CENTRAL SYNAPTIC TRANSMISSION—MICROELECTROPHORETIC STUDIES[1,2]

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This review covers papers published in the past decade which have been concerned with investigations of the chemical sensitivity of single mammalian central neurones using the microelectrophoretic technique. Some studies have merely catalogued the apparent sensitivity of neurones in certain regions to a multitude of drugs, but the basic aim of the majority of investigators has been a comparison of drug-induced responses with excitatory and inhibitory effects produced synaptically, as an essential step in transmitter identification.

Unlike the situation existing at the neuromuscular junction (96, 168, 169) it has not been possible to determine whether the action of an electrophoretically administered substance is localized to particular synapses, or indeed is even restricted to synaptic regions upon central neurones. Nevertheless there has been a tendency in many microelectrophoretic studies to interpret drug-induced alterations of the activity of single neurones in terms of an action at synapses, particularly when neurochemical or histochemical evidence favours such an interpretation. However, there remains a continuing need for caution in so interpreting the excitation or depression of neurones by drugs, even when appropriate alterations in synaptic and induced activity can be produced by "specific" antagonists or potentiators. Furthermore, microelectrophoresis being a relatively new method of investigation, the importance cannot be over-estimated of parallel studies carried out in different laboratories.

It is not intended to present an exhaustive analysis of the function of particular compounds as possible central transmitters, this being covered by recent reviews (20, 27, 28, 49, 52, 56, 71, 90, 127–130, 173, 196, 197, 201, 203,

[1] The survey of literature was completed in Canberra on July 30, 1968.

[2] The following abbreviations are used in this review: ACh (acetylcholine); AV (n. anteroventralis); CL (n. centralis lateralis); CNS (central nervous system); DA (dopamine, 3-hydroxytyramine); DHβE (dihydro-β-erythroidine); DLH (DL-homocysteic acid); GABA (gamma-aminobutyric acid); GLUT (L- glutamic acid); LD (n. lateralis dorsalis); LG (lateral geniculate nucleus); LP (n. lateralis posterior); LSD-25 (lysergic acid diethylamide); MD (n. medialis dorsalis); MG (medial geniculate nucleus); NA (nor-adrenaline); VA (n. ventralis anterior); VL (n. ventralis lateralis); VM (n. ventralis medialis); VPL (n. ventralis posterolateralis); VPM (n. ventralis posteromedialis); 5-HT (5-hydroxytryptamine).

216, 238). Microelectrophoretic studies can only provide evidence that a substance has a transmitter-like action upon certain neurones, and that compounds acting in a specific fashion near synapses can either antagonize transmitter action or enhance it. These findings must be taken in conjunction with the results of other studies, predominantly neurochemical in nature, before a transmitter function can be ascribed to a given compound.

Details will not be discussed of experiments based on changes in behaviour, EEG patterns, or the electrical activity of populations of neurones which follow the deposition of relatively large amounts of substances into discrete brain regions in the form of concentrated solutions or crystals (7, 8, 34, 35, 112, 155, 183). Such experimental observations are difficult to interpret in terms of actions at synapses, but nevertheless provide valuable confirmatory evidence for the presence of specific receptor mechanisms in particular central nuclei, and for the participation of groups of neurones in certain physiological mechanisms, a function which might not be readily detectable at the single cell level.

Microelectrophoretic Techniques

In essence, the microelectrophoretic technique, as applied to the central nervous system, consists of the electrical ejection into the extraneuronal space of possibly pharmacologically active ions from fine glass micropipettes. Simultaneously, extra- or intracellular potentials are recorded from a single neurone, a portion of which is located within the volume of tissue affected by the ejected compound. The blood brain barrier is thus circumvented, and the sites of drug action are restricted to the immediate vicinity of the neurone under observation. Technical details have been described in many publications (see 1, 45, 48, 49, 54, 65, 73, 83, 87, 118, 127, 128, 196, 198, 204, 206, 213). Although microelectrophoresis is primarily useful for substances which are well ionized in water, aqueous solutions in contact with glass usually behave as if positively charged, and electro-osmosis may often be an effective method of administering solutions of poorly ionized substances (133, 152). Such solutions can also be administered by hydrostatic pressure (133) or by diffusion from a "micro-tap" (32), although these methods have a limited application.

The recording of extracellular potentials from neurones is usually carried out with the NaCl-containing centre barrel of five-, seven- or nine-barrel micropipettes, the orifices of all barrels being at the same level and of individual internal diameter 0.8 to 2μm. Arguments have been proposed for using micropipettes of smaller diameter (133, 210, 211), but in practice the high electrical resistance of both recording and drug-containing barrels may introduce difficulties. Although an extracellularly located micropipette may be used to record the response of a large number of neurones (field potentials), the more usual type of record is the all-or-none action potential of a single neurone located within approximately 50μm of the tip. The comparatively large size of these micropipettes introduces a sampling bias in mi-

croelectrophoretic experiments towards larger cells; this may be overcome to some extent by using a parallel-electrode assembly (54) in which a recording microelectrode of 0.5 to 1μm diameter projects less than 10μm beyond the openings of an attached multibarrel drug-containing micropipette. Furthermore, it may be possible to detect drug-induced alterations in the firing of small neurones indirectly by observing the discharge of larger neurones with which they synapse (11, 42, 58).

The excitant or depressant action of an ejected compound upon a single neurone can be assessed directly from oscillographic or photographic records of the action potentials. However, it is usually more convenient to convert these potentials to standard electrical pulses after passage through a continuously monitored voltage gating device, with ultimate computation to provide on-line records of mean firing frequency, post-stimulus and interval histograms (see 30). The continuous plotting of mean firing frequency is of considerable assistance when determining whether maximal effects have been obtained for a given electrophoretic current, and that sufficient time has been allowed between tests for complete recovery. The electrophoretic administration of excitant amino acids, such as DLH or GLUT, provides a ready means of activating otherwise "silent" neurones and achieving a reasonably constant "background" discharge rate upon which to test excitants and depressants, and also provides a standard "response" with which the effects of other substances can be compared. The type of firing used to assess changes in excitability may be very important; low-frequency spontaneous or drug-induced firing may provide more sensitive tests of alterations in postsynaptic excitability than antidromic or supra-threshold synaptic activation. However, some agents may act at the presynaptic level, and hence have effects only on a particular type of synaptic activation.

Extraneuronal drug administration can be combined with intracellular recording by the use of either co-axial (concentric) or parallel micropipettes: the intracellular electrode permits the recording of changes in resting potential; the measurement of synaptic potentials, membrane excitability, and impedance; and the estimation of reversal potentials and of the effects of alterations of intracellular ion concentrations upon synaptic and drug-induced potentials. The co-axial electrode is a development of the "pencil" electrode (48, 77, 104, 105, 229) consisting of a recording microelectrode contained within, and, projecting beyond the orifice of, a single drug-containing micropipette. A more sophisticated development (206) places the recording electrode within the central pipette of a five barrel assembly, thus permitting a study of more than one substance on the impaled nerve cell. Successful investigations of this type have been made using a single or double barrel recording microelectrode attached to the exterior of a single (twin pipettes, 149, 244) or five-barrel micropipette (parallel micropipettes, 54, 70), so that the relationship between the tips remains constant as the pipettes pass through nervous tissue. This arrangement pro-

vides many advantages over the co-axial assembly, although the use of both types of electrode is restricted to relatively large neurones.

In addition to identifying neurones from which action potentials are recorded by their position within the nervous system, and by the antidromic and orthodromic responses to appropriate nerve or tract stimulation, micro-marking techniques have been described which depend upon either local tissue destruction (107, 159) or the electrophoretic deposition of a dye (184, 227). The latter technique has also been used to mark cells intracellularly (121, 126, 222, 228).

Methods used for microelectrophoretic studies in different laboratories are basically very similar, particularly with respect to precautions taken to ensure that alterations in nerve cell activity are not produced merely by current flow or local alterations in tissue pH. It is unfortunate that not all papers include full details of the concentration and nature of solutions contained within micropipettes. In general, there has been a tendency to use too highly concentrated solutions, and it is probable that in some investigations the current used to control the diffusional and hydrostatic efflux of active substances from micropipettes was inadequate for the purpose. To some extent this difficulty can be overcome by using pipettes having small orifices, although a more practical approach is the use of more dilute solutions, with or without the addition of sodium chloride. On occasion, however, retaining currents may have been excessive, and currents in the reverse direction assumed to be ejecting a compound may not have been passed for sufficient time to achieve a suitable concentration in the tip of the pipette, and hence in the tissue surrounding the pipette orifice.

Many of the biological difficulties of microelectrophoretic experiments are common to any microelectrode investigation of the CNS in surgically prepared animals, although the effects of anaesthetics and paralysing agents may be particularly significant. Conflicting results have been reported regarding the effect of some anaesthetic agents on the chemical sensitivity of neurones, particularly by concentrations adequate for "surgical" levels of anaesthesia. After systemic administration most of these agents eventually depress the excitability of neurones with a reduction in "spontaneous" firing and alterations of firing patterns (192, 230). Similar effects have been observed after electrophoretic administration (28, 40, 68, 217). However, an apparently specific depression of the ACh sensitivity of cortical neurones, with minimal reduction in the sensitivity of these cells to excitant amino acids (138), by systemic barbiturates and chloralose was not confirmed in subsequent studies (37, 40). Pentobarbitone, diallylbarbituric acid, methylthioethyl-2-pentyl thiobarbitone, urethane, α-chloralose, nitrous oxide-oxygen mixtures, halothane (40), and methoxyflurane (38) each affected the mean spontaneous firing of cortical neurones and the sensitivity to electrophoretically administered ACh and DLH in a parallel fashion. However, the volatile anaesthetics, particularly methoxyflurane (38), appeared to have the least depressant effects upon cortical neurones when

used in anaesthetic concentrations. In various thalamic nuclei, pentobarbitone reduced the sensitivity of neurones to ACh, NA, and 5-HT, with less effect upon the excitation by GLUT (160, 174, but see 1). As in the cortex, this depressant action upon the chemical sensitivity of thalamic neurones was less with halothane and methoxyflurane (174).

In the caudate nucleus, chloralose and the barbiturates selectively decreased the sensitivity of cells to ACh without affecting that to GLUT, NA, or DA (19). In contrast, anaesthetics, particularly chloralose, enhanced the ability of caudate neurones to respond to stimulation of thalamic and striatal structures (19). Barbiturates also modified the sensitivity of olfactory bulb neurones to ACh, 5-HT, and NA (9).

The depressant effects of systemic anaesthetic agents upon the chemical sensitivity of neurones appears to be minimal in the brain stem (205) and spinal cord (83, 86). Chloralose did not affect the sensitivity of Renshaw cells to ACh (13, 83). The apparent insensitivity of spinal neurones to amines (79) has been ascribed to the effect of pentobarbitone (102, 156, 235, 236); however, recent experiments (63) indicate that anaesthetic doses of pentobarbitone have little or no effect upon the depression by NA of the DLH-induced firing of spinal interneurones.

The possible complicating effects of anaesthetics must thus always be considered in microelectrophoretic experiments. It may be possible to make use of decerebrate or other unanaesthetised preparations, provided that the type of preparation is acceptable on humane grounds, and that the necessary surgical manoeuvre does not interfere with afferent or efferent pathways required in the experimental procedure. However, it may be questioned whether all drug effects observed in unanaesthetised animals are necessarily of physiological significance, as the sensitivity of a cell to excitants or depressants may be abnormally enhanced by the excessive "background" activity of excitatory or inhibitory synapses. If anaesthetics are required, volatile agents may be more satisfactory than barbiturates, although it may be necessary to consider factors other than those associated with the reduction in chemical sensitivity of neurones, such as hypotension and problems associated with anaesthetising paralysed animals with gaseous agents for long periods.

In order to maintain stable recording conditions it is often necessary to paralyse experimental animals, and to provide positive pressure pulmonary ventilation. As the excitability of neurones, particularly those of the cerebral cortex (141), is modified by alterations of tissue CO_2 tension, it may be advisable to monitor blood, tissue, or more conveniently end-tidal, CO_2 levels in such preparations. Although gallamine triethiodide did not modify the sensitivity of Renshaw cells to ACh (84), the amplitude of spinal reflexes (95), the mean spontaneous firing rate of cortical neurones in decerebrate cats, or the sensitivity of these cells to ACh (40), the after-discharge of neurones in the intact and isolated cortex of unanaesthetised cats has been reported to be augmented (113). Furthermore, systemically administered

gallamine increased the mean spontaneous firing rate of neurones in the feline cuneate nucleus (110) and modified transmission through the nucleus (23, 110). In contrast, the spontaneous and synaptic firing of red nucleus neurones were reduced by gallamine (91). Although the enhancement of excitation in the cortex and cuneate may indicate some penetration of the blood brain barrier by gallamine, which when administered electrophoretically excited neurones in many CNS regions (2, 40, 61, 84, 86, 110, 205), the effects which follow systemic administration may also reflect alterations in the activity of peripheral sensory receptors produced by muscle relaxation. Succinylcholine may be more suitable for paralysing animals (110), again provided that indirect central effects consequent upon activation of muscle receptors (171) can be excluded.

The major disadvantages associated with the microelectrophoretic administration of drugs stem from the inability to determine drug concentrations at nerve cell receptors, and the nonuniform distribution of the ejected substance within the tissue as a consequence of its administration from a "point" source. Although the rate of ejection of an ion is related to the electrophoretic current passed through a micropipette, this relationship varies for different substances, is not constant for any one substance in different micropipettes (132, 133), and may not be constant for any one micropipette during its passage through tissue. Furthermore, the concentration of an ejected substance at a given distance from the pipette orifice is not amenable to calculation, except by assuming free diffusion (48, 73, 96, 124, 232), almost certainly an unwarranted assumption in a tissue as complex as the mammalian CNS. However, the relationship between local concentrations of substances ejected from adjacent barrels of a multibarrel micropipette will be roughly proportional to the electrophoretic currents, although perhaps modified, as they would be after any method of administration, by interaction with tissue components.

The nonuniform distribution of drugs within the extraneuronal space raises even more serious difficulties. Firstly, other neurones, glial cells, or even tissue fragments forming a "cap" around the open orifices of micropipettes (1, 248), may prevent electrophoretically administered substances from reaching receptors upon a neurone from which intra- or extra-cellular records are being obtained. Under such circumstances the concentration of the ejected substance may be higher in the vicinity of other neurones, and changes in the activity of these may synaptically affect the cell of major interest. Such indirect actions, and also those resulting from drug effects on glial cells or blood vessels, may of course complicate any microelectrophoretic investigation, but are generally excluded by showing that a large sample of a particular kind of neurone is consistently affected by a substance which has no effect on neighbouring cells.

Secondly, the major sites of intra- and extra-cellular recording presumably being in the vicinity of the soma of a neurone, the concentration of an electrophoretically administered substance may not reach high enough levels

to affect specific receptors if these be located predominantly on dendrites. Furthermore, the levels attained in the vicinity of the soma may be sufficient to produce relatively nonspecific actions, which in turn obscure effects of dendritic origin. Such conditions are particularly likely to occur with neurones of the pyramidal type. Another difficulty associated with the use of co-axial or parallel micropipettes is that areas of membrane affected by ejected substances may be electrically "remote" from the intracellular recording microelectrode, and changes in membrane potential resulting from localized alterations in membrane conductance may not be detected (see 97). Even if relatively distant changes in membrane properties are detected, as an alteration either in membrane potential or conductance, it may not be feasible to compare the equilibrium potential for the associated ionic events with that of synaptic potentials generated by the activation of receptors located in a more uniform fashion over the cell surface, and especially by those located on membrane closer to the site of recording (111).

Spinal Cord

Acetylcholine.—Renshaw cells (100, 247), located ventromedially in the feline spinal grey matter (100, 103, 227, 246) were readily excited by electrophoretically administered ACh (13, 65, 79, 81, 83, 195, 233, 236) and other cholinomimetics, particularly of the nicotinic variety (65, 83). Evidence has been obtained for the presence of both nicotinic and muscarinic receptors on these cells, only the former being involved in synaptic excitation by volleys in motor axon collaterals (66, 79, 84, 85, 98).

On the basis of electrophoretic currents required to produce the same rate of firing, nicotine, carbamylcholine, and tetramethylammonium were more potent excitants than ACh, whereas muscarine, acetyl-β-methylcholine, propionylcholine, and *n*-butyrylcholine were less effective (83). In contrast with other cholinomimetics, the action of ACh was rapid in both onset and offset: this characteristic time course, and its alteration by an anticholinesterase, has been used to identify ACh extracted from brain tissue and administered electrophoretically upon Renshaw cells (195). Specific antagonism has been demonstrated between DHβE, tetraethylammonium, pentamethonium, hexamethonium and (+)-tubocurarine, and the nicotinic receptors of Renshaw cells; and between atropine, hyoscine and related substances, and the muscarinic receptors (84; also 75). Many of the nicotinic antagonists, all of which block the axon collateral synaptic excitation of Renshaw cells (79, 85), excited in high concentrations, and excitation was the only effect of gallamine triethiodide (84). Procaine (75) and glycolic acid esters (80) reduced the sensitivity of Renshaw cells to ACh and excitant amino acids. Electrophoretically administered anticholinesterase agents enhanced the sensitivity of Renshaw cells to ACh (66, 79, 84), prolonged synaptic excitation by axon collateral impulses (66, 85) and, in addition, had direct excitant actions (84). Hemicholinium-3, in concentrations which neither excited Renshaw cells nor reduced their ACh sensitivity, progres-

sively reduced the number of spikes evoked by repetitive axon collateral impulses without influencing the firing evoked by dorsal root volleys (185). All of these results provide convincing confirmatory evidence for the role of ACh as the excitatory transmitter released upon Renshaw cells by axon collateral fibres (98–100, 153).

In contrast with Renshaw cells, other spinal neurones are less sensitive to ACh. Although ACh was reported to have no effect upon motoneurones and interneurones in barbiturate-anaesthetised cats (79), more recent experiments have demonstrated depressant (86, 101, 102, 233, 235) and excitant (86, 235) effects of long latency and duration upon some interneurones in both decerebrate and anaesthetised preparations. These effects, which occurred with approximately 30 per cent of investigated interneurones (235), but cannot be demonstrated in all preparations (86), have yet to be correlated with the operation of particular cholinergic pathways. The depressant actions of carbamylcholine and β-carbomethoxy-ethyltrimethylammonium exceeded those of ACh and acetyl-β-methylcholine; the effects were not blocked by DHβE but were reduced by atropine administered intravenously (86). Gallamine triethiodide excited spinal interneurones with characteristic bursts of high frequency firing; (+)-tubocurarine had both depressant and late excitant actions (86). Both atropine and procaine depressed the spontaneous, synaptic and chemically-induced firing of spinal interneurones with a concomitant reduction in the amplitude of the extracellularly recorded action potential; the threshold of excitability (direct, orthodromic, and antidromic) of the motoneurone membrane was increased in the absence of an alteration in either resting or postsynaptic potentials (75). Procaine also reduced the excitability of intraspinal afferent fibres (82); a similar effect was observed with strychnine although the concentrations were almost certainly higher than those required to block the depression of interneurones by glycine (see later). Hemicholinium-3 did not affect the firing of spinal interneurones by volleys in dorsal root afferent fibres (185). ACh has been reported not to influence the firing of sympathetic (93, 122) or parasympathetic (94) spinal preganglionic neurones.

Amino acids.—Extensive investigations have been made of the sensitivity of spinal neurones to electrophoretically administered amino acids. All neurones were excited by acidic amino acids structurally related to GLUT (76, 78, 87–89, 93, 94, 122) and depressed by neutral amino acids related to glycine and GABA (67–70, 74, 77, 87, 88, 239–243). The depolarization of spinal presynaptic terminals by GLUT or DLH, and the depression of excitability by GABA (82), may be associated with the presence of axo-axonic synapses at the terminal regions of afferent fibres: intraspinal axons appear to be insensitive to amino acids (70, 77, 82, 244).

When administered intracellularly, GLUT has little, if any, effect on the membrane potential of motoneurones (36). However, when administered extracellularly GLUT depolarized these neurones (78), and the reversal potential for the increase in membrane permeability induced by this amino

acid or DLH was at a less depolarized level than that of excitatory synaptic potentials (50). However a direct comparison of the equilibrium potentials for synaptic and amino acid excitation is precluded by the wide distribution of excitatory synapses over the surface membranes of motoneurones (186). It is thus possible that the depolarizing action of these amino acids is identical with that of excitatory transmitters, particularly as alterations of the intracellular ion content of motoneurones which did not influence the amplitude of excitatory postsynaptic potentials were also without effect on the depolarizing action of DLH (71). The action of excitant amino acids is unlikely to be produced by mere chelation of calcium from the vicinity of neurone membranes. Although a series of chelating agents has been shown to excite spinal neurones, the relative effectiveness of each being related to calcium-chelating potency, amino groups are not present in some of the active excitants, and some agents of higher calcium-chelating potency than aspartic acid did not excite interneurones (73).

Of the amino acids present in spinal tissue, L-glutamic and L-aspartic were of approximately equal potency, and were slightly more effective than the D-isomers. The most potent excitants were N-methyl-D-aspartic acid and D-homocysteic acid (89); the naturally occurring amino acids β-N-oxalyl-L-α β-diaminopropionic acid (188) and ibotenic acid (245) were also powerful excitants (125, 231). No evidence has been obtained for the enzymic removal of glutamic or aspartic acids from the vicinity of interneurones (78); furthermore, no specific antagonist has been found for the amino acid excitation of spinal, or indeed of other, neurones (90).

Glycine, GABA and β-alanine hyperpolarize spinal motoneurones, and the membrane permeability increase appears to be identical with that of postsynaptic inhibition: the reversal potentials for the synaptic and amino acid-induced hyperpolarizations appear to be identical, and alterations of intracellular ion concentrations affect both types of hyperpolarization in the same fashion (67, 69, 70, 239, 240, 242–244). The same mechanism is presumably responsible for the depression of the excitability of spinal interneurones and Renshaw cells by these and related amino acids; reports that glycine does not depress the firing of Renshaw cells (242, 243) have not been confirmed (68, 87). The postsynaptic effects of glycine, α-alanine, serine, β-alanine, cystathionine (240), β-amino-*iso*-butyric acid, and taurine were reversibly blocked by strychnine and strychnine-like compounds (68, 70) which suppress spinal post-synaptic inhibition (10, 46, 47, 53, 55). As strychnine did not affect the sensitivity of spinal neurones to "GABA-like" amino acids, including GABA, γ-amino-β-hydroxybutyric acid, 3-aminopropane sulphonic acid, δ-aminovaleric acid, and ε-aminocaproic acid (68), these particular amino acids were considered unlikely to be transmitter substances at strychnine-sensitive spinal inhibitory synapses. On the other hand "glycine-like" amino acids, the postsynaptic actions of which were blocked by strychnine, may indeed be transmitters at these synapses.

Apart from strychnine-like compounds, no other specific antagonists have

been found for depressant amino acids (68). Tetanus toxin, which blocks strychnine-sensitive spinal postsynaptic inhibitions (47), did not affect the postsynaptic action of either glycine or GABA, and presumably has a presynaptic action at spinal inhibitory synapses (62). The depressant effect of glycine on spinal neurones was enhanced by *p*-hydroxymercuribenzoic acid (68) and *p*-chloromercuriphenylsulphonic acid (64). However, in slightly higher concentrations these substances also increased the effectiveness of GABA, β-alanine and L- and D-α-alanine (64). Consequently the mercury derivatives presumably influence amino acid transport mechanisms rather than inhibit specific enzymes associated with the inactivation of glycine in spinal tissue (68).

Electrophoretically administered glycine was generally a more potent depressant of interneurones and motoneurones than GABA (68, 244); the two amino acids were approximately equi-effective upon Renshaw cells (68). The most powerful depressant was 3-aminopropane sulphonic acid (68, 88); muscimol, isolated from *Amanita muscaria* (245), was similar in potency to GABA and was not blocked by strychnine (125).

Catecholamines.—Early experiments in barbiturate-anaesthetised cats failed to demonstrate any action of either NA, adrenaline, DA, or tryptamine derivatives on spinal neurones (44, 79). In decerebrate preparations, however, NA has been reported to have predominantly a depressant action upon spontaneous, synaptic, and chemically-induced firing of some spinal interneurones, particularly of those near the base of the dorsal horn (10, 12, 101, 102, 122, 234, 236). Spinal motoneurones (55, 101, 102, 234, 237), Renshaw cells (10, 101, 102, 233, 236), and some autonomic preganglionic neurones (93, 94, 194, but see 122) were also depressed. Not every cell of these various types of neurone was affected by NA, and although the major action appeared to be depression, excitation has also been observed (233, 236). Both types of effect were generally much slower in onset and offset than the excitation of Renshaw cells by ACh: the depressant potency of (+)- and (−)-NA were similar (102), and that of (−)-adrenaline equal or slightly less (12), whereas DA and isoprenaline were comparatively weak depressants (12) of interneurones. Although NA occasionally hyperpolarized some lumbar motoneurones (55), the effects upon neuronal membrane of this and related substances have yet to be determined. Provided a constant firing rate was maintained by the administration of either DLH or ACh, the depression of the firing of interneurones and Renshaw cells by NA was not markedly reduced by anaesthetic doses of pentobarbitone (63, 102). Consequently the earlier failure to detect a depressant action of NA may have been due to the use of a maximal synaptic discharge in the testing procedure (79) rather than to the effect of the anaesthetic per se.

In a series of α- and β-adrenergic receptor antagonists none was found to block the depression of either interneurones or Renshaw cells by NA (12, 102), and although electrophoretically administered strychnine has been reported not to modify the action of NA upon Renshaw cells (10), subsequent experiments have shown that concentrations of the alkaloid sufficient

to block the depressant action of glycine on these neurones occasionally reduce the effectiveness of NA (63).

Indole derivatives.—The effects of 5-HT upon spinal neurones are complex. Both depressant and excitant effects have been observed in unanaesthetised cats when this substance was administered electrophoretically near some interneurones and Renshaw cells (101, 102, 233, 235). LSD-25 and ergometrine reduce the sensitivity of Renshaw cells to both ACh and DLH (52). An excitatory effect of 5-HT has been reported upon sympathetic preganglionic neurones (93), which was not observed with parasympathetic preganglionic neurones (94).

Dorsal Column Nuclei

No clear excitatory or depressant action of ACh has been demonstrated in the cuneate nucleus of cats (108, 109, 167, 218) or monkeys (108, 109) anaesthetised with pentobarbitone. Nicotine and succinylcholine also are without effect (110); (+)-tubocurarine enhances repetitive firing and gallamine triethiodide has a marked excitatory effect (109, 110).

Cuneate neurones were readily excited by GLUT and its D-isomer which was usually only slightly less effective (108–110, 167, 218). Furthermore, the spontaneous, synaptic, antidromic, and chemically induced firing of cuneate neurones were blocked by GABA and glycine, these amino acids being roughly equipotent (108–110). Adenosine triphosphate and chelating agents such as citric acid and ethylenediamine-tetra-acetic acid were also excitants (109). Noradrenaline, and to a lesser extent adrenaline (109) and DA (109, 167), depressed synaptic and GLUT-induced firing. Both 5-HT and histamine were also found to be weak depressants (109).

Brain Stem, Hypothalamus

Acetylcholine.—Most investigations in this region of the CNS have been carried out on physiologically unidentified neurones. Approximately one third of the "reticular" neurones of the medulla and pons of decerebrate cats were excited by ACh, and one tenth were depressed (24–28, 205). Characteristically the onset of excitation and depression was slow, and outlasted the period of ACh administration; the effectiveness of ACh was reduced by barbiturates (72, 205). Carbamylcholine, acetyl-β-methylcholine, and muscarine had similar actions, the effects of muscarine being very prolonged (25). On the other hand, depressant effects of nicotine were minimal (25); and it has been proposed that ACh may function as both an inhibitory and excitatory transmitter in this region of the CNS, receptors for inhibition being muscarinic in nature, whereas those for excitation have both muscarinic and nicotinic properties (see 28).

Reticular neurones were excited by neostigmine and physostigmine (25, 205), substances which also enhanced the action of ACh. Reports regarding the action of ACh antagonists are conflicting: DHβE has been shown to block excitatory and depressant actions of ACh, and to excite medullary neurones (205); on the other hand, DHβF has been reported to block only

the excitatory effect of ACh (25). Hexamethonium blocked excitant and depressant actions of ACh upon some neurones, and, in addition, had excitant and depressant effects unrelated to those of ACh (25, 205). Although gallamine triethiodide excited reticular neurones (205), some antagonism of ACh effects has been reported (25). Atropine, said to have no significant effect upon the response of ACh-sensitive neurones (205), has been demonstrated to block specifically the actions of ACh, particularly the excitatory effects (25). These findings upon a random sample of "reticular" neurones have yet to be correlated with the effects of ACh antagonists on synaptic activation. However, a high proportion of antidromically identified neurones of the paramedian reticular nucleus were excited by ACh, and this effect was blocked by gallamine (6).

Approximately 10 per cent of neurones investigated in the inferior colliculus were excited by ACh; most of these cells responded to auditory stimulation, but neither this response nor that to ACh was blocked by DHβE (72). Respiratory neurones were generally insensitive to electrophoretically administered ACh (204).

ACh excited approximately 50 per cent of cells investigated in the lateral vestibular nucleus. This firing was not affected by intravenous or microelectrophoretic DHβE, but was blocked by intravenously administered atropine which failed to influence the synaptic firing of the neurones by vestibular or sciatic nerve volleys (248). A similar excitant action of ACh has been reported for neurones of vestibular nuclei and the reticular formation which respond to caloric and galvanic stimulation of the labyrinth; a few cells were also depressed, and the effects of ACh were enhanced by physostigmine (221). Within the feline hypothalamus, ACh has both excitant and depressant effects, particularly in paraventricular and ventral median nuclei (21).

Amino acids.—Brain stem neurones were excited by GLUT (25, 72) and depressed by β-alanine and GABA (27, 72). The hyperpolarization of neurones of Deiters nucleus by GABA was accompanied by an increase in membrane conductance, a decrease in the amplitude of postsynaptic potentials, an increase in spike amplitude, and a block of spike invasion (170, 171). This action of GABA has been related to inhibition of these neurones by Purkinje cell axons (123, 171); in a few instances, electrophoretically administered hydroxylamine increased the amplitude of inhibitory potentials recorded from Deiters neurones in response to cerebellar stimulation (171). The cerebellar inhibition of Deiters neurones was not blocked by strychnine (170, 171).

Catecholamines.—Many brain stem neurones in decerebrate cats were affected by electrophoretically administered (−)-NA; approximately 30 per cent were excited (delayed onset and long duration) and about 20 per cent were depressed, this depression having usually a shorter latency and briefer duration than the excitation (22, 26, 28). A longer lasting depression has also been observed (22). Repeated administration of NA led to a reduction in the excitation. The excitatory, but not the depressant, effect of (+)-

NA was similar to that of the (−)-isomer; DA, adrenaline, and isoprenaline had weak actions (22, 28). Consistent antagonism could not be demonstrated between NA and dibenamine, dihydro-ergotamine, or phentolamine (22, 28). However chlorpromazine, which itself depressed the spontaneous discharge of a high proportion of brain stem neurones, particularly of neurones depressed by NA, consistently antagonized excitation by NA without affecting firing induced by GLUT (29). Amphetamine depressed the firing of approximately 50 per cent of brain stem neurones (28); a smaller percentage appeared to be excited by ergothioneine (3). Within the paramedian reticular nucleus the predominant action of NA was depression (6). A similar depressant effect has been reported for neurones of the red nucleus in unanaesthetised decerebrate cats (91).

Within the lateral vestibular nucleus of decerebrate cats approximately 50 per cent of the neurones were readily excited by NA, and depression was not observed (248). The excitation, of slow onset and prolonged duration, was substantially reduced in a few instances by dichloroisoproterenol which blocked neither the spontaneous nor synaptically induced (vestibular nerve) firing of these neurones. Neither dibenamine, phentolamine, nor isopropyl-NA interfered with excitation by NA; the isopropyl derivative itself had no excitatory effect (248). The main effect of NA in the hypothalamus was depression, particularly in paraventricular and ventral median nuclei (21).

Indole derivatives.—Although 5-HT was reported not to affect brain stem neurones in unanaesthetised cats (72), subsequent studies showed that 40 per cent of these cells in the medulla and pons were excited, and 50 per cent were depressed (28). The predominant action upon neurones of the paramedian reticular nucleus, however, was excitation (75 per cent), only 1 of 20 cells being depressed (6). LSD-25 reduced the spontaneous activity of reticular neurones for prolonged periods (28). Red nucleus neurones were depressed by 5-HT (91), and depression was the main effect observed in the hypothalamus; of 171 neurones tested, the rate of discharge of 22 was depressed and that of only two was enhanced (21).

Other compounds.—Partially purified prostaglandins had both excitatory and depressant effects upon brain stem neurones, particularly on cells of the medial medullary reticular formation (4, 5). When administered electrophoretically, sodium dexamethasone-21-phosphate depressed the spontaneous activity of approximately 14 per cent of cells investigated in the hypothalamus and mid-brain neurones of chloralose-urethane anaesthetised rats (193). The sensitive cells were located in the periventricular grey matter of the third ventricle and the aqueduct; neurones in the cortex, hippocampus, and thalamus were insensitive to the steroid.

THALAMUS

Acetylcholine.—A large majority of neurones of the ventrobasal complex of the thalamus (nucleus ventralis posterolateralis, VPL, and n. ventralis posteromedialis, VPM) of pentobarbitone-anaesthetised cats were sensitive to electrophoretically administered ACh (1, 2, 57). Many cells,

particularly thalamo-cortical relay neurones, were fired by ACh, the sensitivity of these cells being almost equal to that of Renshaw cells. On the other hand, the depolarization of thalamic interneurones, not projecting to the cortex, usually remained subthreshold, and the action of ACh was revealed as a facilitation of the firing induced either synaptically or by an excitant amino acid. In these experiments no specific reduction of the ACh sensitivity by pentobarbitone could be demonstrated. However, such an effect has been reported in more recent surveys of thalamic neurones (160–162, 174), which otherwise essentially confirmed the earlier investigation.

The excitation of ventrobasal thalamic neurones by ACh was slightly slower in both onset and offset than that of Renshaw cells (1, 160), but the latency and duration of firing were both less than those of cortical neurones (see below). Firing was often preceded by an initial depression of spontaneous activity (1, 2, 162). Of the various cholinomimetics compared with ACh, carbamylcholine was a more potent excitant, acetyl-β-methylcholine was approximately as potent as ACh, and propionylcholine, *n*-butyrylcholine, pilocarpine, nicotine, muscarine, and muscarone were weaker (2, 160). The latency of firing by these substances, and the duration of firing after termination of the electrophoretic current, usually exceeded the times observed with ACh. Anticholinesterases such as physostigmine, neostigmine, edrophonium, and 1,5-*bis*-(4-allyldimethylammonium-phenyl)pentan-3-one enhanced the action of ACh upon ventrobasal thalamic neurones and in addition were excitants; this latter effect was minimal with physostigmine (2, 160).

Specific antagonism could be demonstrated between DHβE and ACh on all ventrobasal thalamic cells tested, in the absence of a reduction in amino acid-induced firing (2, 57). The excitant action of higher concentrations of DHβE probably accounts for the failure of subsequent investigators to confirm the consistency of this finding (160). Although some specificity towards ACh receptors could be demonstrated with hexamethonium, (+)-tubocurarine, benzoquinonium, gallamine triethiodide, mecamylamine, atropine, and hyoscine, the action of these agents was complicated by an associated excitation [hexamethonium, (+)-tubocurarine, benzoquinonium, gallamine (2, 160)] or a depression of amino acid excitability [mecamylamine, atropine, hyoscine (2, 160)]. Electrophoretically administered atropine reduced the sensitivity of thalamic neurones to both ACh and DLH or GLUT, but usually firing by the amino acid recovered faster than that by ACh. It was possible, however, by administering atropine sulphate intravenously (0.1 to 10 mg/kg, 51), to block the firing of these cells by ACh without affecting either the spontaneous discharge or firing induced by DLH.

Similar results have been reported for other thalamic nuclei: n. ventralis medialis (VM, 162) n. ventralis lateralis (VL, 92, 162); n. lateralis posterior (LP, 162); n. ventralis anterior (VA, 162); n. centralis lateralis and medialis dorsalis (CL, MD, 162); n. anteroventralis (AV, 162); n. lateralis dorsalis (LD, 162); the dorsal lateral geniculate nucleus (LG, 61, 177, 207,

217); and the medial geniculate nucleus (MG, 226). Within the anterior, medial, and lateral nuclei, the proportion of neurones fired by ACh was highest in VPL, VPM, VM and the ventral half of VL (162, see also 164). Few neurones were fired by cholinomimetics in the LG nucleus of barbiturate-anaesthetised cats (61), but approximately 50 per cent were fired by ACh in animals anaesthetised with nitrous oxide and halothane or methoxyflurane (177), and an even higher percentage in decerebrate preparations (207). As in the ventrobasal complex, ACh also depressed a small percentage of neurones of the LG (177, 207); this effect was not reduced by DHβE (2, 207) or atropine (2). The depressant action of ACh upon MG neurones observed with 40 per cent of cells in animals anaesthetised with nitrous oxide, halothane, or methoxyflurane, has been reported to be abolished by electrophoretically administered strychnine or intravenous picrotoxin (226).

The presence upon thalamic neurones of ACh-sensitive receptors, with mixed nicotinic-muscarinic properties, has led to investigations of possible cholinergic afferent pathways to this region. Neither the spontaneous spindle activity of ventrobasal neurones, nor the synaptic firing induced by peripheral cutaneous nerve or cortical stimulation could be blocked in a specific fashion by either DHβE or atropine administered electrophoretically or systemically (1, 162) in concentrations adequate to block firing induced by ACh. Similar results have been described for the firing of VL neurones by impulses in the brachium conjunctivum (92, 162), although intravenously administered atropine reduced the magnitude of focal potentials evoked in this nucleus by brachium stimulation (106, 162). The firing of LG neurones in response to impulses in the optic nerve fibres was not blocked by either DHβE or atropine (61, 177). In contrast with these essentially negative results, the excitation of ventrobasal and VL neurones by single or repetitive stimulation of the mesencephalic reticular formation was depressed by electrophoretically administered atropine and DHβE (162). Furthermore, similar excitation of LG neurones was suppressed by benzoquinonium (177). In view of the difficulties associated with demonstrating a specific antagonism between DHβE, atropine, or benzoquinonium, and thalamic ACh-receptors, an interpretation of these results in support of the cholinergic nature of fibres projecting to the various thalamic nuclei from the mesencephalic reticular formation requires further confirmation.

Amino acids.—Thalamic neurones were readily excited by DLH and GLUT (1, 89, 92, 160, 177). N-Methyl-D-aspartic acid was a more powerful excitant than DLH, which was in turn more effective than GLUT (1). In a recent study, thalamic neurones located deeper than 6 mm beneath the fornix (in the vicinity of VL) were apparently more sensitive to GLUT, but not to DLH or N-methyl-DL-aspartic acid, than more superficial cells (164). GABA blocked the synaptic and chemically evoked activity of ventrobasal thalamic neurones (2).

Catecholamines.—In a general survey of the sensitivity of neurones in the anterior, medial, and lateral thalamic nuclei of cats anaesthetised with nitrous oxide and halothane or methoxyflurane, NA depressed 63 per cent

and excited 3 per cent of cells in more dorsally located nuclei; the percentage of depressed cells decreased, and of excited cells increased, in deeper nuclei (175). The spontaneous, synaptic, and chemically-evoked (DLH, ACh) firing were depressed by NA; adrenaline and isoprenaline were frequently more effective. The excitant action of NA, more readily apparent in the ventrobasal complex, showed marked variability in different animals and was less apparent with repeated tests, occasionally being replaced by depression. Similar effects were observed with adrenaline. In contrast, DA depressed the excitability of most neurones tested, irrespective of their location in the thalamus; a similar depression was noted in barbiturate anaesthetised cats (2). Many adrenergic blocking agents had depressant effects similar to that of NA, presumably as a consequence of interaction with NA receptors. In addition pronethalol, chlorpromazine, phenoxybenzamine, and phentolamine often excited thalamic neurones. Picrotoxin (1 mg/kg, intravenously), but not strychnine (1 mg/kg, intravenously), blocked both the depressant action of NA and the reduction in GLUT firing produced by repetitive stimulation of the mesencephalic reticular formation (175). However, electrophoretically administered strychnine had been shown to depress the excitability of many thalamic neurones (cf. also 60), and frequently to reduce the depressant action of catecholamines. The administration of pentobarbitone reduced the depressant action of NA upon thalamic neurones without affecting firing induced by GLUT (174).

In cats lightly anaesthetised with nitrous oxide and halothane or methoxyflurane, NA and DA blocked the synaptic, antidromic and amino acid excitation of many neurones in the LG nucleus (176), DA being slightly more potent than NA. The sensitivity of these neurones to catecholamines appeared to be unrelated to the sensitivity to 5-HT. In contrast, in unanaesthetised cats, the major action of NA upon both thalamocortical relay neurones and interneurones of LG was excitation, less than 12 per cent of the cells being depressed (207). Furthermore, in cats anaesthetised with pentobarbitone, DA and NA were found to be comparatively weak depressants of the excitation of LG neurones by impulses in optic nerve fibres. Depression of firing produced by GLUT was not observed (59, 60).

Neurones of the medial geniculate nucleus of cats anaesthetised with nitrous oxide and halothane or methoxyflurane are also sensitive to NA, the firing by GLUT of approximately 60 per cent being depressed (225). Excitation by NA was observed with 10 per cent of the neurones. Strychnine, administered electrophoretically or systemically, blocked both the depressant action of NA and the depression of firing induced by repetitive stimulation of the mesencephalic reticular formation; electrophoretically administered strychnine itself depressed the responses of the neurons to GLUT.

Indole derivatives.—An analysis of the action of 5-HT upon neurones of the LG nucleus in cats anaesthetised with pentobarbitone indicated that this substance (ejected with currents rarely exceeding 20nA), and many structurally related indole and lysergic acid derivatives, depressed the firing of these neurones by optic nerve impulses without affecting either the sensitiv-

ity to GLUT or ACh, or the invasion of the neurones by antidromic impulses (59–61). This effect was of relatively brief latency and short duration, was observed with all LG neurones tested, and was not blocked by the simultaneous electrophoretic administration of 2-bromo-(+)-lysergic acid diethylamide, methysergide, or dibenamine. LSD-25, ergometrine, and methylergometrine had actions similar to those of 5-HT, but of longer duration, and hence were not tested as antagonists. The most potent compounds of this series were 4- and 7-hydroxytryptamine. In contrast with these results, which were interpreted as indicating a specific action of 5-HT at optic nerve excitatory synapses, it has been demonstrated in a more recent investigation using cats anaesthetised with nitrous oxide, halothane, or methoxyflurane, that in addition to blocking the synaptic activation of LG neurones by optic nerve volleys, 5-HT depressed the firing induced by GLUT (one third of cells tested), or by ACh, and occasionally also blocked antidromic invasion (176). In general, the electrophoretic currents used exceeded those required to show a more specific effect of 5-HT in anaesthetised animals (60), and apparently no attempt was made to determine the relative sensitivities of synaptic and GLUT-induced firing to depression by 5-HT and related substances. In decerebrate unanaesthetised cats 5-HT depressed the orthodromic (optic nerve) and spontaneous firing of LG neurones, particularly of geniculo-cortical cells, and excited some geniculate interneurones (207). In this latter investigation, LG neurones were classified on the basis of sensitivity to ACh, NA and 5-HT. In contrast to the depression of the spontaneous firing of LG neurones produced by electrophoretically administered phenobarbitone, nitrazepam tended to fire the neurones in bursts of grouped discharges (217).

Within the ventrobasal complex of pentobarbitone-anaesthetised cats, 5-HT depressed the spontaneous spindle activity of neurones and the firing induced by ACh or GLUT without affecting synaptic excitation by cutaneous nerve volleys (2). Similar effects were observed with DA and 4-HT, there being little difference in the activity of these three compounds as depressants. In a more general survey of the effects of 5-HT in the thalamus of cats anaesthetised with nitrous oxide, halothane, or methoxyflurane, 5-HT depressed the firing by GLUT of the majority of cells tested in more dorsal nuclei, and excited a higher proportion of cells in ventral nuclei (175). Similar effects were observed with LSD-25, methysergide, and bufotenine, and electrophoretically administered strychnine frequently reduced the depressant effects of 5-HT. The predominant effect of 5-HT in the MG [nitrous oxide, halothane anaesthesia (225)] was a depression of spontaneous and excitant amino acid-induced firing, which was reduced by electrophoretically or systemically administered strychnine.

Basal Ganglia

Acetylcholine.—In unanesthetised decerebrate cats, electrophoretically administered ACh excited 75 per cent and depressed 8 per cent of the spontaneously active cells investigated in the head of the caudate nucleus (17,

19). The majority of "silent" cells, which were activated by GLUT, were depressed by ACh. Administration of barbiturates, ether, or chloralose reduced the number of spontaneously active neurones, markedly reduced the percentage excited by ACh, and increased the percentages depressed or unaffected by ACh (see also 165). In a subsequent study, also in decerebrate unanaesthetised cats (165), but using cells activated by DLH, only 20 per cent were influenced by ACh. Cells excited (11 per cent) tended to be located towards the ventricular and ventrolateral surfaces of the head of the caudate; those depressed by ACh were located towards the centre of the nucleus. The excitation and depression of caudate neurones by ACh, of comparatively slow onset and prolonged duration (19, 165), were mimicked by acetyl-β-methylcholine but not by nicotine or tetramethylammonium (165). Confirmation of the predominantly muscarinic nature of the receptors involved was provided by the failure of hexamethonium to influence the effects of ACh, whereas atropine blocked both excitation and depression. Furthermore, particularly after systemic administration, atropine blocked the synaptic excitation and inhibition of caudate neurones produced by stimulation of the thalamic VA nucleus. Neurones excited from this region of the thalamus were always excited by ACh; the activity of those inhibited was always depressed by ACh (165). These results were related to the presence of two different types of neurone in the caudate nucleus, and the enhanced release of ACh from this region resulting from electrical stimulation of VA (163). ACh also excited, or depressed, neurones within the feline globus pallidus-putamen complex; these effects were blocked by atropine, benzoquinonium, and DHβE (250).

Amino acids.—Neurones in the basal ganglia appear to be readily excited by GLUT and DLH (19, 114, 119, 120, 164, 166, 250) and depressed by GABA (19, 114).

Catecholamines and indole derivatives.—The main effect of NA and DA was depression, (17, 19, 119, 120, 166, 249), although excitation was also observed, particularly in the globus pallidus-putamen (250). The depression of some spontaneously active or DLH-excited caudate neurones by DA was blocked by electrophoretically administered phenoxybenzamine (166, 249) which frequently also suppressed the inhibition of these neurones which followed repetitive stimulation of the thalamic nucleus centromedianus. The findings have been related to the possible involvement of DA as an inhibitory transmitter in n. centromedianus-caudate pathways, and as an excitatory and inhibitory transmitter in a substantia nigra-caudate projection (33, 166, 249). Neurones of the globus pallidus-putamen complex have been reported to be excited by NA, DA, and 5-HT, the excitant action of DA being blocked by phentolamine and phenoxybenzamine (250).

Cerebellum

Acetylcholine.—Microelectrophoretically ejected ACh excited cells in the vermal cerebellar cortex (41, 42, 58, 157, 159, 173), and in the fastigial, in-

terpositus, and dentate nuclei (31). Many of the cortical neurones were identified as Purkinje cells by the depth within a superficial folium, the pattern of spontaneous activity, and the responses to activation of various afferent and antidromic pathways (41, 42), or by subsequent histological studies (158). On Purkinje cells, ACh had a long-latency (5 to 50 sec) and long-duration excitant action (41, 157), often preceded by a depression of spontaneous activity (42). Other cholinomimetics excited Purkinje cells, muscarine and carbamylcholine being both more potent and longer-lasting than ACh, while acetyl-β-methylcholine, arecoline, and nicotine were comparable in strength to ACh but had longer durations of action (42, 157, 172). Anticholinesterases (physostigmine, neostigmine, and edrophonium) potentiated and prolonged the firing induced by ACh, and also directly excited cerebellar neurones (42, 157).

In keeping with the greater potency of muscarinic compounds as excitants, atropine (intravenously or electrophoretically administered) reduced or abolished the excitant action of the cholinomimetics on these neurones (41, 42, 58). DHβE also reduced the sensitivity of some Purkinje cells to ACh (41, 42, see also 173), but in addition had an excitatory action when ejected locally (41, 42). The reported efficacy of hexamethonium, gallamine triethiodide, and (+)-tubocurarine as ACh-antagonists at these neurones (173) was not confirmed in subsequent studies: the latter two compounds directly excited Purkinje cells (42).

Essentially similar findings have been reported for the intracerebellar nuclei, in which some 80 per cent of all neurones were found to be sensitive to ACh (31). ACh and acetyl-β-methylcholine usually facilitated firing by GLUT or directly excited these cells with a fairly slow onset, but in a few cells excitation by ACh had a much shorter latency. DHβE failed to block excitation by ACh, but electrophoretic atropine specifically reduced the ACh-sensitivity of some intracerebellar neurones; in other cases nonspecific depressant or excitant actions of atropine were predominant.

Although the bulk of the above findings have been made on Purkinje cells, ACh-sensitive cells have also been reported in the granule cell layer (157, 158, 173). Attempts to confirm these observations using two multibarrel micropipettes in the one cerebellar folium (one pipette being used to excite chemically cells synapsing with a Purkinje cell recorded by the other) showed granule and basket cells to be excited by DLH, but to be insensitive to ACh and carbamylcholine (42, 58). However, these experiments were restricted to superficial folia in the cerebellar vermis, and cholinergic afferents may only supply granule cells of deeply located folia. Nevertheless, intravenous administration of the ACh-antagonists atropine and DHβE (in doses up to 1.2 mg/kg of each) failed to affect the responses of individual Purkinje cells, or the summed surface or subsurface field potentials evoked by stimulation of the parallel-fibre system, the mossy fibre afferents from the ipsilateral external cuneate or lateral reticular nuclei, the climbing fibre afferents from the inferior olive, or the mixed effects of stimulation in the

region of the ipsilateral fastigial nucleus (42). Thus, the sensitivity to ACh of Purkinje cells in the feline cerebellum has yet to be related to synaptic excitation by impulses in cholinergic fibres (209). No studies have been made of the effect of ACh antagonists on the synaptic firing of cells in the intracerebellar nuclei.

Other compounds.—Acidic amino acids excited cerebellar neurones (31, 42, 134, 157, 158), and the cells were depressed by GABA and β-alanine (134). The involvement of GABA as the inhibitory transmitter at Purkinje axon terminals is discussed above. Adrenaline, DA, 5-HT, (173) and NA (248) depressed cerebellar cortical neurones, whereas in the flocculus the predominant action was excitation (248).

Ergothioneine, identified as the "cerebellar excitatory factor" (43), had no excitant action when ejected electrophoretically near single cerebellar neurones in the vermis (42, 58, 144). Strychnine ejected electrophoretically by currents of 10 to 20 nA initially excited cerebellar neurones, but in larger doses produced a local anaesthetic-like effect on the action potentials (42).

Rhinencephalon

Acetylcholine.—ACh, carbamylcholine, and acetyl-β-methylcholine excited, or facilitated the action of GLUT upon some 16 per cent of cells in the amygdala (224), and from 27 to 50 per cent of pyriform cortical cells (154) and hippocampal neurones (14, 15, 116, 117, 215). Excitation by cholinomimetics usually had a long latency (15, 117, 154 but see 215), and persisted for some time after drug ejection had ceased. ACh depressed 4 to 5 per cent of neurones in the pyriform cortex and hippocampus with a rapid onset and offset (15, 117, 154). Most cholinoceptive units of the hippocampus were found to be less than 0.5 mm beneath the dorsal surface (15, 117). Muscarine was a more potent excitant than ACh (154), whereas nicotine was weak (15, 117, 154), but, like ACh, occasionally depressed spontaneous or GLUT-induced firing (15). The predominantly muscarinic nature of ACh receptors on pyriform and hippocampal neurones was also shown by the prolonged reduction in sensitivity to ACh after the electrophoretic ejection of atropine (14, 15, 117, 154). Although the required doses of atropine also reduced spontaneous firing and excitation by GLUT, these latter responses recovered more rapidly than did the sensitivity to microelectrophoretically administered ACh. DHβE blocked the ACh-excitation of some pyriform cortical neurones (154), but was ineffective in the hippocampus (14, 15). This compound also directly excited about half the cells on which it was tested. (+)-Tubocurarine depressed hippocampal cells (117), while dimethyl-(+)-tubocurarine showed both ACh-antagonism and direct excitant actions (14, 15). In contrast with these areas of the palaeocortex, neurones in the diagonal band of Broca were insensitive to ACh and acetyl-β-methylcholine (115).

Amino acids.—Neurones of the pyriform cortex (154), amygdala (224), and hippocampus (14, 15, 115–117, 215, 220A) were readily excited by

GLUT and depressed by GABA. Ejection of GLUT into areas CA1–3 of the hippocampus caused widespread excitation of cells whose synaptic interactions established synchronised waves of activity at 30 to 50 per sec, obscuring unit responses (14, 37). The excitatory effect of GLUT upon cells in the hippocampus, cerebral cortex, thalamus, and subthalamic structures was prolonged and enhanced by systemically, but not electrophoretically, administered thiosemicarbazide; this action was reversed by electrophoretically administered pyridoxal-5-phosphate (219, 220, 220A). Furthermore, thiosemicarbazide prolonged the depressant effect of GABA on hippocampal cells, and again this effect was abolished by electrophoretically administered pyridoxal-5-phosphate (220A).

Catecholamines.—NA and dopamine depressed both synaptic and amino acid-induced firing of rhinencephalic neurones (15, 115-117, 154, 215, 223, 224), DA being the most powerful depressant tested in the pyriform cortex (154). Neither α- nor β-adrenergic blocking agents (dibenamine, phenoxybenzamine, phentolamine, dichloroisoproterenol) affected the depression of pyriform cortical neurones by NA (154).

Indole derivatives.—Microelectrophoretically administered 5-HT also depressed the spontaneous and GLUT-induced firing of cells in the amygdala (224) and pyriform cortex (154, 223), and was more potent than either NA or DA in the hippocampus (15, 115–117, 215). Depression by 5-HT was of short (2 to 5 sec) latency and comparatively prolonged duration. The peripheral 5-HT antagonists (LSD 25, 2-bromo-(+)-lysergic acid, and methysergide) were weak or ineffective in blocking this depression, and were themselves depressants (154, 223). In the hippocampus a few neurones were excited by 5-HT administered with low currents (14, 15, 223). When ejected over a period of several minutes, ergometrine effectively blocked the excitation of hippocampal neurones by GLUT (15).

Other compounds.—In cats anaesthetised with chloralose and urethane, the microelectrophoretic ejection of nitrazepam altered the pattern of spontaneous firing of hippocampal neurones to one of grouped discharges: this action was distinct from the depression of these cells produced by phenobarbitone (217).

Olfactory Bulb

In contrast with the remainder of the rhinencephalon, the predominant action of ACh upon all types of cells in the olfactory bulb of the unanaesthetised rabbit was one of depression (9, 18, 199, 202). Physostigmine had a similar depressant action and prolonged, but did not potentiate, the effect of ACh. Atropine, DHβE, hexamethonium, and chlorisondamine blocked depression of these neurones by ACh (18), without affecting the inhibition of mitral and tufted cells which follows stimulation of the lateral olfactory tract (16, 199). These ACh antagonists also directly depressed mitral cells (18), as did dimethylpiperazinium. Gallamine triethiodide failed to antagonise depression by ACh (9).

Neurones of the olfactory bulb were somewhat less readily excited by GLUT than cells elsewhere in the CNS (9). Other amino acids, more potent as excitants, have apparently not been tested.

The spontaneous firing of most neurones sensitive to NA was depressed by this substance (9, 16, 18, 199, 200, 202). Dibenamine, phentolamine, and tolazoline (16, 18), but not dichloroisoproterenaol (199), effectively reduced both this depressant action of NA and the duration of the recurrent inhibition of mitral cells which followed stimulation of the lateral olfactory tract (16, 18, 199, 200). The firing of mitral cells was also depressed by 5-HT, but LSD 25 and 2-bromo-(+)-lysergic acid diethylamide were more effective antagonists of the depressant action of NA than of that produced by 5-HT (18, 199). The lysergic acid derivatives also reduced the recurrent inhibition of mitral cells (18, 199, 200, 202), an inhibition unaffected by strychnine (199). These results and the effects of intravenously administered reserpine and α-methyl-*m*-tyrosine upon the inhibition evoked by lateral olfactory tract stimulation (199, 200), have led to the suggestion that NA is a transmitter in the olfactory bulb (see 202).

Cerebral Cortex

Acetylcholine.—Some 15 to 20 per cent of the cells sampled in various regions of the neocortex using multibarrelled micropipettes can be excited by ACh and cholinomimetics (40, 127, 129, 131, 135–139, 143, 152, 210, 212–214). Most ACh-sensitive cells occurred in the deep pyramidal cell layers of the sensorimotor, auditory, and visual cortex, and were characterised by spontaneous activity in both anaesthetised and decerebrate preparations (40, 136, 138). Approximately 80 per cent of the physiologically identified pyramidal tract neurones were sensitive to ACh. The excitant action of ACh was occasionally evident only as facilitation of the excitant action of GLUT or other acidic amino acids, and was frequently preceded by a short-latency depression of spontaneous firing (40, 137). The response of a particular neurone to ACh was depressed by small doses of barbiturates, urethane, or chloralose (40, 138, 139), but the overall percentage of cholinoceptive units found in any animal was not simply a function of depth of anaesthesia (40, but see 138, 187). ACh has been shown to depolarize both cortical neurones and "unresponsive" cells which were presumably neuroglia (148).

ACh produced only depression of the spontaneous or amino acid-induced firing of some pericruciate (40, 179, 180, 187) and visual cortical neurones (210, 213). In cats anaesthetised with nitrous oxide-methoxyflurane, as many as 32 per cent of neurones in layers II, III and IV of the pericruciate cortex were depressed by ACh (179, 180), in contrast to some 0 to 5.5 per cent of cells found in studies using barbiturate or chloralose anaesthesia (40, 138, 187).

Characteristically, the excitation of cortical neurones by ACh was of long latency (5 to 20 sec), and prolonged duration (15 to 60 sec) after the

termination of the current. Carbamylcholine, muscarine, acetyl-β-methylcholine, arecoline, pilocarpine, and muscarone were all more potent excitants, and had more prolonged actions than ACh on cortical pyramidal neurones (40, 139). Other choline esters were either weaker excitants than ACh, or were inactive, both as excitants and as antagonists of ACh excitation (139). Earlier findings that nicotine excited cortical neurones in a paroxysmal fashion, irrespective of the effects of ACh (137, 139), were not confirmed in a subsequent study (40) in which nicotine was found to be a weak excitant of ACh-sensitive neurones. The action of ACh was prolonged and potentiated by the anticholinesterases neostigmine and edrophonium, which also enhanced synaptic firing of both sensorimotor (136, 139) and visual cortical neurones (210, 212, 213).

The muscarinic nature of the receptors associated with ACh excitation of cortical neurones indicated by the potency studies was confirmed by a study of the action of ACh antagonists. Intravenous and microelectrophoretic administration of atropine (40, 135, 136, 139) and hyoscine (139) reduced the excitant action of ACh on cortical neurones. When comparatively high ejecting currents were used for atropine, there was a reduction in synaptic firing and in the excitation by amino acids, as well as in the firing induced by ACh (40, 135, 136, 139), but the spontaneous and amino acid responses recovered from the atropine ejection much more rapidly than did the excitation induced by ACh. Electrophoretically ejected atropine also decreased the effect of ACh on cortical neurones depressed by this substance (180, 181, 187). Neither (+)-tubocurarine nor DHβE was an effective antagonist of the ACh excitation of pericruciate neurones (40, 135, 136, 139); they directly excited many of the cells tested (40), as did decamethonium (139). In the visual cortex, DHβE occasionally reduced the synaptic firing of cholinoceptive cells (210). Gallamine triethiodide has been claimed as a specific antagonist of the excitation of Betz cells by ACh (135, 136, 139), but in other studies electrophoretically administered gallamine produced only high-frequency bursts of excitation in both cholinoceptive and noncholinoceptive neurones (40).

The relation of these pharmacological studies on single cortical neurones to possible cholinergic afferent pathways to the cortex is not clear, though ACh may well play some role in the ascending reticular formation, and hence in cortical arousal (127, 130, 131). Many neurones excited by ACh, including Betz cells, were activated by transcallosal, intralaminar, or specific (sensory relay) thalamic stimulation, but many other units excited by these pathways were not affected by ACh (138). There did, however, appear to be an association between ACh sensitivity and the late repetitive firing which followed stimulation of specific thalamic nuclei or the internal capsule (138). Intravenous atropine tended to block this late repetitive response, although it had no effect on the primary response to such volleys (139), or on the average spontaneous firing rate of Betz cells (40, but see 139). Acetylcholinesterase-containing fibres occur in the neocortex, often

terminating in fine networks around the perikarya of pyramidal cells in layer V and polymorph cells in layer VI (151). More diffuse fibrillar staining extends superficially into layer IV (see also 209). It has been suggested that these fibres represent the terminations of the ascending cholinergic activating pathway from the midbrain reticular formation (151, 208).

In contrast with these excitatory cholinergic systems, pharmacological studies in cats anaesthetised with nitrous oxide and methoxyflurane have shown that the long latency, long-duration inhibitions of pericruciate neurones in layers II, III, & IV evoked by repetitive stimulation of the pyramidal tract, the mesencephalic reticular formation, the lateral hypothalamus, and the cortical surface, can be blocked by electrophoretically administered atropine, hyoscine, strychnine, DHβE, and (+)-tubocurarine (179–182). Many of the cells inhibited in this manner were depressed by ACh and other cholinomimetics, including nicotine (182), and this depression was also blocked by strychnine and ACh antagonists (see also 187). These results have suggested the presence of intracortical cholinergic inhibitory interneurones with terminals predominantly in layers II, III & IV (181).

Cortical inhibition.—A number of cortical inhibitory processes have been investigated with particular reference to the effects of strychnine. When administered electrophoretically, in concentrations insufficient to fire the cells, reduce the sensitivity to DLH, or to affect the extracellularly recorded action potentials, strychnine did not block the short-latency recurrent inhibition of Betz cells or the inhibition produced by chemical (DLH) excitation of interneurones in cortical layers III & IV (11, 55). The short-latency inhibition of cortical neurones evoked by direct electrical stimulation of the cortex was not blocked by electrophoretically administered strychnine, picrotoxin, pentamethylenetetrazole, long chain ω-amino acids, atropine, DHβE, (+)-tubocurarine, dibenamine, phenoxybenzamine, or dichlorisoprenaline (142, 145).

Amino acids.—All types of cortical neurones were excited by GLUT and structurally related acidic amino acids, and the relative potencies and time courses of action were similar to those found in tests upon spinal neurones (39, 127, 129, 134, 137, 152, 214). GLUT depolarized cortical neurones (127, 148) but not neuroglial cells (148). As in other CNS regions, no specific antagonist has been found for this excitation by amino acids.

Of the neutral amino acids related to GABA which have been tested on cortical neurones (39, 68, 127, 134, 137, 143, 146, 147, 149, 150, 214), 3-aminopropanesulphonic acid, GABA, γ-amino-β-hydroxybutyric acid, β-alanine, glycine, and taurine were more potent depressants than the majority of other ω-amino and ω-guanidino acids, glycine being less effective than GABA. Long-chain derivatives may have mixed excitant and depressant effects. Cortical neurones in newborn kittens were sensitive to GABA before the cells had much spontaneous activity or were fired by GLUT or ACh (143). Intracellularly injected GABA did not alter the membrane properties of cortical neurones, but the amino acid hyperpolarized these cells when admin-

istered extracellularly (146–148, 150). Hyperpolarization by GABA was associated with an increase in the membrane conductance, and the reversal potential was close to that of inhibitory postsynaptic potentials evoked by surface cortical stimulation. The reversal potentials of both GABA and inhibitory hyperpolarizations were displaced in a depolarized direction by elevation of the intracellular chloride ion concentration (149). GABA may, therefore, well be an important inhibitory transmitter in the mammalian cerebral cortex, but the lack of a specific antagonist precludes a final decision (see also 150). The ineffectiveness of a wide range of pharmacological agents, including strychnine, upon inhibitory processes in the cortex and on the action of GABA has been used as supporting evidence for a transmitter function for the amino acid (145). The depressant effect of GABA, and the inhibition of cortical neurones evoked by surface stimulation, were not prolonged by intravenously or electrophoretically administered hydroxylamine or amino-oxyacetic acid (150). GABA did not hyperpolarize cortical neuroglial cells, although some cells were depolarized (148). The depression of the spontaneous or DLH-induced firing of cortical neurones by glycine and β-alanine was blocked by electrophoretically administered strychnine (68).

The excitation and depression of cortical neurones by brain fractions administered electro-osmotically was to a large extent attributable to the presence of GLUT and GABA, although another substance having a prolonged depressant effect was also present (152).

Catecholamines.—Both excitant and depressant effects have been noted with catecholamines ejected near cortical neurones in anaesthetised (40, 127, 137, 140, 179, 191) and *cerveau isolé* or *encéphale isolé* cats (140, 191). Depressant actions predominated especially in barbiturate-anaesthetised preparations (40, 140), and were of two types: either a short-latency reduction in firing rate similar to that produced by neutral amino acids, or a slow decrease in the size of the extracellularly recorded action potentials with relatively less reduction in firing frequency, resembling the action of local anaesthetics (140). Adrenaline, isoprenaline, DA, and NA caused short-latency depression of synaptic firing and sensitivity to GLUT, but did not affect the antidromic invasion of Betz cells by impulses in the pyramidal tract. In contrast, ephedrine, phenylalanine, and amphetamine had slower local anaesthetic-like actions (127, 140). Dihydroxyphenylalanine was an excitant, as were both adrenaline and amphetamine ejected with large currents. Tyramine and tyrosine were relatively weak short-latency depressants (140). In a later series of *encéphale isolé* or halothane-anaesthetised preparations, NA produced a slowly-developing excitation of as many as 57 per cent of cells in sensitive cats, which was antagonized by dibenamine, and by small intravenous doses of thiopentone (191). Mescaline also excited a number of these neurones (191). On the other hand, in cats anaesthetised with nitrous oxide and methoxyflurane, NA depressed approximately 50 per cent of cells and this depression was blocked by electrophoretically administered strychnine (179).

Indole derivatives—5-HT and related compounds also depressed spontaneous and GLUT-induced firing of pericruciate cortical neurones (40, 140, 179). Tryptamine and 4- and 6-HT were approximately as potent as 5-HT, other tryptamine derivatives being weaker depressants (140). Large amounts of tryptophan, 5-, 6-, and 7-HT were reported to cause paroxysmal excitation (140). More recently, some 30 per cent of neurones in the post-sigmoid and suprasylvian gyri of *encéphale isolé* cats were excited by 5-HT ejected with currents of 5 to 50 nA from solutions of 5-HT bimaleate (189, 190). This excitation was of short latency, and was sometimes revealed only as a facilitation of firing by GLUT. In about half the cells tested, excitation by 5-HT was specifically and reversibly blocked by microelectrophoretically administered LSD-25, 2-bromo-(+)-lysergic acid diethylamide, or methysergide, but these agents had no effect upon the depression of other neurones by 5-HT (190). In contrast, approximately 90 per cent of cells tested in cats anaesthetised with nitrous oxide and methoxyflurane were depressed by 5-HT; this depression was frequently blocked by strychnine (179). Clarification of the difference between these two series of experiments must await identification of the types of neurones which were tested.

The more complex indoles, including ergometrine and LSD-25, characteristically produced a slow fall in the spontaneous or chemically-induced firing rate of cortical neurones with associated changes in the extracellular action potential. Again, certain of these compounds were excitants when ejected with larger currents (140).

Other compounds.—Electrophoretically administered tetrodotoxin, ejected as a cation from dilute solutions in 200 m*M* NaCl, reversibly depressed the sensitivity of cortical neurones to ACh and GLUT, presumably by raising the threshold for spike initiation (178). Ergothioneine has been reported to have no effect on cortical neurones (144).

Conclusion

Few regions of the mammalian CNS remain unviolated by multibarrel drug-containing micropipettes, and a considerable amount of knowledge has been accumulated about the sensitivity of many types of neurones to substances such as ACh, NA, DA, 5-HT, and the amino acids. Furthermore, much is known of the distribution of these substances, and of enzymes associated with their synthesis and inactivation. However, only in a few instances have transmitter-like actions been demonstrated for particular compounds, and reasonably acceptable evidence provided for the identity of transmitters. In many investigations the need for a more rigorous approach is apparent, particularly in experiments using substances having complex pharmacological actions, such as atropine, strychnine, and related compounds, as a means of demonstrating specific antagonism of both drug-induced and transmitter actions. The distribution of an electrophoretically administered antagonist may be quite suitable for blocking in a specific fashion the effect of an excitant or depressant ejected from an adjacent micro-

pipette, yet be inadequate for suppressing the action of the same substance released from synaptic terminals located either uniformly over the surface of a neurone or at regions distant from the site of recording. On the other hand a systemically administered antagonist which penetrates the blood brain barrier may not reach high enough local concentrations to block the action of an electrophoretically administered agent, but may effectively diminish synaptic action. Correct evaluation of the actions of antagonists given systemically also requires that effects upon other neurones located on the excitatory or inhibitory pathway can be excluded.

Experience in microelectrophoretic and associated electrophysiological techniques, even in systems of neurones already well studied, together with a more general appreciation of the advantages and disadvantages of this procedure, will perhaps facilitate its acceptance as an important method of investigation. It is necessary to emphasise, however, that the concentrations of ejected drugs at receptor sites are unknown, and only partially subject to experimental control. Furthermore, many factors such as the concentration and pH of drug solutions, local tissue trauma, tissue pO_2 and pCO_2 levels, and the presence of anaesthetic and paralysing agents, may contribute to the variability of observations made upon single neurones. Thus many problems arise when making quantitative comparisons between results obtained in different portions of the CNS, and particularly when assessing results reported from different laboratories. Some of these factors are amenable to control, and it should usually be possible to select conditions under which the action of an administered substance upon selected and identified neurones can be compared with that of a synaptically released transmitter.

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