be small or non-existent because of duplication of function, or a plasticity which allows Hb-IPN functions to be assumed by other limbic structures.

Many of the findings reported by various workers (see Table 1) are consistent with an inhibitory outflow upon motor activity from the Hb-IPN system. This was suggested by Herrick (1948) on the basis of the obstinate progression syndrome in cats with lesions of the IPN. This syndrome was presumed to be the result of removal of inhibition of motor systems, mediated by the IPN. Similarly, an inability to suppress a response tendency after lesions in the Hb-IPN may account for increased exploratory behavior, decreased rejection of water containing quinine by thirsty rats, and poor performance on passive avoidance tests.

It is possible that the IPN may mediate several different functions. This is suggested by divisions of the nucleus into several regions by cytoarchetectonic (Cajal, 1911; Berman & Bowers, 1967; Ives, 1971), and histochemical techniques (reviewed above, pp.12- 16). Also, workers have implicated the Hb-IPN system in diverse functions (endocrine, visceral, motor, visual, learning, reinforcement, arousal).

The Hb-IPN pathway appears to be one route for efferent outflow from the limbic system; it seems to influence motor and visceral centres in the lower brainstem and spinal cord, and also structures of the ascending reticular activating system (ARAS).

Table 1 Classification of experiments in the literature

Procedure	Effect	Source
Stimulation of the IPN	decrease in respiration	Kabat, 1936
	increase in respiration and heat loss	Cragg, 1959; 1961
	positive reinforcement	Olds & Olds, 1963
	sleep	Faure, 1962
	no sleep	Rossi et al, 1961
Stimulation of the habenula	decrease in respiration	Kabat, 1936
	increase in respiration and heat loss	Cragg, 1959; 1961
	weak positive reinforcement	Olds & Olds, 1963
	'fright-like' reaction, with freezing, arching of the back, vocalization	Reinert, 1963
Lesion of the IPN	obstinate progression (release from motor inhibition ?)	Bailey & Davis, 1942 Herrick, 1948
	decreased rejection of quinine	Donovick et al, 1970
	block of ovulation	Critchlow, 1958; Slusher et al, 1959
	block of electrographic signs of sleep	Jouvet, 1962
	no block of electrographic signs of sleep	Carli et al, 1965
	deficits in brightness discrimination (in rat, not monkey)	Craddock & Thompson, 1971
Lesion of the habenula	increased rejection of quinine	Donovick et al, 1970
	deficits in passive avoidance	Brady & Nauta, 1955;
	enhanced active avoidance	Von Hoesen et al, 1969
	enhanced exploratory behavior	Davis et al, 1966
	increased thyroid-pitutary response to cold	Mess, 1964 Kovacs et al, 1966
Implants of ACh in the brainstem	sleep (blocked by atropine)	Velluti & Hernandez-Peon, 1963; Cordeau et al, 1963
Other	Neural substrates of conditioned learning (?) IPN	Olds et al, 1969
	habenula	Kamikawa et al, 1964

Neuroanatomical tracing of the lower centres which receive efferents from the IPN is difficult because the route is polysynaptic; Lewis and Shute (1967) however, have succeeded in demonstrating by cholinesterase-staining, pathways from limbic structures to the IPN, and pathways from the IPN to the ARAS, whose fibres also contain cholinesterase. In this context the Hb-IPN pathway may be a gate or modulator of limbic effects on motor, visceral, or affective behavior, and limbic influences upon arousal.

The literature lacks electrophysiological studies of the Hb-IPN pathway. It was therefore decided to conduct experiments utilizing macro- and microelectrodes, in order to record the electrophysiological characteristics of the pathway and of IPN cells, particularly with reference to habenular stimulation.

The histochemical data also pose some questions. The habenula, the IPN, and the fasciculus retroflexus have an extremely high level of cholinesterase activity, and the IPN contains choline acetylase in very high concentration. It seems likely that the habenulo-interpeduncular pathway might be cholinergic, which should be reflected in a high IPN neuronal sensitivity to acetylcholine. Hence it was decided to test the sensitivity of IPN units to micro-iontophoretically applied acetylcholine and some related compounds.

In conclusion, although the habenulo-interpeduncular system is anatomically and histochemically prominent, little is known of its electrophysiological or neuropharmacological properties. This motivated the studies to be described.

MATERIALS AND METHODS

Preparation

The habenulo-interpeduncular pathway was studied electrophysiologically in 58 cats (2.3 - 3.0 kg, either sex), using various anesthetics and recording techniques. Twelve cats were anesthetized with α-chloralose (Sigma Corp.), 80 mg/kg given intravenously (femoral vein) after induction with ethyl chloride and ether; 25 cats were anesthetized with Dial compound (diallyl barbiturate acid, monoethyl urea, ethyl urethane, Ciba Co. Ltd.) 0.6-0.7 ml/kg given intraperitoneally; and 21 cats were anesthetized first with ether for the duration of the surgery, and then switched to a combination of oxygen, nitrous oxide, and halothane (Ayerst) or methoxyflurane (Abbott). The blood pressure was monitored via a catheter in the femoral artery and was in the range of 90 to 160 mm Hg. In the later hours of many experiments an intravenous drip (about 0.5 ml/min) of 0.15 M

sodium chloride was given to maintain the blood pressure. In several experiments 10-20 ml Substosan (Poulenc Co.), a plasma expander, were given to replace lost blood. The rectal temperature of the cat was automatically maintained at 37-38°C by means of a heating pad, a rectal thermistor probe, and a regulating circuit. A tracheotomy was done in all cases, and the animals breathed spontaneously.

The head was fixed in a stereotaxic holder for the remainder of the surgery and during the experiment. To allow the placement of electrodes in the habenula (A 8.0, L 0.5, H +5 - +6.0 mm) a portion of the parietal skull was removed bilaterally and across the midline sinus (see Figure 6). The dura was incised and trimmed as closely as possible to the midline venous sinus to prevent deflection of the electrodes by resistant membrane. Bacto agar (Difco), 4% in 0.15 M NaCl, was poured into the area from which the skull had been removed, and allowed to harden. This agar plug was removed a few hours later when the stimulating electrodes were introduced. It served to prevent brain swelling and therefore allowed more accurate placement of electrodes. A chlorided silver plate wrapped in gauze soaked in saline was sewn into the muscles of the neck with a connecting lead to be used as the indifferent or ground lead when recording.

The headholder and the animal were then turned through 180° so that the animal lay on its back, its body supported by a cloth sling. The alignment of head, neck, and shoulders was done with care, to preserve the natural flexion and to avoid venous compression. The lower limbs were placed at a level somewhat higher

than the heart and head to aid venous return.

A path for the ventral approach of electrodes to the IPN (see Figure 6) was prepared as follows. The mandible was exposed and cleaned for a short segment on each side in front of the articulation. It was then transected on both sides, using a rib cutter, and care was taken to coat the stumps with bone wax. A ligature was tied around the chin, and by means of slight tension, the jaw was retracted in a caudal direction over the chest. A tight ligature was placed around the base of the tongue and it too was pulled caudally. The basi-sphenoid bone and the bullae were exposed by an incision through the palate. Bleeding was stopped by pressure and cautery.

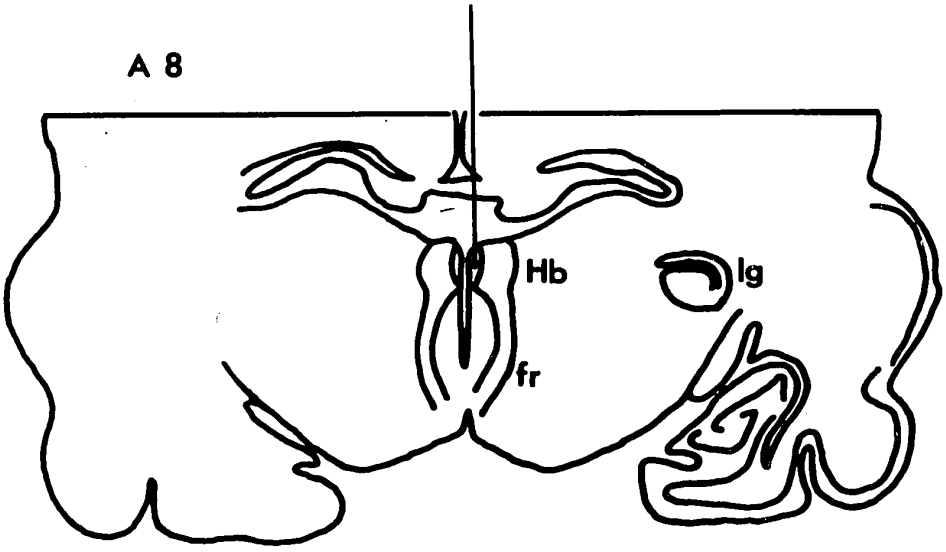
Four small (2-3 mm) holes were made with a hand drill into the porous bone overlying the interpeduncular fossa, through the dense layer of bone into the sinuses, but not entirely through to the underlying brain stem. A mixture of about 1/3 vaseline and 2/3 plasticine in a 2 ml syringe was warmed in a beaker of hot water (approximately 90°C). About 0.1 ml of the warmed mixture was injected into the sinuses via the drilled holes, to create a bloodless field. (This technique was based on one suggested by G. Mandl, personal communication). This injection was essential to the procedure, as any attempt to remove the bone without preinjection of plasticine resulted in a serious blood loss.

After opening the second (or deeper) layer of dense bone, the dura was incised under microscopic control in the midline and at various other points and reflected to the edges of the bone. Whenever

Figure 6 Sites of electrode placement: projection drawings and photomicrographs

Projection drawings (outlines) of histological sections of a cat brain at two anterior planes, X 4. Upper, shows location of electrode in medial habenula nucleus. Lower shows location of electrode in the interpeduncular nucleus. Photomicrographs : Upper shows Prussian blue mark and track through the medial habenula (X 25). Lower left shows Prussian blue mark and track in the interpeduncular nucleus (X 25). Lower right, arrow indicates mark in interpeduncular nucleus made by a HCl lesion (X 63).

cg	central gray	IPN	interpeduncular nucleus
fr	fasciculus retroflexus	lg	lateral geniculate body
Hb	habenular nuclei	rn	red nucleus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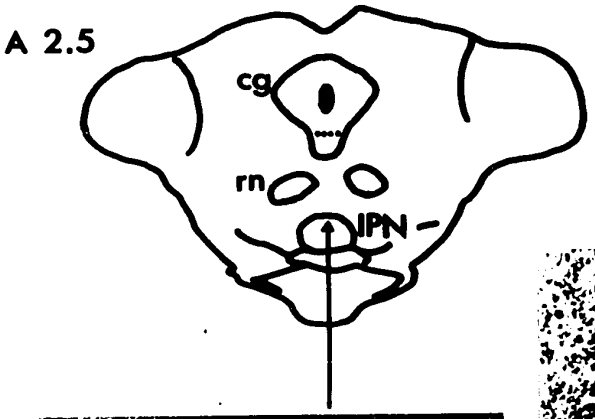
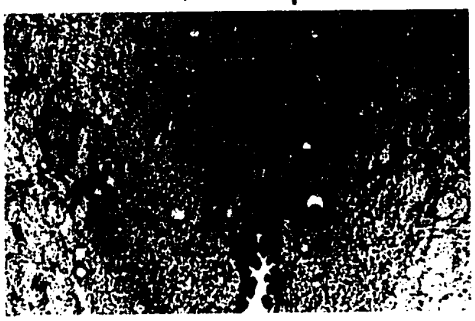
Hb
Ig
fr


A 2.5
cg
rn
IPN



possible the pia was raised with fine forceps and pierced at several locations along the midline (sometimes the course of the basilar artery prevented this), to allow penetrations by the microelectrodes with minimal damage to their tips or "dimpling" of the brain surface. The abundant flow of cerebrospinal fluid (CSF) over the exposed region prevented any drying of the tissue. The amount of spontaneous CSF release was so great that to improve visibility it was necessary to periodically suck-off fluid.

On some occasions the superficial radial nerve was prepared for stimulation. It was dissected for a short length from the surrounding tissue, cut, and the central portion placed on bipolar platinum electrodes. To prevent desiccation, the exposed portion of the nerve was coated with a mineral oil- vaseline mixture, except at the points of electrode contact. For several experiments the olfactory tract was exposed and the overlying dura removed to allow the placement of bipolar stimulating electrodes. In other experiments the effects of peripheral stimulation were studied by inserting steel needle electrodes into some or all of the paws, or the skin of the face.

Stimulation

A Grass S-8 stimulator with radiofrequency stimulus isolation units was used to deliver to the habenula, or other sites, square pulses 0.01 to 0.1 ms wide with an amplitude of 0.1 to 15 volts. On a few occasions the voltage was increased to 25 or 30 volts to study higher threshold events. The usual stimulation frequency was 1 Hz, but two pulses at various intervals, and high frequency trains

of stimuli were also used. Diodes were placed in series with the stimulating leads to minimize distortion of the traces by stimulus artefacts.

Small bipolar stainless steel electrodes (David Kopf) were inserted into the brain stereotaxically to stimulate the habenular region. The vertical separation of the terminal leads was 0.3 to 0.5 mm, and the tip size was 120-140 μ . These electrodes had a DC resistance of 50 KΩ between the two leads with the tip in 0.15 M NaCl, but their capacitance gave an effective resistance of only 9-20 KΩ when tested with a 0.1 ms pulse. These electrodes were also used to record gross potentials in the habenula and the interpeduncular nucleus.

Recording

Several types of recording electrode were used in these studies, including the stainless steel concentric bipolar electrodes mentioned above. These electrodes were also used in a monopolar fashion by recording between one of the leads and the indifferent lead in the neck nuscles. In several experiments, to obtain more localized recording, the tip of the electrode was ground back with a fine stone to produce a lead separation of 0.1 mm.

The following types of electrode were used for extracellular recording of single units: (a) tungsten wire in glass, (b) varnished tungsten wire, (c) varnished stainless steel wire or insect pins and (d) glass micropipettes (single or multi-barrelled).

(a) Tungsten wire in glass. These were prepared by a method close to that described by Schwartz (1965), in which an electrolytically

polished fine tunsten wire is threaded through a tapered glass capillary tube (tip diameter 2μ) and fixed in place so that the wire protrudes 2-5μ (under microscopic control). The preparation of these electrodes is quite time-consuming, but they can be made in quantity and stored for long periods until use.

(b) Varnished tunsten wire. Using thicker tungsten wire and the method of Hubel (1957), electrodes were prepared with several insulating coats of varnish (Formvar, General Electric Co.). These electrodes are easier to prepare, but they have the common disadvantage that they cannot readily be used to mark the position of the tips in the tissue, although the anodal lesions could occasionally be found (see below).

(c) Varnished stainless steel. In order to overcome the problem of marking with tungsten, microelectrodes were made from stainless steel insect needles (Clay Adams) or spring wire (Donald Rope and Wire Ltd.). The needles (or wire) were first sharpened and polished electrolytically in concentrated hydrochloric acid, using about 7-10 volts and direct current (the needle being the anode). The sharpened electrode was then coated with several layers of varnish (Formvar, General Electric Co.). These electrodes had tips of 1-3μ in diameter, and resistances of 8-20 MΩ at 60 Hz, with the tips in 0.15 M NaCl. Prussian blue marks were easily made and located (see Marking Techniques, below).

(d) Glass micropipettes. These were made in the conventional manner, using a Narishige Vertical Puller and Kimax glass capillary tubes, (ID 0.09-1.1 mm), or other (Wesley Coe Ltd., England) 5-barrel

blanks. Double or triple-barrelled pipettes were assembled from single capillary tubes fused with epoxy glue before pulling. In the restricted region of access to the IPN (working through the mouth of the cat), the double and triple-barrelled pipettes were easier to manipulate than the 5-barrelled ones. Also they caused less tissue damage while approaching the recording site, 3-5 mm deep to the ventral surface of the brain. Their recording properties, the flexibility of their shafts, and their small tip size made them more useful for obtaining data from the small cells of the IPN. The disadvantage, however, of too few barrels for test substances in iontophoretic studies, necessitated the use of the bulkier 5-barrelled micropipettes.

The recording barrel was filled with 2 or 3 M NaCl, or 6% Alcian blue dye in 1 M sodium acetate (Lee et al, 1967). The pipettes had tip diameters of 1-8 μ, and resistances (at 60 Hz, with tips in 0.15 M NaCl) ranging from 3-12 MΩ.

Microelectrodes were inserted into the brain under microscopic control. Potentials were led from the recording electrode to a Tektronix 122 preamplifier (time constant 20 ms), preceded by a cathode follower stage when microelectrodes were used. The output from the preamplifier was split before going to a Tektronix 565 oscilloscope. One part was led directly into a Tektronix 2A63 plug-in unit and amplified to allow recording of slow wave activity. A second part was filtered (time constant 2 ms) before entering a Tektronix 3A3 plug-in unit (high band-pass, 800 Hz to 5 KHz). This excluded most slow wave activity and made spike activity more evident. On occasions a beam brightener was used on this beam. The vertical output of this channel was monitored by a loudspeaker-ratemeter unit (Ferch Electronics) and was also directed

into a neurophysiological digital computer (Burns, Mandl and Ferch, 1965), which performed post-stimulus histogram analyses. Records of the ratemeter or computer output were obtained on either a Texas Instruments oscillograph writer or a Grass Model 7 polygraph. A Grass Kymograph camera was used to record the oscilloscope display on high contrast film.

Iontophoresis

Intravenous administrations of drugs are limited in usefulness because of the uncertainty of the site of drug action. In addition, the drug may have difficulty penetrating the blood-brain barrier, or may have deleterious effects on the condition of the animal; hence local microiontophoresis is often the method of choice (Curtis & Eccles, 1958; Krnjevic & Phillis, 1963; Curtis, 1964).

For this technique multibarrel glass micropipettes are filled, by diffusion or centrifugation, with concentrated solutions of the ionized substance(s) to be studied. At least one barrel filled with 2 or 3 M NaCl (or 6% Alcian blue dye in 1 M sodium acetate in some of the present experiments), is retained for recording. If a voltage of the appropriate polarity is applied to a drug-containing barrel, drug ions are ejected from the tip of the pipette, and any effects on cellular activity can be monitored by the recording barrel. The amount of drug ejected depends on the intensity of the current and its duration. Certain precautions, such as the use of retaining voltages to prevent drug leakage from the barrels by diffusion, and the use of control sodium or chloride

currents to assess the effects of anodal or cathodal currents involved in drug ejection, increase the validity of the technique. The drugs tested in this study are noted below, and were used in 1.0 molar concentration.

Table 2 Drugs used in iontophoretic studies

Drug	Source
Acetylcholine chloride (pH 4)	British Drug House
Acetyl-β-methylcholine chloride	Pierce Chemical Co.
Carbamyl choline (carbachol)	Mann Research Labs
Edrophonium chloride (Tensilon)	Hoffman Laroche Ltd.
Neostigmine bromide	Mann Research Labs
Sodium L-glutamate (pH 7)	Matheson, Coleman, & Bell
Gamma-amino butyric acid (GABA) (pH 4)	Montreal Fine Chemicals

Marking techniques

An important adjunct to the stereotaxic method is the verification, at the termination of an experiment, of the position of the electrode tip in the brain. When tungsten microelectrodes were used, an anodal lesion was made at one point in the track by passing 0.4 mA for 1 second, or 0.1 mA for 5 seconds. It was often quite difficult to find these marks, possibly because coagulated tissue was withdrawn with the electrode. Another disadvantage of this technique is that passing such a large amount of current usually damaged the tip and rendered it unfit for further recording.

Location of marks made at the tips of stainless steel electrodes, either the gross bipolar ones or microelectrodes, is somewhat easier. The passage of anodal current deposits in the tissue iron particles, which on subsequent chemical treatment (see below) produce bright blue spots of Prussian blue (ferric ferrocyanide) or Turnbull's blue (ferrous ferrocyanide). The production of these marks is more reliable, but the marks are rather large, and often the microelectrode tip is distorted.

When glass micropipettes were used the position of the tip was marked by Alcian blue dye (Lee et al, 1967) or hydrochloric acid lesions (McCance & Phillis, 1965). About two-thirds of marking attempts with these techniques were successful.

Examples of marks made by some of these techniques are shown in Figure 6.

Histology and reconstruction

At the end of the experiment the cat was killed by an overdose of intravenous barbiturate, magnesium sulphate, or air. The brain was immediately perfused, either through the heart, with the descending aorta tied off, or more usually, through both carotid arteries. First about 250 ml of 0.15 M NaCl were perfused in 1-2 minutes, then 250 ml of 10% formol saline in which 2-3 gm of potassium ferrocyanide had been disolved. Sometimes the solutions recommended by Green et al (1962) were used instead of the simpler mixture, but no differences were noted in the histology after each method. The animal was left in the stereotaxic frame for approximately eight hours, during which time the brain hardened. A scalpel blade mounted in the electrode carrier was used to cut out the relevant

block of tissue. In this way it was quite easy to obtain sections in or close to the plane of the electrode tracks. Frozen sections (50-75 μ ; Reichert microtome) were mounted in series before staining with cresyl violet. The stained sections were examined under the microscope and then projected onto drawing paper by means of a photographic enlarger. The outline of the slice and recognizable nuclear groups, as well as the marks and electrode tracks, were traced out. The electrode tracks were reconstructed in accordance with the written experimental protocol and stereotaxic atlases of the brain (Jasper & Ajmone-Marsan, 1954; Snider & Niemer, 1961; Berman, 1968) to make correlations between the anatomical and electrophysiological or iontophoretic data.

RESULTS

Spontaneous activity: Gross

In cats under various anesthetics (chloralose, Dial, halothane or methoxyflurane with nitrous oxide), slow wave activity can be recorded from both the habenula and the IPN, with either macro- or microelectrodes. The amplitude of these waves is usually 100-400 microvolts (or even more), and their polarity is mainly negative. The waves are not correlated with respiration or cardiac activity. The waves recorded with macroelectrodes are sometimes rather irregular and random (see Figure 7 : F & G). When microelectrodes are used to limit the field of recording the waves are nearly always regular in form and rhythm (see Figures 8: C,D; 9: C-F). This suggests that there may be more-or-less independent foci of such waves in the IPN.

'Spindling' waves are observed only rarely (see Figure 9: F). Such spindles may originate from the pyramidal tract, although its minimal distance from the IPN is 1.5-2.0 mm.

Figure 7 Slow wave activity in the IPN, in different cats, as recorded with macroelectrodes.

A, Chloralose anesthesia B, Deep Dial anesthesia C-E Dial supplemented with 4 mg pentobarbital. In D the habenula was stimulated submaximally at 0.5 Hz. In E the superficial radial nerve was stimulated submaximally at 0.5 Hz F-G Deep Dial anesthesia. In G the habenula was stimulated at 0.5 Hz. The time scale is the same for all traces. Arrow heads mark stimulus artefacts. The vertical calibration is 0.1 mV. Negativity is recorded upwards in this and succeeding figures. Time constant of recording is 20 ms.

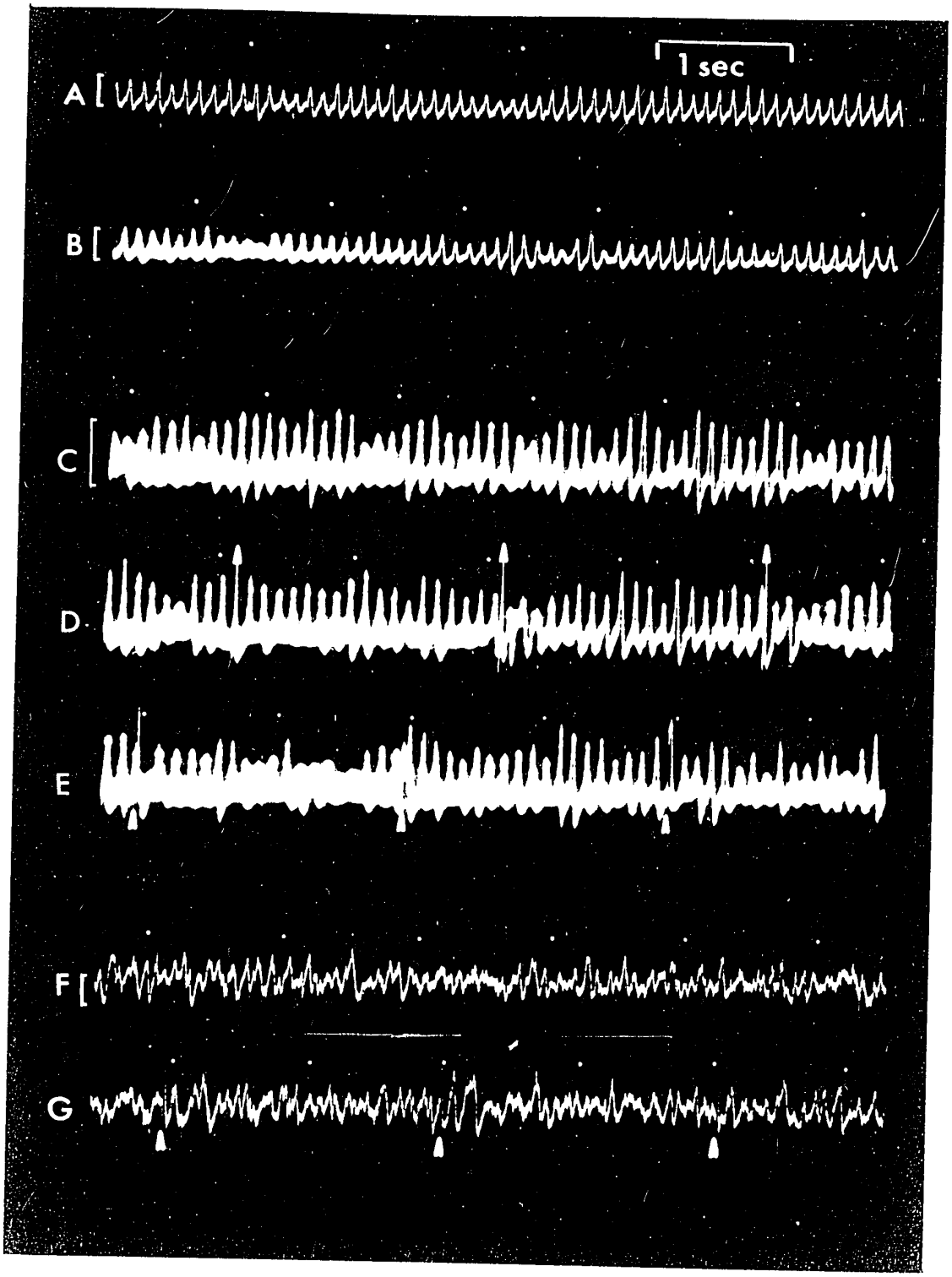

1 sec
A
B
C
D
E
F
G

Figure 8 Slow wave activity in the IPN in different cats under various anesthetics.

A & B are macroelectrode recordings of IPN slow waves on the same time scale in a cat under light Dial anesthesia. In B the habenula was stimulated repetitively at 9.5 Hz. C & D show microelectrode recordings in other cats. The upper trace is recorded via low band pass (80-1000 Hz), the lower trace via high band pass (0.6 - 10 K Hz). In C the cat is under light Dial anesthesia. At the arrow 30 nA L-glutamate is applied continuously by iontophoresis. D is from another cat under methoxyflurane and nitrous oxide anesthesia.

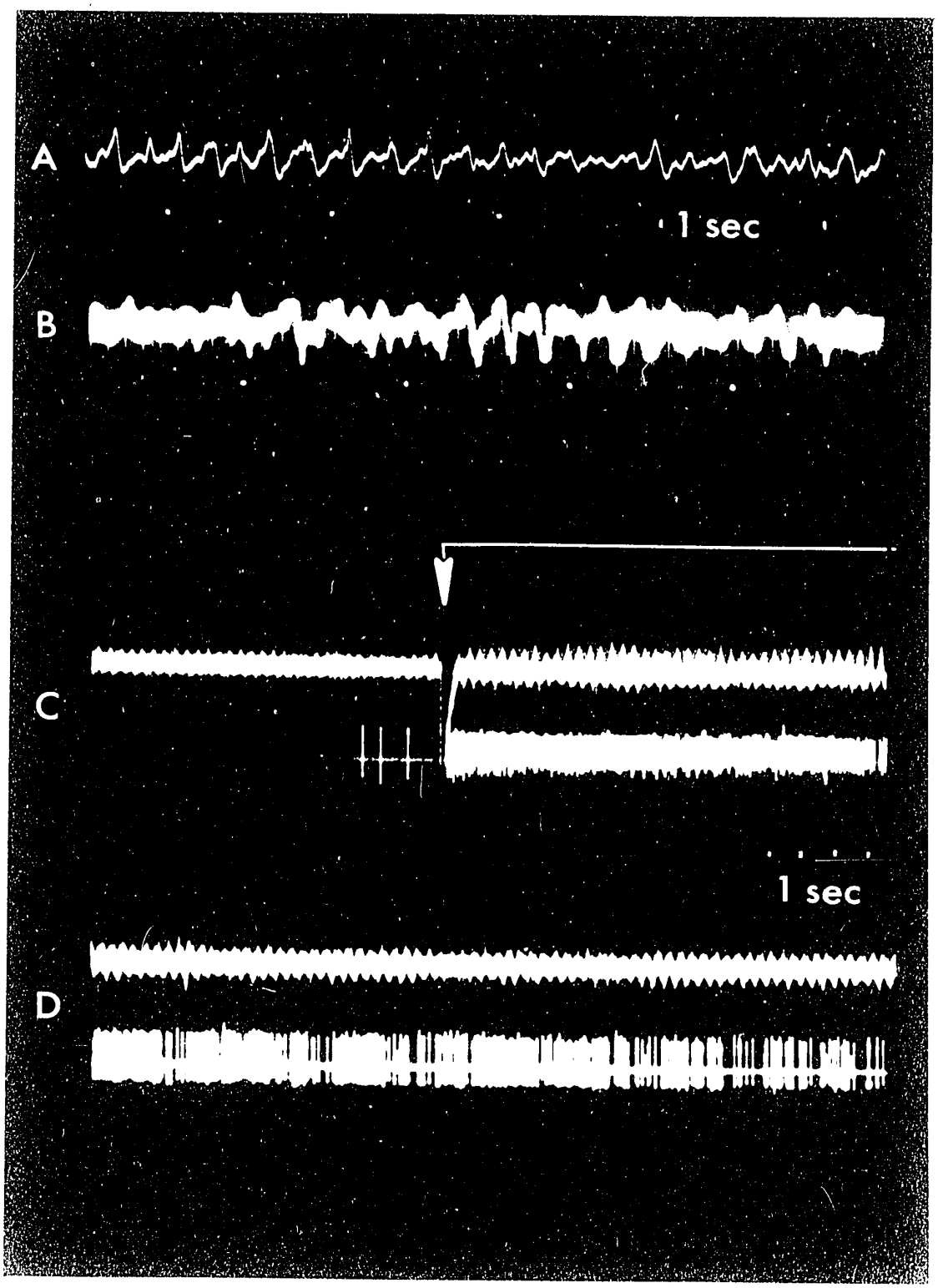
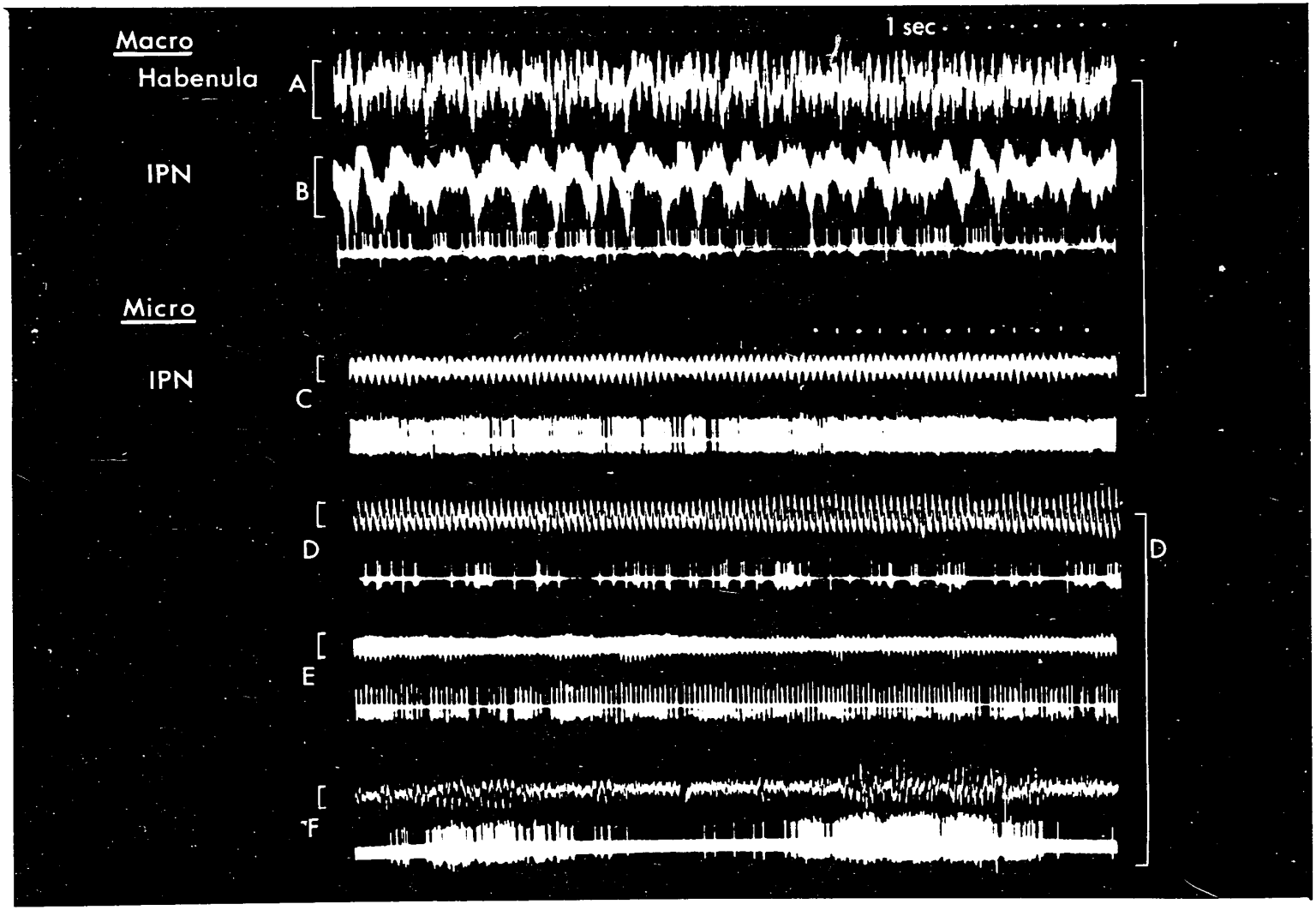

A
1 sec
B
C
1 sec
D

Figure 9 Slow wave activity in the habenula and the IPN

A-B Methoxyflurane anesthesia, microelectrode recording
A. Habenula B. IPN 3 Hz modulated by 0.5 Hz activity
C Halothane anesthesia, microelectrode recording IPN above, low band pass; below, high band pass filter
D-F Light Dial anesthesia; microelectrode recording

Time scales are the same for all traces
Vertical calibration is 0.1 mV


Macro
Habenula
A
IPN
B
1 sec
Micro
IPN
C
D
D
E
F

Anesthetics: effects on wave frequency

Under chloralose or fairly deep barbiturate anesthesia (Dial, 0.7 ml/kg) these waves are quite regular and have a mean frequency of 8-9 Hz, with a range of 7-14 Hz (see Figure 7). Under light barbiturate (Dial, 0.6 ml/kg), or halothane or methoxyflurane with nitrous oxide, the frequency of these spontaneous waves is reduced to 3-4 Hz, but the shape of the waves is the same (see Figures 8 and 9). Most of the experiments were performed under these conditions of anesthesia.

Interjected stimulation

Interjected stimulation at 1 Hz of the habenula, the paws or a peripheral nerve, the olfactory tract or the face, evokes potentials in the IPN, but does not block or reset the rhythm (see Figure 7: D,E,G). Stimulation of the habenula at 1 to 10 Hz also does not block or reset the rhythm. Figure 8 A shows the spontaneous IPN waves. During B the habenula was stimulated repetitively at 9.5 Hz. The last 3 seconds of B show that the 4.5 Hz rhythm remains as in A, although stimulus artefacts are superimposed upon the waves.

Pallidal stimulation

Trembly and Sutin (1962) found in cats under barbiturate that a brief stimulation of the globus pallidus inhibited spontaneous slow wave activity in the ventral tegmental area (adjacent to the IPN) for 300 ms. In the present experiments pallidal stimulation had little or no effect on the IPN spontaneous waves. Bipolar stimulating electrodes were stereotaxically placed in the globus pallidus, according to the atlas of Jasper and Ajmone-Marsan (1954). Pallidal stimulation at 1 Hz,

at low or high intensities, did not block or reset the spontaneous rhythm, although evoked potentials and units were recorded in the IPN.

Effects of barbiturates

In animals under Dial, or chloralose anesthesia, the intravenous administration of small amounts of sodium pentobarbital (2-5 mg total dose), increased the regularity of form of these waves without affecting the frequency. Large doses of pentobarbital (10 mg or more) caused a marked depression of the spontaneous waves in the IPN.

As the recording electrode is advanced out of the IPN about 0.5 to 1 mm towards the central gray, the spontaneous waves diminish and disappear.

Spontaneous activity: Unit

Origin of spikes

Extracellular unit spikes having a duration of 1-2 ms could be recorded with microelectrodes in the IPN. These spikes were probably of mainly cellular origin rather than from fibres, because (a) the signal was dectected for a distance of up to 100 microns, and "held" for long periods (Hubel 1960; Bishop et al 1962), (b) the electrode tip diameters used (1-5 microns) were probably too coarse for any extensive or frequent recording from the small afferent fibres of the fasciculus retroflexus, or from the axons of IPN cells, (c) some units showed repetitive firing which is more typical of postsynaptic responses (cf. Bishop et al 1962), (d) the firing probability (only one half of the units were tested) was increased by L-glutamate, which has not been shown to be excitatory for fibres, and (e) the spikes were mainly negative, or negative-positive,

but not solely positive (cf. Bishop et al 1962). In some cases there are positive spikes of cell origin which are considered due to the location of the electrode at the cell membrane, where the tip inactivates a small portion of membrane. This area never depolarizes, but is a source of current, and hence large positive spikes are recorded (Amassian, 1961; Bishop et al 1962). Recordings of this type are only seen with very fine-tipped microelectrodes.

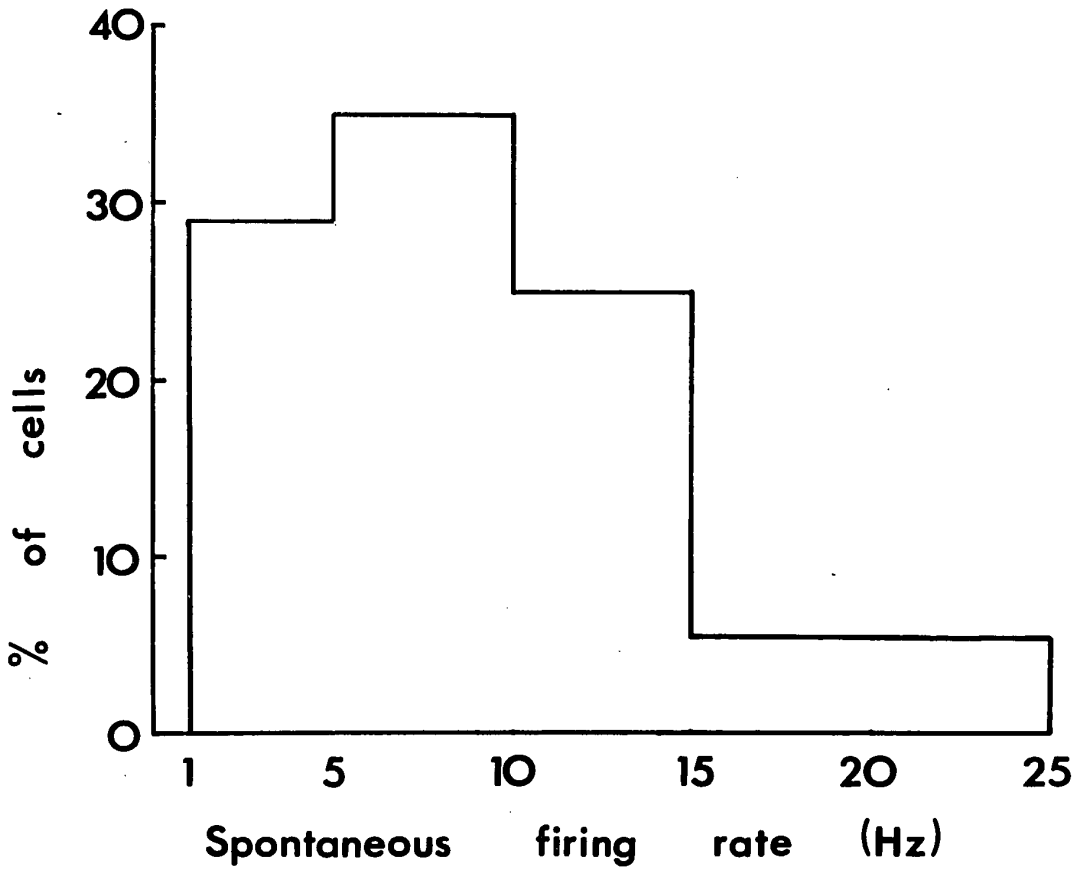
Range of rates of spontaneous firing

In general many IPN units are spontaneously active, at rates from 1-25 Hz, as shown in Figure 10. A few units fire in 'bursts' (e.g. 4-6 spikes at 500 Hz, the bursts being repeated 8-10 times per second), but most units fire regularly or irregularly in trains of single action potentials. The incidence of different rates is similar for cats under Dial or volatile anesthesia.

In initial experiments only a qualitative assessment of spontaneous firing was made. In later experiments the cell firing rate was measured directly with a frequency meter, or a film record of spontaneous firing was made, and the rate counted from that. Some units fired extremely slowly (e.g. less than once every few seconds) and irregularly. These cells were labelled as being spontaneously active. In addition, about two thirds of units which responded with a short burst to a stimulus in the habenula were not spontaneously active. This accounts for the construction of the rates-histogram (Figure 10) from a number of units less than the whole population observed.

Figure 10 Incidence of different spontaneous firing rates of 72 units in the IPN.

Data from cats under Dial and under volatile anesthesia have been combined.


40
30
20
10
0
% of cells
1
5
10
15
20
25
Spontaneous firing rate (Hz)

Relation between spontaneous unit activity and the spontaneous waves

Unit activity recorded in the IPN simultaneously with the spontaneous waves (using the same electrode), and displayed via a high band pass filter (1K to 10 KHz) to eliminate slow activity, at times shows some correlation with the slow waves. Some examples are given in traces E and F of Figure 9; in E the unit fires once on each positive peak of the slow wave. In F the unit fires rapidly during the period of high amplitude slow waves, and less quickly when the slow waves are of low amplitude.

The extent of correlation of unit and slow wave activity was determined by visual inspection, and thus more subtle relations may have been overlooked. The majority of units (approximately 90%) show little or no obvious relation to the slow waves (e.g. see Figure 8: C,D; 9: C,D).

Evoked activity: Gross

The midline IPN extends between rostral levels -0.5 to + 4.5 mm, and laterally it extends (left and right) about 0.5 -1mm. Activity evoked from the habenula was studied mainly in the part of the IPN from A 0.0-3.5mm.

A brief stimulation of the habenula generates similar evoked potentials in the IPN under various anesthetics (chloralose, Dial, halothane or methoxyflurane with nitrous oxide). The sequence is a series of brief positive and negative deflections followed by slower longer duration waves.

Figure 11 Potentials evoked in the IPN by habenular stimulation

Halothane anesthesia; monopolar macroelectrode recording
Submaximal habenular stimulation
Recordings sites separated by steps of 0.5 mm
Vertical calibration is 100 μ V
Negativity is recorded upwards
Time constant of recording is 20 ms
Outline drawing from a histological section through the IPN,
A 2.5 mm showing electrode track
rn red nucleus
cg central gray

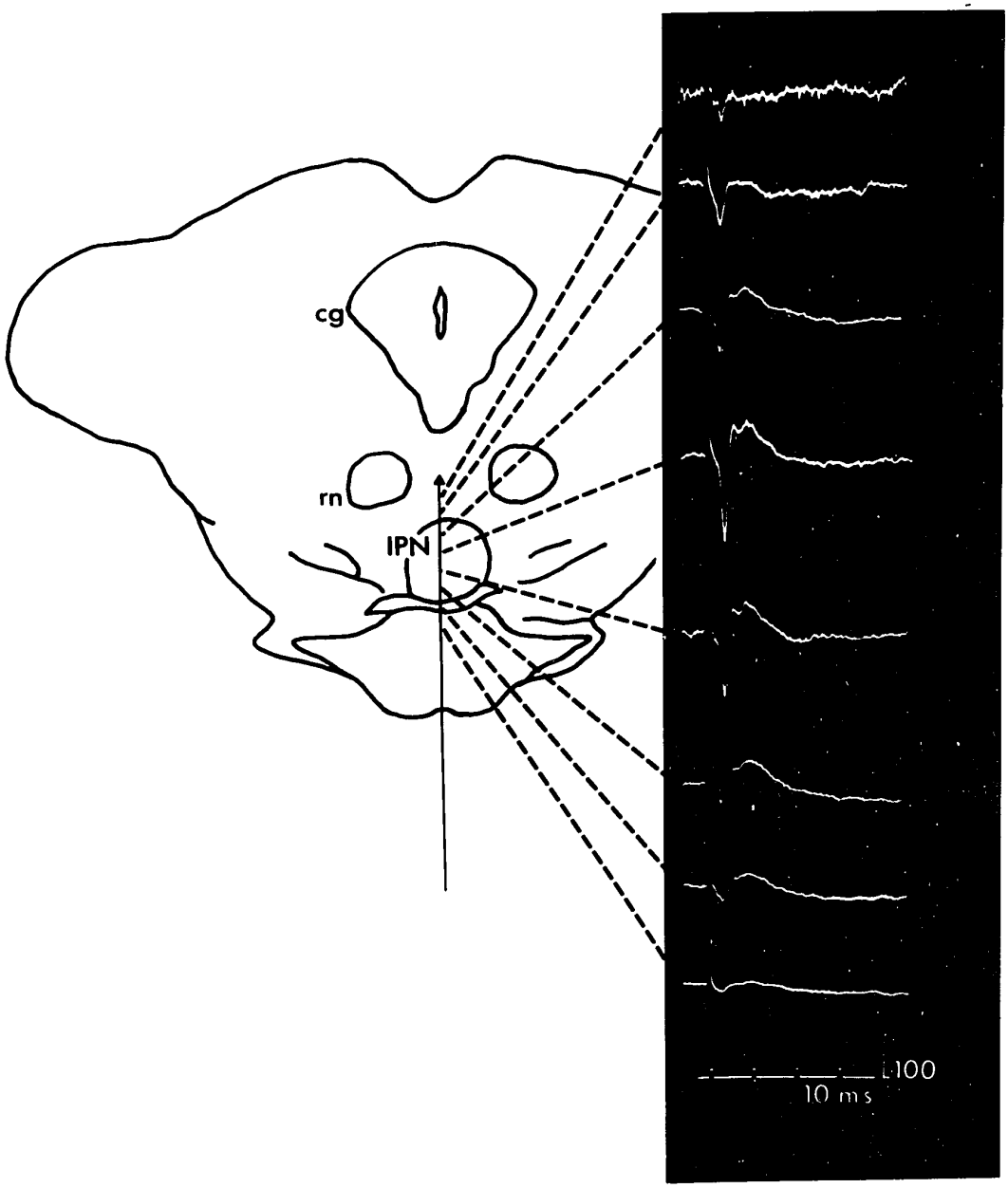

cg
rn
IPN
100
10 ms

Figure 12 Potentials evoked in the IPN by habenular stimulation

Halothane anesthesia; monopolar macroelectrode recording
Different cat from Figure 11; IPN A 3.5 mm
A is most dorsal recording site, J the most ventral
A-E recording sites separated by 0.5 mm, gain for all as in A
E & F are potentials from the same site, but at different gain
F-I recording sites are separated by 0.5 mm, gain as in F
I-J sites are separated by 1.0 mm
Boundaries of nucleus lie at about D and I.

Negativity is recorded upwards
Time constant of recording is 20 ms

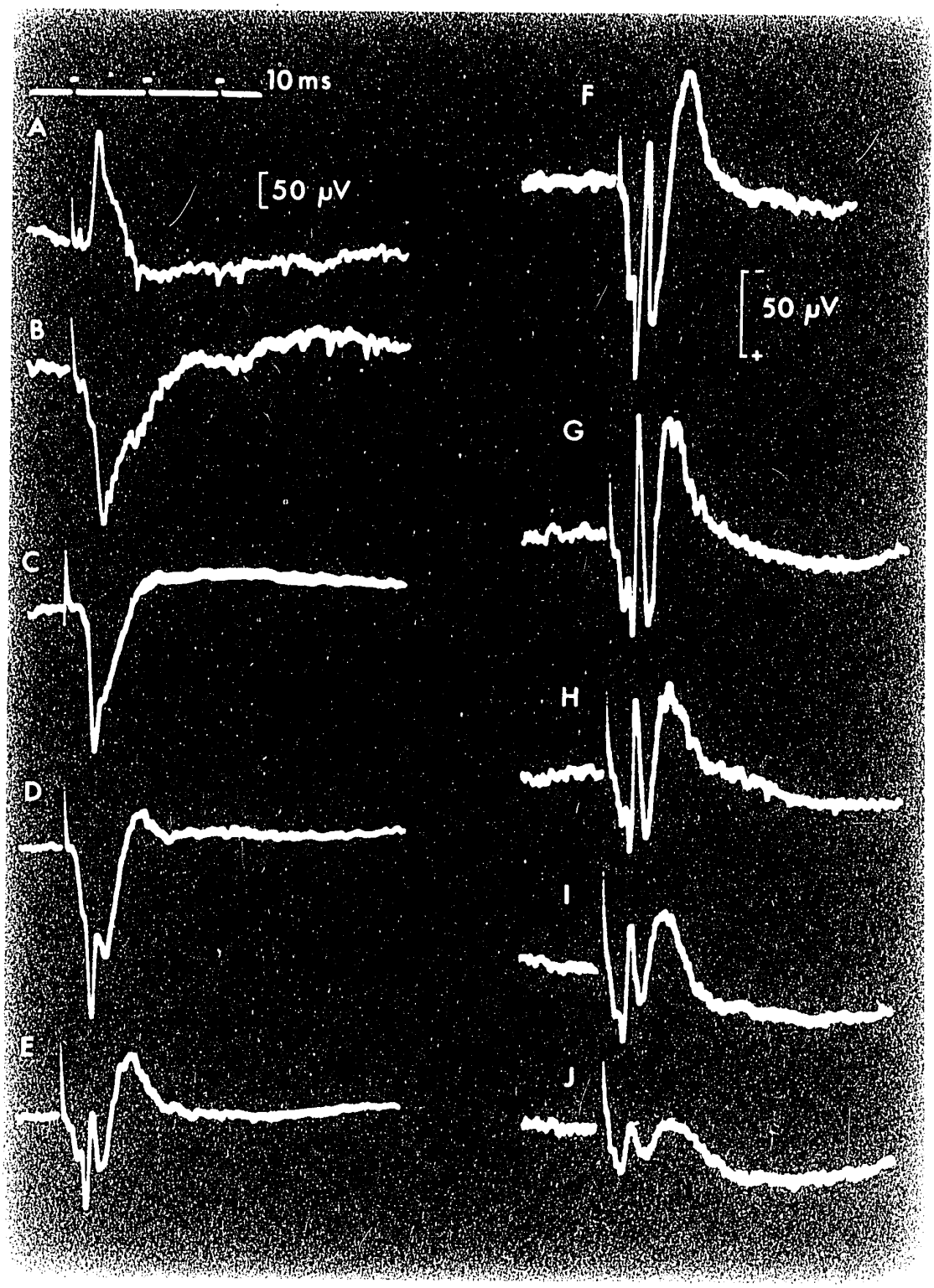

10 ms
A
50 μV
B
C
D
E
F
50 μV
G
H
I
J

Figure 13 Potentials evoked in the IPN by habenular stimulation

Cat under Dial anesthesia. Bipolar macroelectrode recordings at various depths in the IPN, separated by 0.5 mm. A is the most ventral, E is the most dorsal recording site. Horizontal sequences show changes in waves with increasing stimulus intensity, given in volts. Vertical calibrations are all 0.1 mV. F shows the relation between amplitude of potential (mm on film) on the ordinate, and stimulus intensity (volts) on the abscissa, at site B. The open circles represent Wave II, the filled circles, Wave IV. G shows the approximately linear relation between the amplitude of Wave II (abscissa) and the amplitude of Wave IV (ordinate).

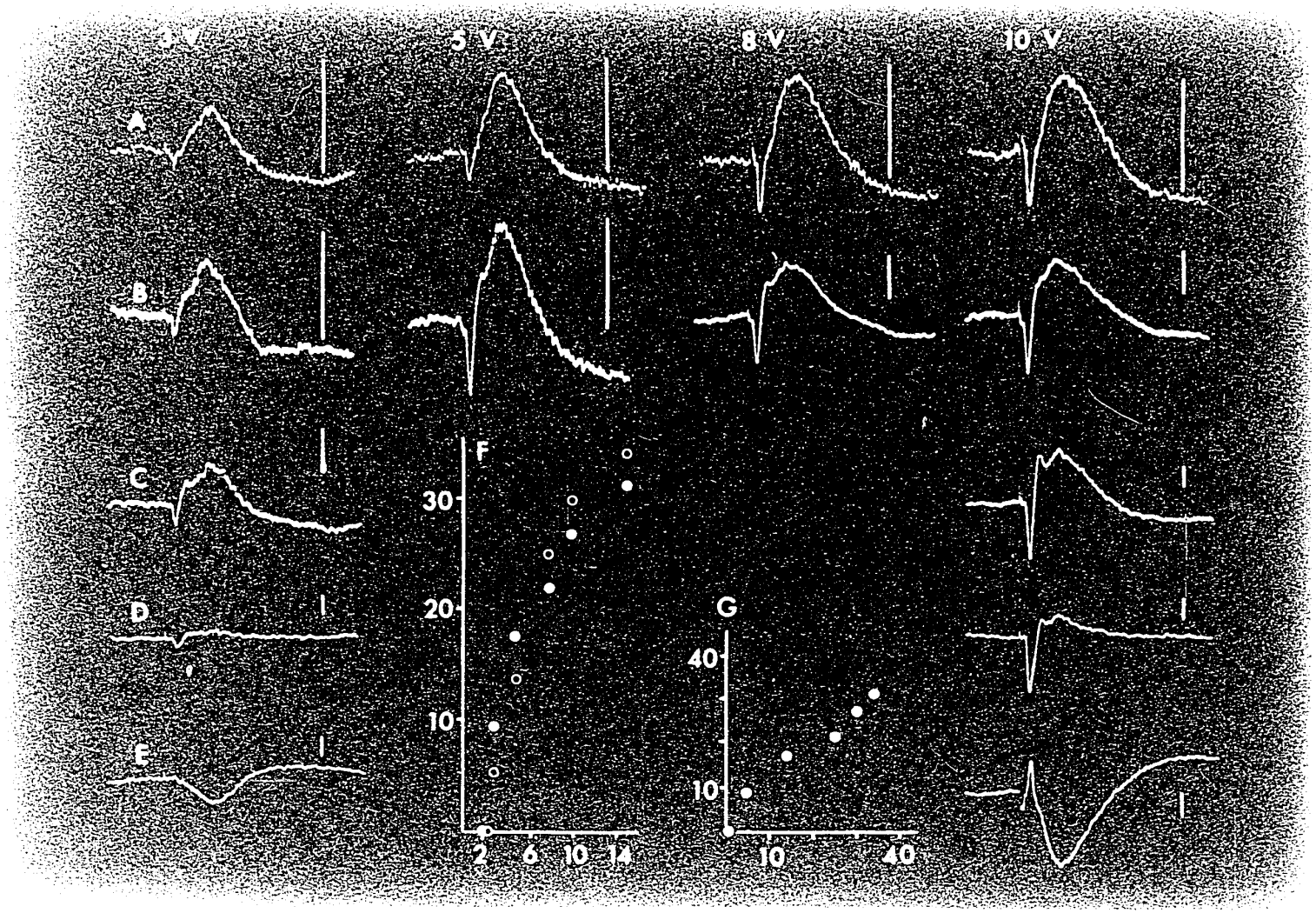
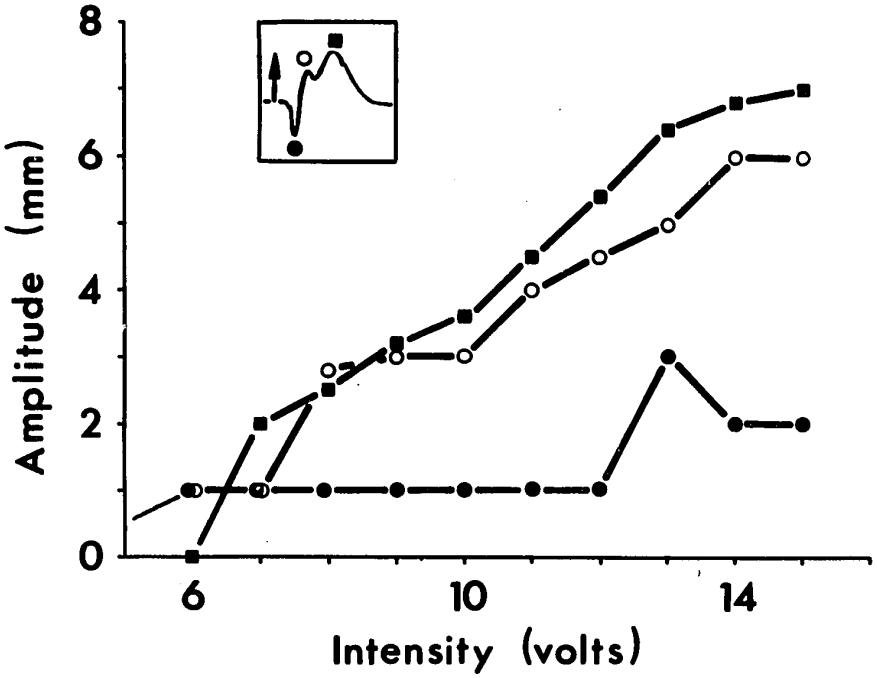
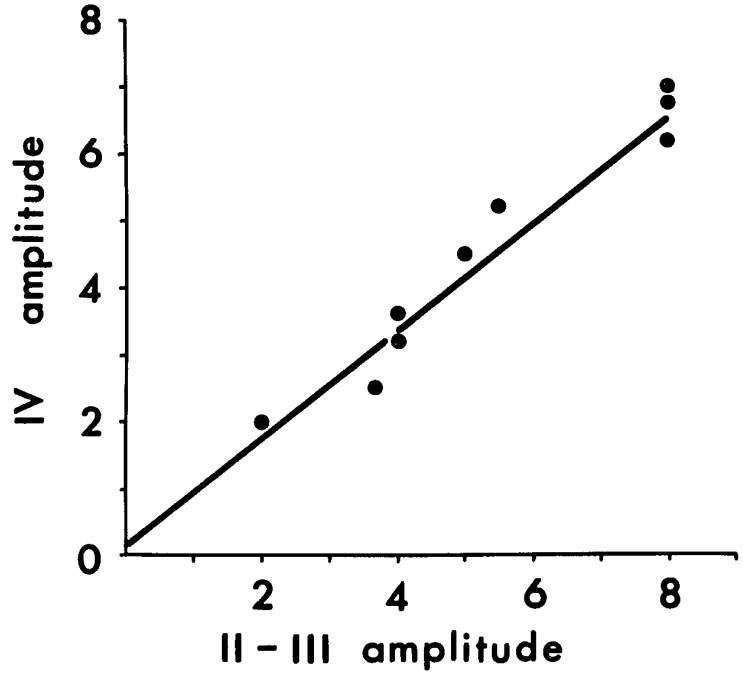

5 V
8 V
10 V
A
B
C
D
E
F
30
20
10
2
6
10
14
G
40
10
10
40

Figure 14 The effect of habenular stimulation intensity on IPN response amplitude

Dial anesthesia; bipolar macroelectrode recording
Insert indicates a typical IPN response to habenular stimulation
The symbols indicate the peaks measured (from the baseline)
Graph shows the amplitudes of these peaks versus habenular stimulus intensity
Lower graph shows the relation of the amplitude of Wave IV (on the ordinate) to the peak to peak amplitude of Waves II-III.
Curve fitted by linear regression statistics


8
6
4
2
0
Amplitude (mm)
6
10
14
Intensity (volts)


8
6
4
2
0
IV amplitude
2
4
6
8
II - III amplitude

Wave I

The first component is a very brief (0.5 ms) low amplitude negative-positive wave of short latency, (1-2 ms following the stimulus artefact). It was not seen at caudal positions in the nucleus (see Figures 11-13).

Waves II and III

The second and third components are made up of a positive-negative sequence, each wave being of brief duration (0.5-1ms) Their initial latency ranged from 3-6 ms, from the stimulus artefact. Commonly there is a positive wave at 3-4 ms and a negative one at 4-6 ms (see Figure 11-13). In rostral recording positions there may be additional waves or inflections, so that there are two positive and two negative peaks (see Figure 12).

In general Waves II-III were more prominent at rostral (A 3.0 mm) than at caudal levels (A 0.0 mm). At each rostral level the amplitudes of these waves were maximal close to the centre of the IPN. However, in a few cats, with monopolar recording at about A 3.0 mm, it appeared that the positive components were enhanced as the electrode approached the dorsal border while the negative components were much diminished, as usual (see Figure 12).

Figures 13 and 14 show the relation between stimulus intensity and amplitude of waves II and III at one position of the recording electrode. In Figure 14 increases in stimulus intensity had more of an effect on the negative components (especially the later slow negative wave) than on the initial brief positivity, but in similar experiments in other cats the early positivity often increased in parallel to the negative ones, with increases in stimulus intensity (see Figure 13).

For each position the latencies and waveforms of II and III were very constant, in contrast to the fluctuations in the slower waves which followed them. Waves II-III follow repetitive stimulation at 100 Hz with minimal decrement in amplitude and no changes in latency. Their amplitudes are often enhanced at stimulation frequencies between 20 and 80 Hz (see Figure 15). Tests at much higher frequencies (e.g. 200 Hz) were not made. But twin shock studies (see Figures 16, 17) show Waves II-III are present at short interstimulus intervals, for example 3-5 ms.

Movements of the stimulating electrode of about 0.5-1.0 mm altered the relative prominence of the negative and positive waves, (not in a predictable manner), but did not detectably change their latencies. Sometimes the threshold was also affected. A larger movement (1.5-2 mm) along the habenulo-interpeduncular tract caused a slight reduction in their latencies.

With macroelectrode recording these waves (II-III) were almost always present, though sometimes of quite low amplitude when compared to the later waves; however, with microelectrode recording it was more common to see the later waves in the absence of II-III, or only a single ill-defined positive potential preceding the later waves.

Wave IV

The fourth component is a slow negative wave, with a peak usually at 8-12 ms after the stimulus artefact, depending to a large extent on the position of the stimulating electrode. Figure 18 shows that the latency of Wave IV is reduced as the stimulating electrode is

Figure 15 Repetitive stimulation in the habenula; frequency following in the IPN

Macroelectrode recording; Halothane anesthesia
Time scale is the same for all records
Left, 1 Hz; Centre, 50 Hz; Right, 90 Hz

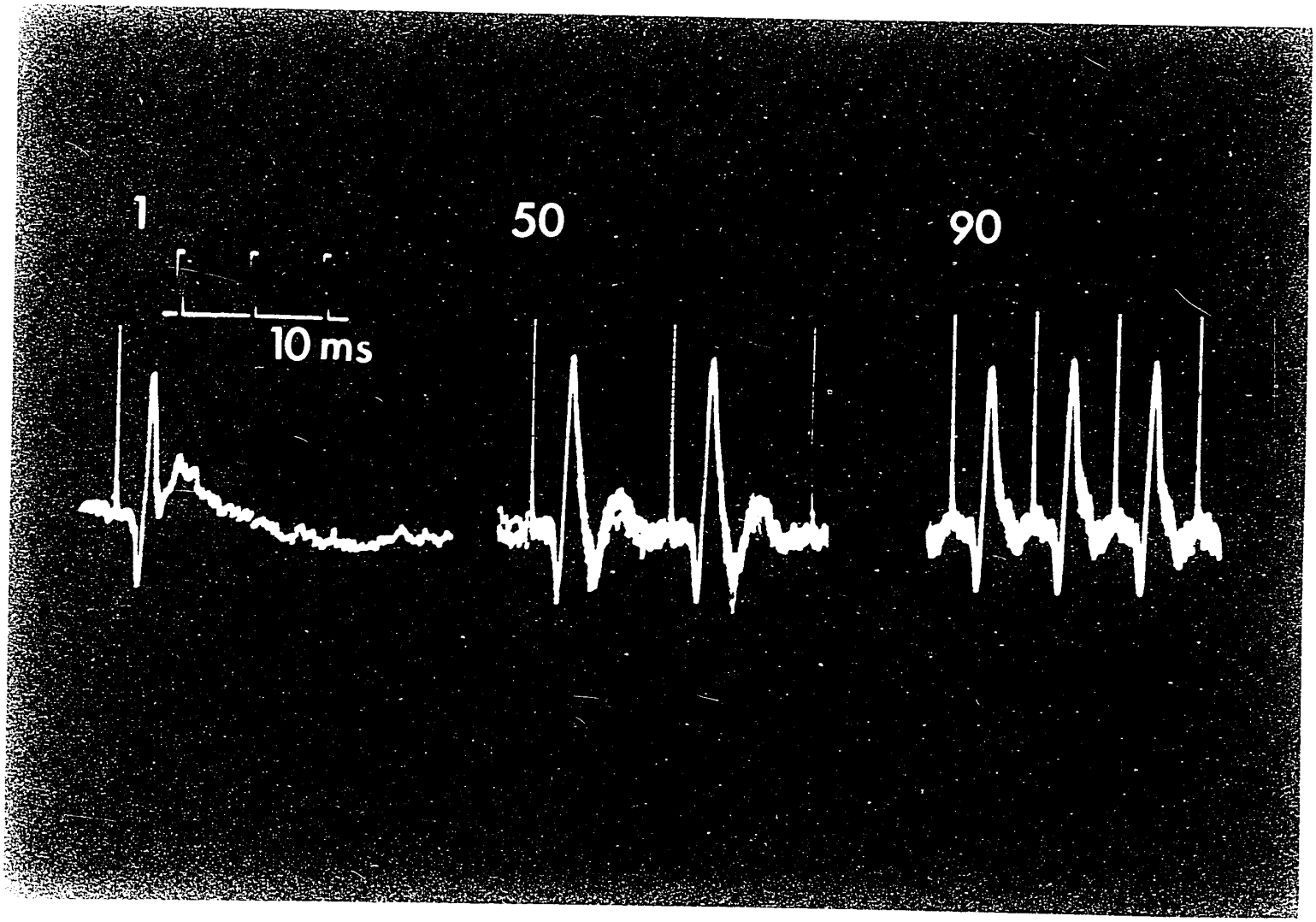

1
50
90
10 ms

Figure 16 Twin pulse studies; stimulating habenula, recording in IPN

Dial anesthesia; macroelectrode recording
Left, interstimulus intervals from 10 ms - 1.8 ms
Right, superimpositions: Top, single stimulus
Centre, intervals 6-1.8 ms
Bottom, intervals 2.5 - 1.8 ms
Time scale is 1 ms
Dot indicates stimulus artefact

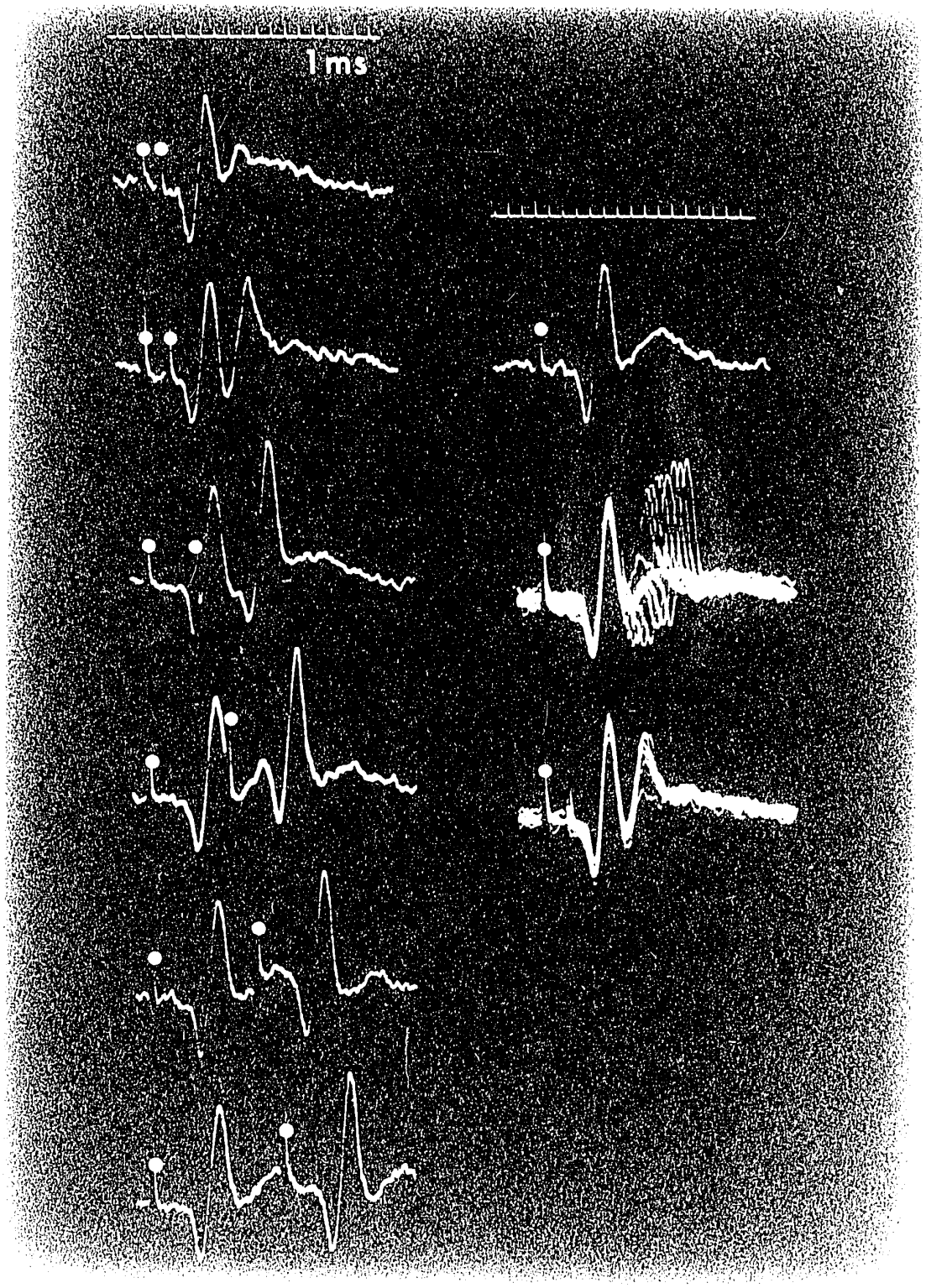

1 ms

Figure 17 Twin pulse studies; stimulating habenula, recording in IPN

Chloralose anesthesia; bipolar macroelectrode recording
C is control trace
Numbers at beginning of other traces indicate interstimulus interval in ms.

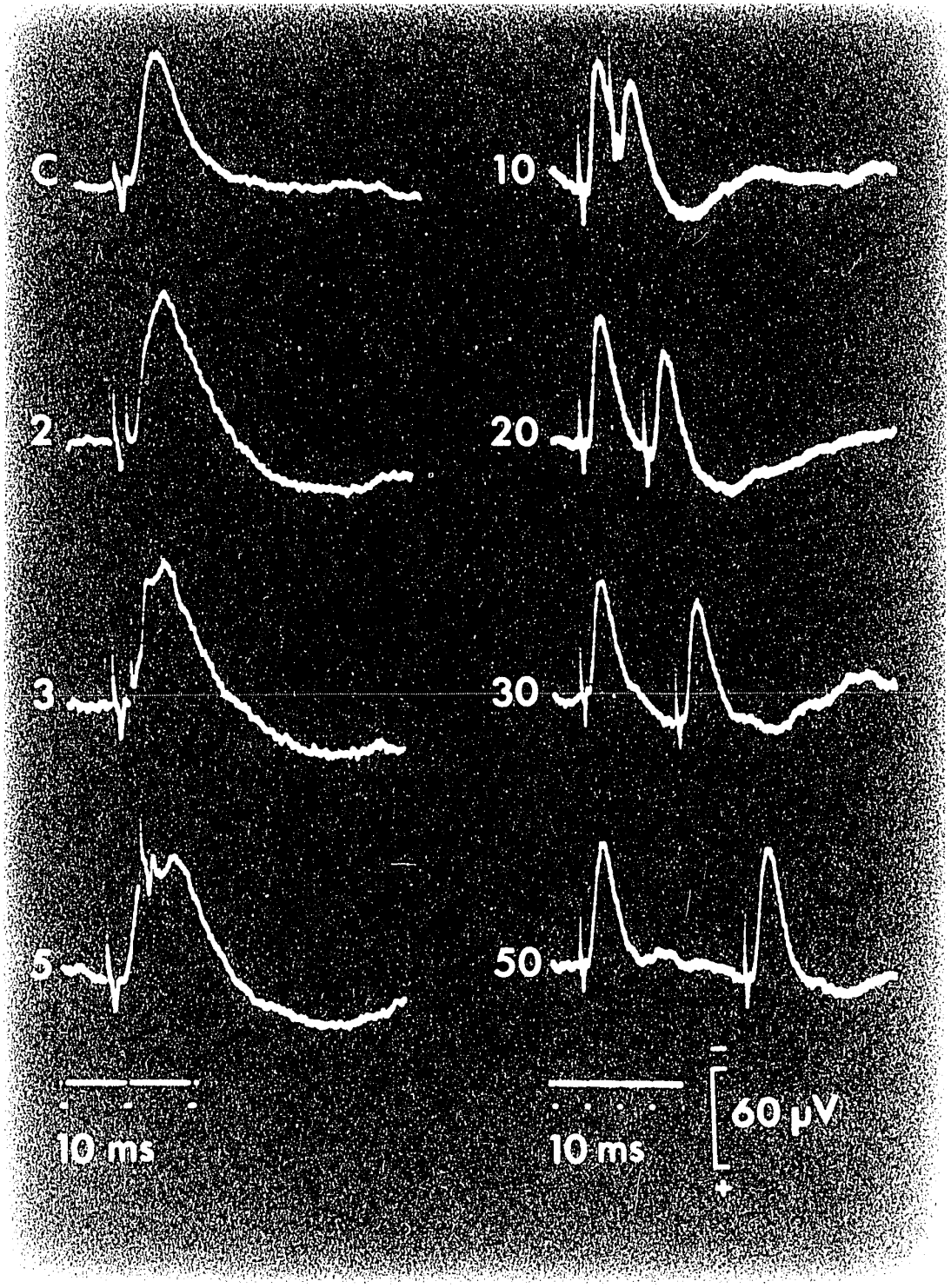

C
2
3
5
10
20
30
50
10 ms
10 ms
60 µV

Figure 18 Effect of changing the position of the stimulating electrode in the habenula on the amplitude and latency of Wave IV in the IPN

Chloralose anesthesia; macroelectrode recording; "N wave" is Wave IV

Ordinate: depth of stimulating electrode (in reference to stereotaxic zero, with superimposition of tracing of histological section drawn to scale of graphs. Areas enclosed by dotted lines indicate the Prussian blue marks made at two points)

Hb habenular nucleus

Abscissa: i. amplitude of slow negative wave (IV) in mm on film record
ii. latency to peak (of IV) from artefact (in ms)

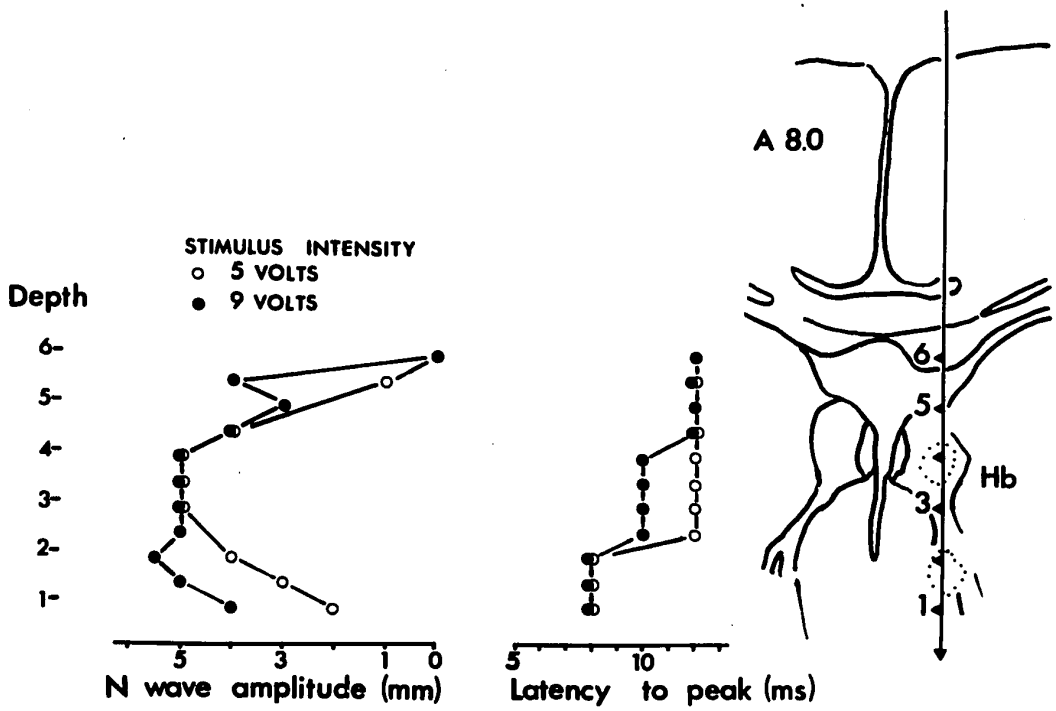
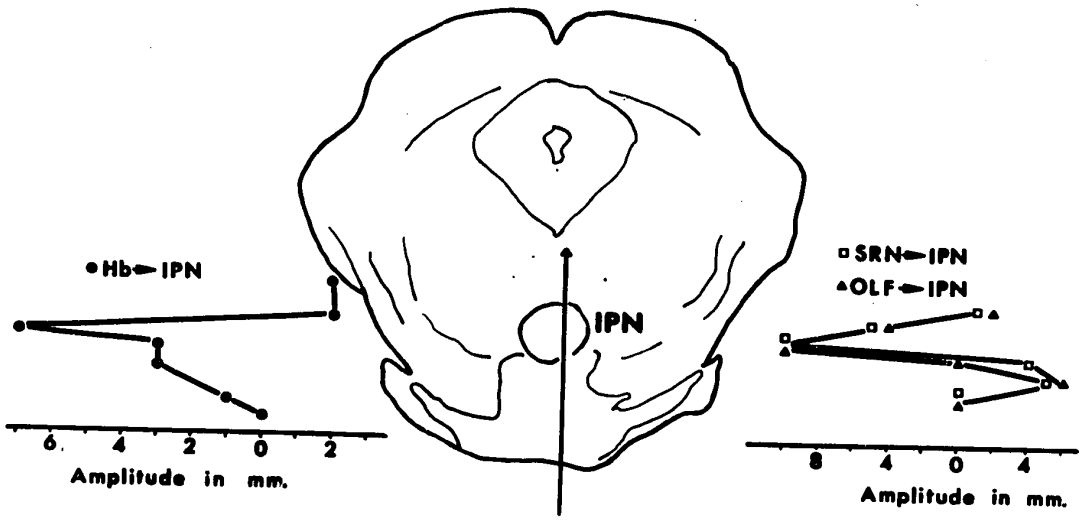

STIMULUS INTENSITY
5 VOLTS
9 VOLTS
Depth
6-
5-
4-
3-
2-
1-
5
3
1
0
N wave amplitude (mm)
5
10
Latency to peak (ms)
A 8.0
6
5
Hb
3
1

Figure 19 Evoked response amplitude at various depths in the IPN to habenula, superficial radial nerve, and olfactory tract stimulation

Chloralose anesthesia; bipolar macroelectrode recording
Response amplitude in mm on film
Polarity reversal at zero on abscissa

Hb habenula
SRN superficial radial nerve
OLF olfactory tract


●Hb⟵IPN
6 4 2 0 2
Amplitude in mm.
IPN
□SRN⟵IPN
▲OLF⟵IPN
8 4 0 4
Amplitude in mm.

moved closer to the IPN along the course of the fasciculus retroflexus. The duration of Wave IV is from 10-20 ms. There is only slight variation in duration with changes in stimulus intensity, although the wave amplitude increases linearly (approx.) with increases in intensity (see Figures 13 and 14). One can see in these Figures that the highest intensity used was still submaximal. In general no more than 15 volts was used for stimulation to ensure minimal spread of stimulating current from the locus of the bipolar electrode in the habenula or fasciculus retroflexus. Usually, as shown in Figure 14, the thresholds of Waves II-III were below those of the later waves, and the relation between the amplitude of II-III and that of IV was linear (approximately).

With repetitive stimulation at frequencies above 20 Hz, the amplitude of Wave IV usually decreased, and its variability increased (see Figure 15, but cf. Figure 27F where Wave IV follows with only slight decrement a short burst at 200 Hz). In two cats under chloralose anesthesia stimulation at 100 Hz for a minute or more resulted in marked post-tetanic potentiation of the Wave IV response to stimulation at 1 Hz. This potentiation lasted only 5-10 seconds.

Wave V

The time constant of recording used in these experiments was not appropriate for recording long duration waves. However there is evidence that the falling phase of Wave IV and the positivity which follows it are not merely a consequence of R-C coupling. For example, in Figure 12 (F-H) the decline of the negative Wave IV is quite rapid and is unlikely to be due to the 20 ms time constant of

recording. Rather it seems that the positivity which develops is genuine. Also in Figure 17, which illustrates a twin pulse study in another cat, the rate of decline of Wave IV, for the control trace and the 2ms-trace, are the same, and too rapid to be merely a result of the time constant of recording. In addition, one can see that the positivity following the twin shocks is markedly greater than that in the control trace. It seems likely that a true positive slow wave (V) follows Wave IV.

Wave V is not always clearly seen after a single habenular stimulus. It is generally of low amplitude, quite variable, and difficult to distinguish owing to the ongoing spontaneous slow waves. Its duration (which can only be estimated) is about 30-50 ms. With repetitive stimulation one can often observe a slow increase in amplitude of Wave V, into a definite wave, but post tetanic potentiation was not seen.

Topography

Figures 11-12 show the potentials recorded monopolarly with a macroelectrode at various depths in the IPN, after a single submaximal stimulus in the habenula. Figures 13, 18 and 19 show the amplitudes of these potentials (from other experiments with bipolar recording) as a function of the position of the recording electrode.

Some generalizations can be made from many such experiments in combination with reconstruction of the electrode tracks from histological sections. Providing that stimulation parameters are relatively low (not above 15 volts, 0.1 ms, 1 Hz) the evoked potentials are mainly confined to the IPN. There is, of course, some electrotonic spread

to the immediate surround. With moderate stimuli Waves I-V could be recorded at 0.5-1 mm from the nucleus in a ventral, dorsal, or lateral direction. With smaller electrodes the potentials are more confined to the nucleus. The best position for the stimulating electrode (in order to generate the largest negative Wave IV) is at the base of the habenula nucleus, close to the origin of the fasciculus retroflexus. The presence of the characteristic evoked potentials appears to be a reliable marker of the IPN position, and was used as such during the experiments.

The potentials were recorded with both monopolar and bipolar electrodes. As illustrated above (see Figures 11-12) monopolar recordings show that the maximal evoked response is close to the centre, in the vertical plane, of the IPN, and it diminishes as the electrode moves towards the peripheries. The evoked potentials do not usually reverse. (Figure 12 shows a series of monopolar recordings at A 3.5 mm at various depths as the recording electrode was moved through the IPN (from a ventral direction). A is recorded at a position about 1.5 mm dorsal to the nucleus. The response appears to be similar to Waves II and III recorded at positions closer to the nucleus (see B,C,D) but of opposite polarity. Also B and C show slow negative potentials corresponding in time of occurrence and duration to the Wave V positivity seen at more ventral positions (see E to I).).

In contrast, when bipolar recording electrodes are used, the potentials always reverse (at approximately the same place for early and late components) at a position close to the centre of the nucleus or about 0.5 mm more dorsal. The reversed potentials were larger (see Figure 13 E), but advancement of the electrode by 0.5 or 1 mm resulted

in their complete disappearance. The most plausible explanation, considering that monopolar recordings rarely show any reversal, is that initially the focal lead is active while the ring is farther away (more ventral) in an inactive or less active area. As the electrode is advanced the ring enters the active region and the focal tends to leave it. This results in an apparent reversal of potentials. As the ring has a larger surface area than the focal lead, it records from a larger region; hence the potentials are of higher amplitude (see Figure 13).

The position of the reversal point is a function of the relation between the lead separation of the recording electrode (in this case 300-500 μ) and the radius and situation of the active region of the nucleus. An estimate of this active region can be made from histological sections which show the IPN radius is from 0.2 to 1.0 mm at various rostral levels. From the present experiments the reversal point appeared to be within 0.5 mm of the anatomical centre of the nucleus.

Recording from the fasciculus retroflexus

In one experiment bipolar macroelectrodes were placed in the habenula and in the fasciculus retroflexus about one half to two thirds of the way between the habenula and the IPN, to study the properties of tract fibres. Habenular stimulation was followed by a single low amplitude positive-negative wave lasting about 2 ms, recorded close to the tract (see Figure 20). In a twin shock study (Figure 20) the tract response to both shocks remained identical until the interval

Figure 20 Potentials evoked in the fasciculus retroflexus by habenular stimulation : twin pulse studies

Methoxyflurane anesthesia; bipolar macroelectrode recording
The number at the beginning of each trace gives the interstimulus interval in ms
Negativity recorded upwards
Time constant of recording is 20 ms

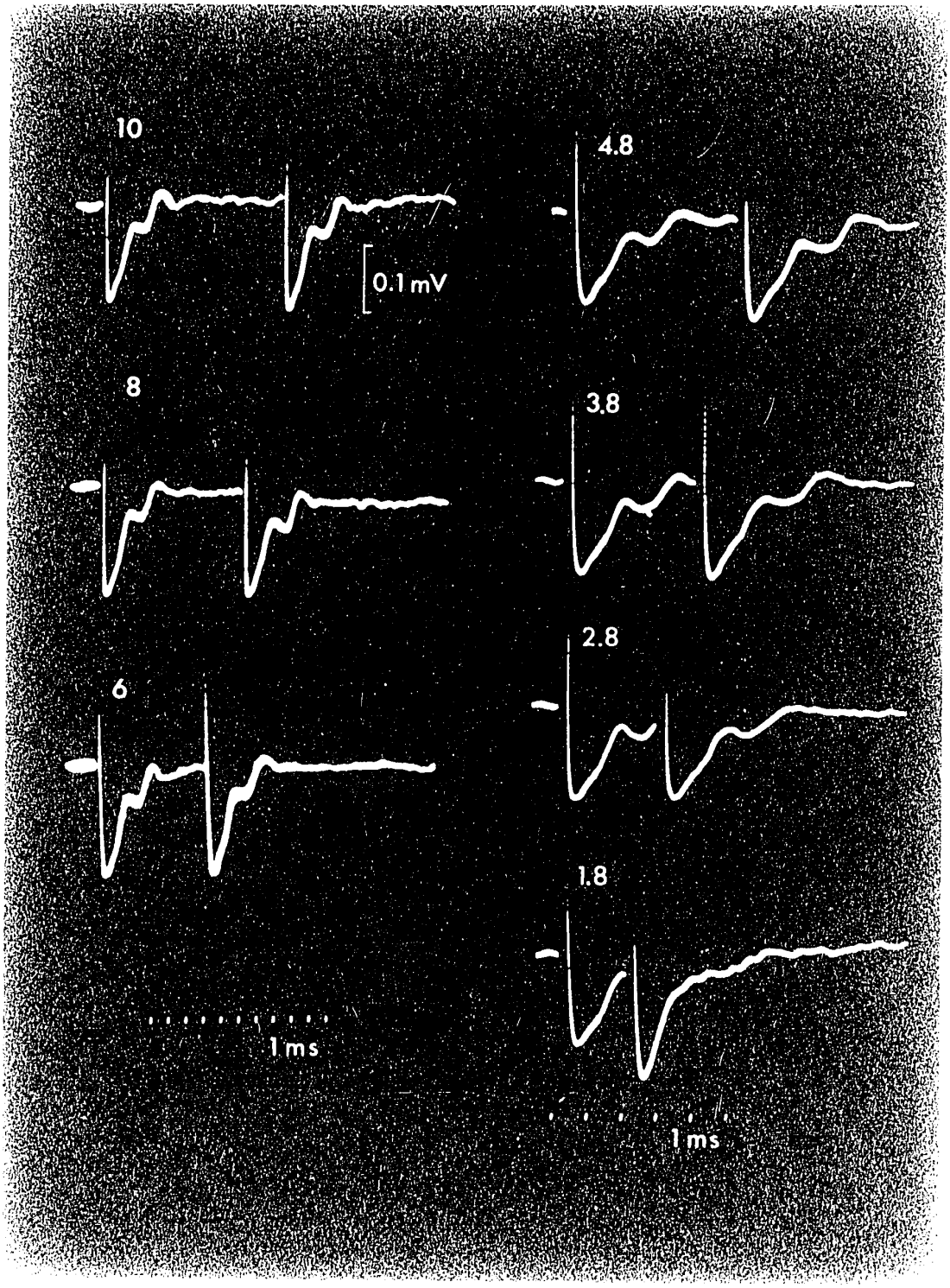
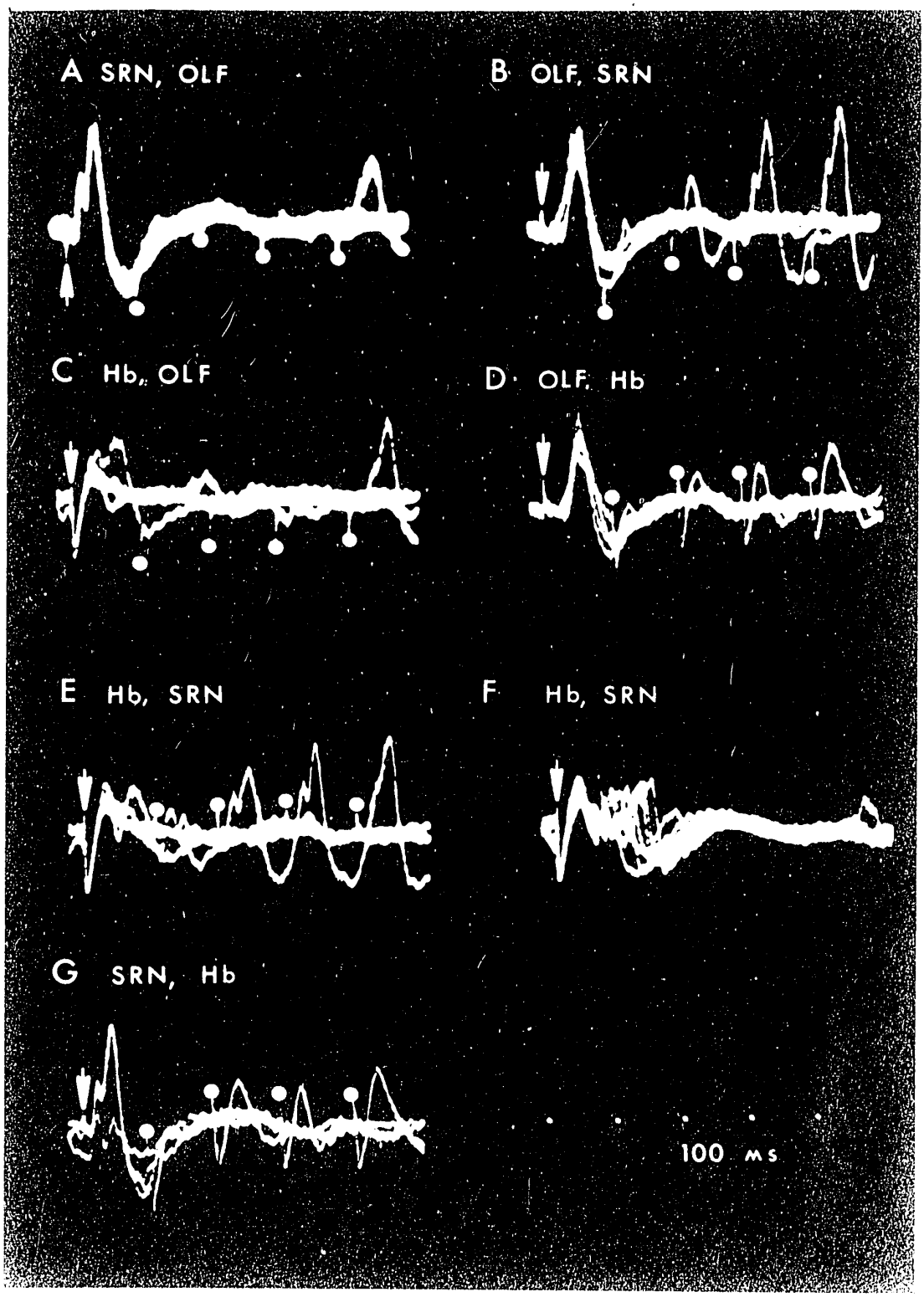

10
0.1 mV
4.8
8
3.8
6
2.8
1.8
1 ms
1 ms

Figure 21 Interaction of evoked potentials in the IPN elicited by stimulation at various sites

Chloralose anesthesia; macroelectrode recordings (bipolar)
SRN superficial radial nerve
OLF olfactory tract
Hb habenula

See text for explanation


A SRN, OLF
B OLF, SRN
C Hb, OLF
D OLF, Hb
E Hb, SRN
F Hb, SRN
G SRN, Hb
100 ms

between stimuli in the habenula was reduced to 4.8 ms. At this point there was a slowing of conduction velocity, as the response to the second shock had a longer latency than before. The increase in latency became more pronounced as the interstimulus interval was reduced to 1.8 ms, at which point the second shock failed to elicit a response.

Stimulation at other sites

Stimulation of the paw or the superficial radial nerve, the face or the olfactory tract also evokes potentials in the IPN, but these have a much longer latency (20-40 ms) and are more variable, probably reflecting their transmission through polysynaptic pathways, than responses to habenular stimulation. These potentials were studied only with bipolar electrodes. Their amplitudes in relation to depth of the recording electrode are shown in Figure 19. The response to stimulation of these sites usually began about 20 ms after the stimulus, reached a peak at 30-40 ms (some times preceded by a lower amplitude earlier peak) and was followed by a lower amplitude potential of opposite polarity. These potentials were unable to follow repetitive stimulation above 3 Hz.

Interaction of inputs

Figure 21 shows a series of evoked potentials recorded with bipolar macroelectrodes in the IPN. In each set A, B, C etc. two sites were stimulated at 400, 300, 300 and 100 ms intervals between the stimuli. (F shows intervals from 90-40 ms). Four traces were superimposed to give the composites shown. At the top left are given the stimulation sites and the order in which they were stimulated. An arrow marks the stimulus artefact of the first stimulus of the pair, and dots the artefacts of the second stimulus. For example, in A, the superficial

radial nerve was stimulated, and the response to this in the IPN is shown at the beginning of the trace after the arrow. Following this SRN stimulus was a stimulus to the olfactory tract at intervals of 400, 300, 200 or 100 ms. The responses in the IPN to this paired stimulation were superimposed and photographed from the oscilloscope screen. Figure 21 shows the response in the IPN to stimuli at various sites (the superimposition of four responses at the beginning of each composite), and the interaction between responses to stimuli at two different sites.

Prior stimulation of the periphery or the olfactory tract has only a slight effect on the response in the IPN to habenular stimulation, probably because of the latter's strong monosynaptic effect on the cells of the nucleus. However, with other combinations of stimulus pairs, the response to the second stimulus is usually reduced with interstimulus intervals of 300 ms or less (reduction to one half of control amplitude occurs with intervals of 200-300 ms). This stands in contrast to multiple stimulations of the habenula, where the response in the IPN is well maintained over the range of 1 to 50 Hz (see Figure 15). The mechanism by which input via one pathway tends to diminsh the effect of imput impinging upon the nucleus via another path may be inhibition, or occlusion etc. Careful study is needed however before more definite statements can be made.

Potentials recorded in the habenula following stimulation in the IPN

Stimulation of the IPN evokes a series of brief positive and negative potentials in the habenula, with latencies from 1-4 ms, occasionally extending to 6 ms. These potentials were able to follow

repetitive stimulation at 100 Hz without decrement. The potentials are most likely antidromic, but may contain a component due to the orthodromic stimulation of interpedunculo-diencephalic fibres (Massopust & Thompson, 1962). Only in a few instances were later, longer duration (10 ms) potentials observed.

Evoked activity: Unit

Usual exploration procedure

For these studies the microelectrodes were stereotaxically positioned in the midline at various rostral levels and advanced through the pons to the region of the IPN, 2 to 5 mm below the surface, depending on the rostral level (the IPN is deeper in its caudal part). In addition to the stereotaxic coordinate information, the presence of the characteristic evoked potentials (I to V described above) at appropriate latencies, low threshold, and high amplitude, was further confirmation of the location of the tip of the electrode within the nucleus. Histological verification of tip position was carried out at the end of each experiment, as described in the Methods section above.

Generally the stimulus in the habenula was repeated at 1 Hz as the microelectrode was advanced through the IPN, and the evoked potentials observed. When a unit was detected the stimulus was withheld for a period of several minutes while spontaneous activity was photographed and/or monitored via the frequency meter. In almost all cases, when the unit could be held for a sufficient period, the unit was found to be spontaneously active. The notable exceptions were 16 "bursting" units (to be described below) which appeared not to be spontaneously active. (Spontaneous unit activity has been discussed above.) Next the relations

between unit firing and habenular stimulation, and other parameters, were tested and recorded. At other times explorations were carried out with only intermittent stimulation.

As described above the evoked potentials tended to diminish at the periphery of the IPN. Exploration was initiated in a ventro-dorsal direction. When the evoked potentials diminished to an arbitrary level owing to the approach of the electrode to the dorsal boundary of the IPN, the position was marked, and the electrode withdrawn. Quite often observations were also made at various points while withdrawing the electrode.

Range of latencies

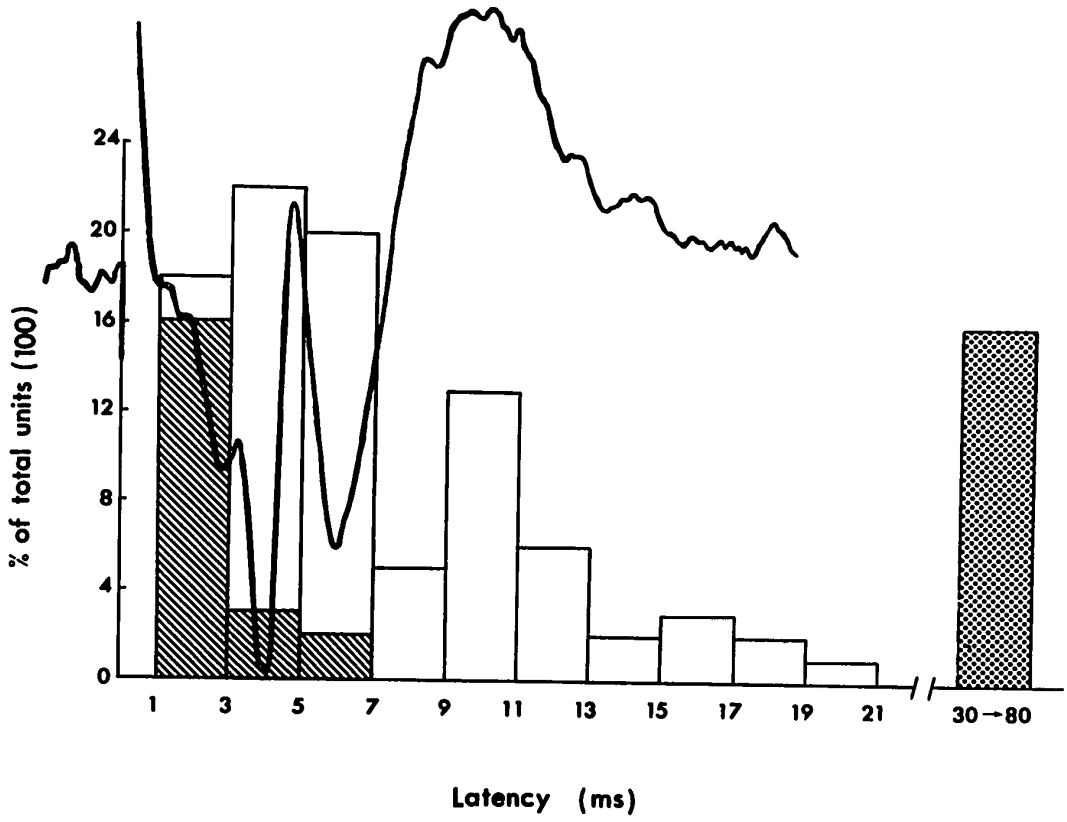
Brief stimulation of the habenula at 1 Hz increased the firing probability of 64% of the 190 units studied in the IPN. Figure 22 shows the range of their latencies. The histogram is based on data from 100 cells. For the remaining 22 units the data are either inadequate, being qualitative only (9 units), or the units showed only a generalized increase in rate after repetitive habenular stimulation. These latter units may be only indirectly or weakly synaptically linked to the habenula. The incidence of latencies was not different for cats under Dial from that for cats under volatile anesthesia. Fewer units were studied under volatile anesthetics (51 cf 71) but there was no significant difference between cats under Dial and those under volatile anesthesia in the number of units per cat.

Relation of unit firing to evoked potentials

One can see from Figure 22, with the superimposed trace of a typical evoked potential, that the majority of units (60%) fired

Figure 22 Incidence of latencies of 100 units in the IPN following a single brief stimulus of the habenula

The subpopulation designated by the hatched columns (1-7 ms) represents the constant latency units.
The eight units of the dotted column at the right (30-80 ms) were long latency units, grouped for ease of presentation.
The superimposed evoked potentials are from a typical experiment -- on the same time scale as the abscissa.


24
20
16
12
8
4
0
% of total units (100)
1
3
5
7
9
11
13
15
17
19
21
30→80
Latency (ms)

during the first 7 ms after the stimulus, a period occupied by Wave I-III. Considering the period extending to the peak of Wave IV, about 12 ms after the stimulus at the latest, 84% of the units are included.

Types of units: i. constant latency

About one third (21) of the units which fired in the first 7 ms after the habenular stimulus were probably excited antidromically, or were preterminal afferent fibres. Thirteen of these were observed in cats under Dial, and eight in cats under volatile anesthesia. They accounted for 11% of all units observed in the IPN. The criteria for distinguishing spikes of cellular rather fibre origin has been discussed above (p. 50). In addition, units were judged to be antidromic on the basis of: (a) short and very constant latency (b) ability to follow high frequency stimulation (200 Hz or more) and (c) cancellation of evoked spikes by preceding spontaneous ones. Apparent collisions could not be conclusive evidence for antidromic activation in this case, because the latencies involved were so short that collision was difficult to distinguish from refractoriness. However Massopust and Thompson (1962) have described in cats an interpedunculo-diencephalic tract which distributes some fibres to the habenula. In addition this criterion was not met by all units as their slow spontaneous rates (2-8 Hz) resulted in a low probability of the collision event, even with prolonged testing.

Eight constant-latency discharges had a very short latency (1-2 ms), corresponding to a conduction velocity of 12-6 m/s. Of these three had small positive spikes, which may indicate a preterminal

afferent fibre origin (e.g. see Figure 23). The remaining units had positive-negative spikes, of which the negative component was the larger. Of these two were tested with iontophoretically applied L-glutamate and were excited, suggesting that they were cells and not fibres. (An outright excitation of identified fibres by L-glutamate has never been observed). Their short latency and ability to follow high frequency stimulation suggests that they may be antidromically activated.

The remaining 13 constant latency discharges (occurring 2-7 ms after the habenular stimulus) usually had negative-positive spikes of 0.6 to 1.5 ms duration. Their mainly negative spikes, their longer latencies, and their excitation by acetylcholine (only a few were tested), suggests that rather than being from afferent fibres, they may be antidromically excited units or units excited by highly efficient synapses. Most units (9/13) followed repetitive stimulation at 100 Hz and short bursts of 4-5 stimuli at 200-700 Hz (see Figures 24 and 25). In Figure 25 D one can see what may be failure of the antidromic impulse to invade the soma after a few high frequency spikes, as only the small A spike remains (cf. Phillips, 1959). Four units with short and very constant latencies were unable to follow high frequencies - one showing failure when the frequency was raised to 10 Hz. This dose not rule out its antidromic activation, as there are other examples of CNS cells unable to follow high frequency stimuli arriving antidromically (e.g. motoneurons, Eccles, 1957).

Figure 23 Short latency units in the IPN evoked by habenular stimulation

A-C Dial anesthesia; stainless steel microelectrode
Single sweeps at various speeds
R-S Dial anesthesia; different cat; tungsten microelectrode
S Superimposition of 5 consecutive sweeps
Probably 2 units; note silent period of about 50 ms after the initial discharge.

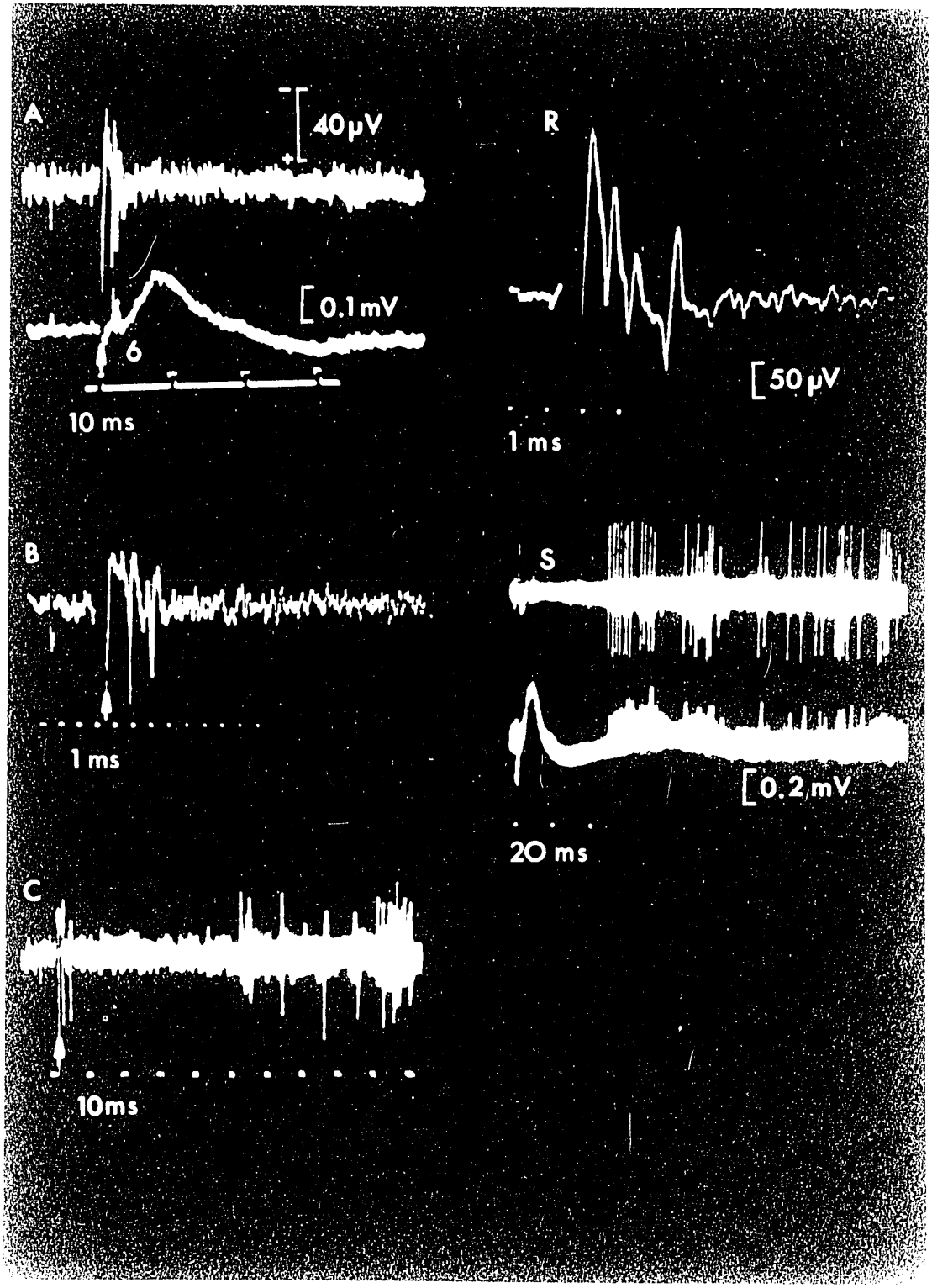

A
40μV
0.1mV
6
10 ms
R
50μV
1 ms
B
1 ms
S
0.2mV
20 ms
C
10ms

Figure 24 Constant latency unit in the IPN evoked by habenular stimulation

A-C halothane anesthesia; glass microelectrode
A. Single sweep
B. Superimposition of 5 consecutive sweeps
C. Habenular stimulation at 110 Hz

Voltage calibration is the same for A, B, & C
Time scale is the same for A & B
Arrows indicate stimulus artefacts

Latency about 6 ms; probably antidromically excited.

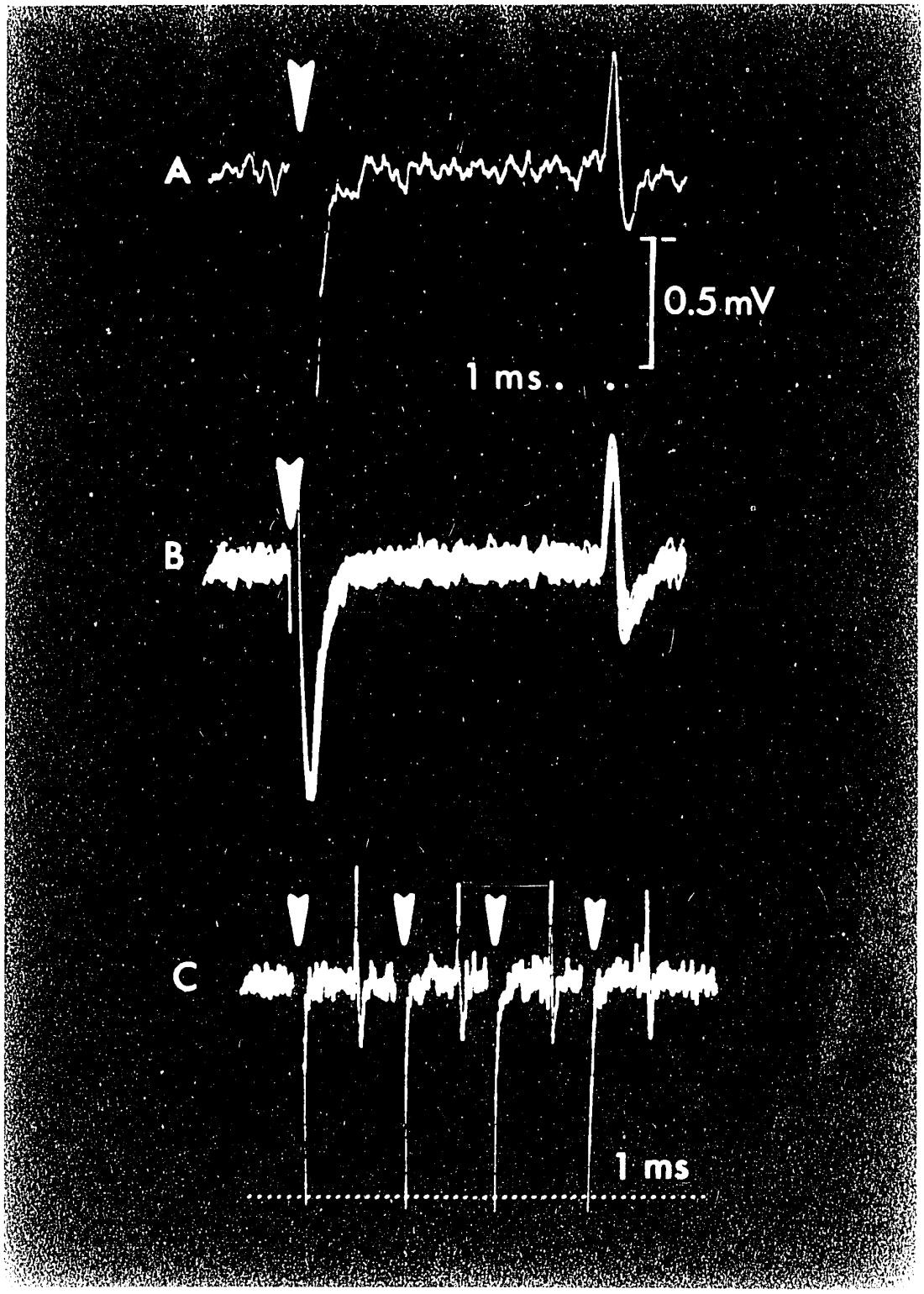

A
0.5 mV
1 ms
B
C
1 ms

Figure 25 Constant latency units in the IPN evoked by habenular stimulation

Dial anesthesia; 5-barrelled microelectrode
A-E one unit : latency 1.8 ms
A-B single sweeps
C superimposition of 5 sweeps
D stimulation at 400 Hz (of habenula)
The time scale for A-D is the same and is shown in C. The time scale in E is 0.5 ms. The vertical calibration applies to all traces.
L-N shows another unit in the same cat at a different rostral level, and a different depth. The latency is about 2.0 ms
L is a single sweep, M shows the superimposition of 5 sweeps and N shows the responses to a train of 5 stimuli at 330 Hz.
The time scale is the same for L-N (1 ms).

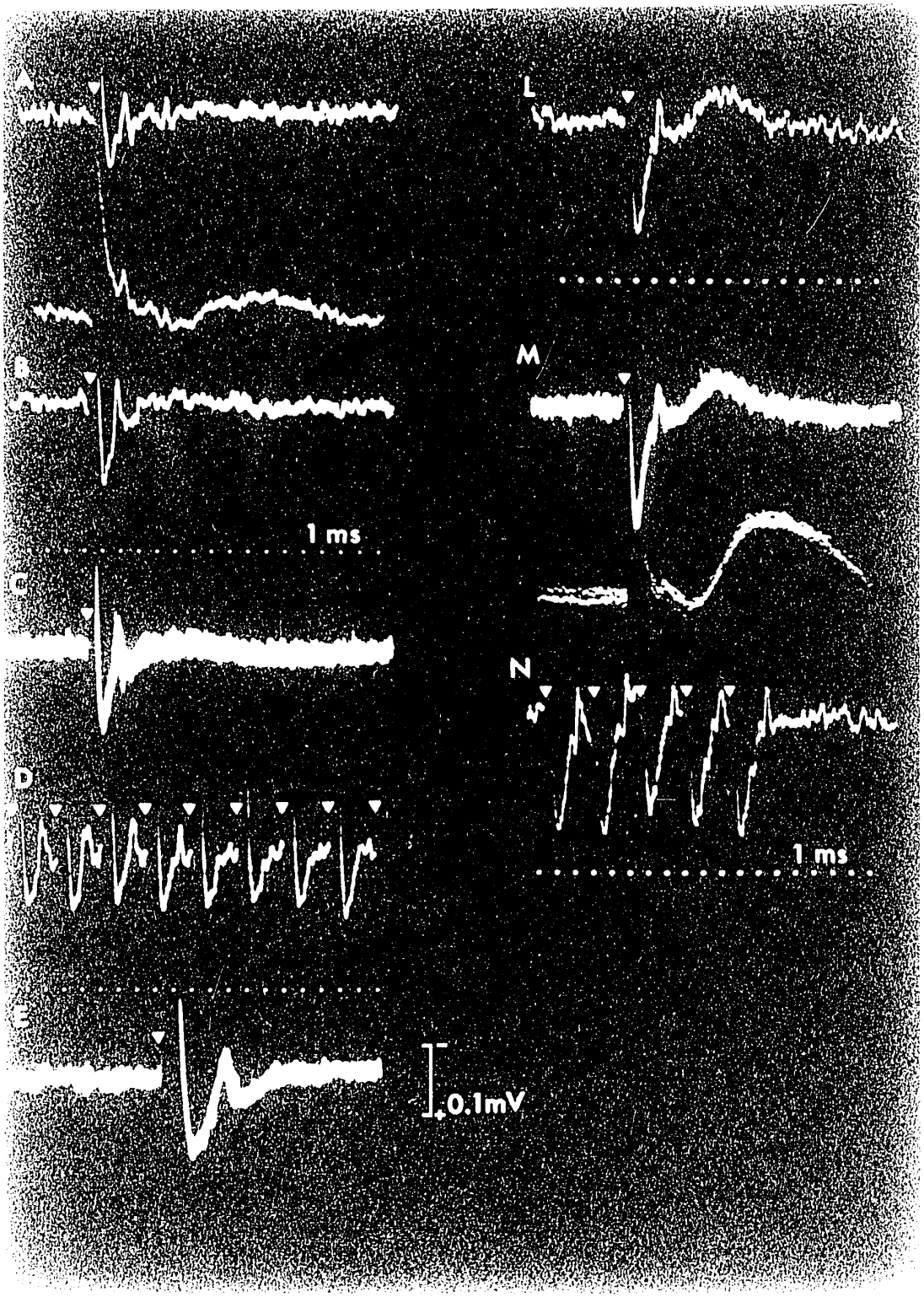
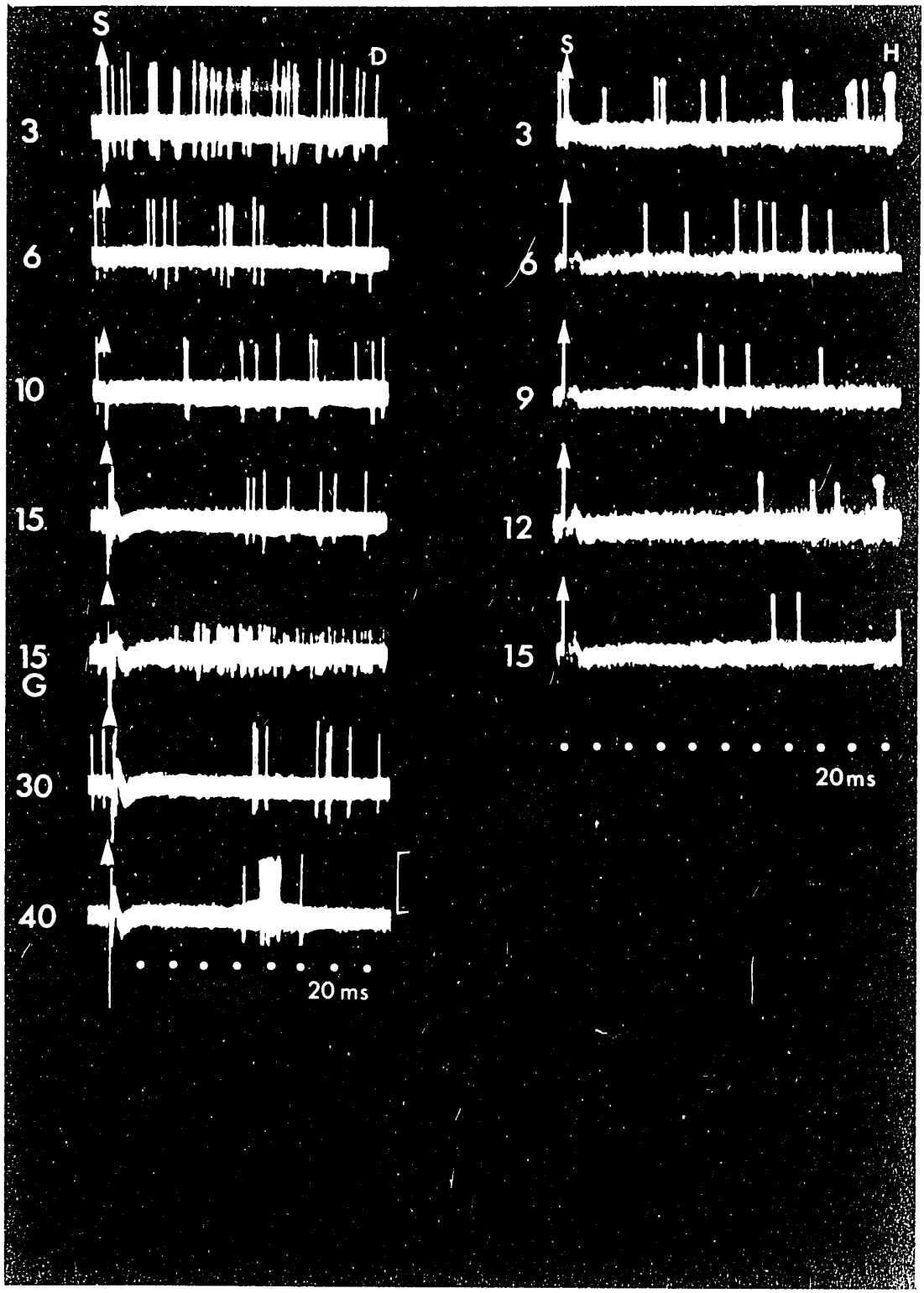

A
B
1 ms
C
D
E
0.1mV
L
M
N
1 ms

Figure 26 Units in the IPN whose firing probability is decreased by habenular stimulation

The sequence at the left shows a unit in a Dial cat which discharged at constant latency (3 ms) when the stimulus intensity was above 12 V. Sequence at the right is a unit showing no initial excitation, from a different cat under halothane anesthesia. Each trace is the superimposition of 5 sweeps. Numbers indicate the stimulus intensity in V. G indicates the continuous iontophoretic application of 30 nA L-glutamate. Vertical calibration, 0.1 mV, same for both units.


S
D
3
6
10
15
15
G
30
40
20 ms
S
H
3
6
9
12
15
20ms

Events following the constant latency spike

For 15 of these units the overall firing pattern seemed little affected by the interjection of the constant latency discharge. Six units however, showed clearly a depression of their spontaneous firing as a silent period for 10-80 ms after the constant latency discharge. This depression increased with greater stimulus intensity and usually occurred even with stimuli at intensities below the threshold for the constant latency spike (see Figure 23: C, S; 26). In Figure 26 the sequence at the left shows a unit which responded with a short constant latency (3 ms) spike to habenular stimulation, above 12 V. This spike followed 4-5 stimuli at 250 Hz. The spontaneous firing of this unit was depressed following the stimulus at intensities below the threshold for the constant latency spike (e.g. 6-10 volts). These units can be driven to fire at intervals much less than the 10-80 ms silent periods. This suggests that the silent period is not due to post-spike depression, but rather to inhibition. (This will be discussed in more detail below, see 'Decrease in probability of firing of IPN units'). In another cat a second unit recorded at the same depth as a constant latency one showed a similar silent period, as if it were affected by the same spike depressing mechanism.

ii. Variable latency units

The remaining 79 units excited by habenular stimulation were probably synaptically evoked. In general this group of units showed quite large fluctuations in latency (± 2 ms with respect to the mean), and they rarely followed high frequency stimulation.

There were two types of synaptic responses of IPN units: either a single spike (56 units) or a short burst of 2-6 spikes at 500-1000 Hz (23 units). Thirty-nine units had short latencies, that is, less than 7 ms, (25 'single spike' units and 14 'burst' units), while 40 units had latencies of 7 ms or longer (31 'single spike' units and 9 'burst' units). There were no burst units with initial latencies in excess of 12 ms (the peak of negative Wave IV).

Each unit showed only one kind of response; even at low intensities of stimulation burst type units fired repetitively, although sometimes the number of spikes in the burst increased with an increase in stimulus intensity. Similarly as the intensity was increased (2-10 times threshold) 'single spike' units failed to fire repetitively. 'Single spike' and 'burst' units were found at all depths in the nucleus, and shared the same range of thresholds, usually 3-12 volts. The description of 'single spike' units to be given applies to units of both short and longer latencies. In the same way, the description of 'burst' units applies to units with latencies from 2-12 ms.

A. 'Single spike' units

Thirty of these units were observed in cats under Dial, while 26 were found in cats under volatile anesthesia. This type of unit accounted for 30% of all units studied in the IPN. These units were spontaneously active at rates of from 2 to 18 Hz.

Figures 27-31 show examples of 'single spike' responses in the IPN following habenular stimulation. The spikes were usually negative-positive (but Figure 28 shows a unit with a positive-negative spike) and had durations of 1-2 ms, while their amplitudes ranged from 0.1-0.6 mV. Figure 29 shows a probable antidromic spike and a

probable synaptic spike from two units recorded at the same depth in the IPN. The threshold of the 'antidromic' spike was 6 volts, while that of the 'synaptic' spike was 11.5 volts. One can see the constancy of the latency of the former, contrasting with the variable latency of the latter, when ten consecutive sweeps at 1 Hz were superimposed. The early spike followed stimuli at 100 Hz with no decrement, while the later spike failed at 70 Hz.

Relation to the evoked waves

'Single spike' units fired with latencies from 2-80 ms after the stimulus in the habenula (see Figure 22); 61% fired in the time up to the peak of the negative Wave IV (12 ms), about 45% during the occurrence of Waves I, II and III (latencies less than 7 ms). From Figure 22 one can see that the longer latency units (over 12 ms) were not grouped at any particular latency. In fact the incidence at any particular latency was low, especially when compared to the earlier time periods. This period (after 12 ms) coincided initially with Wave V and was one of low firing probability.

Events following the 'single spike'

Post-stimulus histograms and photographic superimpositions at slow sweep speeds of responses of these units showed that a rapid return to the spontaneous firing rate followed the peak of early firing. For some units, however, there appeared to be a definite depression of spontaneous firing following the evoked spike (Figures 31 and 32). Figure 32 shows post-stimulus histograms of a unit which fired 6-9 ms after a suprathreshold (5-6 V) stimulus. It did not fire regularly after each stimulus, but the probability of

Figure 27 Synaptically activated units in the IPN; single spikes

A-C: two units of very similar threshold in a Dial cat. A & B are single sweeps (same time scale) at same intensity (7 V).
C is superimposition of 5 sweeps. Upper trace calibration shown in A is 0.1 mV
D-F: another unit in the same cat. D, & E (superimposition of 5 sweeps), have same time scale. F shows response to a burst at 200 Hz.

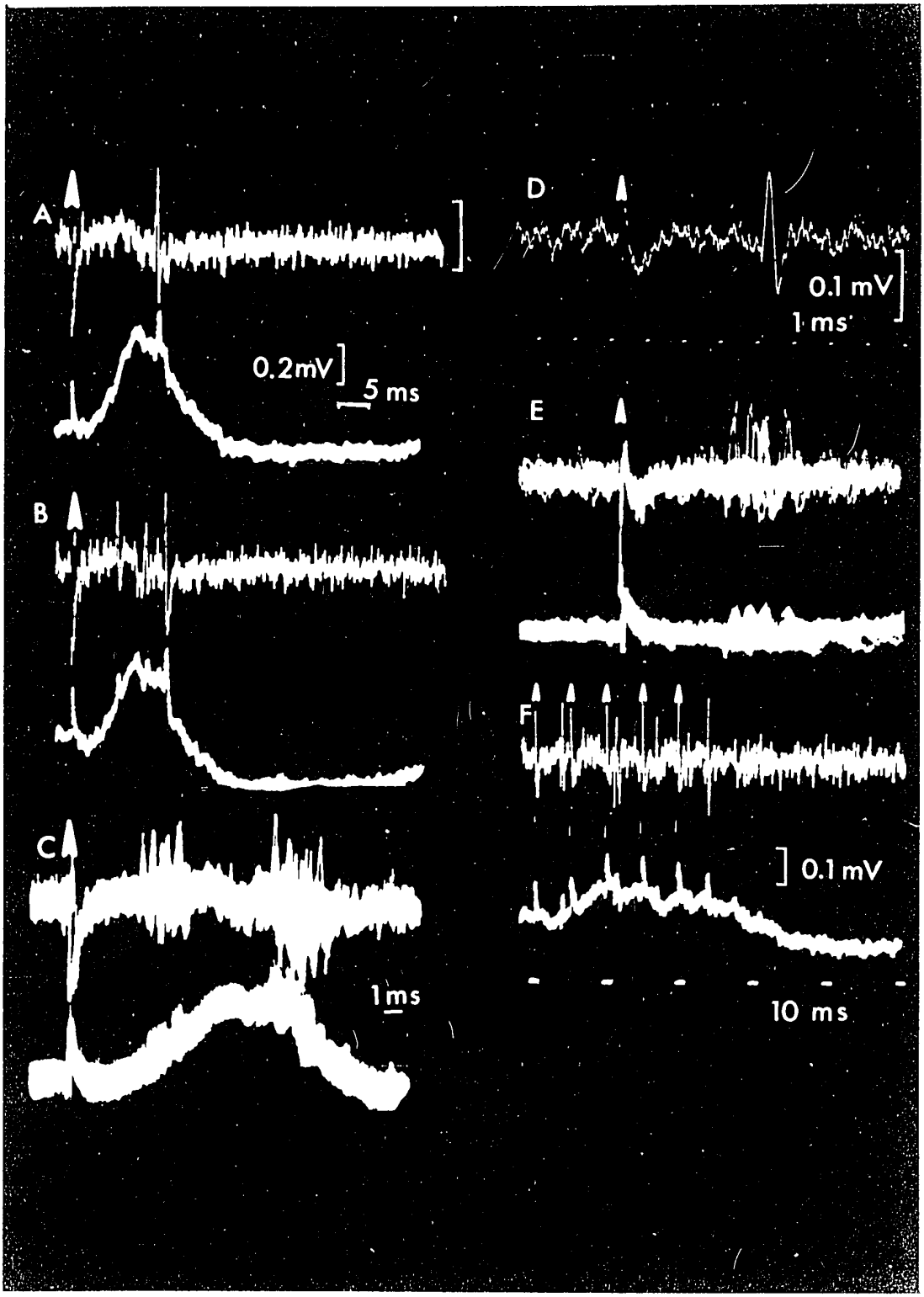

A
0.2mV
5 ms
B
C
1 ms
D
0.1 mV
1 ms
E
F
0.1 mV
10 ms

Figure 28 Synaptically-activated unit in the IPN; single spike

A, single sweep, B, superimposition of 5 sweeps, of a unit in a cat under Dial (glass microelectrode). Same time scale for both. C & D show the response to repetitive stimulation, and have the same time scale.

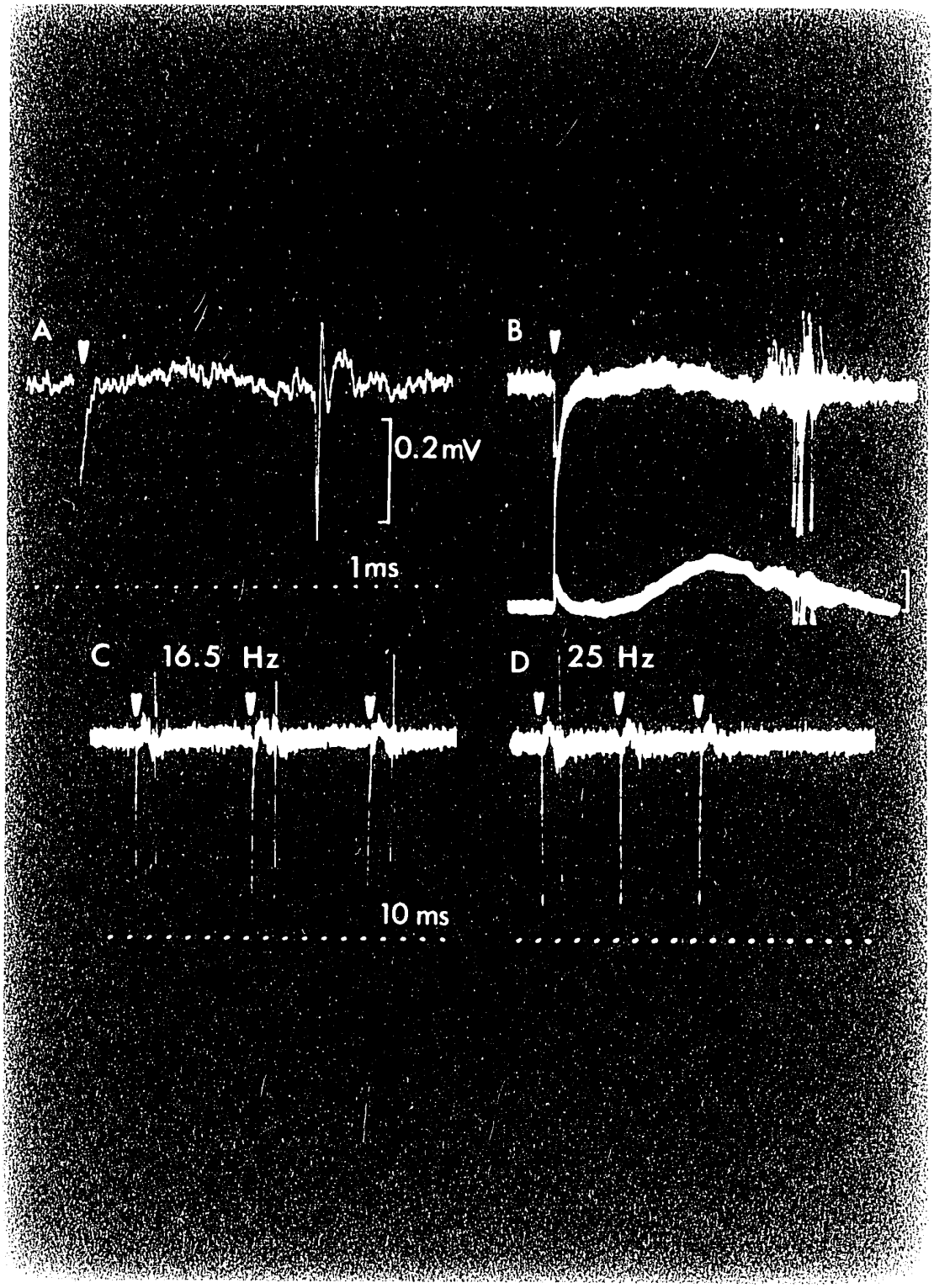

A
0.2mV
1ms
B
C
16.5 Hz
10 ms
D
25 Hz

Figure 29 Constant and variable latency units at the same depth

Two units recorded stimultaneously in a Dial cat, with a stainless steel microelectrode. Threshold of earlier (constant) unit 6 V; other unit, 11.5 V. A, single sweep; B, superimposition of 5 sweeps; C, fast sweep at 8 V. Vertical calibrations in B & C are 0.1 mV.

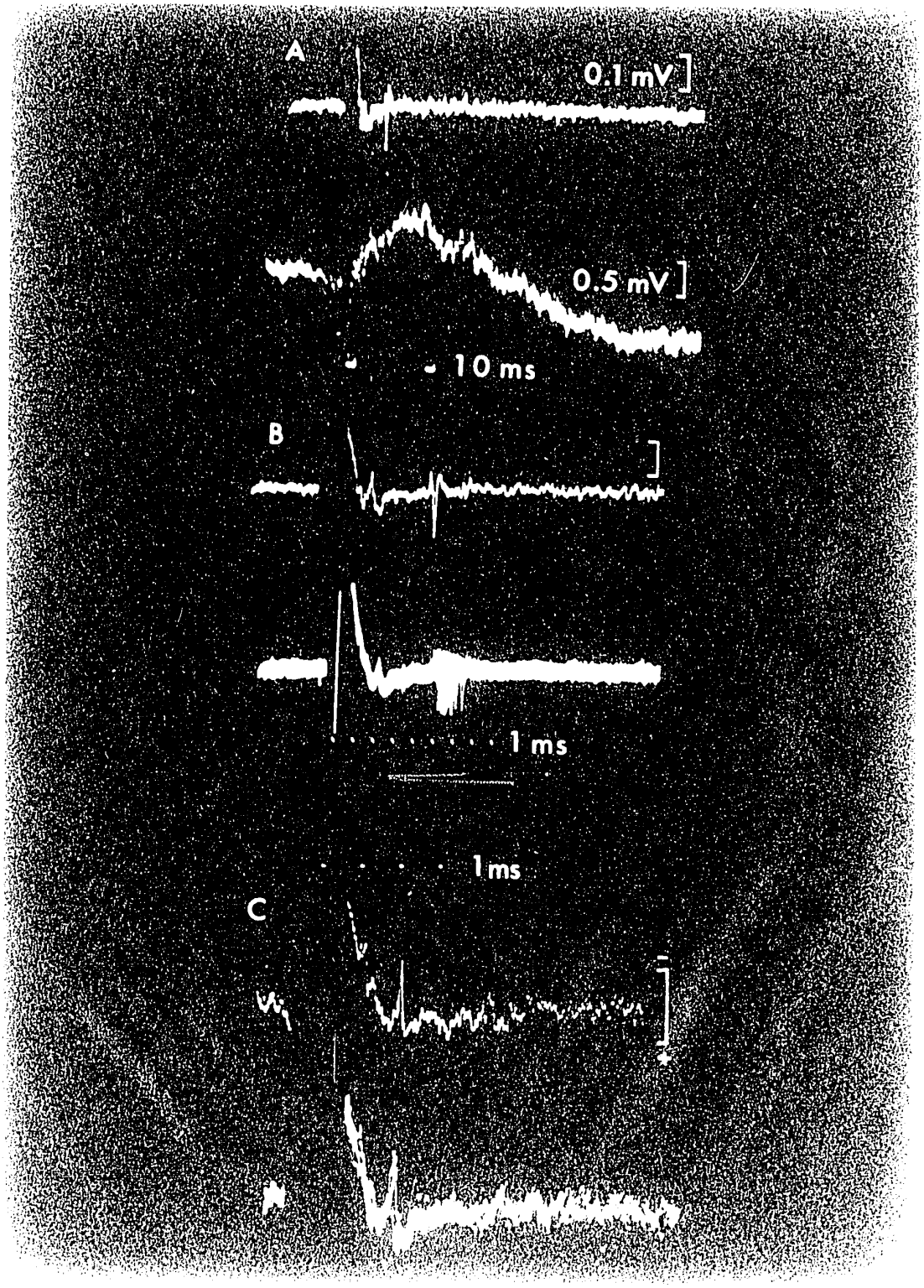

A
0.1 mV
0.5 mV
10 ms
B
1 ms
1 ms
C

Figure 30 Single spike evoked by habenular stimulation

A, single sweep, B, superimposition of 5 traces, of discharge of a unit in a cat under Dial (stainless steel microelectrode). Time scale for A & B is the same. C, superimposition of 3 traces. D, single slow sweep showing silent period. Vertical calibrations are 0.1 mV for upper and lower traces (see B).

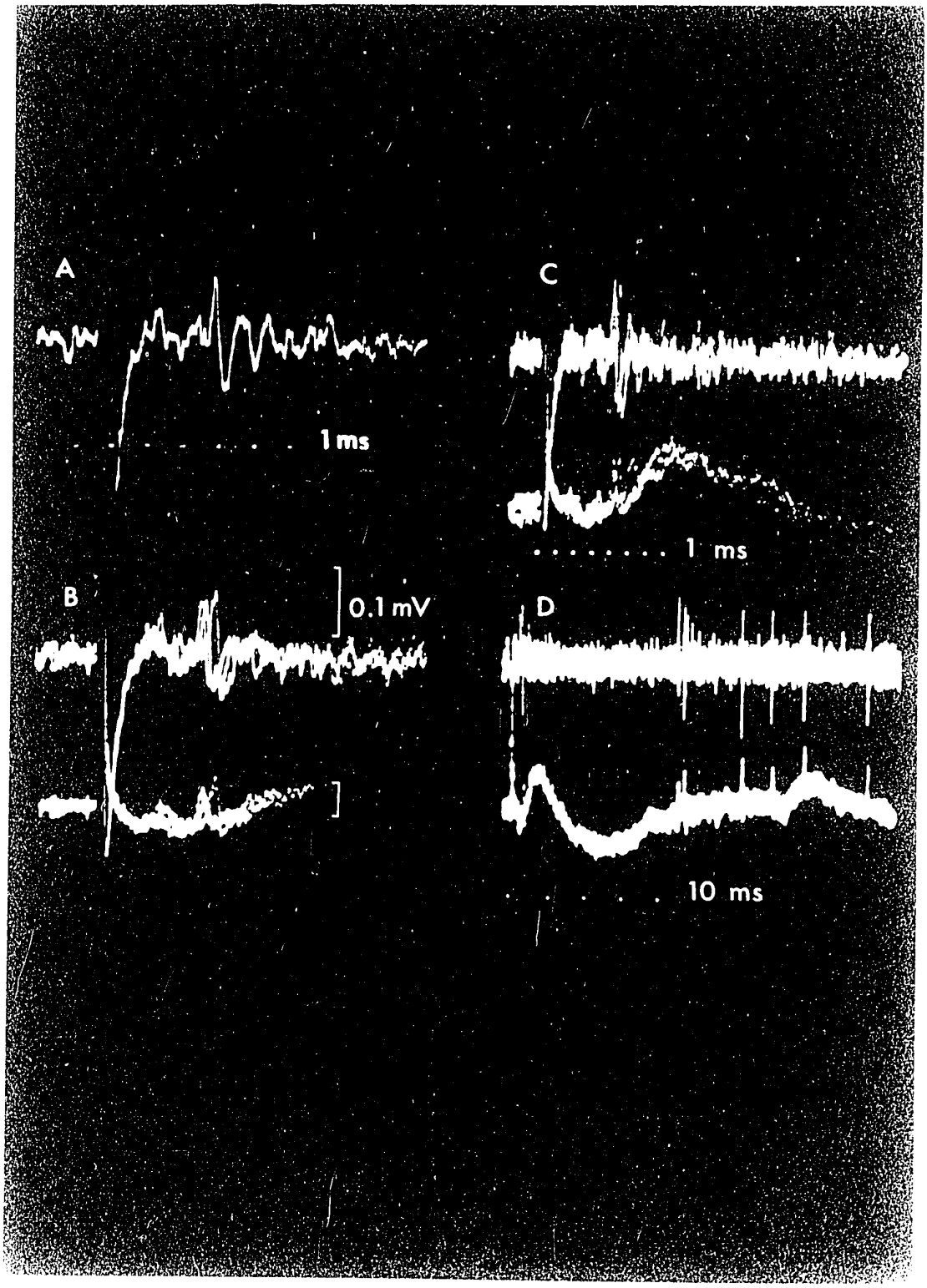

A
1 ms
B
0.1 mV
C
1 ms
D
10 ms

Figure 31 Single spike units in the IPN evoked by habenular stimulation

Two units with similar thresholds (9V) in a cat under Dial A, single sweep; B, superimposition of 10 sweeps (same time scale as in A); C, superimposition of 5 sweeps. Stimulus intensity 15 V. Both units fail at low frequencies (e.g.10 Hz), of repetitive stimulation.

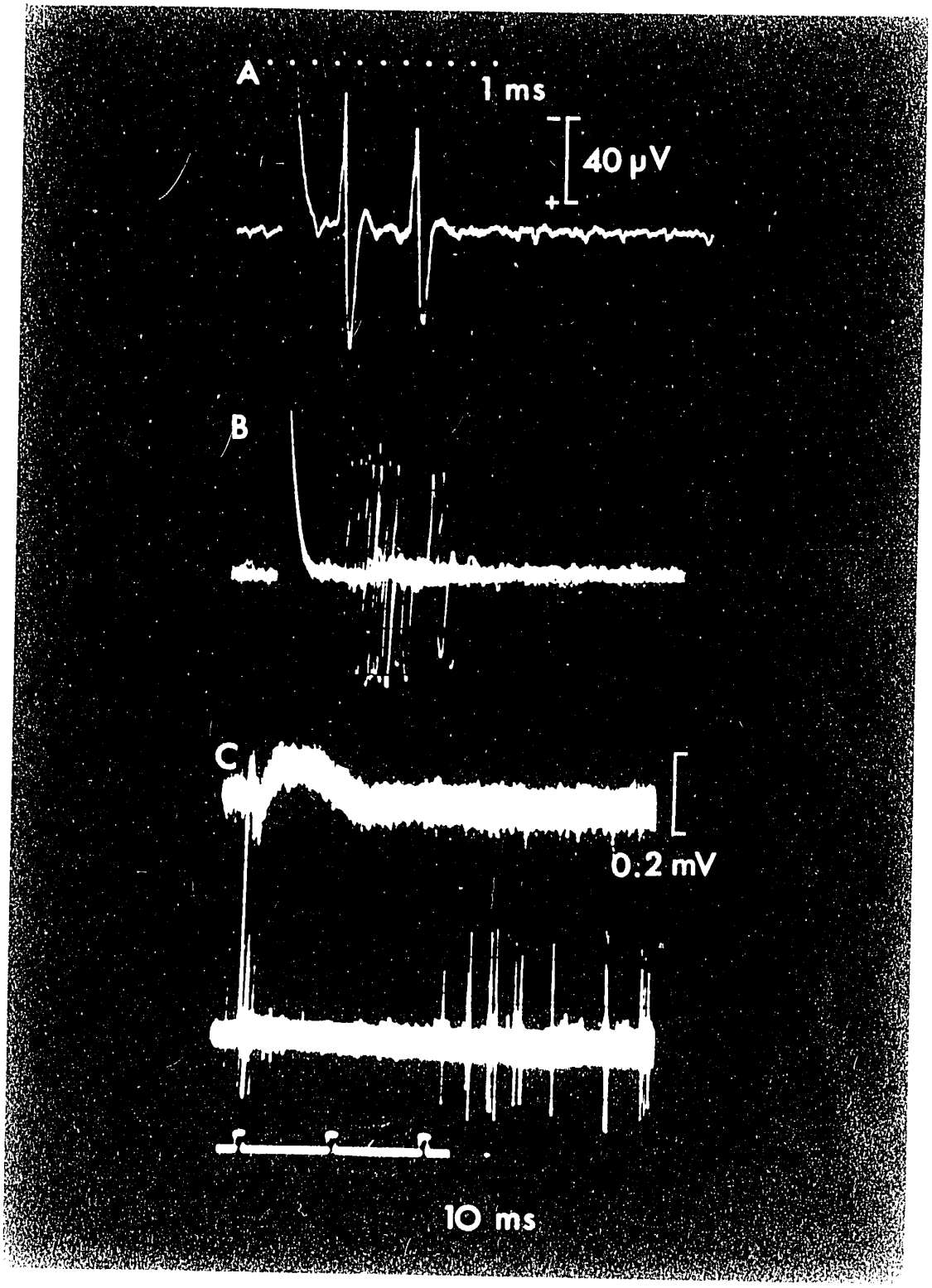
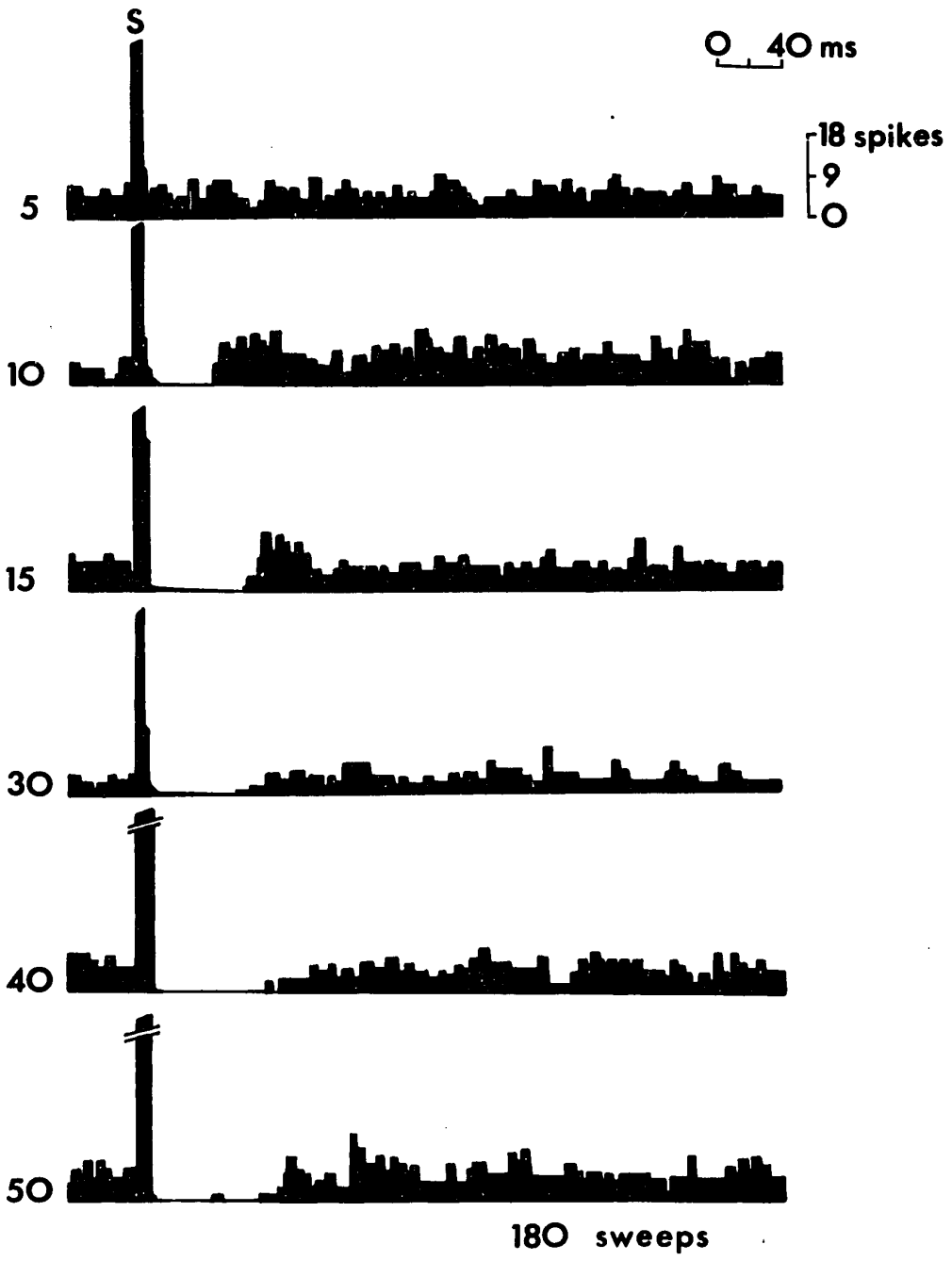

A
1 ms
40 μV
B
C
0.2 mV
10 ms

Figure 32 Post-stimulus histograms of an IPN unit discharge after habenular stimulation.

Penwriter record of digital computer output, for a unit in the IPN of a cat under halothane anesthesia. The intensity of habenula stimulation in volts is given at the beginning of each trace. The unit fires at 6-9 ms after a suprathreshold stimulus (5-6 V). Each trace constitutes the analysis of the post-stimulus period for 180 stimuli at 1 Hz.


S
0 40 ms
18 spikes
9
0
5
10
15
30
40
50
180 sweeps

firing increased with strength of stimulation. The histograms shown are for a period of 400 ms following the stimulus. With stimulus intensities above 10 volts a silent interval follows the early firing. A similar silent period was observed with 16 other units, extending to 140 ms for one unit. As most units could be driven at 30 Hz (or more) for at least two stimuli, and the threshold for depression of the spontaneous discharge was often below that for the early firing, it seems unlikely that the silent periods observed were due to refractoriness or post-activation depression, especially as the stimulation rate for histograms in all cases was 1 Hz. It seems more likely that some inhibitory process was involved.

B. 'Burst' units

Twenty-three units were studied which fired in a burst (2-6 spikes at 500-1000 Hz) after a stimulus to the habenula. Thirteen units were observed in cats under Dial, and ten units in cats under volatile anesthesia. They constituted 12% of all the types of units observed in the nucleus (see Figures 33 and 34).

Spontaneous activity

It has been mentioned previously that about two-thirds (16 of 23 units) of 'burst' units appeared not to be spontaneously active. During the 1-3 minute period of observation in the absence of habenular stimulation they did not fire at all, or perhaps only one or two spikes. Those units which did show spontaneous firing, at rates of 1-6 Hz, fired in irregular trains of single action potentials.

Other features of 'burst' units

This burst type of response was less common than the single variable latency discharge ('single spike' unit). In general the mainly negative (or negative-positive) burst spikes were of low amplitude (50-100 μV). This may partly account for the lower number of 'burst' units than of the larger 'single spike' units. They may also have been missed because of a paucity of spontaneous activity. Often it was difficult to distinguish the spikes of a 'burst' unit from a background of discharge at similar latencies and threshold. It was as if these 'burst' units occurred in clusters.

Relation to evoked waves

The initial latencies of 'burst' units did not exceed 12 ms, the average time to the peak of Wave IV. The burst lasted no more than 5-6 ms, so most discharges were over before the onset of Wave V. Occasionally a small negative wave was seen, superimposed on Wave IV, and corresponding in time to the occurrence of the burst.

Response to repetitive stimulation

Little photographic data was obtained on the responses of 'burst' units as the frequency of the stimulus was raised. Most units appeared unable to follow stimuli above 2 Hz. Small changes in latency of the spikes made interpretation difficult, as one could not be sure if the entire burst followed, or if it was reduced to fewer spikes. In one instance a 'burst' unit was found which could be driven for a short period (30 seconds) at 18 Hz.

Figure 33 Units which fire in a 'burst' after habenular stimulation

A. 'Burst' unit in a cat under methoxyflurane anesthesia, recorded with a stainless steel microelectrode. Left, single sweep; right, superimposition of 5 sweeps.
B. 'Burst' unit in another cat under halothane anesthesia. Single sweep; time base as for A. Recorded with a 3-barrel microelectrode. Spikes have been retouched.
C. Another 'burst' unit in the same cat.

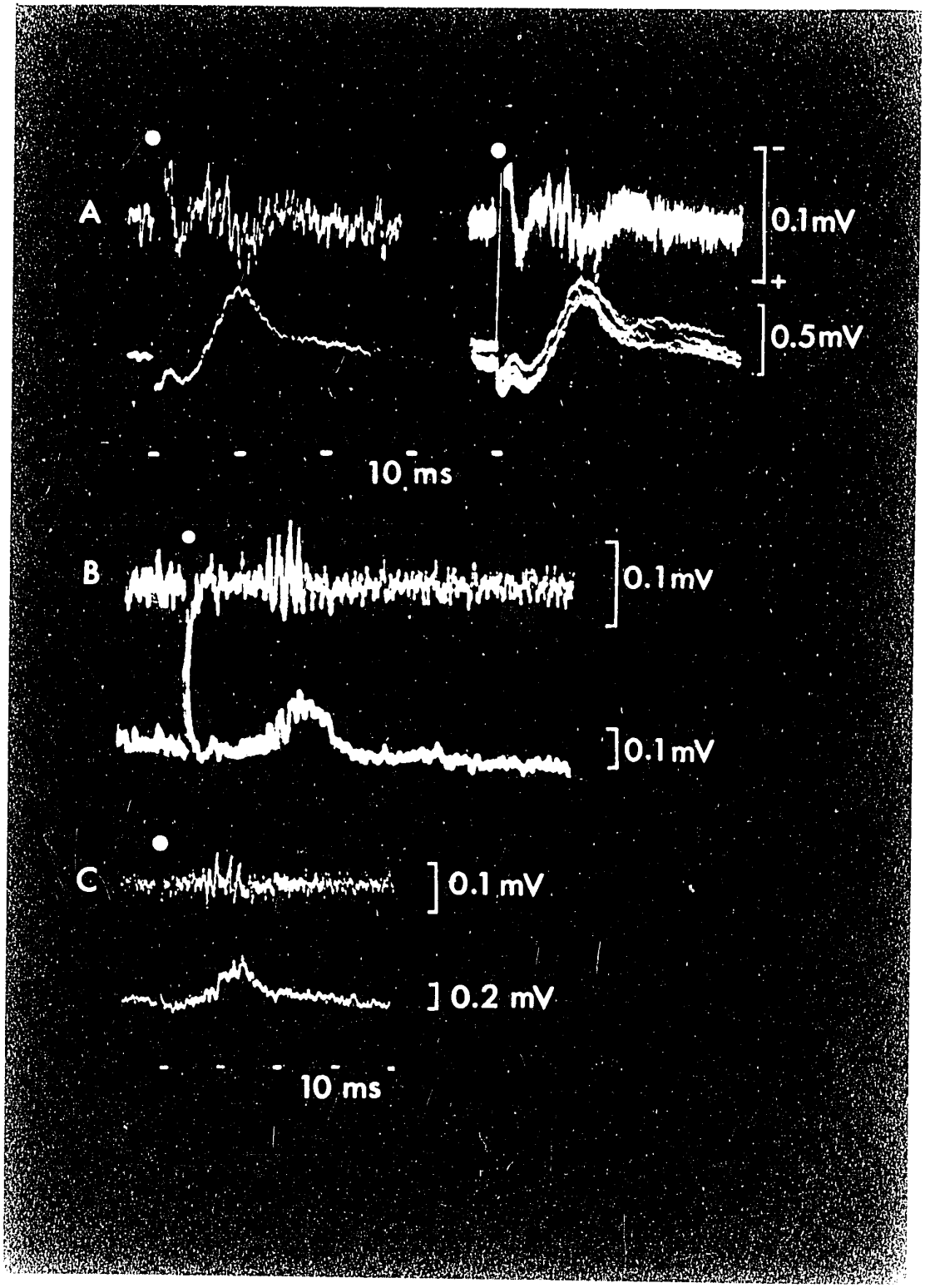
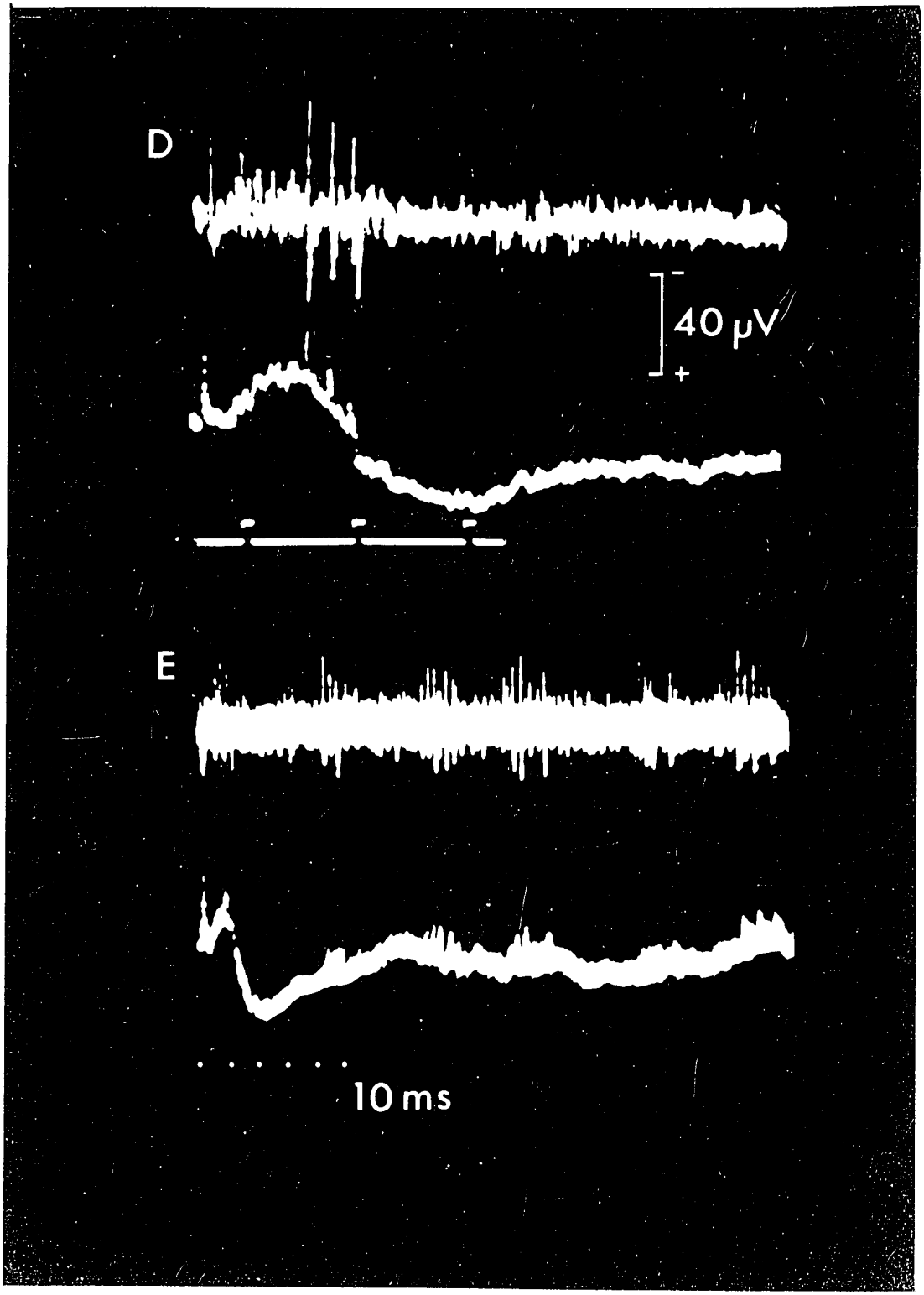

A
0.1mV
0.5mV
10 ms
B
0.1mV
0.1mV
C
0.1 mV
0.2 mV
10 ms

Figure 34 'Burst' response after habenular stimulation

Unit recorded in a cat under halothane anesthesia, which initially showed no spontaneous firing, but discharged in a burst (D) after habenular stimulation. E is taken after a few minutes of repetitive stimulation at 1 Hz. Unit continues to fire spontaneously in 'bursts'. Time scale in D is 10 ms.


D
40 μV
E
10 ms

Relation between 'burst' units and other IPN units

It was quite common to find a larger unit within 20-50 microns of a 'burst' unit. In practically every instance a silent period in the firing of the large unit was seen following the habenular stimulus (and the firing of the 'burst' unit). In all cases the silent period was of much longer duration (20-120 ms) than the period of the burst (2-6 ms).

iii. Decrease in probability of firing of IPN units after habenular stimulation

For some units (69 units) a stimulus in the habenula caused a decrease in firing probability for 10-150 ms, depending upon the stimulus intensity. The mean duration of lowered firing probability at a given intensity was similar for cats under Dial or under volatile anesthesia, but the range of durations was wide for both.

Data were collected from these units in the form of photographic records of superimposed oscilloscope traces (5-10 consecutive sweeps), or as post-stimulus histograms generated by a digital computer (Figure 32). As a rule both techniques were used for each unit, in order to have a visual check of the computer output.

Sequences of excitation and inhibition

Thirty-nine units showed a sequence of initial excitation followed by a prolonged depression of firing. These units have been discussed above; about one-quarter were excited at constant latency (antidromic ?) (Figure 26) and three-quarters were excited at variable latency (presumed synaptic; (Figure 32). In Figure 26 the sequence

at the left shows a unit in which the threshold for depression of the spontaneous discharge was below that of the constant latency evoked spike. This indicates that the silent period is not due to post-spike depression. In one trace (marked 15 G) L-glutamate was applied continuously by iontophoresis causing the unit to fire at a much higher rate. This suggests that the silent period is not due to a stimulus-induced excessive depolarization. The glutamate itself caused some overall depolarization, as seen by the reduced spike amplitude. The silent period was shortened, but not eliminated. This suggests that habenular stimulation causes true post-synaptic inhibition, and not presynaptic inhibition, or merely disfacilitation.

Pure inhibition

For thirty other units there was no evidence of early excitation, and the silent period began soon after the stimulus. The spontaneous rate of firing of these units was not above 20 Hz, so the possibility of a spontaneous spike occurring in a short interval (e.g. 20 ms) after the stimulus was low. In any case it is difficult to make an estimate of the latency of onset of inhibition. These units were found at all depths in the IPN, sometimes in close association with burst units. An example of one of these units is shown in the sequence at the right in Figure 26. The spikes of this group of units were among the largest observed (0.2-1.0 mV), and they were usually mainly negative. Their spontaneous firing accounted for most of the higher rates observed in the nucleus, and the silent period caused by habenular stimulation was easily seen on this background of rapid firing.

Recovery from inhibition

The recovery after a silent period was most often a gradual return to the spontaneous rate. However, in some cases, the silent period was followed first by a brief 'rebound' of higher frequency firing, and then a return to the spontaneous rate. Much more rarely units showed an oscillatory pattern of increased and decreased firing probability.

iv. Unresponsive units

Habenular stimulation had no effect on the firing probability of 20% (38 units) of the IPN cells (Table 3). This may have been due to the unfavorable location of the stimulating electrode. For technical reasons (the midline venous sinus and the design of the electrode carriers) an array of stimulating electrodes could be placed in the habenular region of one side, but bilateral placements were not feasible. (The available histological data show that fasciculus retroflexus fibres may terminate ipsilaterally, contralaterally, or both). The spontaneous firing rates of these unresponsive units ranged from 2-30 Hz. Their spikes were mainly negative with amplitudes of 0.1 to 0.4 mV. These units were found throughout the nucleus interspersed with cells which were influenced by the habenula.

In addition to those units for which the habenular stimulus was inadequate, there may well be units not directly influenced by the habenula.

Summary of responses to habenular stimulation

In summary, for the 190 units studied in the IPN, habenular stimulation increased the firing probability of 64%, decreased the firing probability of 16% (without previous excitation), and had no effect on 20% (see Table 3). Included in the major group of units of increased firing probability are those units which showed a sequence of initial excitation followed by inhibition. These units constituted 21% of the total population and increase to 37% the number of units which showed signs of inhibition after habenular stimulation. Table 4 shows a further classification of units which showed an increase in firing probability to habenular stimulation.

Stimulation at other sites

In several experiments the periphery (paws or face region) was electrically stimulated, but there were no clear effects on the IPN units investigated. For this reason the detailed interaction of inputs at the unit level remains to be studied. In one experiment strong stimulation (50 volts) of the globus pallidus evoked spikes from 2 units at different depths in the IPN. They both had negative-positive spikes; the latency of one was 10-12 ms, the other 50 ms, following the stimulus. In both cases the stimulation also generated a prolonged (40 ms) negative wave, with a peak at 30 ms after the stimulus.

Table 3 Summary of effects of habenular stimulation on IPN unit firing probabilities.

Table 4 Classification of types of units whose firing probability was increased by habenular stimulation.

Constant latency, 'single spike', and 'burst' as described in the text. 'Others' includes units which showed a general increase in firing rate after repetitive stimulation, units for which there were only post-stimulus histograms, or units which could not be placed in one of the other groups.

CHANGE IN IPN CELL FIRING PROBABILITY	NUMBER OF CELLS			% OF ALL CELLS
	DIAL	VOLATILE	TOTAL	
INCREASED	45	38	83	43%
DECREASED	21	9	30	16%
INCREASED-DECREASED	26	13	39	21%
NO CHANGE	13	25	38	20%
	105	85	190	

TYPE OF EVOKED UNIT	NUMBER	% OF ALL UNITS
CONSTANT LATENCY	21	11%
SYNAPTIC: BURST	23	12%
: SINGLE SPIKE	56	30%
OTHERS	22	11%
	122	64%

Chemical sensitivity of IPN units

The chemical sensitivity of IPN units was examined using the microiontophoretic technique (Curtis & Eccles, 1958; Krnjevic & Phillis, 1963a; Curtis, 1964) with double, triple and 5-barrelled micropipettes, having tip diameters from 2-12 μ .

The pipettes were inserted stereotaxically in the midline at anterior planes A0.0-3.5 mm, and advanced to a depth of 3-4 mm below the ventral surface of the brain, where the ventral border of the IPN usually begins. The presence of the characteristic evoked potentials and the type of unit responses to habenular stimulation were further confirmation that the electrode was within the IPN. In several experiments the position of the tip of the electrode was marked by means of Alcian blue dye (Lee et al 1967) or a hydrochloric acid lesion (McCance & Phillis, 1965). Although not all attempts at marking were successful, there was usually sufficient stereotaxic and electrophysiological data to allow reconstruction of tracks and the classification of units as within or outside of the IPN. No attempt was made to identify histologically the specific cell from which data were obtained.

The substances tested and their sources, are given in Table 2 of the Methods section above. Time did not permit an extensive general survey of various substances. Instead, investigation was restricted to the unit reactions to L-glutamate or acetylcholine (ACh) and some related compounds. ACh was of particular interest because

of the intense choline acetylase activity in the IPN, and the strong cholinesterase staining in the habenula, the fasciculus retroflexus, and the IPN, as revealed by histochemical studies (described above, page 15 of the Introduction).

Spontaneous activity

All units studied were spontaneously active. The incidence of different spontaneous rates is given above for all units in Figure 10. Cholinoceptive units (those which were excited by ACh) had a wide range of spontaneous rates, with a pattern of incidence similar to that of the total population. Units which were insensitive to ACh also had a wide range of spontaneous firing rates. Cholinoceptive units tended to fire quite regularly or irregularly, in single spikes rather than in bursts. The effect of iontophoretically applied substances was tested on spontaneously firing units only, that is, L-glutamate was not used to raise their excitability, although it was found to be a potent excitatory agent for most units. It is possible, therefore, that the number of ACh sensitive units was underestimated. However, data were more easily correlated with those from the population of units previously studied with metal and single glass microelectrodes, as the experimental conditions were much the same.

The responses to ACh

The effects of iontophoretically applied ACh (as a cation) on the firing patterns of IPN units were observed and compared with the effects of control anodal currents of sodium ions.

The application of ACh often caused a slow acceleration in firing rate in IPN units which persisted for 2-20 seconds after the cessation of the drug current (see Figures 35 ii, iii; 36 B-E; 37 B & D; 38 A-C; 41 E; 42 A, B; 43). Usually there was some initial depression of the spontaneous firing (probably anodal depression as ACh is released by an outward current) which delays the onset of increased firing. For two-thirds of the units this delay was from 2-5 seconds, while it was longer (5-25 seconds) for the remaining third (see Figures 35 iii; 36 B; 38).

Figure 38 shows a unit in which the spontaneous firing was depressed markedly during the application of drug current. The firing began to accelerate showly during the application and was much increased over the control rate for a period of 10-15 seconds after the cessation of the ACh current. This rapid firing is not entirely due to post anodal exaltation as the control anodal current does not give rise to a rebound with the termination of the sodium current (trace D).

Figure 36 shows in another unit increased excitation with higher amounts of ACh. ACh 150 nA caused the unit to fire extremely rapidly. The spike amplitude appeared to increase; this may be a result of movement of the cell towards the electrode. The unit did not fire for about 2 seconds after the cessation of the ACh current. This may have been a result of depression due to excessive depolarization caused by the large amount of ACh and the rapid firing rate.

Figure 35 The effects of iontophoretically applied compounds on units in the IPN

All currents in this and succeeding figures are in nanoamperes. Current applications are indicated by a white line below each trace. When there are two beams the upper is recorded with a 20 ms time constant and the lower with a 2 ms time constant.
Traces i and ii are of a unit in a cat under Dial anesthesia.
Traces iii-v are of a unit in a different cat under methoxyflurane anesthesia. Trace v shows the spontaneous activity of this unit.
The time scale is the same for all traces.

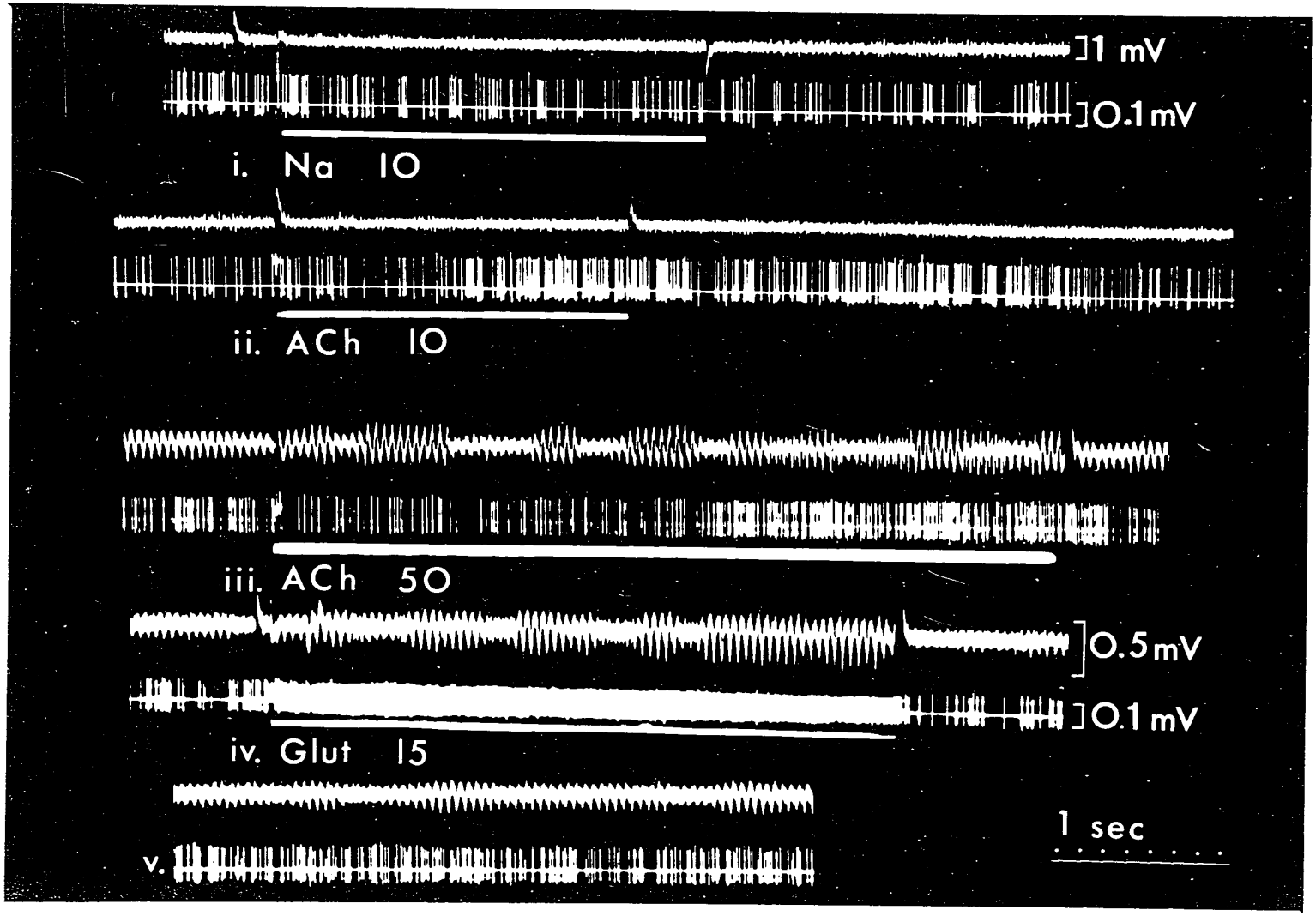

1 mV
0.1 mV
i. Na 10
ii. ACh 10
iii. ACh 50
0.5 mV
0.1 mV
iv. Glut 15
v.
1 sec

Figure 36 Effects of ACh on firing of a unit in a cat under Dial anesthesia

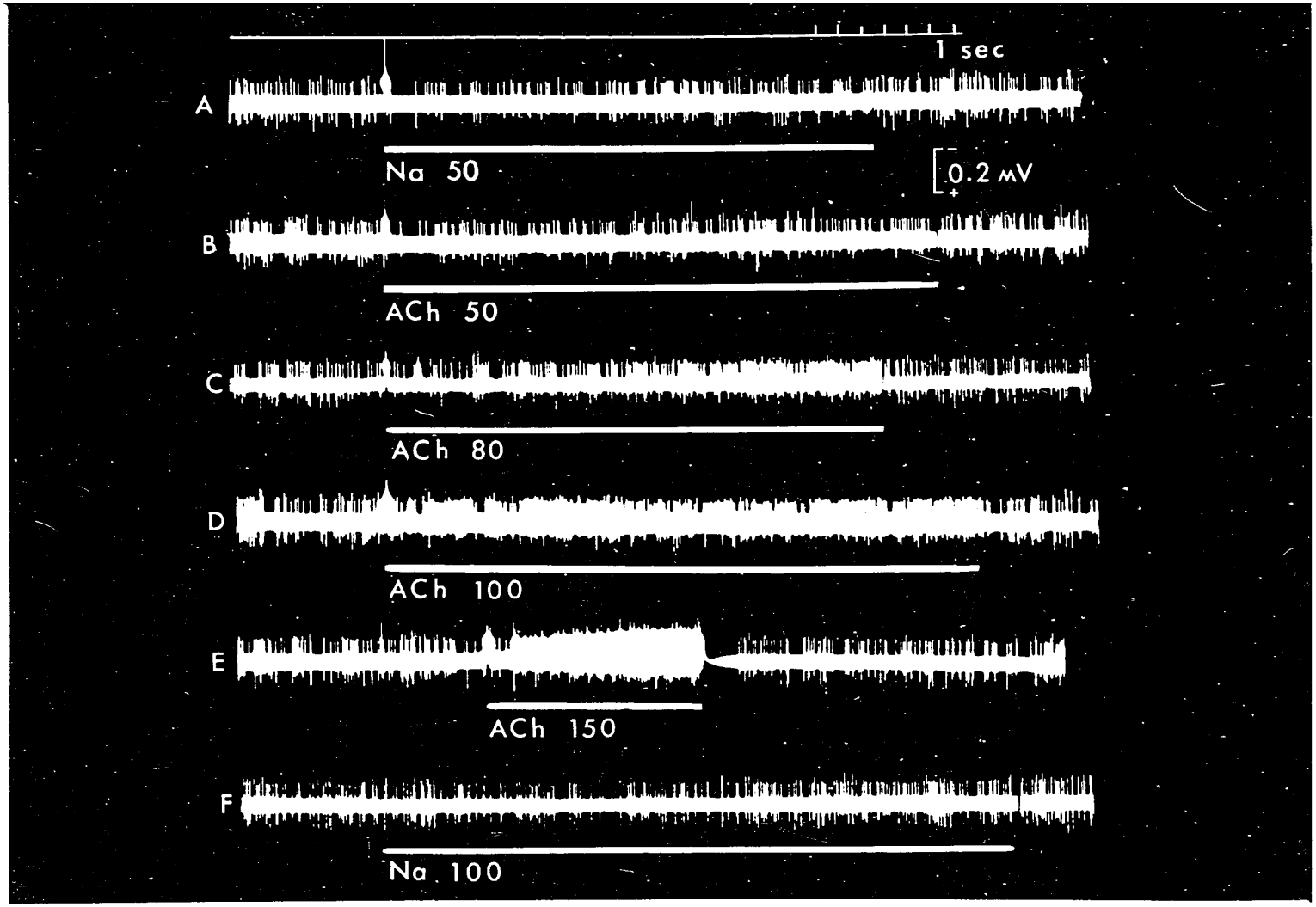

1 sec
A
Na 50
0.2 mV
B
ACh 50
C
ACh 80
D
ACh 100
E
ACh 150
F
Na 100

Figure 37 The effects of L-glutamate and ACh on firing of a unit in another cat under Dial anesthesia.

This unit was inhibited by habenular stimulation.

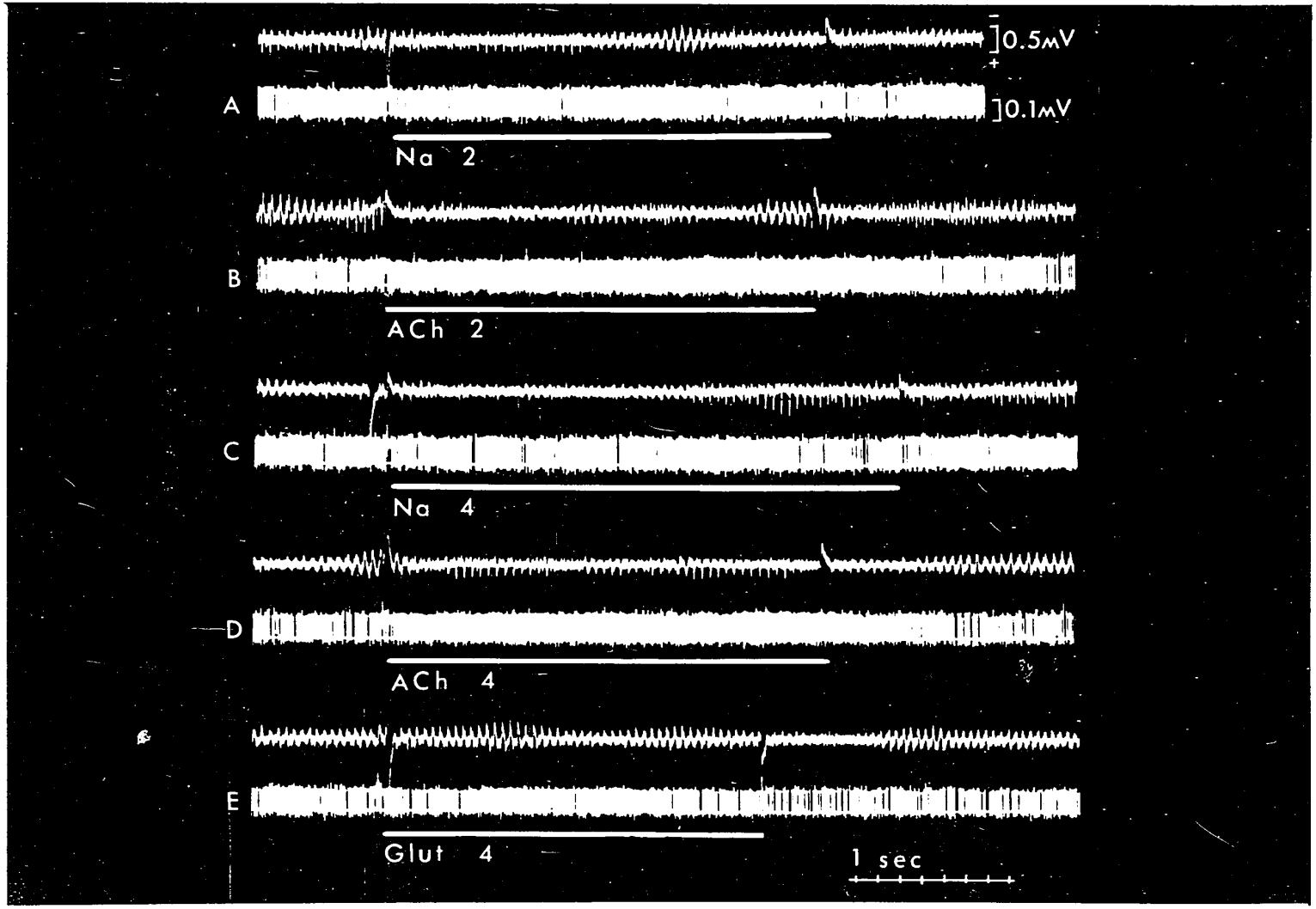
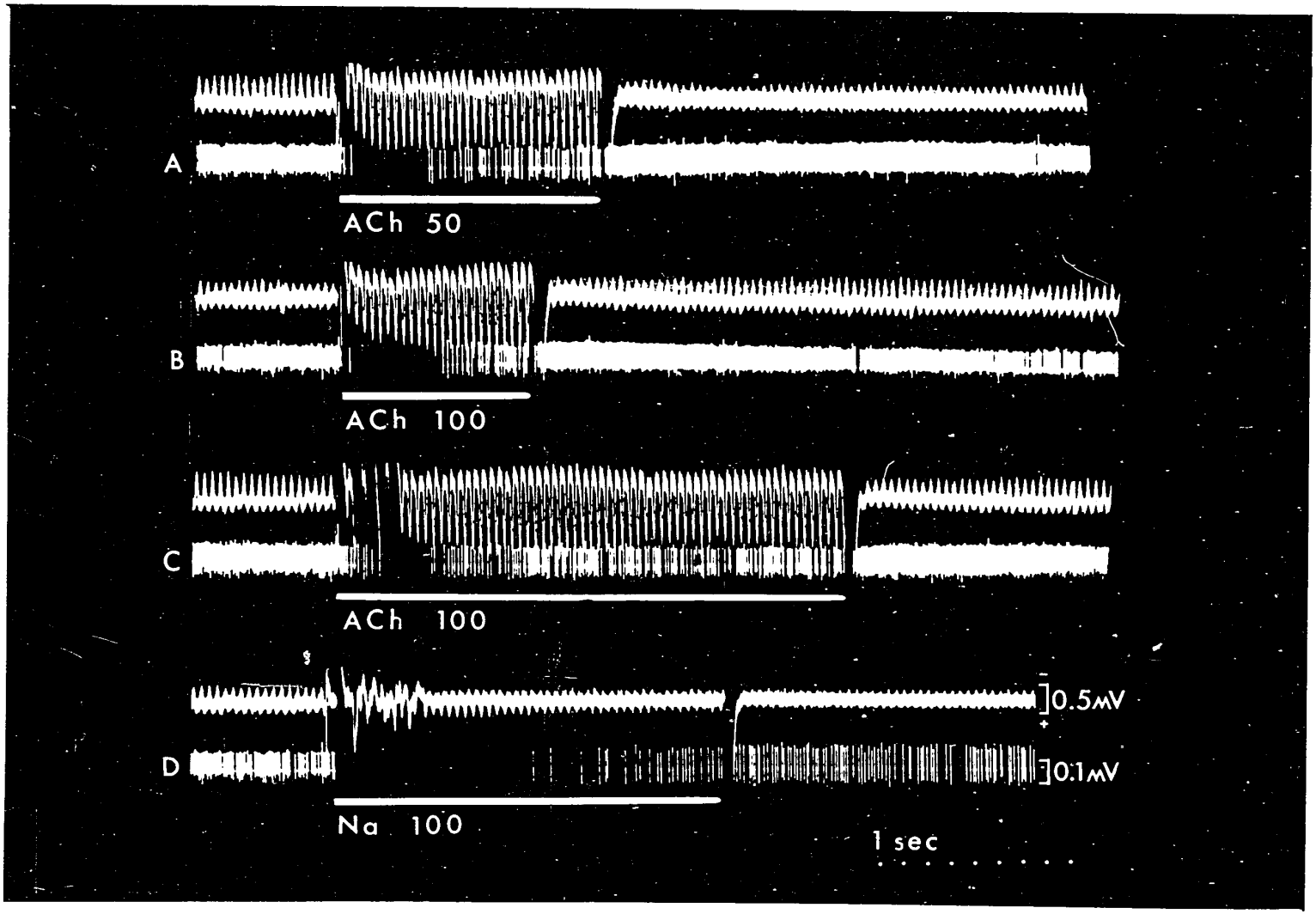

0.5mV
0.1mV
A
Na 2
B
ACh 2
C
Na 4
D
ACh 4
E
Glut 4
1 sec

Figure 38 Effects of ACh on another unit in a cat under Dial anesthesia

This unit was excited by habenular stimulation at a latency of 5-9 ms. Recovery from hyperpolarization caused by Na 100 occurs about 15 seconds after the end of the trace shown.


A
ACh 50
B
ACh 100
C
ACh 100
0.5mV
D
0.1mV
Na 100
1 sec

The threshold iontophoretic current for these excitatory effects of ACh ranged between 2 and 50 nA, but in general it was between 20 and 40 nA.

In 3 cats low currents (e.g. 8 nA) of L-glutamate or ACh depressed the spontaneous firing rate of cells and sometimes entirely blocked their firing for various lengths of time (see Figures 39-42). In lower doses these substances were excitatory (e.g. Figure 41 E; Figure 42 A,B), and the units gave the typical excitatory responses to L-glutamate or ACh. However at slightly higher doses the spike disappeared abruptly (not via a graduated fall in amplitude such as occurs with inactivation by excessive depolarization), and returned only after the end of the drug application (see Figure 39 C; Figure 40; Figure 41 F; Figure 42 D). Usually, after recovery, the spikes were at first larger than the spontaneous ones, and were generated at a slower rate. Some examples are shown where total block was not produced, but ACh applications evoked larger and slower spikes, as if the unit were hyperpolarized. Figure 42 shows a series of ACh applications of equal current given close to the same unit. The responses vary from the typical excitatory one (traces A, B), to something which resembles afterhyperpolarization or inhibition (trace C), to total block of the spike (trace D). The extent of block seems to correspond to the amount of increased firing previously induced by ACh; greater blocking follows higher degrees or longer durations of excitation.

Figure 39 Blocking of unit firing in the IPN by ACh in a cat under Dial anesthesia

C & D are a continuous sequence showing the blocking of the spike after ACh application, and recovery about 30 seconds after cessation of the ACh current.
E, F Habenular stimulation (15V, 1 Hz) appears to depress the spontaneous firing of this unit. Time scale is 10 ms.
F is a superimposition of 5 sweeps.

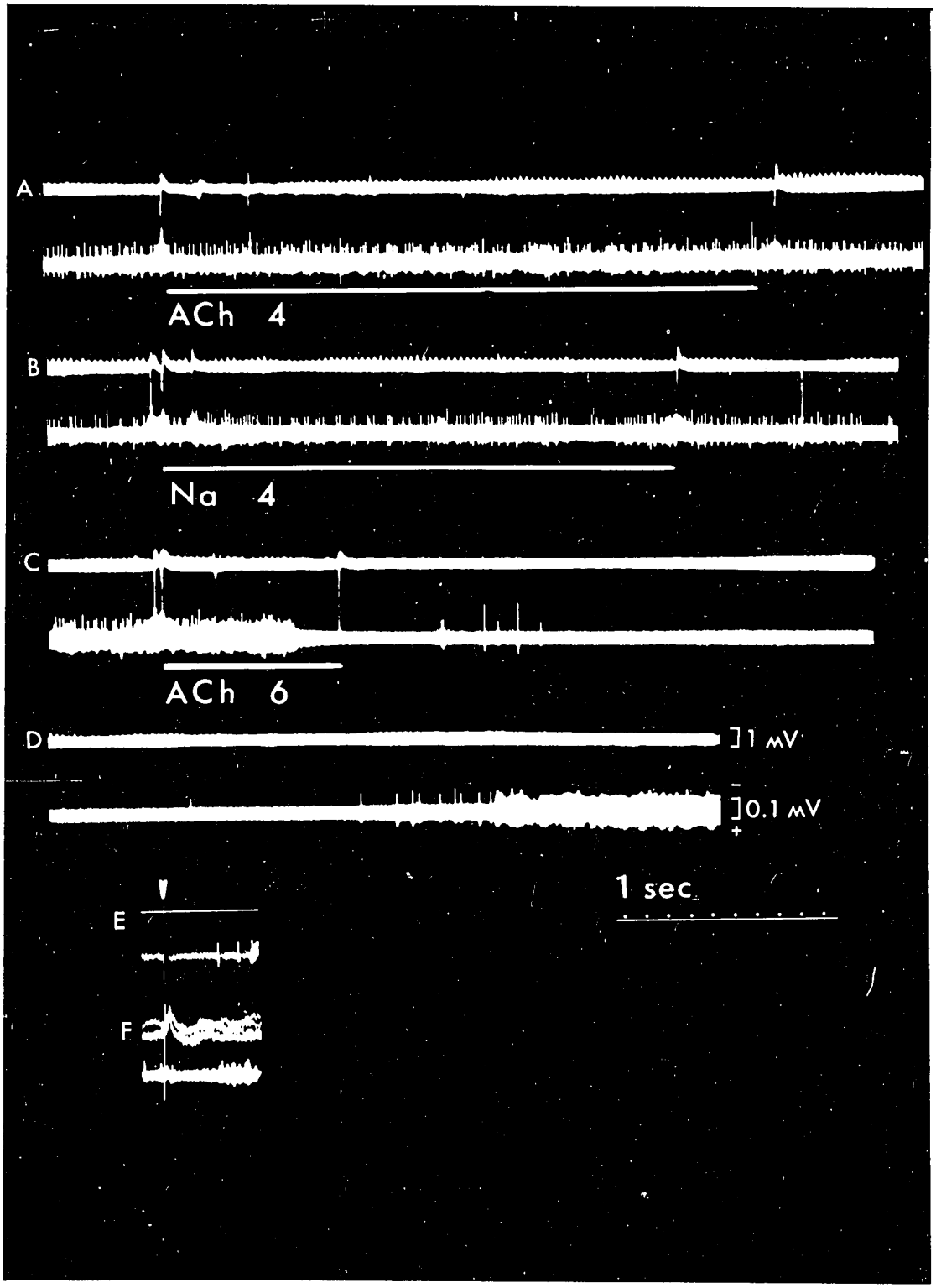

A
ACh 4
B
Na 4
C
ACh 6
D
1 mV
0.1 mV
1 sec
E
F

Figure 40 Blocking effects of L-glutamate and ACh on units in the IPN

At least two units in a cat under Dial anesthesia, which appear to be blocked by brief applications of L-glutamate or ACh. Traces 2, 3 and 4 are a continuous sequence. After ACh 40, firing of units returned about 1 minute following the end of the trace. Block could also be produced with ACh 25.

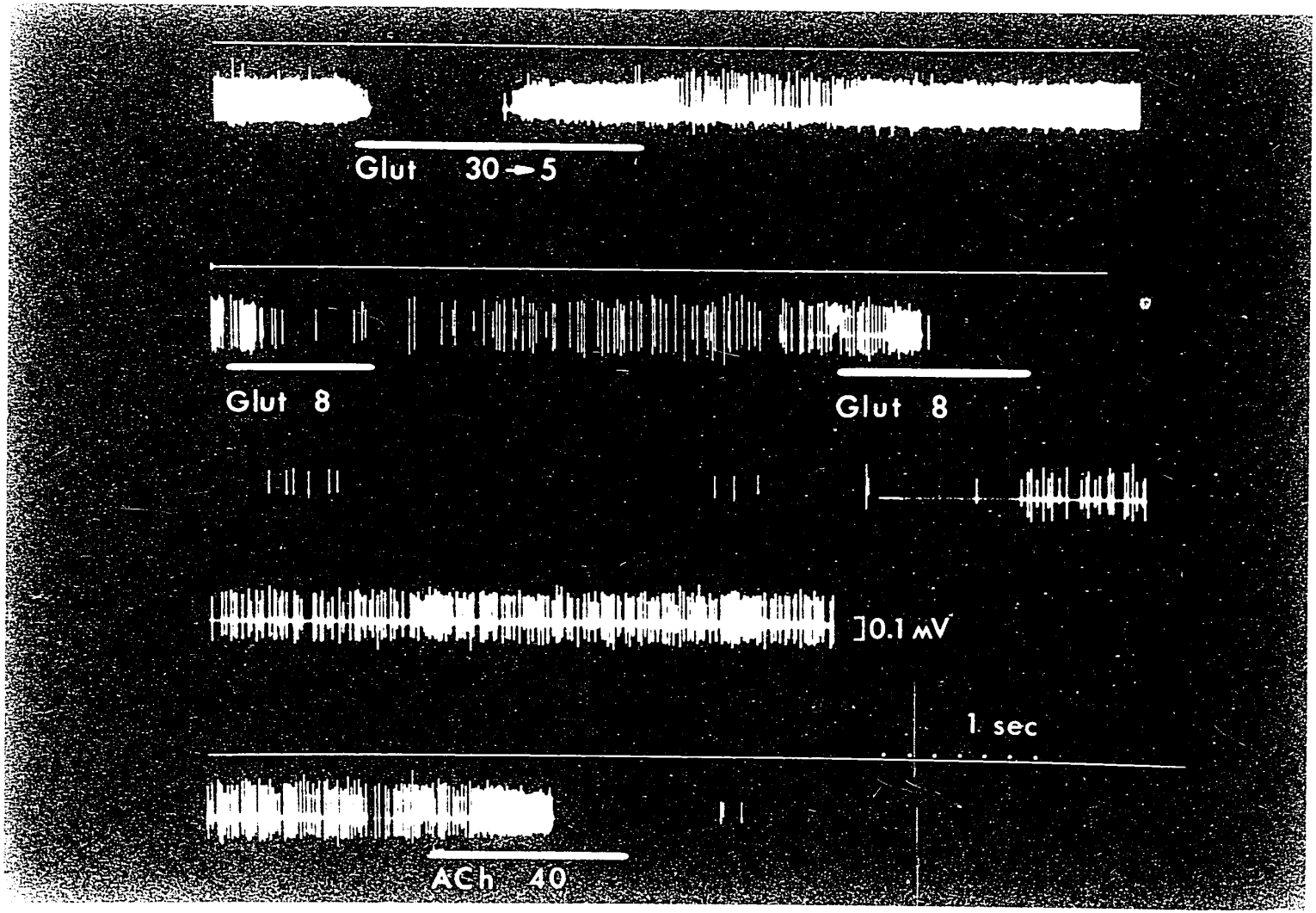

Glut 30 → 5
Glut 8
Glut 8
0.1 mV
1 sec
ACh 40

Figure 41 Dual effects of ACh on unit firing in a cat under Dial anesthesia

Traces F and G are a continuous sequence. This unit was unresponsive to habenular stimulation (up to 20 V).

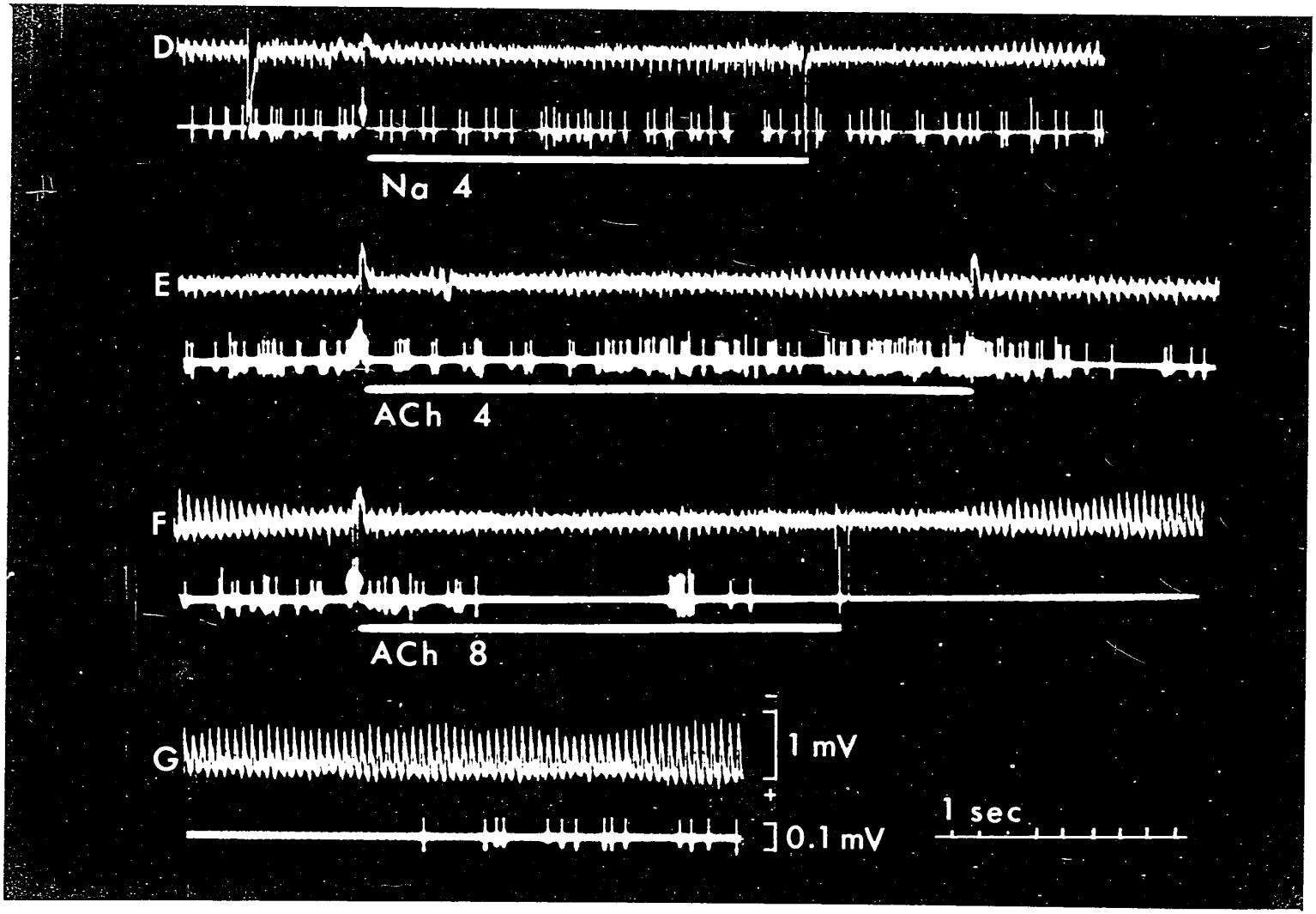

D
Na 4
E
ACh 4
F
ACh 8
G
1 mV
0.1 mV
1 sec

Figure 42 Different periods of application of ACh

This unit, from a cat under Dial anesthesia, showed early excitation followed by later inhibition after habenular stimulation (see F: time scale is 10 ms).

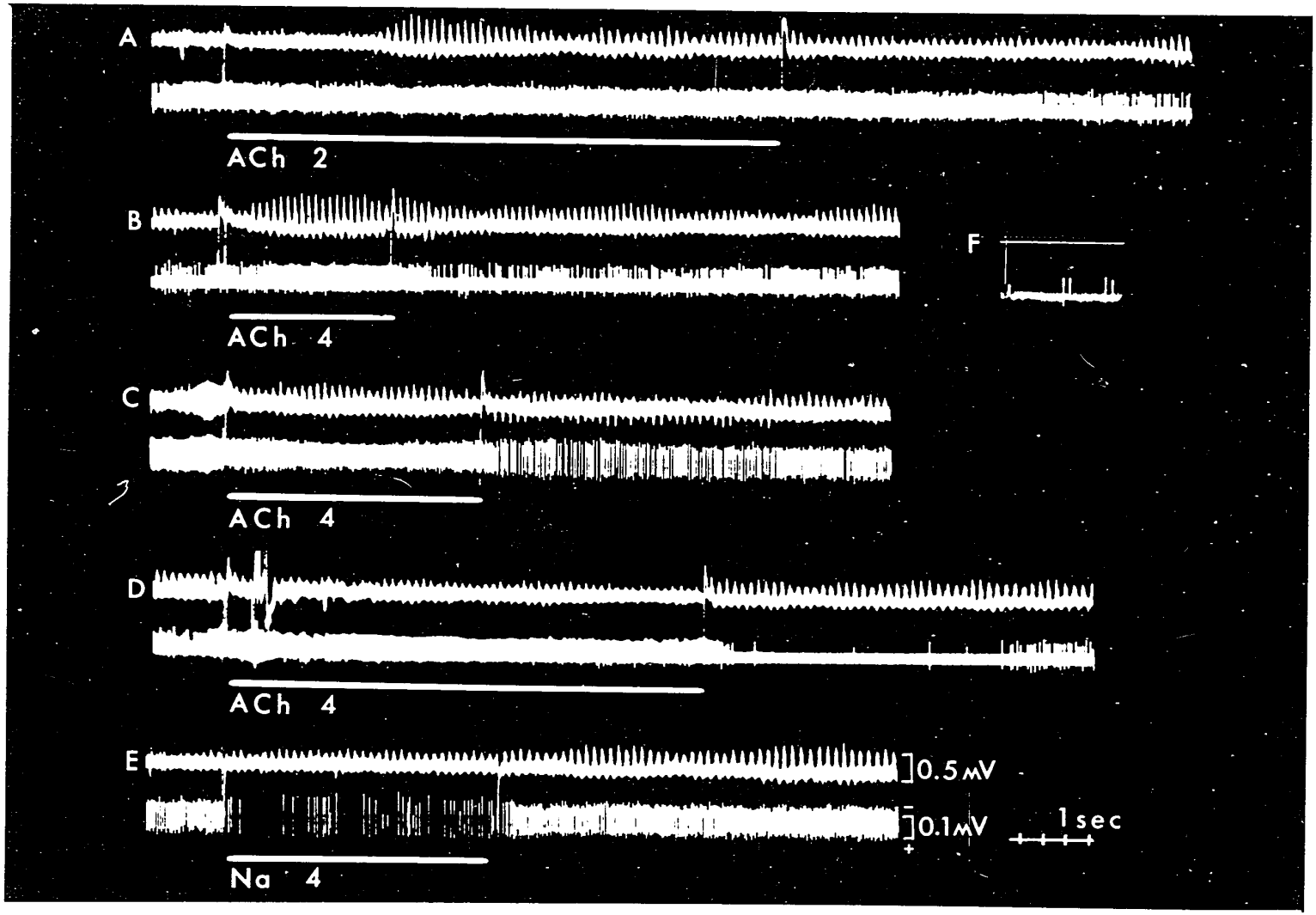

A
ACh 2
B
ACh 4
F
C
ACh 4
D
ACh 4
E
0.5 mV
0.1 mV
1 sec
Na 4

Table 5 shows the results of these studies on 81 units. It is seen that 77% of the units were excited by ACh, that is, their firing probability was increased. This was in contrast to a less marked effect, or more usually an anodal depression of firing, by sodium currents.

Table 5 enumerates two units as being depressed by ACh application. In both cases the evidence was greater depression of unit firing by ACh than by equal currents of sodium ions (anodal current), but in both instances the effect was very mild. It seems probable that these units were insensitive to ACh and that the slight difference between current and ACh effects was owing to the different spatial relation of the ACh and sodium barrels to the unit. This would increase to 23% the proportion of units unresponsive to ACh, (see Table 5). Units classified as unresponsive showed either no change in firing pattern to ACh applications, or responses indistinguishable from those to control anodal currents.

The response of cholinoceptive units to habenular stimulation

Table 6 shows the responses of units to habenular stimulation and their responses to iontophoretically applied ACh. The incidence of responses to habenular stimulation is not statistically different from that of the population of units not tested iontophoretically. These percentages are given at the bottom of the table for comparison. Many units (81%) showed an increase in firing probability to both ACh and habenular stimulation. Two thirds of these units were 'single spike' units and one third 'burst' units. Some showed sequences of initial

Table 5 The effects of iontophoretically applied acetylcholine on the firing probability of units in the IPN

e.g. Increased means that the firing probability of the units increased as a result of iontophoretic application of ACh.

ANESTHETIC	NUMBER OF CELLS			
	Firing Probability			
	Increased	Decreased	No change	Total
Dial	32	1	9	42
Volatile	30	1	8	39
Combined data	62 (77%)	2 (2%)	17 (21%)	81

Table 6 Responses to iontophoretically applied acetylcholine of IPN units typed for their responses to habenular stimulation.

ACh	+	firing probability increased by ACh
ACh	0	firing probability unaffected by ACh
Hb	+	firing probability increased by habenular stimulation
Hb	-	firing probability decreased by habenular stimulation
Hb	0	firing probability unaffected by habenular stimulation

Combined data shows the grouped data for each category of response to habenular stimulation for cells tested iontophoretically for ACh sensitivity.

IONTOPHORETIC EFFECT	NUMBER OF UNITS			
	Hb +	Hb -	Hb 0	TOTAL
ACh +	21 (81%)	14 (88%)	4 (50%)	39
ACh 0	5 (19%)	2 (12%)	4 (50%)	11
Combined data	26 (52%)	16 (32%)	8 (16%)	50
Cells not tested by iontophoresis	96 (68%)	22 (16%)	22 (16%)	140

excitation followed by later inhibition. A considerable number of units (88%) showed an increased firing rate to ACh but decreased firing probability after habenular stimulation (pure inhibition).

The response to L-glutamate and GABA

In agreement with previous observations on the brain (Hayashi & Nagai 1956; Curtis & Koizumi 1961; Krnjević & Phillis 1963 a,b), L-glutamate is a potent excitatory agent for most units of the IPN. The intense firing response to glutamate typically is of short latency and usually ends abruptly with termination of the current (see Figure 35 iv). It is quite different from the typical response to ACh. The high firing rate sometimes leads to slight depolarization of the unit as evidenced by a reduced spike amplitude, and sometimes complete (but reversible) block. Among other things, L-glutamate was useful as a tool to test the specificity of atropine blockade of ACh, and to discriminate between inhibition and disfacilitation. GABA was tested less frequently, but on all occasions low amounts (e.g. 4-10 nA) easily blocked unit activity, whether spontaneous or evoked by glutamate or ACh. It was not tested on synaptic activity evoked from the habenula.

Pharmacology of the response to ACh

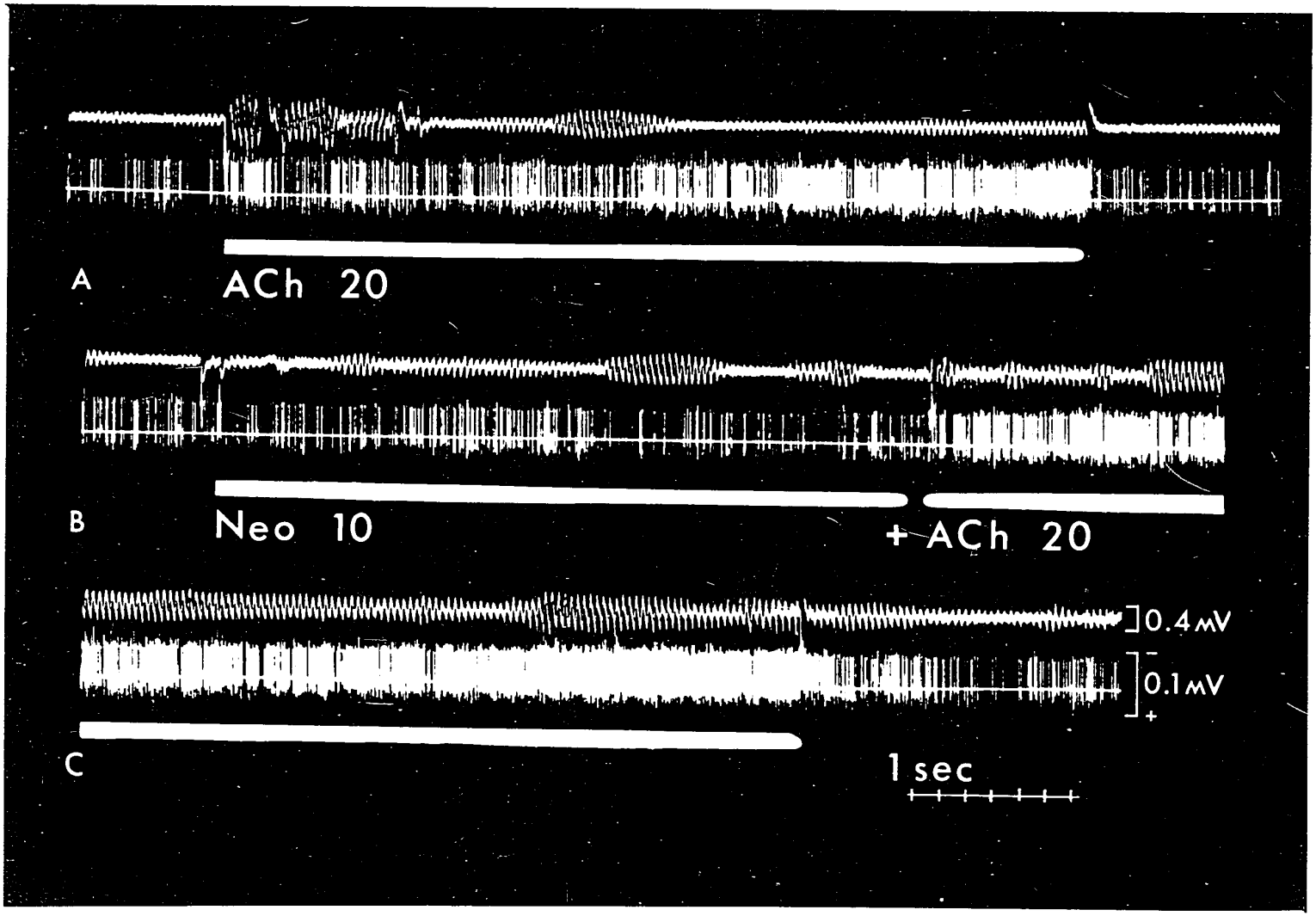
Two other choline esters, carbamylcholine (carbachol), and acetyl-β-methyl choline chloride were tested on a small number of units in a few cats. Their effects on a unit were never as strong as that of ACh itself, and much of the time the effects were very weak. Currents up to 80 nA were used to release them, but perhaps an adequate concentration close to the units was not achieved. Also, the units tested were not strongly excited by ACh, so conditions were not optimal for revealing weak agonists.

The anticholinesterase drugs neostigmine bromide (prostigmine) and edrophonium chloride (Tensilon) applied iontophoretically (as cations) potentiated the effects of ACh released from another barrel. Neostigmine gave much more consistent results than Tensilon, which often depressed the unit, thus increasing the difficulty of interpretation. Neostigmine by itself had an excitatory action at doses above 30 nA, but in doses low enough to have no direct excitatory effect on the unit it reduced the latency of the acceleration of firing produced by ACh applied concurrently from another barrel (Figure 43). Also the maximal firing rate was increased, and the afterdischarge was prolonged. Though it was not tested systematically on many units, neostigmine appeared to have little effect on synaptic transmission induced by habenular stimulation.

Atropine sulphate injected intravenously (0.3-1.0 mg/kg) blocked the effects of iontophoretically applied ACh on cholinoceptive units for twenty minutes (0.3 mg/kg) to an hour (0.6 mg/kg or more). The general condition of the animal was usually unaffected by such injections. In unit studies, during the first 3-4 minutes following the atropine injection the spike height and spontaneous firing were reduced, and sometimes L-glutamate was less potent as an excitatory agent. (This was probably due to the local anesthetic action of atropine (Curtis & Phillis 1960; Krnjević & Phillis 1963 b). The response to ACh was reduced (low dose) or entirely blocked (high dose atropine). Shortly thereafter the response to L-glutamate usually regained its control strength and ACh action was completely blocked,

Figure 43 The potentiation of ACh excitation by neostigmine

IPN unit of a cat under methoxyflurane anesthesia. B & C are a continuous sequence. The termination of the white line indicates the end of ACh, but not neostigmine application.


A
ACh 20
B
Neo 10
+ ACh 20
0.4 mV
0.1 mV
C
1 sec

while the spontaneous activity remained low. When recovery began, twenty to fifty minutes after the atropine injection, it was rapid, and the usual responses were obtained within a few minutes. Usually there was no effect on evoked waves (I-V), or on evoked unit responses (from the habenula), either with low or high doses of atropine. Atropine decreased the duration of inhibition of one unit which showed a sequence of initial excitation followed by later inhibition after habenular stimulation. This effect was short-lived; it lasted 3-4 minutes after 0.6 mg/mg atropine had been injected intravenously, whereas the simultaneous blockade of iontophoretically applied ACh lasted 52 minutes.

DISCUSSION

Spontaneous activity

In cats under various anesthetics one can record with either macro- or microelectrodes fairly regular spontaneous slow-wave activity, ranging in frequency from a mean of 9 Hz in cats under chloralose to a mean of 3 Hz in cats lightly anesthetized with Dial, or under halothane or methoxyflurane with nitrous oxide.

Origin

The origin of these spontaneous waves is unclear. Trembly and Sutin (1962) have recorded in cats under barbiturate anesthesia rhythmical slow waves in the ventral tegmental area of Tsai (VTA), which is adjacent to the IPN. This activity appears to be mediated by a recurrent pathway through the subthalamus and rostral dienceph-alon to the VTA (Gahm & Sutin, 1968), as lesions along its course abolish VTA slow wave activity. According to Gahm and Sutin the

lateral habenula is involved, and the pathway to VTA closely follows the habenulo-interpeduncular tract. Perhaps the latter path is the one described by Nauta (1958) as the habenulo-tegmental path from the lateral habenula to the VTA.

Although the mechanism responsible for the rhythmical activity in the VTA is unknown, several lines of evidence suggest a 'pacemaker' below the level of the diencephalon. The rhythm is synchronous on the two sides, and stimulation of the caudal brain stem or peripheral nerves evokes bilaterally symmetrical responses in the VTA. These data indicate a common input to the left and right pathways. In contrast, within the diencephalon these bilateral pathways remain independent, since stimulation or lesions in this region affect rhythmical and evoked activity only in the ipsilateral VTA (Gahm & Sutin, 1968).

Trembly & Sutin (1962) thought that the source of rhythmic activity recorded in the VTA might be in the IPN, as a phase reversal occurred in the VTA lateral to the dorsal margin of the IPN. The wave amplitude, however, was maximal lateral to the IPN. Gahm & Sutin (1968) subsequently suggested that the slow wave activity might be generated by large cells at the border between the IPN and the VTA.

The IPN waves recorded in the present experiments did not show the barbiturate dependence of the VTA waves which "do not appear with anesthetics other than barbiturates or during natural sleep" (Gahm & Sutin, 1968). However it seems that these authors

did not use gaseous anesthetics, and recorded from cats under chloralose on perhaps as few as three occasions. Their testing may not have been adequate entirely to rule out the possibility of seeing comparable waves in the absence of barbiturates.

The lower frequency of spontaneous IPN waves in non-paralysed animals under Dial, halothane or methoxyflurane is consistent with the observations of Trembly & Sutin (1962) and Gahm & Sutin (1968), in that frequency of the VTA rhythm decreased with increasing depth of anesthesia. These authors recorded from animals paralysed with gallamine triethiodide under sodium pentothal, or pentobarbital, or local anesthesia, and it is likely that the anesthesia in their experiments was lighter than in the present studies.

It is somewhat disconcerting that many IPN units showed no obvious correlation between their firing pattern and the spontaneous slow waves, though these units were influenced by habenular stimulation. This lack of correlation suggests that the origin of the slow wave activity may not be within the IPN, or, that different populations of cells are involved; one giving the unit firing (and which is influenced by the habenula) and the other giving the waves, and which is possibly only weakly influenced by the habenula (interjected stimulation of the habenula does not block or reset the rhythm). The recording sites in the present unit studies were mainly confined to medial parts of the IPN; perhaps units giving rise to the waves are located in the lateral regions. Cajal (1911) has described

cells in the lateral regions which project to the tegmentum. Lewis & Shute (1967) described connections of the cholinesterase-containing neurons of the IPN with those of the VTA. It could be that spontaneous oscillations arise in these lateral IPN cells and are transmitted to the VTA (giving rise to the rhythmical activity seen by Trembly & Sutin (1962) and Gahm & Sutin (1968). The rhythm of the waves may be generated either (a) locally, e.g. by recurrent collaterals (c.f. thalamic rhythm, Andersen & Sears, 1964), or (b) at some other site, and transmitted to the IPN by a path other than the fasciculus retroflexus (c.f. Cajal's (1911) second group of afferent fibres of unknown origin which distribute to large groups of cells). These latter fibres may be part of the recurrent pathway described by Gahm and Sutin (1968).

An observation more difficult to reconcile with the idea that the IPN and VTA spontaneous waves have a common source is the lack of effect of pallidal stimulation on IPN waves, whereas a single stimulus of appropriate strength blocks for 300 ms waves in the VTA and other sites in the recurrent pathway. However pallidal stimulation inhibits the rhythm only in the ipsilateral VTA, and does not affect responses in VTA evoked by stimulation of thalamic or epithalamic (e.g. habenular) regions (Gahm & Sutin, 1968). Pallidal inhibition may not be distributed to the IPN. If the wave frequency depends on input to the IPN, as the IPN is a single midline structure receiving bilateral input, it may be that the effects of unilateral pallidal stimulation are masked by the continuing electrical acitivity arriving from the contralateral input.

Other possible sources of rhythmic input to the IPN are the hippocampus and the septum. The theta rhythm of the hippocampus is often in the range of 3-5 Hz (Green & Arduini, 1954), which corresponds to the frequency seen in the IPN. Petsche et al (1962) have demonstrated in the rabbit that specific cells limited to the medial septum are responsible for driving the hippocampal theta rhythm. A substantial proportion of hippocampal pyramidal (efferent) neurons (Green & Machne, 1955) and septal neurons (Gogolak et al, 1968) fire in bursts which are synchronized with the theta rhythm. Hence the neuronal output from these limbic structures may be synchronized with theta activity (Komisaruk, 1970). The habenula receives input from these areas via the fornix and stria medullaris (Nauta, 1958); and, although no direct pathways between the septum or the hippocampus and the IPN have yet been described, except possibly that of Trembly and Sutin (1961), presumably a rhythmical input to the habenula could be relayed to the IPN via the fasciculus retroflexus. Green and Arduini (1954) reported that, in unanesthetized cats, immobilized with tubocurarine, appropriate afferent stimulation evoked theta type activity in the region "of the habenulo-peduncular tract". (It is not clear from their terminology or their description if they mean the tract from the habenula to the VTA, or to the IPN, or both).

One might expect that stimulation of the habenula would disrupt IPN activity dependent on a relay in the habenula. In fact, interjected stimulation of the habenula (at 1-10 Hz) had little

effect on IPN waves. The stimulation was unilateral, however, and perhaps input via the contralateral fasciculus retroflexus was sufficient to maintain the IPN rhythm. Alternatively, there may be extra-habenular pathways, as yet not described, which mediate the rhythmical theta activity.

Various differences exist between the hippocampal theta activity as described by Green and Arduini (1954) and the IPN spontaneous waves seen in the present experiments. For example, the IPN rhythm was spontaneous and usually continuous for periods of at least 30 minutes. In contrast, the theta rhythm, although it does occur spontaneously for quite long periods (30 sec in the short time segments shown in the records of Green and Arduini (1954)), is more commonly a transient (3 to 5 sec) response characterizing hippocampal arousal following afferent stimulation. Also the theta rhythm in response to the usual stimuli was abolished by anesthetic doses of pentobarbital (Green & Arduini, 1954), whereas the IPN waves were seen under various anesthetics and were somewhat accentuated by low doses of pentobarbital.

There is little overlap, except perhaps at the habenula, between the circuit proposed by Gahm and Sutin (1968), mediating the oscillatory activity recorded from the VTA, and the pathways connected with the septum and hippocampus carrying theta activity. In addition, the source of theta rhythm appears to be the medial septum (Petsche et al, 1962), while Gahm and Sutin (1968) present evidence suggesting that the 'pacemaker' for VTA activity is below the level of the diencephalon. It is rather doubtful then that the VTA activity and the theta rhythm are synonomous, or have a common source.

Evoked activity

Unit discharges: i. Excitation

Habenular stimulation excited 64% of the 190 units studied in the IPN. These formed two groups: those having constant latency of discharge (21 units) and those having a variable latency (79 units). Evidence presented above in the Results indicates that the constant latency discharges were most probably from (a) afferent fibres of the fasciculus retroflexus, (b) antidromically activated cells, or, less likely, (c) units excited orthodromically by synapses of high efficacy.

The group of discharges with variable latency were probably synaptically activated. There were two sub-classes of responses in this group: a single spike discharge (56 units), or a short high-frequency burst (2-6 spikes at 500-1000 Hz; 23 units). Each unit gave only one type of response; even at low intensities of stimulation it was extremely rare for 'burst' units to fire only a single spike, while strong stimuli (2-10 times threshold) failed to cause repetitive firing of 'single spike' units. The thresholds for each type of response ranged from 3-15 V.

The 'single spike' units were usually spontaneously active, at rates from 1-15 Hz, whereas the 'burst' units rarely fired spontaneously. In general the spike amplitudes of 'single spike' units was 100-400 μV, while the 'burst' units had smaller spikes (50-100 μV). The 'burst' units often failed to follow stimulation at rates above 3 Hz, whereas the 'single spike' units generally followed repetitive stimulation at 10-40 Hz, or even more; and several of these units followed short bursts of 3-5 spikes at 100-200 Hz. It therefore seems likely that the 'single spike' and 'burst'

responses arise from separate populations of cells.

'Burst' type units

These units were found at various depths in the IPN, in some instances in close proximity to units which showed signs of inhibition. The high-frequency (500-1000 Hz) 'burst' pattern of firing resembles that described for a variety of postulated inhibitory interneurons, including the Renshaw cells of the spinal cord (Eccles et al, 1954; 1961), and some cells of the cerebellum (Eccles et al, 1966), the ventrolateral thalamus (Andersen et al, 1964a; Marco et al, 1967; Steriade et al, 1971), the hippocampus (Andersen et al, 1969), the cerebral cortex (Stefanis, 1969), and the ventromedial hypothalamus (Murphy & Renaud, 1968; 1969).

While conclusive cell-marking of putative inhibitory interneurons has not yet been described for any CNS region, there is a large amount of indirect evidence (histological) pointing to specific cell types within various regions as inhibitors of other cells within the same local area. Characteristics common to most types of putative inhibitory interneurons are: smaller size than the cells they inhibit, short axons with local multi-branched terminal arbors, and axon terminations as pericellular nests about the somata of the larger cells in the region (Cajal, 1911; Lorente de Nó, 1934; Walberg, 1957; Andersen et al, 1964a, b; 1969; Kuypers & Tuerk, 1964; Kubota et al, 1965; Colonnier, 1966; Eccles et al, 1966; Hamori et al, 1966a, b; Szentagothai 1967; 1968; Thomas & Wilson, 1967; Murphy & Renaud, 1969).

Cajal's account of the IPN (1911), from Golgi studies in the cat, describes in the "deep"zone, small stellate or Golgi II cells with multi-branched processes radiating in all directions, and a larger type of cell similar to that found in the "superficial" zone. The axons of the smaller cells form numerous arborizations in regions near their cell bodies.

Comparing the histological data and the firing patterns of the IPN 'burst' units with the descriptions (both histological and electrophysiological) of presumed inhibitory interneurons given by other authors (references above) leads to the proposal that 'burst' units may be the small cells described by Cajal (1911) and that they may serve to inhibit the larger cells of the region. Evidence showing inhibition of one unit following 'burst' firing of another unit, and no inhibition with failure of 'burst' firing, would be more conclusive of a functional relationship. This inhibition could be 'feed-forward' inhibition as opposed to 'feedback' inhibition by recurrent collaterals, as 'burst' units appear to be directly excited by habenular stimulation. However, recurrent collaterals (described for the larger cells of the IPN by Cajal, 1911) may also activate this same pool of inhibitory interneurons.

Inhibition of IPN units after habenular stimulation

The various types of units which appeared to be inhibited by habenular stimulation have been described above in the Results. These units formed 37% of all those observed, and about one half of them showed pure inhibition. The latency of onset of inhibition was very short; spontaneous firing was very rare within a short interval

(1-20 ms) after the stimulus, but these units did not fire spontaneously at rates over 20 Hz. Since there was a reduced firing-probability in the absence of stimulus-induced excitation, it was probably the result of true inhibition. The duration of inhibition was related to the stimulus strength; this is a further indication of its synaptic nature. The short latency of inhibition suggests that some fibres of the fasciculus retroflexus might directly inhibit these cells. However the conduction path is rather short, and one cannot rule out the interpolation of inhibitory interneurons.

The other units responded to habenular stimulation with sequences of initial excitation followed by inhibition. It seemed unlikely that the decreased probability of firing was due to post-activity depression because the threshold for depression of the spontaneous discharge was often below that of the evoked spike. It seems more likely that inhibitory interneurons are interpolated between (a) the terminals of fasciculus retroflexus fibres and these IPN neurons and/or the terminals of these IPN neuron axon collaterals and the same or other IPN neurons (recurrent inhibition).

Identification of the source of evoked potentials

The potential fields surrounding groups of neurons are a manifestation of the electrical activity of individual cells. Within certain neuronal populations, such as the hippocampus or the lateral geniculate body, there is a preponderance of cell somata in one area, with the majority of the dendrites oriented in a single direction (Renshaw et al, 1940; Bishop & O'Leary, 1942). A field is set up

across the responding cells with its polarity dependent upon the orientation of the majority of cells (Lorente de Nó, 1947) and an evoked response may be interpreted relatively easily in terms of the response of components of the individual neurons (c.f. Lomo, 1971).

Extracellular records from a simultaneously firing population of less highly oriented neurons cannot be interpreted in terms of activity represented by a single 'average' or statistical neuron (Fatt, 1957). The potentials recorded represent the algebraic summation of individual neuronal sources and sinks. Hence it is more difficult to ascribe the resulting potentials to particular neural events such as invasion of dendrites, etc.

Organization of the IPN

Cajal (1911) described the IPN as being rather similar to the molecular layer of the cerebellum because the dendrites of the larger cells lie mainly in parasagittal planes, at right angles to the transversely looping fibres of the fasciculus retroflexus (which are somewhat analagous to the parallel fibres of the cerebellum). He speculated that the fibres may make synaptic contact with the dendritic spine processes seen on the larger cells, but he did not suggest how the small stellate cells might be activated. He also described recurrent collaterals branching from the axons of the larger cells which eventually project to the tegmentum, while the axons of the smaller cells appeared to terminate close to their cell of origin in an extensive terminal arbor. However dendrites and cell bodies do not appear to be arranged in any regular layers, so as to simplify the interpretation of the recorded potentials.

The IPN is unusual among CNS structures in that it seems to be a fairly homogeneous midline structure, but receives bilateral inputs. It seems probable that the high density of intertwined transverse fibres, from the fasciculus retroflexus and elsewhere, may lead to considerable convergence on the same neurons.

Activity evoked in the IPN by habenular stimulation

Stimulation of the habenula evoked in the IPN potentials which were similar under the various anesthetics. These potentials have been described in the Results above, and assigned the notation of Waves I to V. One can make some tentative speculations as to the mechanism of generation of these various waves, keeping in mind the limitations imposed by the irregularity of neuronal arrangement within the nucleus, and possible inhomogeneities in the conduction medium which may distort the potentials recorded.

The early waves

Waves I, II and III are brief negative and positive waves which occur in the period from 1-7 ms after a habenular stimulus, with latencies of 1-2 ms, 4-5 ms, and 5-6 ms respectively. At rostral recording positions (A 3 to 3.5 mm) an additional positive-negative sequence is also seen at a latency of 2-3 ms.

Unit studies indicated that 60% of the evoked discharges started during the first 7 ms. In view of their brief duration, their spike-like appearance, and their coincidence with the time of initial discharge of many units, it seems likely that Waves I to III represent summated unit spikes, and are thus 'population spikes'.

Supporting evidence comes from the observation that with microelectrodes Waves I to III were most often inconspicuous; the restricted field of recording and the localized nature of extracellular unit spikes limits the number of units 'seen' by the electrode and thus 'population spikes' might be less evident.

Wave I

Wave I was not obvious in microelectrode recordings and was not always seen with gross recordings, perhaps because of its low amplitude and its close association with the stimulus artefact. As it had the shortest latency, it probably represents presynaptic activity of the fasciculus retroflexus fibres and/or antidromic spikes.

Wave II and III

Wave II and III probably represent the population spikes of synaptically activated units in the IPN, as they corresponded in time to the occurrence of the 'variable latency' unit spikes. The additional positive-negative component seen in more rostral positions at a latency of 2-3 ms overlaps with the discharge of both constant latency (presumably presynaptic or antidromic) and variable latency spikes, and most likely represents a mixture of both types of spikes. There was only a slight tendency for the population of constant latency units to be located at rostral rather than at caudal levels of the nucleus, so this cannot explain why the additional waves were seen only at rostral levels of the nucleus. However only twenty-one such units were studied.

The later waves

Waves IV and V, in contrast to the earlier potentials, had a long duration (IV-20 ms; V-30 ms or more), were prominent in both macro- and microelectrode records, and showed more variability in superimposed traces.

Wave IV

Wave IV is seen as a negative wave with monopolar recording and appears to represent excitatory postsynaptic potentials (EPSP's) in the nucleus (c.f. Andersen et al, 1964 a). It attains its peak amplitude at 9-12 ms after the habenular stimulus (depending on the position of the stimulating electrode), and in records where the early waves are not obvious, the negative shift from the baseline begins about 4-5 ms after the stimulus.

Wave IV has several characteristics of a post-synaptic potential: growing in size with increased stimulus intensity (not all-or-none), temporal summation at low frequencies of repetitive stimulation (e.g. 10-20 Hz), post-tetanic potentiation, and increased variation or decrement in amplitude at higher frequencies (30 Hz or more), presumably owing to synaptic failure.

Generation of Wave IV

The simplest explanation is that Waves II-III and IV are generated by the same cells and the same input; the postsynaptic spikes, arising from the cells bodies, are manifested as the population spikes II-III, while the EPSP's that initiate these spikes persist in the dendrites of the same cells, giving rise to Wave IV. The

relation between the amplitude of Waves II-III and that of IV was usually approximately linear, which tends to support this hypothesis. The main argument against this hypothesis is that, occasionally, different thresholds for Waves II-III and IV were observed. These differences may be spurious, arising as a result of recording positions more favorable for recording responses from groups of dendrites, or cell bodies. Alternatively the threshold differences may be genuine, indicating that different fasciculus retroflexus fibres terminate on different regions (e.g. proximal and distal dendrites, or cell body) of the same cell.

Other possibilities are that Wave IV may represent EPSP's in second order neurons synaptically excited by those which fire intially; or EPSP's in a population of high threshold cells which do not often give rise to spikes (c.f. latencies of evoked unit firings: the majority are less than 7 ms).

Wave IV could also represent EPSP's in neurons activated by slower-conducting fibres of the fasciculus retroflexus. Ariens Kappers et al (1936) have described two types of fibres, an inner bundle of unmyelinated fibres and an outer group of myelinated ones. The following considerations point against the hypothesis of the slower unmyelinated fibres mediating the slow Wave IV:

(i) The thresholds for Waves II-III and IV were usually the same, and the occasional differences never exceeded 2 V. This suggests that only one group of fibres were involved.

(ii) The threshold of unmyelinated fibres is 50-100 times that of fast myelinated fibres, (Erlanger & Gasser, 1937), though perhaps only 15 times that of small myelinated fibres (Erlanger & Gasser, 1930). The range of stimulating intensity used as generally 2-10 times the threshold of Waves II-III, so it is doubtful that enough unmyelinated fibres would have carried impulses (at slower conduction velocities) which arrived later and generated Wave IV.

(iii) The conduction path from the habenula to the IPN is at least 12 mm. Since many response latencies were quite brief (2-3 ms, corresponding to a conduction velocity of 6-4 m/s), the group of fibres involved were probably the myelinated ones.

At the entry zone one might expect some temporal and spatial despersion of afferent impulses owing to the branching and looping of the fasciculus retroflexus fibres within the nucleus. This would account for, in part, the relatively wide range of latencies of unit discharge observed.

One last possibility is that Wave IV may be mediated by a pathway other than the fasciculus retroflexus. This is rather unlikely in view of the similar thresholds of Waves II-III and Wave IV, and the approximately linear relation between their amplitudes. It is necessary to isolate the habenulo-interpeduncular pathway from other systems in order to determine whether later events are mediated by other centres and tracts, or represent activity within the IPN only.

Wave V

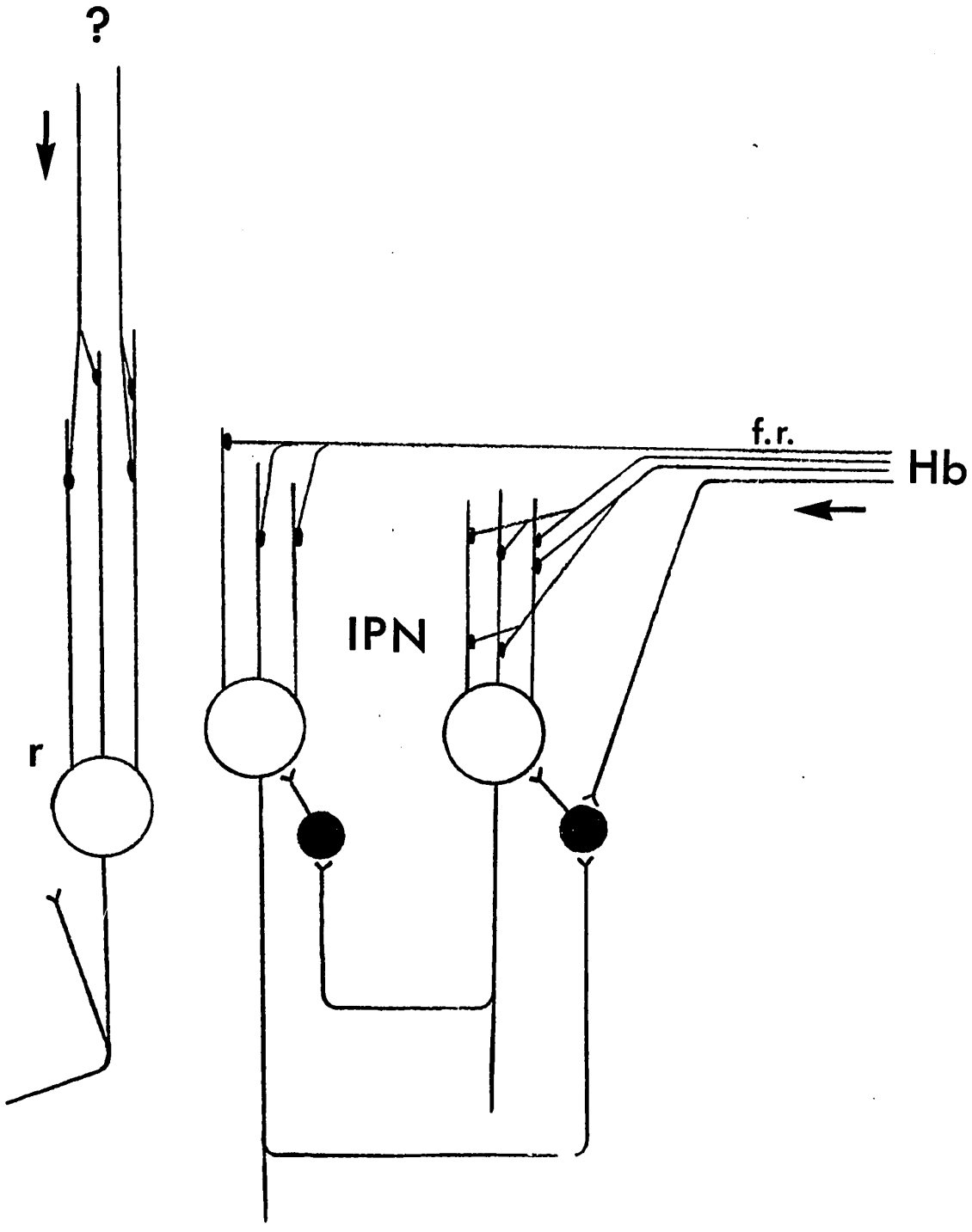
Thus far consideration has been given only to excitatory events represented by waves in the IPN. However the late and long-duration positive Wave V is reminiscent of similar waves associated with inhibitory post-synaptic potentials (IPSP's) elsewhere in the CNS (motoneurons, Brock et al, 1952; Coombs et al, 1955; hippocampus, Spencer & Kandel, 1961; thalamus, Andersen & Eccles, 1962). Also unit studies indicated that 37% of the population showed signs of inhibition after habenular stimulation. Wave V may therefore represent IPSP's in the nucleus, perhaps partly masked by the initial excitation.

The time course of inhibition of unit firing was often much longer (by a factor of 2 or more) than the duration of Wave V. This may have been partly because the time constant of recording (20 ms) was too short to record the full duration. In addition the size and duration of extracellular potentials is usually related to the rate of change of membrane potential. Moreover, if the membrane potential is relatively close to the reversal potential for the IPSP, as is usually the case, the hyperpolarization due to inhibition and the resultant current flow are likely to be small, and result in a relatively small extracellular wave.

Wave V could be mediated by interneurons located somewhere other than the IPN, but it is quite likely that the small stellate cells seen in histological studies function as the inhibitory interneurons within the IPN, and are activated by fibres of the fasciculus retroflexus or by recurrent collaterals of IPN neurons.

Figure 44 A model of synaptic arrangements within the IPN

See text for explanation.


?
f.r.
Hb
IPN
r

Model for the generation of waves and spikes in the IPN

Figure 44 illustrates a simple model of synaptic arrangements in the IPN which could account for most of the observations. The 'parallel' fasciculus retroflexus fibres (f.r.) within the nucleus excite IPN neurons, generating EPSP's in the dendritic tree and spikes in the cell bodies. The latter are seen as Waves II-III population spikes, while the EPSP's persisting in the dendrites give rise to Wave IV. Inhibitory interneurons are shown as black-bodied neurons. These may be the units which gave 'burst' discharges. They are shown in this figure as activated either by fasciculus retroflexus fibres, or by recurrent collaterals of IPN neurons. A second group of afferents of unknown origin (c.f. Cajal, 1911) are shown terminating on a more lateral neuron (r). Activity of a population represented by this neuron gives rise to the spontaneous rhythmic waves, which are projected to the ventral tegmental area and other regions.

Chemical sensitivity of IPN neurons

A previous study of choline acetylase activity in various regions of the mammalian brain has revealed that the IPN has one of the highest concentrations of enzyme (Lewis et al, 1967). The activity is about ten times that observed in the caudate nucleus, for example. In addition the habenula, the fasciculus retroflexus, and the IPN stain intensely for acetylcholinesterase (Shute & Lewis, 1963; Krnjević & Silver, 1966; Lewis & Shute, 1967). This histochemical evidence is strongly suggestive of a cholinergic

pathway (c.f. Lewis et al, 1967). The present studies, although they show that the majority (about 80%) of IPN neurons are excited by ACh, do not clearly indicate a simple transmitter action of ACh in the habenulo-interpeduncular pathway.

Unit responses

The typical response of 62 IPN units (77%) to iontophoretic applications of ACh was characterized by a relatively slow acceleration of the firing rate, sometimes preceded by initial depression of spontaneous firing (for 2-10 seconds), and a short (2-5 seconds) after-discharge following the cessation of the iontophoretic current. The increased firing was potentiated by concurrent iontophoretic applications of neostigmine and blocked by intravenous administration of atropine sulphate (0.3-1mg/kg or more). This suggests that ACh has a muscarinic action on cells in the IPN (Dale, 1914; 1938). Unfortunately studies were not done with drugs which block nicotinic effects of ACh, so it is not certain that only muscarinic-type receptors are present on IPN cells. However the delayed type of response to ACh and blockade by atropine are more characteristic of muscarinic than nicotinic actions of ACh (Dale, 1914; 1938; Krnjević & Phillis, 1963 a,b).

The firing probability of most of these cells was increased after a short latency by L-glutamate and was strongly depressed by GABA (only a few units were tested). This agrees with previous observations in various regions of the brain (Hayashi & Nagai, 1956; Curtis & Koizumi, 1961; Krnjević & Phillis, 1963 a,b).

A small number of IPN units (8) were excited by low doses of L-glutamate and ACh (2-6 nA), but slightly higher doses abruptly blocked their firing for various periods, seemingly not by a mechanism of excess depolarization. This suggests a phenomenon of indirect inhibition such as that described by Martin et al (1970), or Zieglgansberger & Herz (1971). At low doses the effects of glutamate or ACh are produced solely on the cells from which one is recording. With higher doses there may be diffusion to afferent terminals or nearby small cells, (from which one is not recording), which are in synaptic relation with the cell being monitored. In the present situation it appears that the small cells are excited by L-glutamate or ACh and release substances or generate activity that is inhibitory to the monitored cell. This supports the notion that the small stellate neurons close to larger neurons in the IPN are inhibitory interneurons (excited by L-glutamate and ACh), as proposed above.

The relation between responses of units to ACh and responses to habenular stimulation is not clear. Over 80% of units excited by stimulating the habenula (including some constant latency spike units, variable latency single spike units, and burst units) were excited by ACh; but over 85% of units inhibited by the habenula were also excited by ACh. It appears that inhibition is not mediated by ACh, but that inhibitory interneurons may be excited by ACh. This suggests that cholinergic fibres of the fasciculus retroflexus

directly excite both primary IPN neurons and inhibitory interneurons (burst units). It could be that primary IPN neurons themselves are both excited by ACh and are cholinergic, releasing ACh at their terminations (e.g. within the IPN at recurrent collateral endings on other IPN neurons, perhaps inhibitory interneurons).

The response of IPN units to ACh was not particularly strong, and had a fairly long latency. Little effect of ACh or related compounds (e.g. neostigmine, edrophonium,atropine) on unit responses to habenular stimulation, or on evoked waves I to V was seen. In addition, most cells were excited by ACh regardless of whether they were excited or inhibited by habenular stimulation. These data indicate that it is unlikely that ACh mediates short latency synaptic events, or serves as a conventional transmitter at habenulo-interpeduncular junctions. One consideration is that the cholinergic input normally may not lead to spike generation, but may alter cell excitability by a non-propagated effect on membrane potential or permeability. Some non-cholinergic fibres in the fasciculus retroflexus which may be obscured by the intense general cholinesterase staining, may mediate the early synaptic events.

Perhaps a closer relation between ACh excitation and synaptic events will be elucidated in further studies, especially if care is taken to activate all types of fibres of the fasciculus retroflexus. It may be that unmyelinated fibres, which may not

have been stimulated in these studies, are the cholinergic ones. Olivier (1971, personal communication) has demonstrated histochemically in cats that one can distinguish two groups of fibres in the fasciculus retroflexus on the basis of their acetylcholinesterase staining; one group stains intensely and is unmyelinated, while the second group, of myelinated fibres stains less intensely.

Relevance of present findings to habenulo-interpeduncular function

As summarized in the Introduction, efferent pathways from the IPN appear to influence descending activity in motor and visceral centres of the brain stem and spinal cord, and ascending structures of the ventral tegmental pathway of the ascending reticular system.

The spontaneous rhythmic waves recorded from the IPN and habenula may be conducted to these sites electrotonically from some source, and have little relevance for the activity of these nuclei. In support of this view is the lack of correlation between patterns of spontaneous unit firing and the spontaneous slow waves, and the ineffectiveness of interjected unilateral habenular stimulation in blocking or changing the spontaneous waves.

Alternatively, as considered above, the spontaneous waves may arise in the lateral cells of the IPN and be transmitted to the habenula and other regions via the interpedunculo-diencephalic pathway (Massopust & Thompson, 1962), the ventral tegmental pathway of the ascending reticular system (Lewis & Shute, 1967), or the recurrent diencephalic pathway of Gahm and Sutin (1968). (The rhythm of these waves may be generated in the IPN, or externally, as discussed above).

It seems possible that the IPN waves regularly observed may represent a sample at one site of reverberating activity in a circuit. The histochemical studies of Lewis and Shute (1967) have revealed cholinesterase-containing links via the IPN between the hippocampal projection system and the ventral tegmental pathway of the ascending reticular system, which also stains for cholinesterase. This latter gives off fibres to the septum from which originate afferents to the hippocampus, thus completing the circuit. Activity in this circuit might serve to maintain a level of arousal or excitability, which in turn would determine the sensitivity of these structures to afferent input, and their level of tonic output.

The muscarinic excitatory action of ACh applied microiontophoretically to IPN units is more consistent with a relatively slow facilitation of excitability, rather than a rapid and brief conventional transmitter action (c.f. Krnjević, 1969). This cholinergic facilitation may be the role played by the cholinesterase-containing fibres of the Hb-IPN pathway, and this link with the ascending reticular system, so that the IPN may produce changes in arousal or excitability in response to activity of limbic structures, transmitted by many and varied inputs to the habenula (and thence via the fasciculus retroflexus), or directly to the IPN. Presumably the descending efferents from the IPN could also carry this facilitation, but as experiments have suggested an inhibitory influence of the IPN upon motor activity, presumably there is some interposed relay whose output is inhibitory. Other functions of this cholinergic facilitatory system could be to potentiate repetitive

discharges necessary for consolidation of learning and memory (Gerard, 1955; Penfield & Milner, 1958), or the development of conscious processes (Libet, 1965; Libet et al, 1967).

Neuropharmacological studies have indicated that the transmitter involved in brief synaptic actions at Hb-IPN junctions is most likely not acetylcholine. There were populations of IPN units purely excited by habenular stimulation, while other units were purely inhibited, or initially excited then inhibited. These data are probably the substrate for diverse functions of different populations of cells within the IPN; the cytoarchetectonic and histochemical inhomogeneity of the IPN has been discussed in the Introduction. Thus far there has been no fine mapping of terminations of different afferent inputs within the habenula, or the termination within the IPN of fasciculus retroflexus fibres from discrete areas in the habenula. While the spontaneous IPN slow waves and the cholinesterase-containing pathways appear to subserve prolonged widely dispersed facilitation, the brief synaptic actions may transmit a different, more localized type of information which functions to activate or inhibit particular sequences of internal responses and/or externally-manifested behavior. The two systems most likely interact, but little experimental evidence for a relation between the ongoing slow waves and evoked unit responses was obtained.

The phylogenetic constancy of the habenulo-interpeduncular complex in vertebrates implies a basic function for these nuclei; this appears to be the linking of motivational states to motor and

visceral responses. In lower vertebrates the motivational states might be generated by crude detectors associated mainly with olfactory structures, and the efferent outflow directed mainly upon motor and visceral responses more-or-less organized as reflexes of the brain stem and spinal cord. As evolution proceeded and vertebrates became equipped to detect and integrate other stimuli in addition to olfactory ones, and the processes of learning and memory developed, allowing more varied and complex responses, the limbic system and paleo- and neocortices emerged. The linking function of the habenulo-interpeduncular system has become entwined in the myriads of limbic interconnections and response tendencies, and it may serve to arouse the additional capacities of learning, memory and ideation, which function to generate and control the eventual behavioral output (both motor and visceral), superimposed on the pre-existing brain stem and spinal reflexes.

SUMMARY

1. The habenulo-interpeduncular pathway was studied * electrophysiologically in 58 cats under various anaesthetics (chloralose, Dial, halothane or methoxyflurane with nitrous oxide). Bipolar stimulating electrodes were placed stereotaxically in the habenula by dorsal insertions, while a surgical procedure was developed to enable stereotaxic placement of extracellular recording electrodes in the interpeduncular nucleus (IPN), using a ventral approach.

2. * Spontaneous rhythmic oscillations, ranging from 3-4 Hz to 9-10 Hz under different anaesthetics, were recorded in the habenula or the IPN with macro- or microelectrodes. Many IPN units fired spontaneously at rates of 1 - 25 Hz. There was little obvious

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* Asterisk denotes claim to original work by the author.

correlation between the rhythmic waves and the spontaneous firing patterns of IPN units. Possible origins of the rhythmic waves are discussed.

3. * Stimulation of the habenula at 1 Hz under various anaesthetics evoked in the IPN brief positive-negative changes in potential (Waves I to III), followed by slower negative (Wave IV) and positive (Wave V) deflections. These waves were mainly confined to the IPN when moderate stimulus intensities were used. The possible origins of these potentials are discussed and the following tentative conclusions are reached: (a) Wave I represents activity of afferent fibres and/or antidromic spikes (b) Waves II-III appear to be 'population' spikes (summated unit spikes) of IPN cells excited synaptically by habenular stimulation, (c) Wave IV is generated by excitatory synaptic potentials, perhaps from the same cells which fire initially giving rise to Waves II-III, and (d) Wave V, a prolonged positive wave, probably represents inhibitory synaptic potentials in cells of the IPN.

4. * IPN units excited by habenular stimulation (64%) formed two major categories: (i) units evoked at constant latency, which were considered to be from afferent fibres and/or antidromically excited cells, and (ii) units excited at variable latencies (mean latency ± 2.0 ms), which were thought to be synaptically excited. Synaptically excited units showed two main types of discharge after habenular stimulation: (a) a single spike, or (b) a 'burst' of 2-6 spikes at 500-1000 Hz. Evidence is presented

which suggests that these two types of response arise from separate populations of cells.

5. A consideration of the firing patterns and histological descriptions of putative inhibitory interneurons elsewhere in the CNS leads to the suggestion * that units giving the 'burst' discharge after habenular stimulation serve to inhibit the larger cells of the nucleus and are likely the small short-axoned cells described in the IPN by Cajal (1911).

6. * After habenular stimulation the firing probability of some IPN units (37%) was depressed for 20-150 ms, depending upon the stimulus intensity. Sixteen percent of the units showed pure inhibition, while the remainder (21%) were initially excited and then depressed. Several lines of evidence indicate that this depression was most likely due to post-synaptic inhibition, rather than to post-activity depression or disfacilitation. Presumably this inhibition is mediated by inhibitory interneurons -- perhaps the 'burst' units (see point 5) -- and may be a 'feed-forward' type of inhibition, or a 'feedback' inhibition by recurrent collaterals. Direct inhibition by fibres of the fasciculus retroflexus is also a possibility, especially in cases where the latency of inhibition is very short.

7. * Twenty percent of the population of IPN cells were unaffected by habenular stimulation. This may have been due to inadequate stimulation, or to the existence of a group of cells not directly influenced by habenular stimulation.

8. * A simple model of possible synaptic arrangements within the IPN is proposed, based on histological information (Cajal, 1911) and on electrophysiological data from the present experiments.

9. * The chemical sensitivity of IPN neurons to L-glutamate, acetylcholine (ACh) and some related compounds, was investigated using the micro-iontophoretic technique with multi-barrelled pipettes. IPN units excited by ACh (77%) showed, at rather long latency, (2-10 seconds), an acceleration of firing rate which persisted for 2-20 seconds after cessation of the drug current. Concurrent applications of neostigmine via another barrel, shortened the latency of accelerated firing, increased the maximal firing rate, and prolonged the after-discharge. Intravenous administration of atropine sulphate (0.3-1.0 mg/kg) reversibly blocked the effects of ACh for 20 minutes to one hour. Thus the ACh receptors on IPN units appeared to be of the muscarinic type, although the presence of nicotinic-type receptors cannot be ruled out.

10. * Over 80% of IPN units **either** excited or inhibited by habenular stimulation were excited by ACh. Neither atropine nor neostigmine appeared to affect the evoked waves (Waves I to V), or unit responses to habenular stimulation. These findings, and the relatively long latency responses of IPN units to ACh, appear to preclude ACh as a simple excitatory transmitter in the habenulo-interpeduncular pathway. Rather, ACh release from fasciculus retroflexus fibres may modulate the excitability of IPN neurons by non-propagated effects on membrane potential, or permeability.

11. The relevance of these findings to the possible function of the habenulo-interpeduncular pathway is discussed.

BIBLIOGRAPHY

Adey, W.R., Dunlop, C.W., Killam, K.F. & Brazier, M.A. (1960) Investigations of the action of thiosemicarbazide on the cerebellar cortex of the cat. in Inhibition in the nervous system and gamma-aminobutyric acid, edited by Roberts, E., Baxter, C.F., Van Harreveld, A., Wiersma, C.A.G., Ross Adey, W., and Killam, K.F. Oxford: Pergamon Press, pp. 317-323.

Adey, W.R. & Tokizane, T. (1967) editors, Structure and Function of the Limbic System. Progress in Brain Research, Vol. 27. Amsterdam: Elsevier.

Akagi, K. & Powell, E.W. (1968) Differential projections of Habenular Nuclei. J. comp. Neurol. 132, 263-274.

Albers, R.W. & Brady, R.O. (1959) The Distribution of Glutamic Decarboxylase in the Nervous System of the Rhesus Monkey. J. Biol. Chem. 234, 926-928.

Amassian, V.E. (1961) Microelectrode studies of the cerebral cortex. Int. Rev. Neurobiol. 3, 67-136.

Anand, B.K. & Brobeck, J.R. (1952) Food intake and spontaneous activity of rats with lesions in the amygdaloid nuclei. J. Neurophysiol. 15, 421-430.

Andersen, P., Eccles, J.C., Schmidt, R.F. & Yokota, T. (1964)a Slow potential waves produced in the cuneate nucleus by cutaneous volleys and by cortical stimulation. J. Neurophysiol. 27, 78-91.

Andersen, P., Eccles, J.C. & Loyning, Y. (1964)b Location of post-synaptic inhibitory synapses on hippocampal pyramids. J. Neurophysiol. 27, 592-607.

Andersen, P., Eccles, J.C. & Loyning, Y. (1964)c Pathway of post-synaptic inhibition in the hippocampus. J. Neurophysiol. 27, 608-619.

Andersen, P., Eccles, J.C. & Sears, T.A. (1964)d The ventrobasal complex of the thalamus: types of cells, their responses and their functional organization. J. Physiol. 174, 370-399.

Andersen, P., Gross, G., Lomo, T. & Sveen, O. (1969) Participation of inhibitory and excitatory interneurons in the control of hippocampal cortical output. in The Interneuron ed. M.A.B.Brazier, UCLA Forum Med Sci No. 11, Los Angeles: U. of California Press, pp. 415-465.

Andersen, P. & Sears, T.A. (1964) The role of inhibition in the phasing of spontaneous thalamo-cortical discharge. J. Physiol. 173, 459-480.

Ariens-Kappers, C.U., Huber, G.C. & Crosby, E.C. (1936) The Comparative Anatomy of Vertebrates, including Man. New York: Macmillan. 2 vols.

Asdourian, D., Stutz, R.M. & Rocklin, K.W. (1966) Effects of thalamic and limbic system lesions on self-stimulation. J. comp. physiol. Psychol. 61, 468-472.

Bailey, P. & Davis, E.W. (1942) The Syndrome of Obstinate Progression in the cat. Proc. Soc. exp. Biol., N.Y. 51, 307.

Bailey, P. & Sweet, W.H. (1940) Effects on respiration, blood pressure, and gastric motility of stimulation of orbital surface of frontal lobe. J. Neurophysiol. 3, 276-281.

Bard, P.A. (1928) A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system. Amer. J. Physiol. 84, 490-515.

Berman, A.L. (1968) The Brain Stem of the Cat. Madison: U. of Wisconsin Press.

Berman, A.L. & Bowers, S.R. (1967) A cytoarchitectonic analysis of the interpeduncular complex of the cat. Anat. Rec. 157, 213.

Bishop, G.H. & O'Leary, J.L. (1942) The polarity of potentials recorded from the superior colliculus. J. cell. comp. Physiol. 19, 289-300.

Bishop, P.O., Burke, W. & Davis, R. (1962) The identification of single units in central visual pathways. J. Physiol. 162, 409-431.

Boyd, E.S. & Celso, M.B. (1970) Effect of some brain lesions on septal intracranial self-stimulation in the rat. Amer. J. Physiol. 219, 734-741.

Boyd, E.S. & Gardner, L.C. (1967) Effect of some brain lesions on intracranial self-stimulation in the rat. Amer. J. Physiol. 213, 1044-1052.

Brady, J.V. & Nauta, W.J.H. (1955) Subcortical mechanisms in emotional behavior: the duration of affective changes following septal and habenular lesions in the albino rat. J. comp. Physiol. Psychol. 48, 412-420.

Broca, P. (1878) Anatomie comparée des circonvolutions cerebrales. Le grand lobe limbique at la scissure limbique dans la série des mammifères. Rev. Anthrop., S.2, 1, 385-498.

Brock, L.G., Coombs, J.S. & Eccles, J.C. (1952) The recording of potentials from motoneurones with an intracellular electrode. J. Physiol. 117, 431-460.

Brodal, A. (1947) The hippocampus and the sense of smell. A review. Brain 70, 179-222.

Brodal, A. (1963) in The Rhinencephalon and Related Structures. Progress in Brain Research, Volume 3, ed. Bargmann, W. & Schadé, J.P. Amsterdam: Elsevier.

Brown, J.O. (1964) The nuclear pattern of the non-tectal portions of the midbrain and isthmus in the dog and cat. J. comp. Neurol. 78, 365-406.

Burgi, S. & Bucher, V.M. (1955) Uber einige rhinencephale Verbindungen des Zwischen- und Mittelhirns. Dtsch. Z. Nervenheilk. 174, 89-106.

Burns, B.D., Ferch, W. & Mandl, G. (1965) A neurophysiological computer. Electron. Engng. 37, 20-24.

Ramon y Cajal, S. (1911) Histologie du système nerveux de l'homme et des vertébrés. Paris: A. Maloine, 2 volumes.

Cannon, W.B. (1927) The James-Lange Theory of Emotion: A Critical Examination and an Alternative Theory. Amer. J. Psychol. 39, 10-124.

Cannon, W.B. (1929) Bodily changes in pain, hunger, fear and rage. An account of recent researches into the function of emotional excitement. New York: Appleton.

Carli, G., Armengol, V. & Zanchetti, A. (1965) Brain stem-limbic connections and the electrographic aspects of deep sleep in the cat. Arch. ital. Biol. 103, 725-750.

Carlsson, A., Falck, B. & Hillarp, N.A. (1962) Cellular localization of brain monoamines. Acta physiol. scand. 56, suppl. 196, 1-28.

Collins, E.H. (1954) Localization of an experimental hypothalamic and midbrain syndrome simulating sleep. J. comp. Neurol. 100, 661-691.

Colonnier, M.L. (1966) The structural design of the neocortex. in Brain and Conscious Experience, ed. J.C. Eccles. New York: Springer, pp. 1-23.

Coombs, J.S., Eccles, J.C. & Fatt, P. (1955) The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. J. Physiol. 130, 326-373.

Cordeau, J.P., Moreau, A., Beaulnes, A. & Laurin, C. (1963) EEG and Behavioral Changes following microinjections of Acetylcholine and Adrenaline in brainstem of cats. Arch. ital. Biol. 101, 30-47.

Cowan, W.M., Guillery, R.W. & Powell, T.P.S. (1964) The origin of the mamillary peduncle and other hypothalamic connexions from the midbrain. J. Anat., Lond. 98, 345-363.

Craddock, S.N. & Thompson, R. (1971) A discrete interpeduncular-central tegmental region critical for retention of visual discrimination habits in the white rat. J. comp. physiol. Psychol. 76, 39-50.

Cragg, B.G. (1959) A Heat-loss Mechanism involving the Habenular, Interpeduncular, and Dorsal Tegmental nuclei. Nature, Lond. 184, 1724.

Cragg, B.G. (1961)a The connections of the Habenula in the Rabbit. Exp. Neurol. 3, 388-409.

Cragg, B.G. (1961)b The role of the habenula in the respiratory response of the rabbit to warmth or to restraint. Exp. Neurol. 4, 115-133.

Craigie, E.H. (1930) Observations on the brain of the humming bird (Chrysolampis mosquitus Linn and Chlorostilbon caribaeus Lawr). J. comp. Neurol. 45, 377-482.

Critchlow, V. (1958) Blockade of ovulation in the rat by mesencephalic lesions. Endocrinology 63, 596-610.

Curtis, D.R. (1964) Microelectrophoresis. in Physical Techniques in Biological Research, Volume V. Electrophysiological Methods, Part A. ed. W.L. Nastuk, New York: Academic Press, pp. 144-190.

Curtis, D.R. & Eccles, R.E. (1958) The excitation of Renshaw cells by pharmacological agents applied electrophoretically. J. Physiol. 141, 435-445.

Curtis, D.R. & Koizumi, K. (1961) Chemical transmitter substances in brain stem of cat. J. Neurophysiol. 24, 80-90.

Curtis, D.R. & Phillis, J.W. (1960) The action of procaine and atropine on spinal neurones. J. Physiol. 153, 17-34.

Dahlstrom, A. & Fuxe, K. (1964)a A method for the demonstration of monoamine containing nerve fibres in the central nervous system. Acta physiol. scand. 60, 293-295.

Dahlstrom, A. & Fuxe, K. (1964)b Evidence for the Existence of Monoamine-containing neurons in the central nervous system. I. Demonstration of Monoamines in Cell Bodies of Brain Stem Neurons. Acta physiol. scand. 62, suppl. 232.

Dale, H.H. (1914) The action of certain esters and ethers of choline and their relation to muscarine. J. Pharmacol. exp. Therap. 6, 147-190.

Dale, H.H. (1938) Acetylcholine as a chemical transmitter of the effects of nerve impulses. J. Mt. Sinai Hosp. 4, 401-429.

Davis, K.B., McIver, A.H. & Nielson, H.C. (1966) The effect of cold stress and habenular lesions on avoidance learning, open-field, and exploratory behavior. Proc. Amer. Psychol. Ass. 1, 21-22.

Delgado, J.M.R., Roberts, W.W. & Miller, N.E. (1954) Learning motivated by electrical stimulation of the brain. Amer. J. Physiol. 179, 587-593.

Donovick, P.J. & Burright, R.G. (1968) Water consumption of rats with septal lesions following two days of water deprivation. Physiol. Behav. 3, 285-288.

Donovick, P.J., Burright, R.G., Kaplan, J. & Rosenstreich, N. (1969) Habenular lesions, water consumption and palatability of fluids in the rat. Physiol. Behav. 4, 45-47.

Donovick, P.J., Burright, R.G. & Zuromski, E. (1970) Localization of quinine aversion within the septum, habenula and interpeduncular nucleus of the rat. J. comp. physiol. Psychol. 71, 376-383.

Eccles, J.C. (1957) The Physiology of Nerve Cells. Baltimore: The John Hopkins Press.

Eccles, J.C. (1969) The Inhibitory Pathways of the Central Nervous System (The Sherringtonian Lectures, IX) Springfield: Thomas.

Eccles, J.C., Eccles, R.M., Iggo, A. & Lundberg, A. (1961) Electrophysiological investigations on Renshaw cells. J. Physiol. 159, 461-478.

Eccles, J.C., Fatt, P. & Koketsu, K. (1954) Cholinergic and inhibitory synapses in a pathway from motor-axon collateral to motoneurones. J. Physiol. 126, 524-562.

Eccles, J.C., Llinás, R. & Sasaki, K. (1966) The inhibitory interneurones within the cerebellar cortex. Exp. Brain Res. 1, 1-16.

von Economo, C. (1931) Encephalitis Lethargica: its sequelae and Treatment. Translated from original (Wien, 1918) by K.O. Newman. London: Oxford Press.

Edinger, L. (1899) The Anatomy of the Central Nervous System of Man and of Vertebrates in General. (Trans. W.S. Hall). Philadelphia: F.A. Davis Co.

Endröczi, E. and Lissak, K. (1960) The role of the mesencephalon, diencephalon and archicortex in the activation and inhibition of the pituitary-adrenocortical system. Acta physiol. acad. sci. Hung. 17, 39-55.

Erlanger, J. & Gasser, H.S. (1930) The action potential in fibers of slow conduction in spinal roots and somatic nerves. Amer. J. Physiol. 92, 43-82.

Erlanger, J. & Gasser, H.S. (1937) Electrical signs of nervous activity. Philadelphia: University of Pennsylvania Press.

Fatt, P. (1957) Sequence of events in synaptic activation of a motoneurone. J. Neurophysiol. 20, 61-80.

Faure, J., Bensch, C. & Didier, V. (1962) Role d'un systeme mesencephalo-limbique dans la "phase paradoxale" du sommeil chez le lapin. C.R. Soc. Biol., Paris 150, 70-73.

Flexner, L.B., Flexner, J.B., Roberts, R.B. and de la Haba, G. (1964) Loss of recent memory in mice as related to regional inhibition of cerebral protein synthesis. Proc. Nat. acad. Sci. Wash. 52, 1165-1169.

Forel, A. (1872) Beitrage zur Kenntniss des Thalamus opticus. Sitzungsber. d. Wiener Acad. Bd LXVI, III (Abtheil.).

Forel, A. (1877) Untersuchungen uber die Haubenregion U.S.W. Ach. f. Psychiat. 7, 393-495.

Friede, R.L. (1959) Histochemical investigations on succinic dehydrogenase in central nervous system. II. Atlas of midbrain of guinea pig including pons and cerebellum. J. Neurochem. 4, 290-303.

Friede, R.L. (1961) Histochemical investigations on succinic dehydrogenase in central nervous system. V. The diencephalon and basal telencephalic centres of the guinea pig. J. Neurochem. 6, 190-199.

Friede, R.L. (1961) A histochemical atlas of tissue oxidation in the brain of the cat. New York, Hafner Publishing Co.

French, J.D. (1960) The reticular formation. in J. Field (ed.) Handbook of physiology Vol.II Washington D.C.: American Physiological Society, pp. 1281-1305.

Fuxe, K. (1965) The distribution of monoamine terminals in the C.N.S. Acta physiol. scand. 64, suppl. 247.

Gahm, N. and Sutin, J. (1968) The relation of the subthalamic and habenular nuclei to oscillating slow wave activity in the midbrain ventral tegmental area. Brain Res. 11, 507-521.

Ganser, S. (1882) Vergleichend-anatomische studien uber das Gehirn des Maulwurfs. Morph. Zahrb. 7, 591-725.

Gastaut, H. (1953) A propos des fonctions non olfactives au rhinencephale. J. Physiol. Paris 45, 117-120.

van Gehuchten, A. (1894) Contribution á l'etude du systeme nerveux des teleosteens. Cellule 10, 255-295.

Gerard, R.W. (1955) The biological roots of psychiatry. Am. J. Psychiat. 112, 81-90.

Gloor, P. (1960) Amygdala. in J. Field, H.W. Magoun, V.E. Hall (Ed.) Handbook of Physiology, Section I: Neursphysiology Vol.II. Washington, D.C., American Physiological Society, pp. 1395-1420.

Gogolak, G., Stumpf, C., Petsche, H. & Stere, J. (1968) The firing pattern of septal neurons and the form of the hippocampal theta wave. Brain Res. 7, 201-207.

Grace, J.E. (1968) Central nervous system lesions and saline intake in the rat. Physiol. Behav. 3, 387-393.

Grant, R. (1950) Emotional hypothermia in rabbits. Amer. J. physiol. 160, 285-294.

Green, J.D. & Ardnini, A.A. (1954) Hippocampal electrical activity in arousal. J. Neurophysiol. 17, 533-557.

Green, J.D. & Machne, X. (1955) Unit activity of rabbit hippocampus. Amer. J. Physiol. 181, 219-224.

Green, J.D., Mancia, M. & Baumgarten, R. (1962) Recurrent inhibition in the olfactory bulb. I. Effects of antidromic stimulation of the lateral olfactory tract. J. Neurophysiol. 25, 467-488.

von Gudden, B. (1881) Mitteilung über das Ganglion interpedunculaire. Arch. f. Psychiat. 11, 424-427.

Guillery, R.W. (1959) Afferent fibres to the dorso-medial thalamic nucleus in the rat. J. Anat. Lond. 93, 403-419.

Hamori, J. & Szentagothai, J. (1966)a Identification under the electron microscope of climbing fibres and their synaptic contacts. Exp. Brain Res. 1, 65-81.

Hamori, J. & Szentagothai, J. (1966)b Participation of golgi neuron processes in the cerebellar glomeruli: an electron microscope study. Exp. Brain Res. 2, 35-48.

Hanaway, J., McConnell, J.A. & Netsky, J. (1971) Histogenesis of the substantia nigra, ventral tegmental area of Tsai, and interpeduncular nucleus: an autoradiographic study of the mesencephalon in the rat. J. comp. Neurol. 142, 59-74.

Hatton, G.I. (1965) Retention of discrimination and advoidance habits following lesions in the interpeduncular nucleus. J. comp. physiol. Psychol. 59, 331-334.

Hayashi, T. & Nagai, K. (1956) Action of ω-amino acids on the motor cortex of higher animals, especially γ-amino-β-oxybutyric acid as the real inhibitory principle in brain. Abstr. XX int. physiol. Cong. 410.

Haymaker, W., Andersen, E., Nauta, W.J.H. (1969) ed. The hypothalamus. Illinois: C. Thomas.

Hernandez-Peon, R., Chavez-Ibarra, G., Morgane,P.J. and Timoiaria, C. (1963). Limbic cholinergic pathways involved in sleep and emotional behavior. Exp. Neurol. 8, 93-111.

Herrick, C.J. (1921) Introduction to Neurology, (2nd edition). Philadelphia: W.B. Saunders.

Herrick, C.J. (1933) The functions of the olfactory parts of the cerebral cortex. Proc. Nat. Acad. Sci., Wash. 19, 7-14.

Herrick, C.J. (1948) The Brain of the Tiger Salamander. Chicago, Illinois: Univ. of Chicago Press.

van Hoesen, G.W., MacDougall, J.M., Mitchell, J.C. (1969) Anatomical specificity of septal projections in active and passive avoidance behavior in rats. J. comp. physiol. Psychol. 68, 80-89.

Hubel, D.H. (1957) Tungsten microelectrode for recording from single units. Science 125, 549-550.

Hubel, D.H. (1960) Single unit activity in lateral geniculate body and optic tract of unrestrained cats. J. Physiol. 150, 91-104.

Huber, G.C. & Crosby, E.C. (1928) Somatic and Visceral connections of the Diencephalon. in The Vegetative Nervous System. Res. publ. Assoc. Res. Nerv. ment. Dis. 9, 199-248.

Huber, G.C. & Crosby, E.C. (1929) The nuclei and fibre paths of the avion diencephalon, with consideration of telencephalic and certain mesencephalic centres and connections. J. comp. Neurol. 48, 1-226.

Ives, W.R. (1971) The interpeduncular nuclear complex of selected rodents. J. comp. Neurol. 141, 77-94.

Jansen, J. (1930) The brain of myxine glutinosa. J. comp. Neurol. 49, 359-507.

Jasper, H.H. and Ajmone-Marsan, C. (1954) A stereotaxic atlas of the diencephalon of the cat. Natl. Res. Council of Canada (Ottawa).

Johnston, J.B. (1902) The brain of petromyzon. J. comp. Neurol. 12, 1-86.

Jouvet, M. (1962) Recherches sur les structures nerveuses et les mechanismes responsables des differentes phases du sommeil physiologique. Arch. ital. Biol. 100, 125-206.

Kaada, B.R. (1960) Cingulate, posterior orbital, anterior insular and temporal pole cortex. in J. Field, H.W. Magoun and V.E. Hall eds. Handbook of Physiology, Section I: Neurophysiology, Vol.II, Washington D.C. American Physiological Society, pp. 1345-1372.

Kaada, B.R., Andersen, P. & Jansen, E. (1954) Stimulation of the Amygdaloid Nuclear Complex in Unanesthetized cats. Neurology 4, 48-64.

Kabat, H. (1936) Electrical stimulation of points in the forebrain and midbrain: the resultant alterations in respiration. J. comp. Neurol. 64, 187-208.

Kamikawa, K., McIlwain, J.T., Adey, W. (1964) Response patterns of thalamic neurons during classical conditioning. Electroenceph. Clin. Neurophysiol. 17, 485-496.

Killam, K.F., Dasgupta, S.R. & Killam, E.K. (1960) Studies of the action of convulsant hydrazides as vitamin B6 antagonists in the central nervous system. in Inhibition in the nervous system and gamma-aminobutyric acid, pp. 302-316, ed. Roberts, E., Baxter, C.F., Van Harreveld, A., Wiersma, C.A.G., Ross Adey, W., Killam, K.F. Oxford: Pergamon Press.

Klüver, H. & Bucy, P.C. (1939) Preliminary analysis of functions of the temporal lobes in monkeys. Arch. Neurol. Psychiat., Chicago 42, 979-1000.

Knyihar, E. & Cisillik, B. (1970) Localizations of inhibitors of the acetylcholine and GABA-synthesizing systems in the rat brain. Exp. Brain Res. 11, 1-16.

Koelle, G.B. (1954) The histochemical localization of cholinesterases in the central nervous system of the rat. J. comp. Neurol. 100, 211-236.

Komisaruk, B.R. (1970) Synchrony between limbic system theta activity and rhythmical activity in rats. J. comp. physiol. Psychol. 70, 482-492.

Kovacs, S., Sandor, A., Vertes, Z. & Vertes, M. (1966) The effect of stimulation of the habenular nucleus on pituitary-thyroid function. Acta physiol. hung. 30, 39-45.

Krnjević, K. (1966) Physiological aspects of the limbic system. A review by...a recording for the Hoffman-LaRoche Fdn. Montreal, MG-4392

Krnjević, K. (1969) Central cholinergic pathways. Fed. Proc. 28, 113-120.

Krnjević, K. & Phillis, J.W. (1963)a Acetylcholine sensitive cells in the cerebral cortex. J. Physiol. 166, 296-327.

Krnjević, K. & Phillis, J.W. (1963)b Pharmacological properties of acetylcholine sensitive cells in the cerebral cortex. J. Physiol. 166, 328-350.

Krnjević, K. & Silver, A. (1966) Acetylcholinesterase in the developing forebrain. J. Anat., Lond. 100, 63-89.

Kubota, K., Sakata, H., Takahashi, H. and Uno, M. (1965) Location of the recurrent inhibitory synapse on cat pyramidal tract cell. Proc. Japan Acad. 41, 195-197.

Kuypers, H.G.J.M. & Tuerk, J.C. (1964) The distribution of the cortical fibres within the nucleus cuneatus and gracilis in cat. J. Anat.,Lond. 98, 143-162.

Lee, B.B., Mandl, G. & Stean, J.P.B. (1969) Microelectrode tip position marking in nervous tissue: a New Dye Method. Electroenceph. Clin. Neurophysiol. 27, 610-613.

Lewis, P.R. and Shute, C.C.D. (1967) The Cholinergic Limbic System: Projections to the hippocampal formation,medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. Brain 90, 521-529.

Lewis, P.R.,Shute, C.C.D. & Silver, A. (1967) Confirmation from choline acetylase analyses of a massive cholinergic Innervation to rat hippocampus. J. Physiol. 191, 215-244.

Libet, B. (1965) Cortical activation in conscious and unconscious experience. Perspect. Biol. Med. 9, 77-86.

Libet, B.,Albers, W.W., Wright, E.W. & Feinstein, B. (1967) Responses of human somatosensory cortex to stimuli below threshold for conscious sensation. Science, N.Y. 158, 1597-1600.

Loeb, C., Magni, F. & Rossi, G. F. (1960) Electrophysiological analysis of the action of atropine on the central nervous system. Arch. ital. Biol. 98, 293-307.

Lomo, T. (1971)a Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the Dentate area of the Hippocampal Formation. Exp. Brain Res. 12, 18-45.

Lomo, T. (1971)b Potentiation of monosynaptic EPSP's in the Perforant Path-Dentate Granule Synapse. Exp. Brain Res. 12, 46-63.

Lorento de No, R. (1934) Studies of the structure of the cerebral cortex II. Continuation of the study of Ammonic system. J. Psychol. Neurol., Leipzig 46, 113-177.

Lorente de No, R. (1947) Action potential of the motoneurons of the hypoglossus nucleus. J. cell. comp. Physiol. 29, 207-287.

Lubar, J.F., Boyce, B.A., Schaefer, C.F. (1968) Etiology of polydipsia and polyuria in rats with septal lesions. Physiol. Behav. 3, 289-292.

Lyon, M. & Harrison, J.M. (1959) The effects of certain neural lesions in the rat on the reaction to a noxious stimulus. J. comp. Neurol. 111, 101-131.

MacLean, P. (1949) Psychosomatic disease and the visceral brain. Psychosom. Med. 11, 338-353.

MacLean, P. (1952) Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (visceral brain). Electroenceph. Clin. Neurophysiol. 4, 407-418.

MacLean, P.D. (1955) The Limbic System ("Visceral Brain") in Relation to Central Gray and Reticulum of the Brain stem. Psychosom. Med. 17, 355-366.

MacLean, P.D., Horowitz, N.H. & Robinson, F. (1952) Olfactory-like responses in piriform area to non-olfactory stimulation. Yale J. Biol. Med. 25, 159-172.

MacLean, P.D., Ploog, D.W. & Robinson, B.W. (1960) Circulatory effects of limbic stimulation, with special reference to the male genital organ. Physiol. Rev. 40, 105-112.

Malmo, R.B. (1961) Slowing of the heart rate after septal self-stimulation in rats. Science 133, 1128-1130.

Manocha, S.L. and Bourne, G.H. (1966)a Histochemical mapping of Succinic Dehydrogenase and Cytochrome oxidase in the Pons and Mesencephalon of Squirrel Monkey (Saimin Sciureus). Exp. Brain Res. 2, 230-246.

Manocha, S.L. and Bourne, G.H. (1966)b Histochemical mapping of Monoamine Oxidase and Lactic Dehydrogenase in the Pons and Mesencephalon of Squirrel Monkey (Saimin Sciureus). J. Neurochem. 13, 1047-1056.

Marburg, O. (1944) The Structure and Fibre Connections of the Human Habenula. J. comp. Neurol. 80, 211-233.

Marco, L.A., Brown, T.S. & Rouse, M.E. (1967) Unitary responses in ventrolateral thalamus upon intranuclear stimulation. J. Neurophysiol. 30, 482-493.

Martin, A.R., Wickelgren, O. & Beranek, O. (1970) Effects of iontophoretically applied drugs on spinal interneurones of the lamprey. J. Physiol. 207, 653-665.

Massopust, L.C. and Thompson, R. (1962) A new interpedunculodiencephalic pathway in rats and cats. J. comp. Neurol. 118, 97-105.

McCance, I. & Phillis, J.W. (1965) The location of microelectrode tips in nervous tissue. Experientia 21, 108-109.

McCleary, R.A. (1961) Response specificity in the behavioral effects of limbic system lesions in the cat. J. comp. physiol. Psychol. 54, 605-613.

McCleary, R.A. & Moore, R.Y. (1965) Subcortical Mechanisms of Behavior. New York: London (Basic Books).

Mess, B. (1964) Changes in thyroidal cold response of heat-adapted rats following bilateral lesions of the habenular nuclei. Acta Physiol. Acad. Sci., Hung. 24, 299-304.

Mettler, F.A. (1945) Fibre connections of the corpus striatum of the monkey and baboon. J. comp. Neurol. 82, 169-204.

Mettler, F.A. & Mettler, C.C. (1941) Role of the neostriatum. Amer. J. Physiol. 133, 594-601.

Meynert, T. (1872) The brain of mammals. Stricker's manual of Histology. New York: Wm. Wood & Co.

Milhaud, M. & Pappas, G.D. (1966)a The Fine Structure of Neurons and Synapses of the habenula of the cat with special reference to subjunctional bodies. Brain Res. 3, 158-173.

Milhaud, M. & Pappas, G.D. (1966)b Postsynaptic bodies in the habenula and interpeduncular nuclei of the rat. J. Cell Biol. 30, 437-441.

Mitchell, B. (1963) Connections of the Habenula and of the Interpeduncular Nucleus in the cat. J. comp. Neurol. 121, 441-453.

Moruzzi, G. & Magoun, H.W. (1949) Brain stem reticular formation and activation of the EEG. Electroenceph. Clin. Neurophysiol. 1, 455-473.

Murphy, J.T. & Renaud, L.P. (1968) Inhibitory interneurons in the ventromedial nucleus of the hypothalamus. Brain Res. 9, 385-389.

Murphy, J.T. & Renaud, L.P. (1969) Mechanisms of Inhibition in the Ventromedial Nucleus of the Hypothalamus. J. Neurophysiol. 32, 85-102.

Nauta, W.J.H. (1946) Hypothalamic regulation of sleep in rats: An experimental study. J. Neurophysiol. 9, 285-316.

Nauta, W.J.H. (1956) An experimental study of the fornix system in the rat. J. comp. Neurol. 104, 247-272.

Nauta, W.J.H. (1958) Hippocampal projections and related neural pathways to the mid-brain in the cat. Brain 81, 319-340.

Nauta, W.J.H. & Mehler, W.R. (1966) Projections of the lentiform nucleus in the monkey. Brain Res. 1, 3-42.

Nielson, H.C., McIver, A.H. and Boswell, R.S. (1965) Effect of Septal Lesions on Learning, Emotionality, Activity, and Exploratory Behavior in Rats. Exp. Neurol. 11, 147-157.

Nielson, H.C. & McIver, A. (1966) Cold stress and habenular lesion effects on rats behaviors. J. appl. Physiol. 21, 655-660.

Olds, J. (1958) Self-stimulation of the brain. Science 127, 315-323.

Olds, J. & Milner, P. (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. comp. physiol. Psychol. 47, 419-427.

Olds, J., Mink, W.D. & Best, P.J. (1969) Single unit patterns during anticipatory behavior. Electroenceph. Clin. Neurophysiol. 26, 144-158.

Olds, M.E. & Olds, J. (1963) Approach avoidance analysis of rat diencephalon. J. comp. Neurol. 120, 259-315.

Ostfeld, A.M., Machne, X. & Unna, K.R. (1960) The effects of atropine on the electroencephalogram and behavior in man. J. Pharmacol. exp. Therap. 128, 265-272.

Ostrowski, K. & Barnard, E.A. (1961) Application of isotopically labelled specific inhibitors as a method in enzyme cytochemistry. Exp. Cell Res. 25, 456-468.

Palay, S.L. (1956) Progress in neurobiology. I. Neurochemistry Structure and Function in the neuron, S.R. Korey and J.I. Nurnberger, eds., Hoeber Harper, pp. 64-82.

Papez, T.W. (1937) A proposed mechanism of emotion. Arch. Neurol. Psychiat., Chicago 38, 725-743.

Penfield, W. & Milner, B. (1958) Memory Deficit Produced by Bilateral Lesions in the Hippocampal Zone. Arch. Neurol. Psychiat., Chicago 79, 475-497.

Petsche, H., Stumpf, Ch. & Gogolak, G. (1962) The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. I. The control of hippocampus arousal activity by the septum cells. Electroenceph. clin. Neurophysiol. 14, 202-211.

Phillips, C.G. (1959) Actions of antidromic pyramidal volleys on single Betz Cells in the cat. Q.Jl.exp. Physiol. 44, 1-25.

Pitts, R.F., Magoun, H.W. & Ranson, S.W. (1939) Localization of the medullary respiratory centres in the cat. Amer. J. Physiol. 126, 673-688.

Pribram, K.H. & Kruger, L. (1954) Functions of the "Olfactory Brain". Ann. N.Y. Acad. Sci. 58, 109-138.

Raisman, G. (1969) A comparison of the mode of termination of the hippocampal and hypothalamic afferents to the septal nuclei as revealed by electron microscopy of degeneration. Exp. Brain Res. 7, 317-343.

Rall, W., Burke, R.E., Smith, T.G., Nelson, P.G. & Frank, K. (1967) Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. J. Neurophysiol. 30, 1169-1193.

Ramon-Moliner, E. (1962) An Attempt at Classifying Nerve Cells on the Basis of Their Dendritic Patterns. J. comp. Neurol. 119, 211-227.

Ranson, S.W. (1939) Somnolence caused by hypothalamic lesions in the monkey. Arch. Neurol. Psychiat., Chicago 41, 1-23.

Ranson, S.W. & Ingram, W.R. (1932) Catelepsy caused by lesions between the mammillary bodies and third nerve in the cat. Amer. J. Physiol. 101, 690-696.

Reinert, H. (1963) Defense reaction from the habenular nuclei, stria medullaris and fasciculus retroflexus. J. Physiol. 170, 28-29P.

Renshaw, B. (1946) Central effects of centripetal impulses in axons of spinal ventral roots. J. Neurophysiol. 9, 191-204.

Renshaw, B., Forbes, A. & Morison, B.R. (1940) Activity of isocortex and hippocampus; electrical studies with microelectrodes. J. Neurophysiol. 3, 74-105.

Riss, W.,Halpern, M. & Icalia, F. (1969) Anatomical aspects of the evolution of the limbic and olfactory systems and their potential significance for behavior. Ann. N.Y. Acad. Sci. 159, 1096-1111.

Roberts, E. & Eidelberg, E. (1960) Metabolic and neurophysiological roles of γ-aminobutyric acid. Int. Rev. Neurobiol. 2, 279-332.

Rossi, G.F., Favale, E., Hara, T., Giussani, A. & Sacco, G. (1961) Researches on the nervous mechanisms underlying deep sleep in the cat. Arch. ital. Biol. 99, 270-292.

Routtenberg, A. (1968) Hippocampal correlates of consumatory and observed behavior. Physiol. Behav. 3, 533-535.

Scharrer, E. (1945) Capillaries and mitochondria in neuropil. J. comp. Neurol. 83, 237-243.

Schwartz, S.T. (1965) An analysis of resting activity in the cuneate nucleus. Ph.D. thesis, Albert Einstein College of Medicine, Yeshiva University, New York.

Shimizu, N. & Nagaaki, M. (1957) Histochemical studies of succinic dehydrogenase of the brain of mice, rats, guinea pigs and rabbits. J. Histochem. Cytochem. 5, 334-345.

Shimizu, N., Nagaaki, N. & Masaaki, O. (1959) Histochemical studies of monoamine oxidase of the brain of rodents. Z. Zellforsch. 49, 389-400.

Shute, C.C.D. & Lewis, P.R. (1963) Cholinesterase-containing systems of the brain of the rat. Nature, Lond. 199, 1160-1164.

Slusher, M.A. & Critchlow, V. (1959) Effect of midbrain lesions on ovulation and adrenal response to stress in female rats. Proc. Soc. exp. Biol., N.Y. 101, 497-499.

Smaha, L.A. (1968) Efferent fibre connections of the interpeduncular nucleus of the cat. Anat. Rec. 160, 430. (Abstract)

Smith, W.K. (1945) The functional significance of the rostral cingular cortex as revealed by its responses to electrical excitation. J. Neurophysiol. 8, 241-255.

Snider, R.S. & Niemer, W.T. (1961) A Stereotaxic Atlas of the Cat Brain. Chicago: The University of Chicago Press.

Spenser, W.A. & Kandel, E.R. (1961) Hippocampal neuron responses to selective activation of recurrent collaterals of hippocampofugal axons. Exp. Neurol. 4, 149-161.

Spiegel, E.A., Miller, H.R. & Oppenheimer, M.J. (1940) Forebrain and rage reactions. J. Neurophysiol. 3, 538-548.

Stefanis, C. (1969) Interneuronal mechanisms in the cortex. in The Interneuron, ed. M.A.B. Brazier, UCLA Forum Med. Sci. No.11. Los Angeles: U. of California Press, pp. 497-526.

Steriade, M., Apostol, V. & Oakson, G. (1971) Clustered firing in the cerebello-thalamic pathway during synchronized sleep. Brain Res. 26, 425-432.

Szentagothai, J. (1967) Models of specific neuron arrangements in thalamic relay nuclei. Acta Morphol. Acad. Sci., Hung. 15,113-124.

Szentagothai, J., Flerko, B., Mess, B. & Malasz, B. (1968) In: Hypothalamic control of the Anterior Pituitary. Budapest: Akademiai Kiado.

Taber, E. (1961) The cytoarchitecture of the brain stem of the cats. I. Brain stem nuclei of cat. J. comp. Neurol. 116, 27-29.

Terzuolo, C.A., Sigg, B. & Killam, K.F.(1960) Effect of thiosemicarbazide on responses of spinal motorneurons. in Inhibition in the nervous system and gamma-amino butyric acid, Ed. by Roberts, E.,Baxter,C.F., Van Harreveld, A., Wiersma, C.A.G., Adey, W.R., Killam, K.F. Oxford: Pergamon Press, pp. 336-337.

Thomas, R.C. & Wilson, V.J. (1967) Recurrent interactions between motoneurons of known location in the cervical cord of the cat. J. Neurophysiol. 30, 661-674.

Thompson, R. and Hawkins, W.F. (1961) Memory unaffected by mamillary body lesions in the rat. Exp. Neurol. 3, 189-196.

Thompson, R., Langer, S.K. & Rich, I. (1964) Lesions of the limbic system and short-term memory in albino rats. Brain 87, 537-542.

Thompson, R. & Myers, R. (1971) Brainstem mechanism underlying visually guided responses in the rhesus monkey. J. comp. physiol. Psychol. 74 Monograph 479-512.

Thompson, R. and Rich, I. (1961) Transitory behavioral effects of interpeduncular nucleus damage. Exp. Neurol. 4, 310-316.

Trembly, B. and Sutin, J. (1961) Septal projections to the dorsomedial thalamic nucleus in the cat. Electroenceph. clin. Neurophysiol. 13, 880-888.

Trembly, B. & Sutin, J. (1962) Slow wave activity in the ventral tegmental area related to barbiturate anesthesia. Exp. Neurol. 5, 120-130.

Usher, D., Kasper, P. & Birmingham, M. (1967) Comparison of pituitary-adrenal function in rats lesioned in different areas of the limbic system and hypothalamus. Neuroendocrinology 2, 157-174.

Valenstein, E. (1968) The Anatomical locus of Reinforcement. in Progress in Physiological Psychology, V.2. Ed. E. Stellar and J.M. Sprague. New York: Academic Press.

Wakefield, C. (1968) Connections and Morphology of the Habenula. M.Sc. thesis, Dept. of Anatomy, Ottawa, University of.

Walberg, F. (1957) Corticofugal fibres to the nuclei of dorsal columns. An experimental study in the cat. Brain 80, 273-287.

Watkins, J.C. (1968) Metabolic derangements and other causative factors in toxic convulsions. Biochem. J. 106, 4P-7P.

Wells, J. & Sutin, J. (1963) Trigeminal and pretectal influences upon slow wave activity of the ventral tegmental area in the cat. Exp. Neurol. 7, 355-365.

Wilson, V.J. & Burgess, P.R. (1963) Disinhibition in the cat spinal cord. J. Physiol. 13, 386-398.

Woodburne, R.T. (1943) The nuclear pattern of non-tectal portions of midbrain and isthmus in the opposum. J. comp. Neurol. 78, 169-190.

Yamadori, T. (1969) Efferent fibres of the Habenula and Stria Medullaris Thalami in Rats. Exp. Neurol. 25, 541-558.

Zieglgänsberger, W. and Herz, A. (1971) Changes of Cutaneous Receptive Fields of Spino-Cervical-Tract Neurones and other Dorsal Horn Neurones by Microelectrophoretically Administered Amino Acids. Exp. Brain Res. 13, 111-126.