AN INVESTIGATION OF THE NATURE OF PUTATIVE SYNAPTIC TRANSMITTERS IN THE RAT CORPUS STRIATUM

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ABSTRACT

The role of the biogenic amines dopamine and acetylcholine, and the amino acids glutamic, aspartic and gama-amino-butyric (GABA) acids in the rat corpus striatum has been investigated by using microiontophoretic application of these and related substances to striatal cells in conjunction with stimulation of the corticostriate afferent pathway.

In keeping with the findings of earlier workers, application of dopamine (DA) and acetylcholine (Ach) to striatal neurones resulted in both excitatory and depressant effects. The frequency of encountering excitatory effects was markedly lowered by the use of barbiturate anaesthesia (Dial CIBA), especially excitations produced by Ach. For this reason a penthrane - air anaesthesia system was developed and used in this study.

Cortical stimulation generally resulted in depression of glutamate induced striatal neurone firing, usually following either a brief excitation (28 ms) or after an equally brief latency (30 ms). Striatal units that were initially inhibited by cortical stimulation had a higher probability of being depressed by catecholamines, otherwise there did not appear to be any relationship between the response to stimulation and the responses to Ach, DA and noradrenaline (NA).

D-amphetamine, iontophoretically applied, was found to depress almost all striatal cells to which it was applied. Apomorphine (APO), iontophoretically applied, exhibited dopamine agonist actions. Spiroperidol, a butyrophenone which antagonises the behavioural effects of DA and APO, when administered systemically (i.p.), antagonised the depressant actions of Ach, DA, NA and APO on striatal cells and markedly increased the number of cells excited by DA and NA but not by APO or Ach. This effect did not appear to be mediated through a direct effect on dopamine receptors.

Bicuculline methyl iodide ejected at current levels sufficient to block GABA induced depression of striatal cell firing, caused a marked increase in cellular excitability and enhanced the initial excitation resulting from cortical stimulation, suggesting the presence of an intrinsic GABA mediated inhibition of striatal neurones.

Glutamic acid di-ethyl-ester (GDEE), reported to be a glutamate antagonist, reversibly blocked striatal cell firing ($\bar{x}$ = 12ms latency), in response to cortical stimulation, as well as excitatory amino acid induced excitation of the same cells with little alteration in spontaneous firing. This suggests very strongly that the excitatory amino acids; glutamic or aspartic, function as the transmitter in the excitatory fine-fibre cortico-striatal projection.

GENERAL INTRODUCTION

The corpus striatum, which is taken to include the caudate nucleus and putamen, remains one of the more enigmatic regions of the mammalian brain, despite the large amount of research effort that has been focussed upon its neuroanatomy, physiology and pharmacology. Virtually all of the ideas concerning its physiological function remain largely speculative.

The striatum accounts for approximately 10% of the total brain volume in mammals and increases in anatomical complexity as one ascends the phyllogenic scale. In rodents the striatum is a single entity, the division into a separate caudate and putamen by the coalescing internal capsule fibres becomming more marked in the higher mammals.

Neuroanatomically the striatum is remarkably unstructured. Golgi studies have shown that it consists largely of small interneurones which have relatively extensive globular dendritic fields and short axonal processes[15]. The cells comprise in excess of 90% of the total neuronal population. Other cell types account for a further 7 - 8% and only 2 - 3% of the striatal neurones; large diameter spindle shaped cells, have axons which leave the striatum. These efferent cells do not appear to have any particularly obvious relationship with the remaining striatal interneurones, and appear to be scattered more or less randomly throughout the striatum. Fine poorly myelinated or unmyelinated axons, which appear to be striatal afferents, form a network of fibres which are intersected by the dendritic trees of the interneurones in a manner somewhat analagous to the cerebellar granule cell fibres.[16,17].

This apparently unstructured aspect of the striatum is one of the root causes for the difficulties encountered in divining its function and physiology

since such an amorphous structure is not readily amenable to the same e experimental manipulations as are the cerebral cortex, hippocampus or cerebellum.

The striatal outflow appears to project to only two regions of the brain, the globus pallidus and the pars-reticularis of the substantia nigra [29]. A striato- cortical projection has been suggested by many workers but no compelling evidence for the existance of such a pathway has yet emerged. In fact the striato - pallidal projection appears to consist of collaterals of the striato-nigral fibres[28]. No direct projection either from the striatum, pallidum or S.N. to motor nuclei has been described, although this system continues to be referred to as the extra-pyramidal motor system.

In contrast to the restricted outflow, the striatum receives a wide convergence of inputs. Afferent fibres derive from the entire ipsilateral cortex and from the sensori-motor area of the contralateral side, and are somatopically distributed within the nucleus,[3,4,26,27]. as are the fibres from intralaminar thalamic nuclei. Fibres also project from the substantia nigra[1,10,30] and a number of smaller mesencephalic nuclei. The diffuse, unmyelinated dopaminergic projection from the substantia nigra pars-compacta have been the focus of a great deal of research following the demonstration of dopamine involvement in the 'extra pyramidal' symptoms of Parkinson's disease and related pathologies. The development of the fluorescent histochemical techniques for visualising catecholamine pathways by Anden et al[1]. has served to intensify this interest. Recently the presence of a parallel larger diameter non-dopaminergic nigro-striatal pathway has also been described [10]. Striatal afferents also derive from the vestibular,[24] auditory and possibly from the raphe nuclei as well.

Because of this wide convergence of input and the very restricted nature of the striatal outflow, the striatum is generally held to perform some sort of integrating function - presumably of 'slow' activity[7]. Striatal units have been described which fire in advance of ramp type voluntary movements in monkeys[8,18]. Divac has suggested on the basis of phyllogeny that the corpora striatum represent vicarious cerebra[9], and that by analogy they may perform functions analogous to the cerebral hemispheres. However the restricted nature of the striatal outflow poses a considerable interpretational problem, since striatal activity per se does not appear to exert a direct effect on motor pathways. Striatal stimulation results in the inhibition of pallidal neurones[22,28] which normally appear to have a relatively high tonic discharge rate. Behaviourally such stimulation results in a 'freezing' reac tion following several seconds delay, but does not result in discrete motor responses[6,32].

Pallidal neurones project in turn to the mid-brain tegmentum; sub-thalmic nucleus and the intralaminar thalamic nuclei among other sites, while the projections from the substantia nigra do not appear to be known with any certainty. From behavioural studies it would appear that there is a caudal projection from the substantia nigra to the tegmental reticular formation and possibly a decending spinal projection, since nigral stimulation has been reported to augment spinal root to root reflexes[31]. Striatal stimulation also gives rise to mossy and climbing fibre responses in the cerebellum which appear to be mediated by the globus pallidus and substantia nigra[11].

The presence of a possible striato-pallidal-thalamic-cortex-striatum pathway has been held to imply the presence of a functional negative feedback loop - the 'caudate loop' ; although the evidence is largely

circumstantial. A similar striatal - nigral - striatal loop involving the dopaminergic nigro-striatal fibres and the GABA containing striato-nigral pathway has also been postulated. The presence of the parallel excitatory nigro - striatal projection complicates matters however[10].

Stimulation of striatal afferent pathways seldom gives rise to action potentials from striatal cells[2,13] yet virtually all cells from which intracellular records have been made respond to cortical and thalamic stimulation with EPSPs followed by IPSPs. The failure of striatal inter-neurones to fire has been presumed to be due to a constitutional peculiarity of the cell membrane or due to the presence of tonic inhibition[19]. For this reason the striatum has long been known as one of the 'silent' areas of the brain. Intra-striatal stimulation also results in a similar EPSP-IPSP sequence to that produced by afferent path stimulation, the latency of the locally induced response indicates that it is polysynaptically mediated[19].

The relatively homogeneous structure of the striatum, and , at least in the cat and rat relatively diffuse distribution of the afferent fibres makes interpretation of local field potentials and their drug induces changes exceedingly difficult.

One technique that holds promise for aiding the understanding of striatal function is that of iontophoresis. Ideally the technique attempts to mimic the effects of post-synaptically released transmitter substances on the cell under observation, the neuronal response generally being inferred from changes in firing rate or pattern, although intracellular recording yeilds far more information. Allowing for the fact that there are associated with the neuronal membrane a large variety of other structures;

glia, astrocytes, parts of other neurones etc., which can greatly affect the access of the applied substances to the target cell; many results int the CNS obtained by using this technique have been remarkably consistent with results obtained from neurochemical and pharmacological studies.

Since the effect, excitation or depression, of an iontophoretically applied substance is generally assessed by its effect on the spontaneous or stimulus induced neuronal discharge, the 'silence' of striatal neurones necessitates the simultaneous application of an excitant compound, traditionally glutamic acid, to raise the firing rate of the cell under investigation sufficiently to observe drug induced changes.

Since the striatum contains among the highest concentrations of dopamine[12] and acetylcholine esterase in the CNS, both dopamine and acetylcholine have been applied iontophoretically to striatal neurones in the hope of determining their possible roles. The results obtained to date have been equivocal, though cells inhibited by nigral stimulation appear to have an increased probility of being depressed by dopamine[5]. The relative importance of acetylcholine in the striatum is inferred from the high levels of acetylcholine esterase and choline acetylase present[20,23] and the behavioural effects of intrastriatal injections of cholinergic agonists such as arecoline and oxytremorine[21] and of antagonists such as atropine, even though the pharmacological specificity of these substances has not been completely established.

The striatal efferent neurones appear to be the source of the acetylcholine esterase activity in the striatum (Deadwyler pers.com.) and produce prodigious amounts of the enzyme, sufficient it appears to account for the high concentrations reported. Yet these same neurones appear to release GABA at their nigral and pallidal terminals[25]

Much of the speculation as to the role of ACh and dopamine in the

striatum has been based on evidence obtained from the behavioural responses to intrastriatally administered drugs following unilateral striatal or nigral lesions. Cholinomimetics tend to produce responses which are reciprocal to those produced by dopamine or dopamine agonists such as apomorphine which tend to cause the animal to rotate towards the side opposite the lesion (contralateral rotation) [21] . Thus the butyrophenone tranquillisers such as haloperidol cause the animal towards the lesioned side, indicating that it in some manner blocks the actions of dopamine. Because of the gross nature of the lesioning and the relative ansence of a defined neuronal architecture it is exceedingly difficult to relate the drug effects observed in these studies to specific functional groups of cells.

Other possible transmitter substances ; glutamic acid and GABA have also been reported to be present in unusually high concentrations in the striatum [14] but have not attracted any attention. Since both these substances have been demonstrated to be putative neurotransmitters in other systems this lack of attention seems remarkable.

In the light of the foregoing, then it was decided to investigate the nature of the synaptic substances which might be involved in the cortico-striatal pathway. Since striatal cells receiving cortical afference might-also recieve a particular pattern of other inputs, particularly nigral and intrastriatal; the pharmacological response pattern of these striatal cells responsive to cortical stimulation was also investigated.

In carrying out these investigation it became necessary to develop a number of new techniques for anaesthesia, iontophoresis of drugs and for data processing. These appear in the appendix at the end of the dissertation.

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ALTERATIONS BY ANAESTHETIC AGENTS OF THE RESPONSES OF RAT STRIATAL NEURONES TO IONTOPHORETICALLY APPLIED AMPHETAMINE, ACETYLCHOLINE, NORADRENALINE AND DOPAMINE.

ABSTRACT

The effect of two anaesthetic agents, penthrane and di-allyl barbiturate (Dial , CIBA) was investigated on the responses of rat striatal neurones to iontophoretically applied noradrenaline (NA), dopamine (DA), acetylcholine (ACh) and d-amphetamine (d-AMPH).

At both high (50mg/Kg) and low (35 mg/Kg) dose levels of Dial there was a highly significant (p 0.1%) change in the nature of the responses to all the substances tested. This change was primarily a decrease in the frequency of excitatory responses observed to catecholamines and to ACh, compared to those observed under penthrane.

While the dosage of Dial also affected the nature of the responses it was to a much lesser degree, d-AMPH responses were not affected and were almost purely inhibitory.

Under both anaesthetics, there was no evident correlation between the responses to d- amphetamine and to the catecholamines.

INTRODUCTION

Amphetamines have been postulated by many workers to exert their behavioural effect through the release of presynaptically stored catecholamines[2,4]. The initial enhanced exploratory behaviour seen in mammals following d-amphetamine treatment has been ascribed to the release of noradrenaline, while the stereotyped behaviour seen at higher dose levels has been postulated to involve striatal dopaminergic pathways[6].

Local iontophoretic application of d- amphetamine (d-AMPH) to rat brain stem neurones[2] causes excitation while in cat caudate it depresses glutamate induced firing[7]. These observed differences can be ascribed to differences in the recording site, species and the nature of the anaesthetic agents used; halothane in the rat and Dial - urethane in the cat. In addition the effects of amphetamine were paralleled by those of noradrenaline (NA)[2] and dopamine (DA)[7], findings which would tend to support the hypothesis that catecholamines mediate the activities of amphetamine.

Since anaesthetic agents have been shown to have profound effects on the pharmacological responses of CNS neurones[3,12,14,19] t was of interest to determine the concommitant effects of anaesthetics on the responses to iontophoretically applied d-AMPH and catecholamines of rat caudate nucleus neurones and to see whether the reported positive correlation between the responses to catecholamines and d-AMPH persists. Such information could help elucidate the mechanism of action of d-AMPH in the caudate nucleus.

METHODS

Hooded rats of both sexes (250 - 500 gm) were used for all experiments. In the initial barbiturate series (15 rats) Dial (di-allyl barbiturate) was given intraperitoneally at a dosage of 50 mg/Kg. In the second series (18 rats) Penthrane (methoxyfluorane - Abbot) was initially given, with air, at a level of 1% for 20 to 40 minutes following halothane induction (1 ml in a closed container). After this period the penthrane level was slowly tapered off to approximately 0.07% and maintained at this level for the remainder of the experiment. At this level the animals were in early stage 3 anaesthesia (corneal reflex still present). Since rats are obligate nose breathers, and since the animals were breathing spontaneously, a rubber snout mask constructed from a gum rubber dropper teat was used to avoid the necessity for tracheal intubation or tracheostomy. The snout mask did not increase the respiratory dead space more than 0.1 ml. The gas mixture was delivered from a simple laboratory constructed anaesthesia machine which permitted the accurate metering and mixing of the fresh and penthrane saturated air streams.

A third series of 3 rats were given Dial at 35mg/Kg following halothane-air induction. This dose gave an anaesthesia level comparable to that acheived under penthrane once the animal was induced with halothane, but was not of itself adequate to induce anaesthesia.

In all series, recording was not commenced for at least one hour following surgery. Haert rate was monitored during the penthrane anaesthesia and would remain between 270 and 320 beats per minute throughout the experiment. The rat, mounted in a stereotaxic frame, lay on a DC operated heating pad[17], a rectal thermistor probe being used to regulate

the animals temperature at 37.4°C.

The skull was opened and a small trephine hole (2 mm in diameter) was made using a stereotaxic drill to allow insertion of the microelectrode in the region of the head of the caudate (A 8.5 mm, L 2.5 mm [11]). Most of the exploratory tracks were confined to the body of the striatum.

A miniature 7 barrel micropipette [18] (tip diameter 5 - 9 microns) was used for extracellular recording and drug application. The centre recording barrel and one or two lateral barrels were filled with 2 M NaCl. These and the drug containing barrels were filled by centrifugation immediately before use.

Drugs used were d-amphetamine sulphate (0.2M, pH 5.6), dopamine HCl (0.2M, pH 4), l-noradrenaline HCl (0.2M, pH 3.5) and in the second and third series of animals, acetylcholine HCl (0.2 M, pH 3.5), partly as a further index of the anaesthetic's effects. Glutamate (as Na glutamate) (0.2M.pH 7) was used to excite ' silent' cells or to increase the firing rate of spontaneously firing cells. Metered iontophoretic injection currents were supplied from a 6 channel FET constant current source[15]. Current controls (+30 to +50 nA passed through a lateral NaCl barrel) were performed on the majority of cells, the responses of any cell which showed significant current sensitivity were disregarded, although current sensitive cells were seldom encountered. Retaining currents used were between -10 to -20 nA.

Neuronal activity was amplified, monitored on an oscilloscope, the Y output of which passed via a window discriminator to an epochal rate-meter[16]. The ratemeter output was plotted with a chart recorder to provide a permanent record of changes in firing rate. The epoch time chosen varied from 0.5 to 3 seconds depending upon the firing rate and firing pattern of the cell under scrutiny.

RESULTS

The most immediately obvious difference between the anaesthetic regimes used was the markedly increased probability (at least 20 fold) of finding, under the light anaesthetic doses, striatal cells which were either spontaneously firing (1 - 5 spikes per second), or which could be excited by low current (2 - 15 nA) applications of glutamate ions. Virtually all cells encountered required some glutamate 'drive' to increase the spontaneous firing rates to a level which permited the recording of drug effects (rates greater than 3 - 5 spikes per second).

This incerased probability of encountering responsive cells under penthrane is not reflected in the ratio of the number of cells tested to the number of animals used (Table 2) because this data was collected as part of another study involving a far more intensive investigation of the responses of each cell encountered.

Quite noticeable differences in the effects of the concentrations of the two anaesthetic agents on the striatal cell firing constancy were evident. Under Dial (50 mg/Kg) cells exhibited a relatively constant discharge rate over prolonged periods (up to one hour), whereas under both penthrane and Dial (35 mg/Kg) many of the cells showed both random and cyclical changes in firing rate, presumably reflecting changing levels of presynaptic drive. Under both anaesthetics striatal cells exhibited two different patterns of firing, both of approximately equal occurrence; irregular firing and bursting; but there appearred to be no particular relationship between firing pattern and the cell's response to applied drugs.

The responses of striatal neurones to the iontophoretic application of d- AMPH, Ach, NA and DA were markedly affected by the anaesthetic agent used. Under Dial virtually no excitatory responses to catecholamines

or to d-AMPH were observed (Tables 1 and 2), whereas under penthrane both excitatory and depressant responses were observed (Table 3).

Under Dial anaesthesia (50 mg/Kg), in agreement with the findings of Feltz and DeChamplain[7] , 88% of the cells responding to d-AMPH were depressed, and under either dose level of this anaesthetic, there appears to be a strong correllation between d-AMPH depression and depression by both DA and NA as can be seen from Tables 1 and 2. Of those cells tested under Dial (50 mg/Kg),76% were depressed by DA and 65% depressed by NA. A very similar picture can be seen for the lower (35 mg/Kg) dose level (Table 3.).

However under penthrane, the frequency of occurrence of excitatory responses increases dramatically for all compounds tested. d-AMPH caused excitation in 10% of the 85 cells tested and depression in 46%, but no correlation between the responses of cells affected by d-AMPH and the responses to DA or NA was evident. The proportion of cells under penthrane responding with either excitation or depression to d-AMPH was considerably reduced (56%) in comparison to the proportion under Dial (50 mg/Kg) - 98%. Of the 102 cells tested under penthrane 36% were excited by DA and 45% (of 97 cells tested) excited by NA.

Both excitatory and depressant responses to ACh were found under penthrane anesthesia, in substantial agreement with the findings of other workers in the caudate nucleus[1,13], and in the rat brain stem[3]. The frequency of encountering, cells excited by ACh was somewhat lower than that reported by Bloom et al[1] for the encephalé isolé cat caudate This may merely reflect species difference or a difference resulting from the use of penthrane.

The effect of an intraperitoneal injection of sodium thiopental (10mg/Kg) on one ACh excited unit in a penthrane anaesthetised rat, in agreement

with the findings of Bloom et al.[1] and Bradley and Dray[3], this resulted in the supression of the excitatory response after a 20 minute delay. This delay was presumably due to the injection site employed.

In the third series of rats (Dial 35 mg/Kg), the frequency of excitatory responses to ACh were much reduced in comparison to that observed under penthrane (Table 3), a phenomenon which has been reported by others[3].

A Chi-square analysis was performed on the data to verify the observed difference between the effects of the anaesthetic agents used and the effects of the two dose levels of Dial on the responses to iontophoretically applied drugs. With the responses observed under penthrane as the control all drug responses under both levels of Dial differed significantly, $p < 0.1\%$. However between the two dose levels of Dial there was no significant difference in the nature of the responses to dAMPH and the difference between the nature of the responses to both DA and NA were barely significant; at the $p = 0.1\%$ limit.

DISCUSSION

While in this study under penthrane anaesthesia, a considerably higher proportion of cells were excited by catecholamines than had been reported for the caudate of the encephale-isole cat(11%[5];16%[1]), the proportion was lower than reported for cells in the penthrane anaesthetised cat putamen (44% [20]). However, in the present study there was no similarity to the predominantly excitatory responses to catecholamines and d-AMPH found in the brain stem of the halothane anaesthetised rat [2], or to the almost purely depressant responses to catecholamines observed in the cerebral cortex of halothane and penthrane anaesthetised cats[8] and rats[10]

These differences in responses should make one cautious about extrapolating drug response phenomena observed in one area of the brain to other areas.

It is evident that the nature of the anaesthetic agent can have a very profound effect on the nature of the results obtained and thus on the interpretation placed upon them. The depressant actions of the barbiturates on CNS neurones has been well documented [3,12,14,19], and the rather dramatic change in the nature of the responses observed in this study can only reinforce the conclusion that barbiturate anaesthetics have no place in single cell neuropharmacological studies.

Unfortunately it was not possible to perform a set of comparison studies on artificially ventilated curarised animals to provide a further comparison with the penthrane study described here. However the level of penthrane was kept as low as possible throughout the experiment as indicated perviously.

Several studies have indicated that the actions of d-amphetamine are perhapes not mediated by catecholamines. Depletion and destruction of

catecholamine neurones by intraventricular injections of 6-OH dopamine have been shown to leave the depressive effect of iontophoretically applied d-AMPH on the caudate nucleus neurones of the barbiturate anaesthetised cat unchanged[7]. Furthermore it has been reported that depletion of central catecholamine terminals by 6-OH dopamine does not affect the characteristic behavioural responses induced by d-AMPH[9].

The finding of an absence of correlation between d-APMH depression and the responses to monoamines (and ACh) in penthrane anaesthetised rat caudate cells reinforces the suggestion that amphetamines may exert their own intrinsic actions on CNS neurones which are not mediated by other transmitter agents. However this observed intrinsic action of iontophoretically applied d- amphetamine may not underlie the behavioural effects of amphetamine observed at theraputic dose levels.

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TABLE 1. Neuronal responses in animals anaesthetised with Dial (50 mg/Kg)

		NA				dAMPH			
		+	o	-	NT	+	o	-	NT
DA +	1	.	.	1	.	.	1	.	.
DA o	11	1	4	4	3	.	3	7	1
DA -	38	3	7	25	3	2	.	36	.
Not tested(NT)	3	.	1	.	2	.	.	3	.
Total cells	53								

NOTE The responses are tabulated against the responses to dopamine (DA) for purposes of comparison. As described in the text acetylcholine was not employed in this part of the study. Excitatoty responses are shown as +, no response o, and depression -. Cells in the column NT (not tested) must be subtracted from the total number of cells when making comparisons between drugs.

TABLE 2. Neuronal responses in penthrane anaesthetised animals.

		NA					dAMPH				ACh			
		+	o	-	±	NT	+	o	-	NT	+	o	-	NT
DA +	37	24	8	4	1	.	6	10	14	7	16	11	4	4
DA o	38	9	16	9	2	2	.	16	15	6	9	17	7	5
DA -	27	7	2	12	.	5	1	11	10	5	12	4	7	4
NT	2	2	.	.	.	.	1	.	.	1	1	1	.	.
Total cells	104													

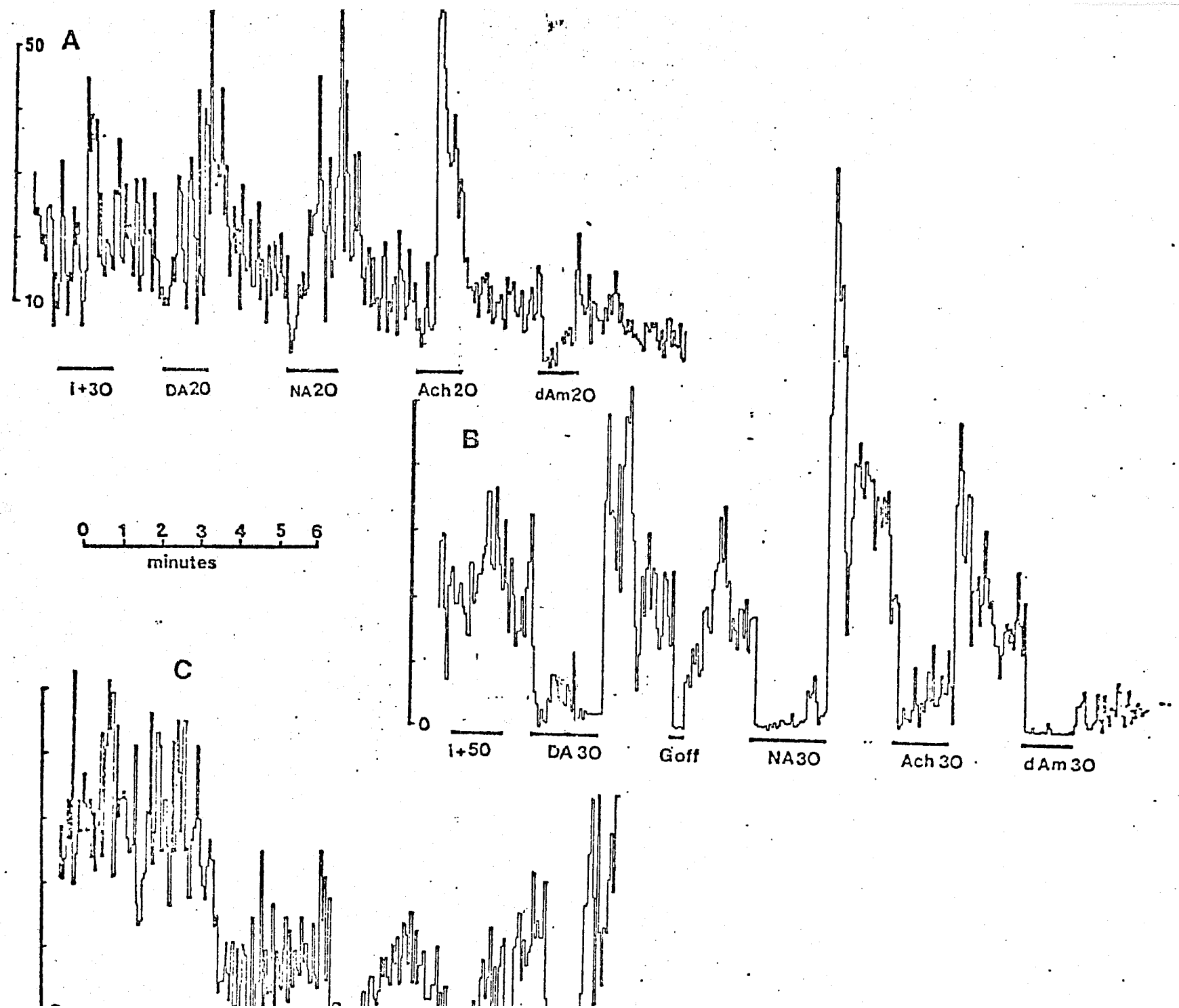
NOTE. The responses are tabulated in the same manner as Table 1 with the addition of an additional column, ± , to signify cells which gave biphasic or inconsistent responses to the drugs employed.

TABLE 3. Neuronal responses in animals anaethetised with Dial (35 mg/Kg)

		NA				dAMPH			ACh		
		+	o	-	NT	+	o	-	+	o	-
DA +	4	.	1	.	3	.	3	1	3	1	.
DA o	6	.	.	5	1	.	1	5	3	3	.
DA -	36	.	2	34	.	.	2	34	2	.	34
Total Cells	46										

FIGURE 1 Ratemeter records from three striatal neurones showing the nature of the observed responses to iontophoretically applied ACh, dAMPH NA and DA. Traces A and B were recorded from two different penthrane anaesthetised animals. The responses illustrayed in A show etrong excitatory responses to all but dAMPH, while cell B is strongly inhibited by all the substances applied. Cell C is from a Dial (35 mg/Kg) anaesthetised animal and is also depressed by all the substances applied to it.

The epoch time used in all traces was 3 seconds, and the vertical axis represents 50 counts per epoch. Current controls are shown as i+30 (or +50); G off signifies glutamate off. All currents are +ve and in nanoamperes (nA) Cell recording parameters - A; 3370 um (depth from the cortical surface) glutamate -7 nA, B; 3300 um, glutamate - 10nA, C 4280 um, glutamate - 10 nA. Unless indicated otherwise on the records the glutamate was applied for the entire duration of the record.


A
50
10
I+30
DA20
NA20
Ach20
dAm20
B
0
I+50
DA30
Goff
NA30
Ach30
dAm30
0 1 2 3 4 5 6
minutes
C

EFFECT OF SYSTEMICALLY ADMINISTERED SPIROPERIDOL (R 5147) ON THE RÈSPONSES OF RAT STRIATAL NEURONS TO IONTOPHORETICALLY APPLIED APOMORPHINE, DOPAMINE, ACETYLCHOLINE AND NORADRENALINE.

SUMMARY

Apomorphine, which is considered on the basis of behavioural studies to act as a dopamine agonist within the corpus striatum, was applied iontophoretically in conjunction with dopamine (DA), noradrenaline (NA) and acetylcholine (Ach) to rat striatal cells to seek possible confirmation of this agonist action at the cellular level.

Systemically administered spiroperidol, a putative butyrophenone dopamine antagonist (1 mg/Kg , a dose level adequate to produce catalepsy in rats) caused a highly significant increase in the number of cells responding with excitation to NA and DA. Spiroperidol also caused an increase in the number of cells unresponsive to apomorphine by reducing the proportion of cells depressed by it. Spiroperidol had little effect on the proportion of cells which were unresponsive to NA and DA, but increased the proportion of cells which did not respond to ACh without changing the proportion excited by ACh.

Apomorphine appears to show weak dopamine agonist actions in the striatum when applied iontophoretically, and while these effects are antagonised by spiroperidol they are probably mediated by other non-dopaminergic mechanisms since Ach effects were also antagonised.

INTRODUCTION

Evidence for the dopamine agonist actions of apomorphine has accumulated from a number of studies[1,8,9,10] Ernst has described structural similarities between apomorphine and other substances which behave as dopamine agonists, and dopamine, which may underlie their similarities of action[9]. In the guinea pig vas deferens apomorphine at low dosages[12] has been shown to be a competetive agonist of dopamine.

In the brain apomorphine , dopamine and other dopamine agonists such as d-amphetamine elicit similar responses when injected into the striatum [8,9,10,22,23]. Systemic injection of apomorphine in rats produces a stereotypy which appears to be identical to that produced by amphetamine[9,10], and it is thought that this stereotyped behaviour is mediated through basal ganglia dopaminergic pathways. Reserpine potentiates the responses to apomorphine suggesting an action on a dopaminergic system which has become supersensitised through chemical denervation.

Neuroleptic drugs of the butyrophenone family; haloperidol, spiroperidol (R 5147) and spiramide (R 5808) amongst others, are claimed to antagonise the effects of dopamine and presumed dopamine agonists such as apomorphine at central dopamine receptor sites[1,21], to a greater degree than the phenothiazine compounds such as chloropromazine. Chloropromazine exhibits a wide spectrum of effects such as adrenergic blockade and local anaesthetic actions. In the normal animal this presumed dopamine antagonism results in a catalepsy which appears analagous to the ataxia- akinesia of classical Parkinsonism which is associated with a loss of dopaminergic terminals in the striatum[2]. Haloperidol also blocks apomorphine induced depression of nigral (pars compacta) cell firing when both substances are administered intravenously[4] although this effect may not have been mediated through dopaminergic terminals.

Since it would be extremely convenient to have a specific dopamine agonist to aid in the identification of striatal dopaminergic neurones, it was decided to further investigate whether apomorphine has actions parallel to those of dopamine on striatal neurones when applied iontophoretically, and whether the effects obtained, both to dopamine and apomorphine could be specifically antagonised by spiroperidol.

Thus a study of the responses of striatal cells to iontophoretically applied dopamine, apomorphine, acetylcholine and noradrenaline (which served as a control catecholamine) was carried out and the modification of these responses by spiroperidol investigated.

METHODS

Sixteen hooded rats of both sexes were used for this study. Anaesthesia was induced with halothane and maintained on a penthrane - air mixture delivered from a laboratory constructed anaesthesia apparatus at approximately level 3 (light anaesthesia). Ten animals were used for the initial control series and six other animals were used for investigating the effects of spiroperidol.

The anaesthetic, surgical, iontophoretic and recording procedures have been described in paper 1 of this dissertation [19].

All the drugs employed for iontophoresis were made up in nitrogen gassed distilled water. Because of the very rapid rate of oxidation of apomorphine it was necessary to prepare the solution (60 mg/ml in N_2 gassed normal saline, pH 7) before filling the electrodes by centrifugation. To reduce the amount of adsorbed oxygen contained on the electrode glass especially at the tips where there is a high surface to volume ratio, the electrodes were stored in a nitrogen atmosphere before filling.

Other drugs used were dopamine HCl (DA) Sigma (0.2M, pH 3.5), noradrenaline HCl (NA) Sigma (0.2 M, pH 3.5), acetylcholine HCl (Ach) Sigma (0.2M pH 4.0) and l-glutamate, as the monosodium salt, Sigma (0.2M pH 7). The recording and current control barrels of the miniature 7 barrel microelectrode assembly[18] were filled with 2M NaCl. Ejection currents were usually +30 to +50 nA, retaining currents -15 to -18 nA.

Glutamate ions (-5 to -25 nA) were used to stimulate firing of striatal neurone which are considered to be ' silent' possibly due to a large inhibitory input, apparently intra-striatal in origin[15].

Current controls were performed by passing currents of +30 to +50 nA through the lateral NaCl barrel. Responses of cells which were responsive

either excited or depressed by the current control were rejected.

Recordings were made from the dorso-medial part of the striatum through a 5 mm diameter trephine hole at A 8.0 mm, L 2.5 mm[14].

To minimise the sources of variability in the release of the iontophoretically applied drugs[3], the substances were applied to each cell in the same serial order and for the same duration by means of an automatically timed 6-channel constant current source[18]. Following this the responses of a number of cells to parallel applications of apomorphine (APO) and DA, and APO and NA were recorded to determine whether there was any augmentation or occlusion of the responses.

Unfortunately it proved to be impossible to iontophoretically apply spiroperidol directly onto the cells as has been done with chloropromazine[25]. Spiroperidol is virtually insoluble in water and is sparingly soluble in solutions of low pH such as dilute citric, tartaric and lactic acids (pH 1.5 approx). Attempts at passing spiroperidol or passing current through the current control barrel containing the acid solvent, resulted in a profound excitation of the neurones, presumably due to H^+ ions. In addition the spiroperidol containing electrodes blocked rapidly because of spherical particles of the drug which came out of solution and blocked the tips.

Spiroperidol was therefore administered as a saturated solution in 2% lactic acid[7], given intraperitoneally at 1 mg/Kg about one half an hour prior to recording. Since the related butyrophenone haloperidol has a long duration of action[17], as it appears does spiroperidol, it was felt that at the dose level employed repeating the dose approximately every 4 hours should have maintained an adequate level.

Due to the difficulty of holding cells for sufficiently long periods

of time to perform studies of the responses of single striatal cells before and after spiroperidol injection. Thus the results reported stem from two populations of striatal cells those encountered in the absence and those in the presence of spiroperidol.

RESULTS

In the ten control animals, ACh, DA and NA all showed a preponderence of inhibitory effects on glutamate driven striatal cells (Table 1) a pattern which is consistent with that reported in previous studies[5,13,20,24] (Figure 1). The pattern of responses to apomorphine very closely resemble those of the catecholamines DA and NA, and the time course of the depression of cell firing was similar to that produced by DA and NA. ACh produced a greater proportion of excitatory responses (22/68 cells), than did APO (9/65 cells), DA (11/65 cells) or NA (7/56 cells), a finding also in agreement with other studies.

Spiroperidol produced a marked increase in the number of excitatory responses to DA and NA (Table 2), The difference between the nature of the control responses of each drug and to the responses following spiroperidol were found to be highly significant using a 4 x 4 Chi-square test ($p < 0.01$).

The action of APO appeared to be antagonised by spiroperidol, 32 of 62 cells tested in the spiroperidol treated animals being unresponsive to apomorphine in contrast to 15 of the 68 cells in the control series. The depressant effect of APO was to a large extent antagonised by spiroperidol (15/62 cells inhibited under spiroperidol compared with 44/68 in the controls).. There was little change in the proportion responding with excitation (11/62 under spiroperidol compared to 9/68 in the controls). The proportion of cells not responding to DA and ACh were also substantially increased, while the proportion of cells unresponsive to NA were essentially unchanged.

In 22 cells in which the effects of simultaneous application of APO and DA or NA were investigated there was no augmentation of the depression

of cell firing if already present, by simultaneous application of the two substances, and at most the effects were additive. However if a response to DA or APO was absent, or if the response was excitatory, simultaneous ejection of the two drugs was inhibitory. In no case was excitation or enhancement of excitation by APO observed and in only one cell was there apparent antagonism between APO and DA. In 5 cells in which simultaneous application of NA and APO was carried out, there was additive depression in only one cell. When applying APO and DA or NA simultaneously to a cell the ejection currents used for each substance were half that required for an unequivocal effect by that substance acting alone on the same cell.

There was also a reduction in the average amount of glutamate required to excite striatal cells; ($\bar{x}$ = -12.5 nA compared to $\bar{x}$ = -16.2 nA in the control series.).

The time course of the responses to apomorphine, both excitatory and depressant, were not significantly different from those obtained to DA, NA and ACh.

DISCUSSION

From this study the effects of apomorphine appear to closely parallel the effects of the iontophoretic application of DA and NA on striatal cells in being primarily depressant in nature. Other studies involving the iontophoretic application of DA and NA to striatal cells have led to the idea that DA exerts an inhibitory effect on striatal neurones, although excitatory responses have also been reported[5,13,20,24]. However intrastriatal injections of apomorphine mimic the effect of intrastriatal injections of dopamine [8], while intrastriatal injections of NA are without effect[6]. This suggests that the similarity of the responses observed in this study to DA, NA and APO are non-specific [13] and may not reflect the effect of synaptically released DA on striatal neurones.

Nevertheless in that apomorphine mimics the responses to DA in this study it may be said to be a dopamine agonist.

At dose levels comparable to that at which the neuroleptics spiramide and haloperidol, two butyrophenones very closely related to spiroperidol, would produce marked catalepsy in normal animals[7], spiroperidol appears to antagonise the responses to both APO and DA as reflected in the increase in the proportion of non responsive cells. It also alters the overall nature of the responses to DA and NA to excitation.

This shift in response sign may reflect an antagonism, by spiroperidol of some kind of inhibitory drive at the neuronal level, perhaps through effects on the nigro-striatal dopaminergic pathway. The slight reduction in the amount of glutamate drive required to depolarise the cells under spiroperidol tends to confirm this view. Since there was no change in the proportion of excitatory responses to ACh it would not appear that these changes are due to a direct action on the neuronal membrane.

It is also noteworthy that it was the inhibitory aspect of the responses to APO, DA and NA which were antagonised by spiroperidol (Tables 1 and 2). This agrees quite closely with the current views held on the mode of action of dopamine which is thought to depress striatal cell firing by a hyperpolarising action[11,14], as well as with the idea that spiroperidol and the other butyrophenones block this action. However the parallel effect of spiroperidol on ACh depression is difficult to explain under this scheme.

York[25] has demonstrated that the application of the phenothiazine neuroleptic chloropromazine iontophoretically to monkey striatal cells was able to antagonise the effects of similarly applied dopamine, an effect that appeared to be independent of its local anaesthetic actions. The specificity of this antagonism was not assessed, although it did not antagonise the effects of lDOPA.

From the results of this study APO does appear to have dopamine agonist actions in the striataum, the indeterminate nature of the responses obtained through the use of the iontophoretic application of this substance onto randomly encountered striatal cells does not allow any evaluation of the specificity of action to be made. Similarly despite the powerful behavioural effects of the neuroleptics such as spiroperidol it is not possible from this study to ascribe its actions to a specific antagonism of the synaptic actions of dopamine in the striatum.

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CONTROL		ACH			APO			NA			
		+	o	-	+	o	-	+	o	-	nt
DA +	11	7	1	3	4	3	4	7	2	2	
DA o	15	7	3	5	3	5	6		6	7	2
DA -	39	6	3	30		6	33		3	29	7
nt	3	2		1	1	1	1				3
Total	68	22	7	39	9	15	44	7	11	38	12

TABLE 1. Control responses of striatal neurons to iontophoretically applied acetylcholine(ACH), apomorphine (APO), and noradrenaline (NA) tabulated against the responses to dopamine (DA). Numbers indicate the numbers of cells responding with excitation (+), inhibition (-) or not responding (o). NT indicates cells on which that particular substance was not tested.

SPIROPERIDOL		ACH			APO			NA			
		+	o	-	+	o	-	+	o	-	nt
DA +	23	17	5	1	9	11	2	14	5	4	
DA o	22	8	11	3	2	18	2	4	9	9	
DA -	17	2	4	11		6	11	2	1	13	1
nt											
Total	62	27	20	15	11	35	15	20	15	26	1

TABLE 2 Responses of striatal neurons to the same substances as in Table 1, following intraperitoneal injection of spiroperidol (1 mg/Kg). The increase in the number of both non-responding cells and cells excited by DA, APO and NA is evident.

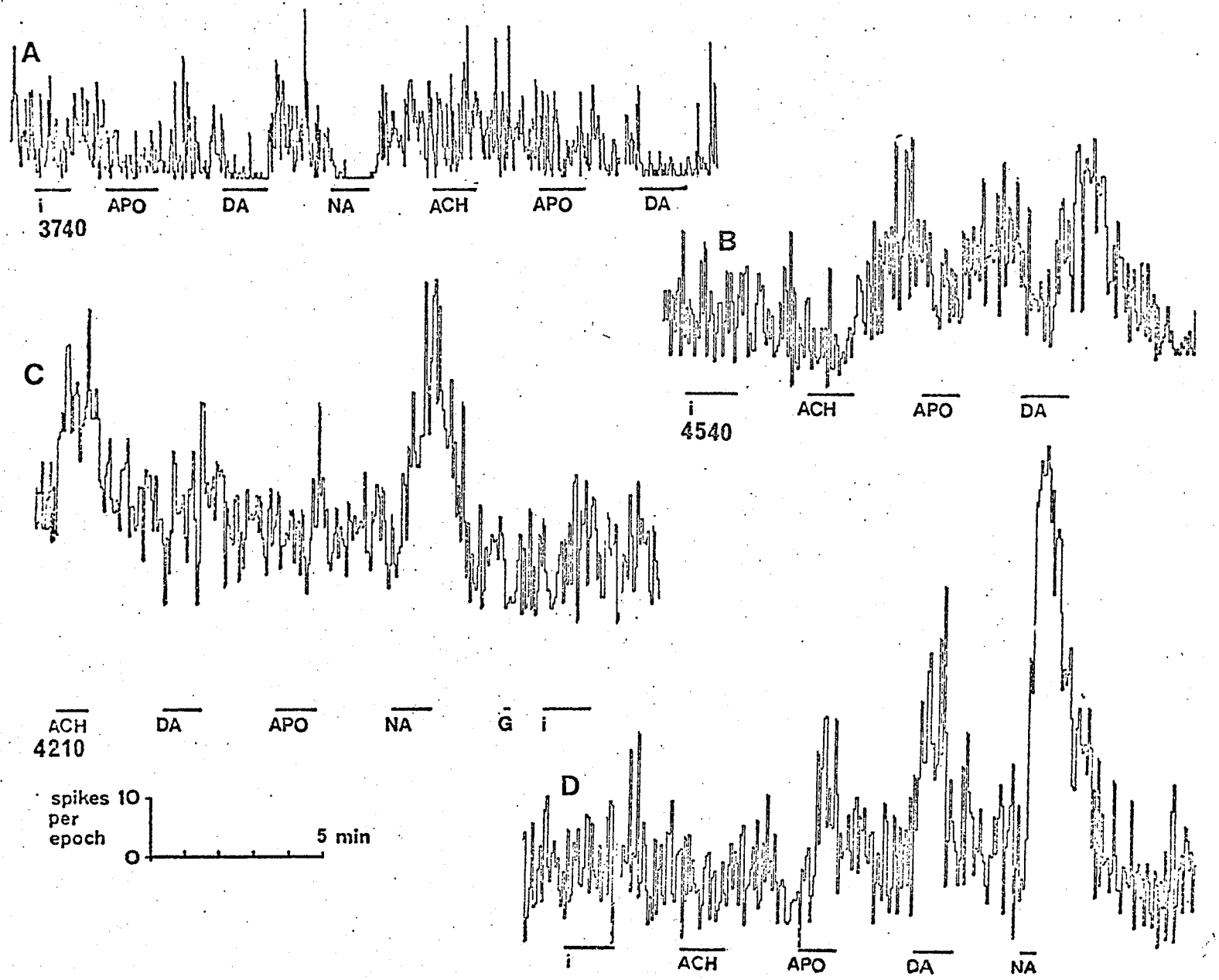
FIGURE 1

Ratemeter records of the responses of four glutamate driven rat striatal cells to iontophoretically applied apomorphine (APO), dopamine (DA), noradrenaline (NA) and acetylcholine (ACH). The same cationic ejection current,+30 nA, was used for all drugs and for the current controls (i). G indicates glutamate (-15 nA) off. Substances were applied for the duration indicated by the horizontal lines under the traces.

Apomorphine was weakly inhibitory on cell A; dopamine and noradrenaline was inhibitory, and acetylcholine was without evident effect. Cell B was considered to be inhibited by all substances, the slow increase in firing rate is characteristic of the changes in cell excitability seen under light penthrane anaesthesia[19].

C and D are records from cells in spiroperidol treated animals, and illustrate the excitatory responses to catecholamines described in the text. In cell D the application of noradrenaline was stopped to prevent the trace from going off-scale.

The numbers under each trace indicate the cell depth in microns below the cortical surface. Counting epochs were 1 second for trace A and 3 seconds for the remainder.


A
i
APO
DA
NA
ACH
APO
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3740
B
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ACH
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DA
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ACH
DA
APO
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G
i
4210
spikes per epoch
10
0
5 min
D
i
ACH
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DA
NA

RESPONSES OF CORTICALLY MODULATED , GLUTAMATE DRIVEN RAT STRIATAL CELLS TO IONTOPHORETIC APPLICATION OF ACETYLCHOLINE, DOPAMINE AND NORADRENALINE

SUMMARY

The nature of the responses of 107 glutamate driven rat striatal neurones to cortical stimulation (4 sites) was recorded. Dopamine (DA) noradrenaline (NA), and acetylcholine (ACh) were applied iontophoretically on to these neurones to examine whether there existed a relationship between the nature of the responses to cortical stimulation and the response to the putative transmitter substances.

Striatal cells responded to cortical stimulation with an initial excitation followed by inhibition of cell firing, or by a delayed inhibition as the dominant response in 65 cells, or by short latency inhibition in 25 cells. Delayed excitation which was independent of the initial response, appeared in 17 cells. The sequence and time course of these initial excitatory - inhibitory responses are in good agreement with previously published intracellular studies. This suggests that the technique of recording the modulation of amino acid induced firing by afferent pathway stimulation is a possible alternative to intracellular recording for investigating the nature of synaptic inputs.

ACh, DA, and NA were found to both excite and depress striatal neurone. firing. Neurones that were initially depressed by cortical stimulation had a greater probability of being depressed by the catecholamines NA and DA. No other relationship between cortical stimulation and drug responses was evident.

The role of either acetylcholine or dopamine in the striatum could not be determined from the results of this study and it is suggested that these substances might act as modulators of cellular activity rather than as classical synaptic transmitters.

INTRODUCTION

The intracellular responses of striatal neurones to cortical stimulation have been investigated by several workers[18,32], and the dominant responses have been described as an initial EPSP followed by a prolonged IPSP. However striatal cells appear to be under a high degree of tonic inhibition which is understood to be due to an intra-striatal inhibitory drive[27]. That striatal cells are seldom observed to fire spontaneously, in either the anaesthetised or awake animals, or to fire in response to afferent stimulation[8] is further evidence for this inhibition.

As a result of this inhibition, the investigator is usually obliged to ' drive ' the neurones by applying small amounts of excitatory amino acids such as glutamic acid, in order to determine the nature of the neuronal response to other iontophoretically applied substances.

Since stimulation of cortical afferent pathways is not usually adequate to fire most striatal cells , the use of glutamate ions to depolarise the cells sufficiently to cause them to fire in response to normally sub-threshold depolarisations could permit observation of synaptically mediated changes in neuronal excitability. Modulation of the amino acid induced 'spontaneous' firing, as determined from post stimulus time interval histograms (PSTH) could therefore be expected to parallel closely the sign and time course of the afferent synaptic input and would appear to be a possible alternative to intracellular recording.

The striatum (caudate nucleus - putamen) recieves a topographically distributed projection from the ipsilateral cortex [9,10,20,21,22,23,39,40], and this projection appears to terminate on striatal interneurones; the pattern of termination showing a high degree of convergence from

other cortical areas[22,23].

One of the prominant neurochemical features of the striatal region is the high concentration of dopamine which is present in fine nerve terminals arising from dopamine containing cell bodies in the substantia nigra pars compacta[1]. This has been demonstrated by fluorescence histochemistry[1,2,17,38], and by other techniques. The striatum also has an extremely high concentration of acetylcholine esterase[7,32], and choline acetylase[31], which are assumed to be related to the presence of high levels of Acetylcholine. It would appear that this acetylcholine is intrinsic to the striatum and is not derived from afferent terminals, although thalamic stimulation has been reported to increase the release of striatal acetylcholine[29]. It appears also that the acetylcholine-esterase arises from the cell bodies of the output neurones, and this localisation can be demonstrated histologically (Deadwyler pers.com.).

Acetylcholine and dopamine have been reported to have both excitatory and inhibitory effects on striatal neurones[5,11,15,16, 30,37,41]. However the frequency of encountering cells which respond to these substances with excitation is reduced by the use of anaesthetic agents such as barbiturates and also by the depth of anaesthesia; the excitatory response to acetylcholine being the most affected[5,37]. Connor[11] has reported that cat striatal cells depressed by nigral stimulation had a high probability of being depressed by iontophoretically applied dopamine.

The EPSP-IPSP response to cortical stimulation has been reported to be a consistent response of all the striatal neurones studied by previous investigators and it is possible that either dopamine or acetylcholine may mediate one or other phase of this response directly or through intrinsic intra-striatal pathways[14,42]. Unfortunately as has been pointed out by

Marco et al[27], the paucity of efferents and small calibre of both the efferent and intrinsic fibres makes it virtually impossible to identify most of the cells encountered as interneurones or output cells by classical methods of antidromic invasion. Thus the nature of the cells investigated must of necessity be assessed on the basis of their responses to afferent stimulation.

The object of this study was to see, using the most favourable anaesthetic conditions possible, if there is any relationship between the responses to cortical stimulation of glutamate driven striatal cells and the responses of the same cells to iontophoretically applied dopamine, noradrenaline and acetylcholine.

METHODS

Twenty seven hooded rats (250 - 400 g) of both sexes, were used in this study. The rats were anaesthetised with a penthrane-air mixture following halothane induction, and were naintained at approximately level 3 anaesthesia[37]. The procedures for anaesthetising, surgery and recording have already been described in detail previously. Briefly, the animal's skull was exposed and a trephine hole drilled at each of the stimulating sites (Fig 1) to permit insertion of the stimulating electrodes. A 2mm diameter trephine hole was drilled over the recording site, and the dura opened to expose the cortex. The overlying cortex was not removed.

Cells were recorded from the medio-dorsal part of the ipsilateral striatum (A 8.0, L 2,5mm, coordinates from Konig and Klippel [24]) at depths from 2.0 mm below the cortical surface to 6 mm below the cortical surface.

Stimulation sites were located at 1; A 12 - 12.5, L 1 - 1.5 mm, 2; A 10, L 2.0 mm, 3; A 4.5 - 5.5, L 2 - 2.5 mm, 4; A 0.5, L 2.5 mm. (Figure 1). Stimulating electrodes were bipolar (0.5 mm separation) stainless steel wires inserted into trephine holes in the skull and held in place with low melting point wax. The electrodes penetrated the dura for approximately 0.5 mm. The stimulating electrodes were connected to RF isolated constant current stimulators, which were in turn connected to a laboratory constructed stimulus driver slaved to a Fabritek 1024 averaging computer.

Stimuli were bursts of 3 pulses (0.3 ms duration, 4mA current) at a pulse repetition rate of 700 impulses per second. Each burst was delivered after a 100 ms delay following the initiation of the aquisition sweep of the Fabritek computer which was time locked to the stimulator. Each of the four sites was stimulated 1.28 seconds after the preceeding site, with a 5 second delay between stimulation of site 4 and the commencement of the next

stimulus cycle. Unfortunately it was not possible to randomise the stimulating sequence but in most cases the cell had returned to control firing level by the end of the sweep period.

The Fabritek averager was used to compute 4 serial post stimulus time interval histograms (PSTH), each having a 1.28 second sweep duration (each PSTH consisting of 256 bins of 5 ms duration each). each representing one of the four cortical sites. The number of stimulus cycles required to produce an adequate PSTH ranged from 64 to 256 depending on the responsiveness of the cell and whether it tended to become depressed by extended stimulation.

Virtually all the cells required some level of glutamate drive to increase the firing rate from less than 1 to 3 to 10 spikes per second to enable ratemeter records of the responses of the neurones to iontophoretically applied substances to be more readily recorded, and to permit the modulation of striatal cell firing by cortical stimulation to be recorded.

Drugs used were dopamine HCl (DA) Sigma (0.2M, pH 4), noradrenaline HCl (NA), Sigma (0.2M, pH 3.5), acetylcholine HCl (ACh) Sigma (0.2M pH 3.5) and glutamic acid as the monosodium salt, Sigma (0.2M, pH 7). The recording and current control barrels were filled with 2M NaCl. Seven barrel micropipettes with 5 - 7 um diameter tips constructed as previously described[36], were filled with the drug solutions by centrifugation immediately before use.

Because of the demonstrated time dependency of drug release from microelectrode barrels[6], the sequence of drug ejection was automatically controlled [35] so that the drugs were ejected in a consistent fashion on to the unit under investigation. At least two complete cycles of drug ejection, with fixed 80 second intervals between sucessive drugs, were applied to each cell.

Intrastriatal injections of noradrenaline do not mimic the effects of similarly applied dopamine in cats and rats[12,13], yet striatal cells have been shown to be depressed by iontophoretically applied dopamine and nor - adrenaline[5,37]. Nor-adrenaline was thus employed as a 'control' catecholamine in this study since its concentration in the striatum is less than one twentieth that of dopamine (Havlicek unpublished observations). Since no physiological role in the striatum has been ascribed to noradrenaline and since intrastriatal injection of NA was without effect, it was used to evaluate the specificity of the responses obtained to the iontophoretic application of dopamine.

RESULTS

Responses to cortical stimulation

The dominant response of both the glutamate driven and spontaneously firing striatal cells to cortical stimulation of the four sites was a depression of cell firing (42/107 cells - Table 1) or initial depression followed by excitation in 17 of 42 cells. Excitation was recorded if there was at least a two fold increase over the control firing level, depression if the level was less than half the control level. A substantial number of cells however did respond with an initial excitation (24/107) , or with delayed inhibition (41/107), (Figure 2, table 1).

The spikes recorded from striatal cells in response to cortical stimulation appear in conjunction with a 2 to 5 mV negative field potential (Fig 3), having a latency of 7 to 10 mS and a duration of 20 to 40 mS. The rising phase of this field potential (20 to 50 times the amplitude of the superimposed spikes) often resulted in a short latency artefact caused by the band pass filter amplifier. This artefact resulted in spurious short latency window discriminator pulses which appeared in the PSTH's as short latency responses that obscured any genuine responses. Thus any initial responses having a latency or duration of less than 15 ms were considered equivocal and were not recorded.

The initial excitation generally had a duration of 20 to 80 mS ($\bar{x}$ = 28 ms), while the initial inhibitory period had a duration of 160 - 250 ms. The subsequent late excitation was characterised by a latency of 75 to 400 ms ($\bar{x}$ 235 ms) and a duration of 30 to 400 ms ($\bar{x}$ = 218 ms) There was little difference in the latencies of the various components of the response between the four stimulation sites, with the exception of the average initial latency, L_1 , for site 4, which was twice that of the other sites (Table 2).

The majority of cells tested appeared to respond to at least 2 inputs. The paucity of responses recorded from site 2 resulted from a faulty stimulating electrode which was later corrected.

In only four of the cells which showed initial bursting behaviour was there any synchronisation of this bursting by the stimulus, (top trace Fig. 5).

Effect of Iontophoretically Applied DA,NA and ACh.

In keeping with previously published reports ACh,NA and DA both excited and depressed glutamate driven striatal cells (Fig 4). Of the 107 cells which responded to both cortical stimulation and to applied substances (Table 2), the responses were essentially the same as reported by previous workers [5,37,41]. Neurones which were excited by DA (30/107) also showed an increased probability of being excited by NA, the 'control' catecholamine (20/30) and by ACh (16/30).

Similarly cells depressed by DA (52/107) were also more likely to be depressed by NA (38/52) and by ACh (33/52) In fact the responses to NA and to ACh are extremely similar.

Correlation between Responses to Cortical Stimulation and to DA, NA and ACh.

The predominant response of striatal cells to cortical stimulation was assessed from the PSTH records (Figs 2, 5.) and the responses of these cells was classified into 4 categories : initial inhibition (-), inhibition followed by excitation (-+), delayed inhibition (del -) and excitation followed by inhibition (+-). A response was tabled under these categories if the inhibitory and or excitatory change was greater than 30% of the firing

level during the initial 100 ms control period preceeding stimulation(see illustration Table 2).

Cell responses to cortical stimulation were initially tabulated under the 4 categories described above, and these responses further tabled against the responses to the iontophoretically applied drugs (Table 3). As can be seen from this table that cells inhibited by cortical stimulation (-) were more likely to be depressed by catecholamines, while cells giving a biphasic response (-+), were more likely to be depressed by ACh.

Evoked Responses

Evoked responses were initially recorded in conjunction with the PSTH's and evoked potentials were recorded from 24 cells (Figure 5 lower trace of each pair of traces). The responses were essentially similar to those described by Liles[26], in that they are multiphasic, having an initial negativity of short duration followed by a positivity having a duration of approximately 70 to 200 mS. In some units, not necessarily bursters this was followed by an oscillatory response. It is also evident that there is a strong correlation between the initial negative component of the evoked potential and the excitatory responses as indicated by the PSTH and also between the evoked potential positivity and the period of inhibition.

DISCUSSION

There was a very close correspondence between the time course of the initial excitatory phase of the response to cortical stimulation; the period of inhibition and the long latency excitation as observed from the PSTH records obtained in this study, and the striatal responses described by previous workers[8,18,26] from both extracellular and intracellular studies. This correspondence indicates that the technique of observing the modulation of glutamate induced cell firing could be an exceedingly useful tool for investigating the effects of the afferent input on neurones which are 'silent' such as those in the striatum.

There appeared to be no significant differences between the responses to the four cortical stimulating sites. The presence of a high degree of convergence of cortical inputs on the striatal cells observed in this study presumably reflects the more primitive nature of this region in rats, in essential agreement with the findings of Webster[39]. In the cat, as might be expected on anatomical grounds[40], there appears to be less convergence and evoked potential studies appear to support this [26]. Due to the PSTH time resolution used (5 ms per bin) and because of the presence of the artefact referred to previously, it was impossible to analyse the latencies of the initial excitatory responses to cortical stimulation.

From anatomical, histochemical and pharmacological studies[1,2,3,38,41], there has been established the presence of an extensive, diffuse dopaminergic nigro-striatal projection. In comparison with other pathways the diffuse nature of this projection is extraordinary; each fibre appearing to terminate in about 5×10^5 terminals[5]. While the degree of branching of these fibres is unknown, it would appear that each cell body in the pars compacta of the substantia nigra probably gives rise to a large number of collaterals[17].

It appears highly likely that such a fine calibre projection might be better suited to the release of substances which have a controlling effect on the excitability of a large population of cells, since the high degree of branching would not appear to be suitable for the discrete transmission of information. Connor has reported [11], that 27% of cat striatal cells were inhibited by nigral stimulation after a latency of approximately 15 ms. These cells were preferntially depressed by dopamine. Feltz[14] however has demonstrated the presence of a non dopaminergic nigro-striatal pathway which has an excitatory drive on striatal cells and which is not affected by destruction of the dopaminergic pathway by 6-hydroxydopamine. The inhibition described by Connor may also have been mediated by intra striatal neuronal pathways , as these appear to have an inhibitory effect on striatal cells.

It is generally accepted on the basis of other iontophoretic studies[11,16, 37,41], pathological evidence, and by analogy with the responses to dopamine in other systems, that dopamine plays an inhibitory role in the striatum. If this is so one would be led to expect that since the striatum has a relatively uniform structure histologically[20], the dominant response of striatal neurones to iontophoretically released dopamine would be depression of cell firing. Marco et al [27] recording intracellularly have observed that the IPSP recorded following intrastriatal stimulation (1.5 mm distant from the recording site) often blocked the EPSP resulting from the same stimulus, although in most instances the response observed was an EPSP (10 ms latency). followed by a prolonged IPSP (12 - 22 ms latency). The latencies strongly suggest that both components were polysynaptically mediated.

No catecholamine containing neurones have been demonstrated in the striatum, only terminals. Thus it would appear unlikely that a polysynaptically

mediated release of dopamine is responsible for the IPSPs observed by Marco et al. or the depression demonstrated in this study. Why those striatal cells which were inhibited by cortical stimulation should also be depressed preferentially by catecholamines remains a mystery.

While the latencies reported by Marco et al do not exclude the possibility of the involvement of the striato -nigral - striatal pathway (12 to 22 ms for a distance of 1.5 mm between the recording and stimulating electrodes), it would seem unlikely that this loop is involved in the present study. Two reasons can be given to support this, the paucity of output neurones in the striatum (2 to 3% of the total[20,21]) and the paucity of excitatory responses observed to cortical stimulation.

The increase in the probability of ACh depressing cells which showed a biphasic response to cortical stimulation (-+ Table 3), may point to the involvement of interneuronally released ACh, but the sample of 15 cells in this category is too small to allow any definite conclusions to be drawn.

It is evident that iontophoretically applied dopamine and acetylcholine exert very much the same spectrum of effects on striatal neurones (Table 1) and these effects appear to be to a certain degree independent of the nature of the response to cortical stimulation (Table 3).

This suggests that either the original assumption of a purely inhibitory role for striatal dopamine is an oversimplification or that iontophoretic application of dopamine in the striatum does not mimic its physiological actions. The evidence from this study that DA and NA both appear to evoke the same spectrum of responses from glutamate driven cells is disturbing in the light of Cools' finding that intrastriatal injections of NA or saline were without behavioural effect, while injections of DA produced contraversive

turning[12]. This suggests that the motor effect of dopamine may not be mediated directly through striatal synaptic mechanisms. Herz and Zieglgänsberger conclude from their study[16] that the depression of striatal cell firing by iontophoretically applied biogenic amines is 'non specific' in nature. They suggest that the "specificity" of these substances may be more dependent on their characteristic distribution in the brain than of different basic effects'. While this suggestion is not supported by Cools' findings, the results of the present study support it.

While high levels of acetylcholine esterase[7,32] and choline acetylase[28] have been demonstrated in the striatum, the nature of the role of ACh in this area is equally obscure. Recently it has been demonstrated that the acetylcholine-esterase activity in the striatum originates from about 2% of the total cell population, which appear to be output neurones. These cells synthesize prodigious amounts of acetylcholine esterase which can be visualised following DFP and cyclohexamide treatment of the animal (Deadwyler pers. comm.). This finding only serves to complicate the problem.

Substances such as atropine which appear to act via a muscarinic cholinergic mechanism have marked anti-Parkinsonism effects without having any effect on dopamine reuptake[34]. The lability of the observed striatal cell excitatory responses to iontophoretically applied ACh and their tendency to convert to inhibitory responses in the presence of even sub anaesthetic doses of barbiturates[5,37]; as well as the similarity of the effects of ACh observed in this study to those produced by DA and NA do not allow any conclusion to be made about the role of striatal ACh to be made from the data presented here.

Evidence from other physiological and clinical studies have suggested that striatal DA may play a much more subtle role as a ' modulator ' of

other presumed striatal neurotransmitters; ACh, GABA and 5 hydroxytryptamine[4]. The term modulator can be taken to include 'non synaptic' effects on either single neurons or on entire populations , which could be a hormone like action on cell metabolism, or it could be manifested as a general change in neuronal excitability mediated through the release of another substance. Alternatively it could act on the ionic enviroment, perhaps through an action on glial cells[25]. Such effects tend to be very difficult to demonstrate by conventional neuropharmacological techniques.

Hull et al[19] have also suggested this modulator role on the basis of the finding that there was no change in the average firing rate of striatal cells following ipsilateral interruption of the dopaminergic nigro striatal pathway , although the lesion resulted in a diminuition of the DA concentration of that side.

Since the responses reported from this study agree well with previous findings, it would appear unlikely that the use of glutamate ions to raise the excitability of the striatal neurones in this study is in any way responsible for the nature of the responses observed.

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		NA				ACh			
		+	o	-	NT	+	o	-	NT
DA +	30	20	33	7	.	16	5	8	1
DA o	23	4	7	12	.	5	10	8	.
DA -	52	6	4	38	4	7	11	33	1
NT	2	.	.	.	2	2	.	.	.
	107	30	14	57	6	30	26	49	2

TABLE 1.

Tabulation of the responses of cortically modulated striatal cells to noradrenaline (NA) and acetylcholine (ACh) against the responses to dopamine (DA). No account has been taken of the nature of the response of these neurones to cortical stimulation. The tendency for DA stimulated cells to be stimulated by NA and ACh is evident, as is the converse, the tendency for DA depressed neurones to be depressed by NA and ACh. NT = not tested.

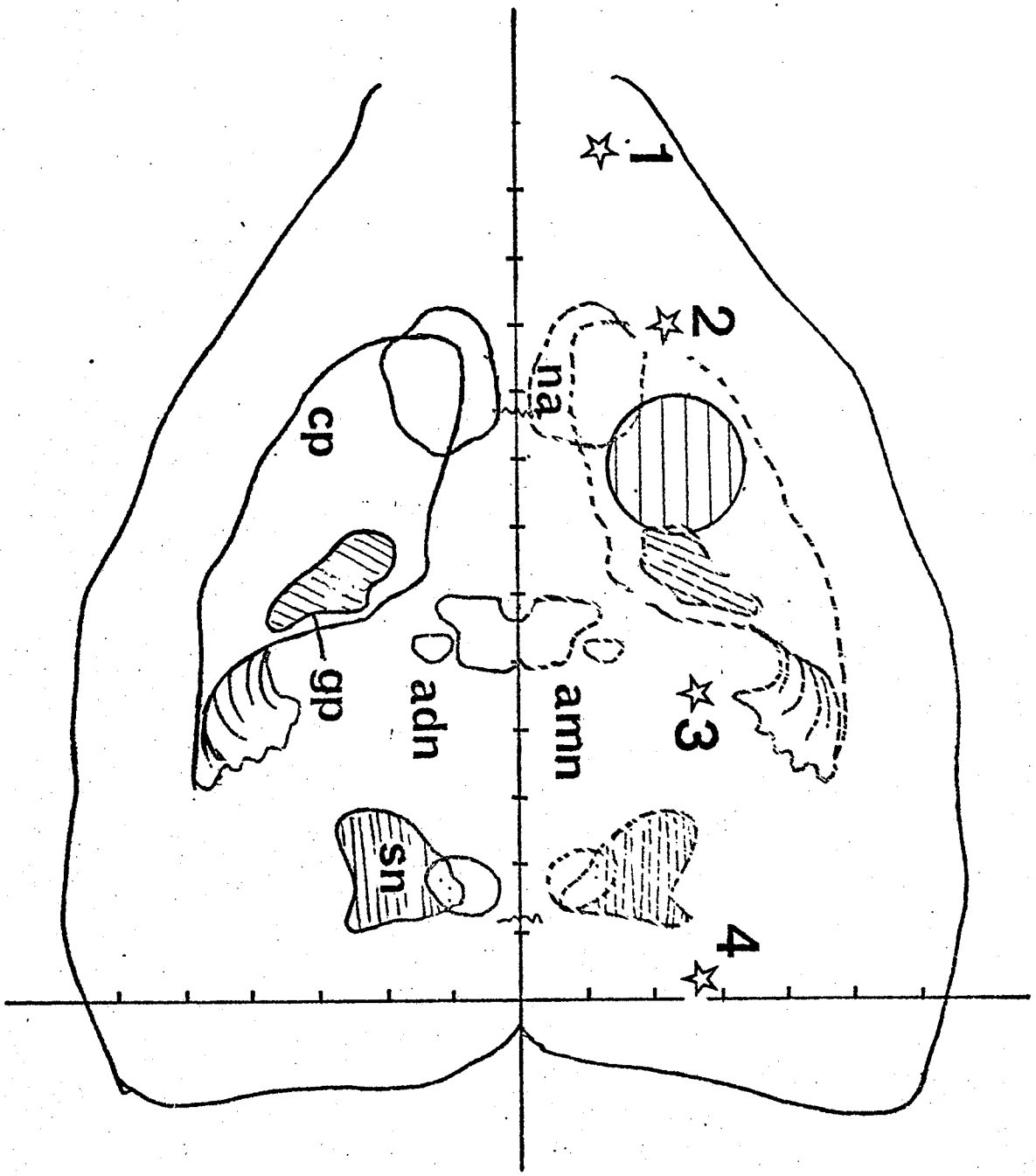


FIGURE 1

Diagrammatic dorsal view of the rat brain indicating the position of the various extrapyramidal structures, the location of the cortical stimulating sites (stars) and the recording site (shaded circle). The axes are those used in Konig and Klippel's atlas[24], and are in millimteres. Abbreviations used; cp - caudate-putamen (corpus striatum); na - nucleus accumbens; gp - globus pallidus; sn - substantia nigra; amn - nucleus antero-medialis (thalamus); adn - nucleus antero-dorsalis (thalamus).

Site	e_1 initial excitation	L_1 initial latency	i initial inhibition	L_2 latency of excitation	e_2 delayed excitation
1	24 ms	28 ms	223 ms	261 ms	233 ms
2	35 ms	21 ms	163 ms	183 ms	248 ms
3	23 ms	23 ms	248 ms	278 ms	210 ms
4	30 ms	48 ms	201 ms	218 ms	184 ms

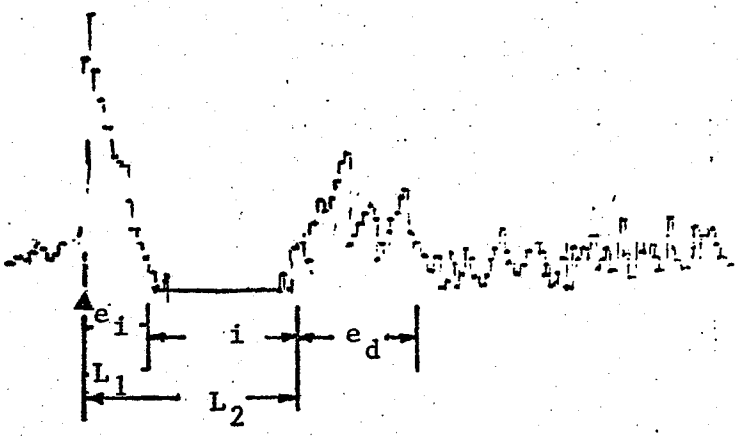


TABLE 2

Mean latencies of the components of the striatal cell response to cortical stimulation. The diagram illustrates the manner in which the time periods listed in the table are measured. As can be seen the periods of initial excitation (e_1) and initial latency (L_1) are measured in the same manner except that the initial latency period includes both excitatory and non-excitatory delays. All latencies are measured from the start of the stimulus (arrow).

FIGURE 2.

Six typical post stimulus time interval histograms from glutamate driven striatal cells to illustrate the normal range of response patterns encountered following cortical stimulation of the four stimulation sites.

Four classes of responses are illustrated here; initial excitation followed by inhibition (+-) 3180 (=depth of the cell below the cortical surface in um), sites 1 and 3; excitation alone (+) 3780,site 4.; delayed inhibition (del -) , 4080,site 1; inhibition alone (-) 3780, site 3. As can be seen most of the units show an inhibitory - excitatory sequence (2750, sites 3,4; 3510, sites 3,4.). Onset of stimulation is indicated by the arrow heads. Each histogram represents the summation of 64 responses. Stimuli were bursts of 3 pulses, 0.3 mS duration, 4mA intensity, burst rate of 700 / sec. Each histogram represents 1.28 seconds.

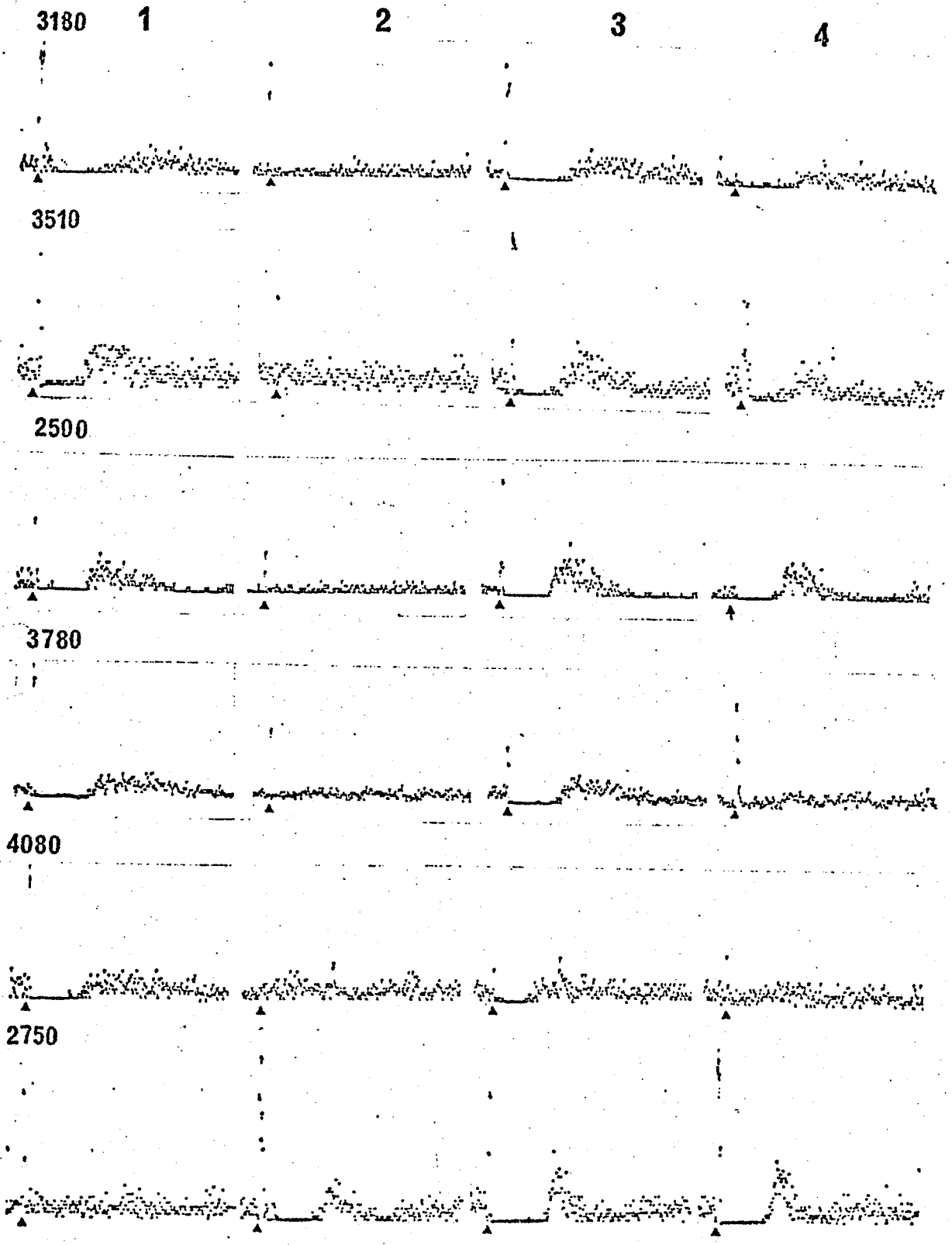

3180
1
2
3
4
3510
2500
3780
4080
2750

FIGURE 3.

Striatal field potentials associated with the excitatory response to single shock cortical stimulation.

The short latency negative (upward deflection) response can be seen to have 100 to 200 uV spikes riding on the peak of the negativity (8 - 12 ms latency). Six responses are illustrated here. Graticule calibration: vertical; 1 mV/cm, Horizontal 5ms/cm. The recording amplifier was DC coupled and stimuli were single 0.3 ms 4 mA pulses.

Cortical Response	n.	DA +	DA o	DA −	DA nt	NA +	NA o	NA −	NA nt	ACh +	ACh o	ACh −	ACh nt
−	25	7	3	15	.	5	2	18	.	11	3	11	.
−+	17	6	2	9	.	6	.	11	.	1	7	9	.
del−	41	11	9	20	1	13	7	18	3	11	10	20	.
+−	24	6	9	8	1	6	5	10	3	7	6	9	1
Total	107	30	23	52	2	30	14	57	6	30	26	49	1

TABLE 3

Drug responses tabulated against the responses of the striatal cells to cortical stimulation (see Fig. 2). It is evident that the only significant relationship between the responses to cortical stimulation and the responses to iontophoretically applied DA, NA and ACh is that units inhibited (-) by cortical stimulation are more likely to be depressed by DA and NA. Cells having a biphasic response to stimulation (−+) tended to be more likely to be depressed by ACh. nt = not tested.

FIGURE 4

Ratemeter records from two typical neurons which are respons ive to cortical stimulation; both cells were driven by -15nA of glutamate. Cell A had a rapid firing rate, each counting epoch being 1 second. It can be seen that this cell was excited by DA, NA and ACh, and was unresponsive to the 50nA current control (i50). Cell B had a slower firing rate (3 second epochs) and was inhibited by NA and DA, but excited by ACh. The rapid cessation of cell firing following stopping glutamate ejection (G), illustrates the sensitivity of this cell to glutamate. Vertical calibration 20 counts per epoch.

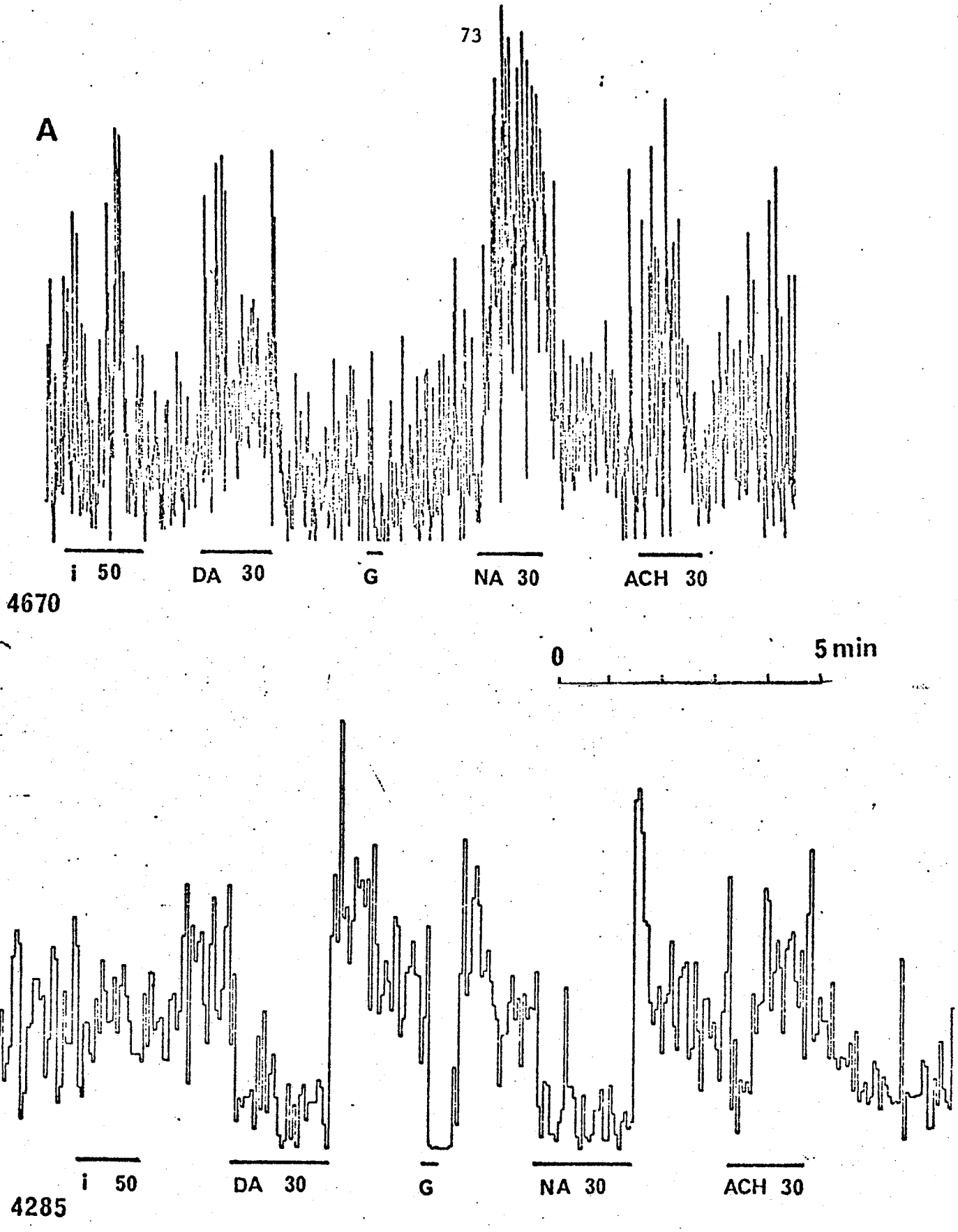
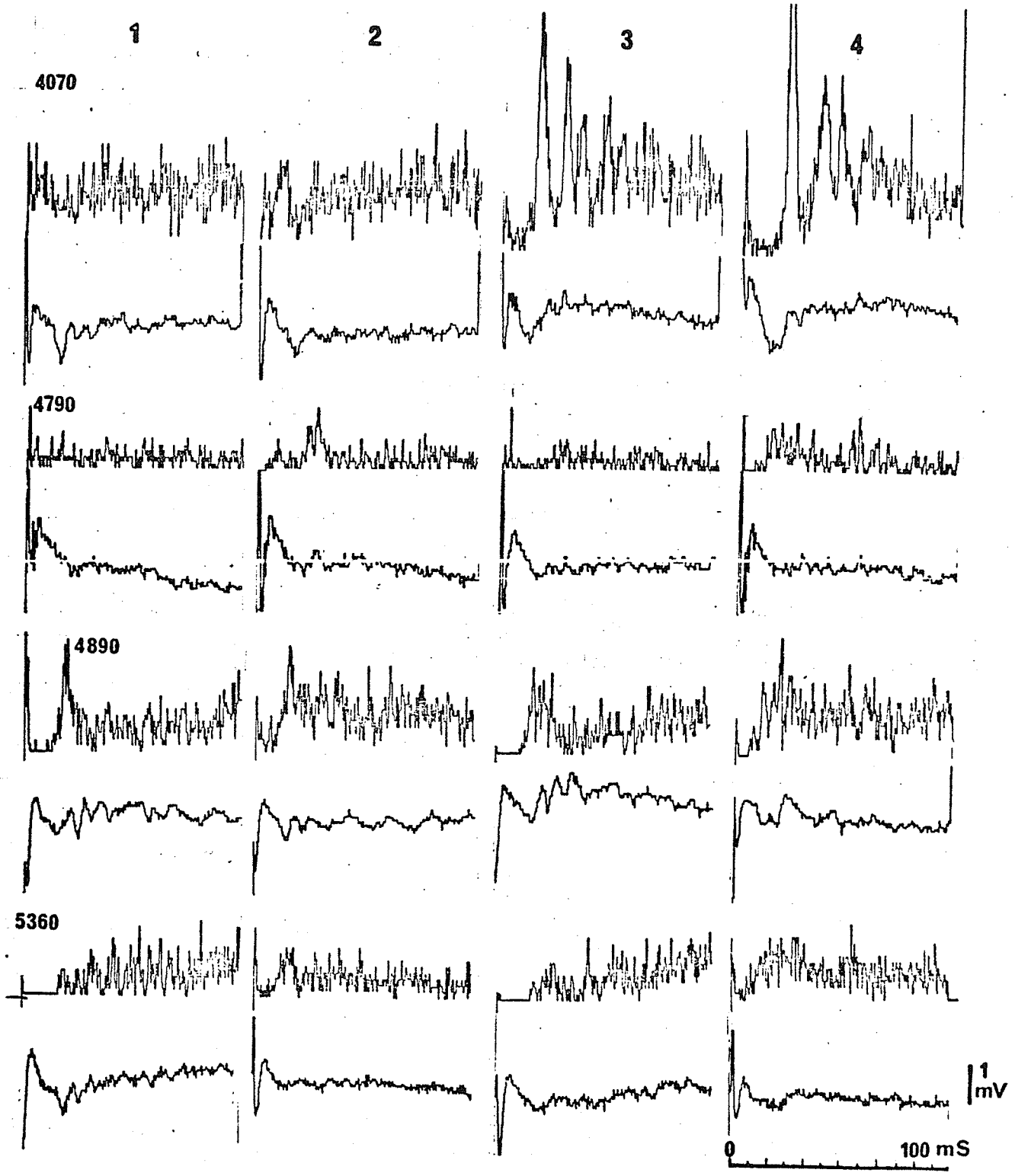

A
i 50
DA 30
G
NA 30
ACH 30
4670
0
5 min
B
i 50
DA 30
G
NA 30
ACH 30
4285

FIGURE 5

Comparison between PSTH and evoked potentials of glutamate driven striatal neurons to stimulation of four cortical sites. Both the PSTH (upper trace of each horizontal block of four traces), and the evoked potential (lower trace) have the same time scale and have been recorded from the same electrode. The evoked potentials were DC coupled into the averaging computer.

It can be seen that in most cells the stimulus is followed by an initial negativity (downward deflexion) which correlates with an initial excitation as indicated by the PSTH. The subsequent positive potentials appear to correlate with an inhibition of cell firing. The difference in appearance between these PSTHs and those illustrated in figure 2 stems from the use of a different recorder to plot the averager output.

The fine deviations on the evoked potential traces do not represent nerve spikes, and each is the summation of 32 sweeps.


1
2
3
4
4070
4790
4890
5360
1 mV
0
100 mS

ANTAGONISM OF CORTICAL EXCITATION OF STRIATAL NEURONS BY GLUTAMIC ACID DI-ETHYL ESTER: EVIDENCE FOR GLUTAMIC ACID AS AN EXCITATORY TRANSMITTER IN THE RAT STRIATUM.

SUMMARY

Rat striatal cells that were excited by cortical stimulation were found to respond to cortical stimulation with an average latency of 12 ms and each response contained a variable number of spikes. The stimulus-response ration was generally less than 1:1

Iontophoretic application of glutamic acid di-ethyl-ester (GDEE) a substance reported to be a glutamate antagonist, at currents of +50 to +125 nA, in the vicinity of neurones excited by cortical stimulation ,almost totally supressed the excitation in 90% of the cells, and this suppression was fully reversible. All cells were excited by glutamate. GDEE also suppressed neuronal excitation produced by aspartate and d-l homocysteic acid.

Bicuculline methyl iodide (BMI) ejected iontophoretically near the cells at dose levels sufficient to block GABA induced depression of cell firing,(+ 100 nA) , caused a marked increase in neuronal excitability ; an increase in the number of spikes generated in response to cortical stimuli, and an increase in both spontaneous and glutamate induced firing. BMI did not affect the duration or the intensity of the long latency inhibition which followed cortical stimulation .

It is concluded from this study that an excitatory amino acid, either aspartic or glutamic may function as the transmitter in the corticostriate projection, and that GABA may function as a tonic inhibitory transmitter in the striatum.

INTRODUCTION

The presence of a corticostriate projection of considerable size, described in various animals[4,5,15,36], has aroused much attention, and has been subjected to electrophysiological analysis by a number of workers[1,12,22 23,29]. One dominant characteristic of this cortico-striate input is that it appears to be excitatory, yet striatal cells which respond to cortical stimulation do not usually do so with spikes but with a complex EPSP - IPSP sequence as recorded intracellularly[1,10] and as determined from evoked potential studies[22].

From anatomical data it appears that the majority of striatal cells are interneurones[14,16] whose axons are short and appear to terminate intrastriatally. These interneurones , which have extensive dendritic fields (approximately 500 um in diameter), are reported to recieve intrastriatal afferents on all segnments of the dendrites. Extra striatal afferents, which appear to be primarily cortical, thalamic and nigral in origin mostly terminate on the more proximal segments of the interneurones[17,19]. This differential distribution of the afferent terminals is associated with a differentiation in the nature of the synaptic boutons between those found on the proximal and distal dendritic segments.

Hull and Buchwald[1,12] have described the nature of the synaptic response to afferents arising externally to the striatum as being excitatory, while intrastriatal afferents appear to be predominately inhibitory[18,19,20,24]. Since the latter predominate[19] striatal interneurones, in the absence of any external excitatory drive, can be considered to be in a state - of tonic inhibition. This explains the low probability of encountering spontaneously firing cells in the striatum , a phenomenum reported by numerous workers. However despite this inhibitory input, some striatal

cells will respond to cortical stimulation with spike discharges. Liles[23] has reported units in the cat striatum which could follow cortical stimulation rates of over 100 per second, while maintaining a 1:1 stimulus-spike relationship and appeared to be orthodromically driven. It is assumed that this corticostriate projection is monosynaptic but the axons appear to be very fine diameter on the basis of anatomical evidence[4,5,14,16,18]. Since there are no reports of short latency cortical responses following striatal stimulation and as the fibres are very fine diameter, the likelihood of antidromically driving the corticostriate pathway to confirm this appears to be remote.

Of the putative transmitter substances which have been described as occurring in the striatum , dopamine appears to be predominately inhibitory [2,10,34]; dopamine induced excitations are generally slow in onset and decay, are not very consistent and are influenced by the nature and level of anaesthesia employed[2,34]. Similarly acetylcholine, which appears to be present in the striatum in high concentrations[3,28], exerts both inhibitory and excitatory effects on striatal cells following iontophoretic application[2,10,25,34] and is even more sensitive to the anaesthetic agent used. Sub anaesthetic doses of barbiturates block the ACh excitatory responses[2,34].

Glutamic acid, a potent neuronal excitant , is present inhigh concentrations in the striatum, 10 to 12 uM/g[13], a level in concentration in the CNS second only that found in the cortical ectosylvian gyrus 12.4 uM/g.

One well described characteristic of the response to iontophoretically applied glutamate, or of related aspartic acid , is the rapidity of the onset of excitation and the rapidity of cessation of its effects once the the glutamate ejecting current is turned off. In addition the consistency

with which these excitatory effects can be elicited, regardless of the anaesthetic conditions, is another characteristic of the responses to excitatory amino acids.

Besides the ubiquity of its occurrence, which appears to dampen the enthusiasm of many investigators for considering glutamate as a transmitter candidate, one main problem in establishing identity of action between glutamate and the unknown CNS excitatory transmitter has been the lack of an effective antagonist. L glutamic acid di-ethyl ester (GDEE) has been proposed as a possible antagonist of glutamate actions in the CNS. Haldeman and McLennan [7,8] have demonstrated that GDEE can reversibly block synaptic activation of cat thalamic relay neurones, cuneate nucleus neurones and spinal cord interneurones . In addition GDEE reversibly antagonises the effects of iontophoretically applied glutamate, aspartate and its excitatory analogue d-l homocysteic acid and cysteic acid on these neurones without affecting the excitatory response to acetylcholine.

The results reported in this study are an extension of an investigation aimed at establishing the pharmacological characteristics of striatal cells recieving cortical drive. It was of interest to determine whether glutamic acid might be the transmitter involved, especially since striatal cells are often exquisitely sensitive to iontophoretically applied glutamate[34].

METHODS

Thirteen hooded rats of both sexes, 250 - 400 g weight, anaesthetised with a penthrane air misture following halothane induction, were used for these experiments. The gas mixture was supplied from a simple laboratory constructed anaesthesia machine (see appendix) which permitted the accurate control of the penthrane bevels. A flow through mase-mask delivered the anaesthetic gasses and eliminated the necessity for tracheostomy. The animals were maintained at stage 3 anaesthesia.

The skull was exposed with the animal in the stereotaxic frame, and 3 or 4, 0.75 mm diameter burr holes drilled in the skull to accomodate the stimulating electrodes. The multibarrel microelectrode assembly was passed through a 2 mm diameter trephine hole located in the region of A 8.0, L 2.5 mm [21], and the exploratory recording tracks were confined to the body of the striatum.

Miniature 6 or 7 barrel micropipette assemblies with a tip diameter of 4-8 um [34] were used for extracellular recording and drug application. The centre recording barrel and one lateral current control barrel were filled with 2M NaCl.

The remaining barrels were filled with glutamate, as the mono sodium salt (GLUT) Sigma (0.2M,pH 7); aspartate , as the Na salt (ASP) (0.2M, pH 8); d-l-homocysteic acid (DLH) Calbiochem (0.2M,pH7); L-glutamic acid di-ethyl-ester (GDEE) Sigma (0.2M,pH4) and in several experiments acetyl-choline (ACh) Sigma (0.2M,pH 3.5) was used. GABA (0.2M, pH 3.5) and bicuculline methyl iodide (courtesy of Dr S.F.Pong), as a saturated solution (20°C) in 0.5M NaCl, pH approximately 7, were also used.

To reduce possible oxidative breakdown of the drugs in the barrels

during the experiments, all electodes were stored prior to filling in N_2. Solutions such as bicuculline which were freshly made up before experiment were made up with N_2 gassed solutions and the other drug solutions were stored frozen under N_2. The electrodes were filled by centrifugation immediately before use.

The details of the experimental and recording procedures have been given in previous communications[31,32,33,34]. Briefly the extracellularly recorded cell firing was amplified using a variable band-pass amplifier (normally 100Hz-10 KHz bandpass) and fed into a monitor oscilloscope. The Y output of the oscilloscope passed via a window discriminator to an epochal ratemeter[31] which generated an analogue voltage proportional to the number of spiked fired during the counting epoch. This output voltage was displayed on a chart recorder (Fig 4). The window discriminator output pulses were also fed to the Fabritek 1042 signal averager which was used to compute post-stimulus time-interval histograms (PSTH).

Four stimulating sites were used (Fig 1); these were located at: 1; A 12-12.5, L 1-1.5, 2; A 10 ,L 2.0, 3; A 4.5 - 5.5, L 2-2.5 , 4; A 0.5, L 2.5. according to the coordinates of Konig and Klippel's atlas[21].

Cortical stimulation was delivered via parallel bipolar 36g. stainless steel stimulating electrodes which had a tip separation of 0.5mm; the electrodes projecting through the dura for approximately 0.5 mm. The electrode wires were mounted in a short metal tube which fitted snugly into the burr holes drilled in the skull, and which was helf in position with wax.

Stimuli were delivered from an RF isolated constant current stimulators which in turn were driven from a laboratory constructed stimulus controller -driver which was slaved to the Fabritek 1042 Signal Averaging computer

operating in the PSTH mode.

The stimuli consisted of single 0,3 ms pulses, 2 - 6mA intensity, which were above threshold for the responses.which were delivered at intervals ranging from one to 10 per second depending on the unit under scrutiny when performing PSTH analyses. Units were revealed by advancing the microelectrode assembly slowly through the striatum while stimulating the cortical sites either sequentially (1-2-3-4) or at a single site. Once a unit was encountered which responded consistently to cortical stimulation the following procedure was normally followed:-

A control PSTH (64 to 259 sweeps, depending on the excitability of the unit under investigation) were made of the response to stimulation of the most effective cortical site. During this control PSTH a cationic current equal to the GDEE ejecting current (+50 - +125 nA) was passed from the lateral NaCl barrel. Next GDEE was applied with the same current and after an initial period of 3 - 5 minutes had elapsed a second PSTH was made, GDEE being ejected during this PSTH. After a recovery period of up to 10 minutes following the GDEE application a 'recovery' PSTH was made in the same manner as the control. All PSTH's of each series contained the same number of sweeps.

Following the PSTH series the responses of the unit to applied glutamate and other substances were tested , and the efficacy of GDEE in antagonising these resoposes was determined. The majority of units could be held for the 1 to 1.5 hours required to complete the test sequences.

The effects of bicuculline methyl iodide[26,27] and GABA on the nature of the responses was also briefly investigated as described in the results section.

RESULTS

Responses to Cortical Stimulation

While there appear to be no intra- or extracellular studies onnthe responses of rat striatal cells to cortical stimulation, comparable to those carried out by Liles on cats[23] and by Buchwald et al [1]; the nature of the responses observed in this study appear to be essentially similar.

Although the number of cells responding with excitation to two or more cortical inputs was not specifically determined, at least a third of the 38 neurones encountered appeared to do so. The number of spikes generated in response to the single stimulus pulse was quite variable, from 1 to 5 or more(Fig 2) in contrast to Liles findings[23]. Also the number of spikes was to some extent a function of both the stimulus strength and repetition rate. The majority of units responded on less than a 1:1 basis and the response ratios, while not specifically examined tended to vary somewhat during the experiment. It is possible that the responses recorded in some cases may have been derived from more than one unit, and while there was some variability of spike height, it was no more marked than that which has been observed during intracellular studies[24].

Cells were often encountered that fired irregularly in response to stimulation at first, but with continuing stimulation (at 3/s) the firing would become less erratic and often the number of spikes per response would incerase. Similar responses to intra-striatal stimulation have been reported by Marco et al [24]. Some cells tested showed a distinct post-tetanic potentiation of firing following a stimulus burst. Unfortunately for lack of suitable recording apparatus it was not possible to analyse these characteristics in greater detail.

Latencies as determined from PSTHs ranged from 4 - 20 mS ($\bar{x}$ = 12 mS)

and varied according to the stimulation site (Table 1). The latencies of firing as may be observed from Fig. 3 did not exhibit a high degree of constancy (approxiamtely $\pm$ 1-2 ms variability), and the latency of the later spikes were even less predictable as is evident from the shape of the PSTH. Changes in stimulus parameters and the application of GDEE or the current control did not alter the latency significantly.

Effect of GDEE on Cortically Evoked Firing.

Since the Fabritek did not have the facility to integrate the PSTH, in order to compare responses it was necessary to rate the magnitude of the PSTH on a 1 to 5 scale as is illustrated in the diagram in Table 2.

In the majority of the 38 units tested GDEE produced a powerful and reversible reduction in the number and frequency of spikes generated in response to cortical stimulation as is evidenced by the PSTHs (Fig 3).

In less than 10% of the remainder of the cells tested were the number of spikes produced unchanged by application of GDEE. Of those units unaffected most of these responded with a latency of less than 5 ms. In only one cell did GDEE produce a slight enhancement in the response to stimulation. All the cells tested were also excited by application of glutamate ions (-10 to -50nA) The retaining currents used to prevent leakage of amino acids were approximately +15 nA.

Recovery from the effects of GDEE was generally rapid, full responsiveness returning in about 2 to 3 minutes. A chi-square test of the difference in the frequency of the distribution of the number of responses of each magnitude before, during and after GDEE application was made, using the magnitude criteria described earlier. Between the control and recovery periods there was no significant difference, while between the GDEE and

either control period the difference was highly significant (p 0.01)

Effect of GDEE on Amino acid Induced Excitation.

GDEE was effective in antagonising the excitation produced by iontc phoretically applied glutamate, aspartate and d-l homocysteic acids (Fig 4). and in agreement with Haldeman and McLennan's findings[7,8] required ejecting currents from between +50 to +125 nA to acheive this effect. Cationic control currents of the same magnitude either had no effect or enhanced the response slightly. There was little difference in the ability of GDEE to block the responses of the same magnitude to the three excitant amino acids tested on striatal cells (Fig 4). However on several cortical cells tested glutamate evoked excitation was antagonised to a greater degree than that produced by d-l homocysteic acid which is in agreement with previous studies[7,8].

Spontaneous firing ,when present was in most cases unaffected by GDEE or the current control, even with ejectioncurrents of +125 nA. None of the cells tested were sufficiently strongly or consistently excited by ACh to permit the determination of the effects of GDEE on ACh excitation.

The Effect of Bicuculline Methyl Iodide on the Responses to Cortical Stimulation

The effect of bicuculline methyl iodide (BMI) [26,27], was tested on 8 units, primarily to see whether either of the inhibitory responses which are observed in striatal neurones following cortical or intrastriatal stimulation[1,24]; the short and long latency inhibitions, might be mediated by GABA.

GABA applied iontophoretically to glutamate driven or spontaneously

firing striatal cells inhibited the firing (to 50% or less of the control level) at ejection currents of +10 - +20 nA.in all cells tested. BMI was able to block this GABA induced depression with application currents of +80 to +125 nA in 5 out of the 7 cells tested.

In all units, BMI applied with currents of +80 to + 125 nA produced a great increase in spontaneous activity, the cells frequently began to fire in paroxysmal bursts. This increase in excitability is reflected in the increased amplitudes of the PSTHs generated in response to cortical stimulation.when BMI was simultaneously applied. (Fig 5).

However there was no evidence of any antagonism of the long latency inhibition by BMI (Fig 6) in any of the cells since the time course and latency remained unchanged in an addional 5 cells which were examined with a longer PSTH timebase (1.28 s), It can be seen that BMI besides increasing the overall activity of the cell, markedly enhanced the magnitude and duration of the initial excitatory response without significantly altering the time course or intensity of the long latency inhibition resulting from cortical stimulation.

DISCUSSION

The degenerating fibre tracts which can be demonstrated terminating in the striatum following cortical lesions are comprised of fine poorly myelinated axons[37,37] the majority less than 1 um in diameter. Such fibres are generally considered to have conduction velocities in the range of 0.5 to 1 meter per second.[35], Assuming that in the rat that the caudato-petal fibres course ipsilaterally in the callosum and then enter the striatum via the internal capsule bundles, as would appear to be the case from Websters' study [36,37], then an approximate estimate of the path length from sites 2 or 3 to the recording locus would be 8 mm. Assuming a mono-synaptic pathway [36], a latency of 12 ms would give a conduction velocity of 0.8 m/s, a figure that agrees fairly well with previous studies[23].

The observed variability of the latency of these spike responses is further evidence for the existance of these fine fibre pathways. The fine diameter of these axons makes them very difficult to stimulate anti-dromically and the lack of striatal evoked cortical responses reported in the literature confirms this. The assumption that the cortico-striate pathway terminates monosynaptically on striatal cells derives from both histological and neurophysiological evidence[12,23,24].

Since GDEE blocks both the synaptically and glutamate induced excitation of striatal cells, this would suggest that an excitatory amino acid such as glutamic acid, may be involved as the excitatory transmitter in the cortico-striatal pathway.

However as has been repeatedly pointed out, there are many dangers inherent in the use of antagonists to evaluate possible neurotransmitter cansisates. Non-specificity of effect, local anaesthetic actions, intrinsic actions and non-reversibility of action amongst others tend to obscure the

desired effect. GDEE appears to have few drawbacks as a glutamic acid antagonist antagonist; while it is neither highly specific or potent, it does tend to preferentially antagonise responses to glutamate over those to the other excitatory amino acids[7,8], the cortical data reported in this study, and this action is reversible. GDEE does not appear to have a local anaesthetic effect, in this study the spontaneous firing of the majority of cells was not affected and nor was there a noticable change in spike amplitude[10], even with ejection currents of +125 nA. Although GDEE has been found to cause a block of excitation of spinal motoneurones by increasing membrane conductance[38] in a manner similar to GABA, the lack of change in spontaneous activity of striatal cells observed in this study suggest that this effect might be peculiar to the spinal cord. Future intracellular studies on striatal cells in conjunction with GDEE will be required to give an unequivocal answer. Yarborough (pers. comm,) has confirmed that GDEE applied to rat cortical cells antagonised amino acid induced excitations without affecting ACh induced excitation.

The data presented here is further evidence for the supposition that either glutamic or aspartic acids function as excitatory transmitters in the CNS, and that one of these substances is probably the transmitter in the cortico-striate pathway; although until a more selective antagonist becomes available, it will be difficult to determine which.

While it is not known whether GABA functions as an inhibitory transmitter in the striatum, the finding that bicuculline methyl iodide applied with ejections currents of sufficient magnitude to block GABA induced depressions of striatal cell firing, causes a marked enhancement of both striatal cell activity and the cortically evoked excitations, suggests that a GABA mediated inhibitory drive may be present. This enhancement

of activity may be due to a local effect of the bicuculline rather than GABA antagonism since the mode of action of bicuculline as a convulsant is not understood. Curtis et al[6] observed a similar slight enhancement of Renshaw cell firing during iontophoretic application of bicuculline, but the responses of the Renshaw cells to glycine, d-l homocysteic acid and acetylcholine was unaffected. Bicuculline depresses cat cortical cells[11].

Since GABA is taken up selectively by both striatal terminals and neurone bodies[9], it is tempting to suggest that a possible source of striatal GABA would be the terminals of the intrinsic striatal plexus[17,19], which are thought to mediate the IPSPs produced by intra-striatal stimulation[24]. However BMI in this study did not affect the inhibitory responses obtained, but caused a marked enhancement of the excitation (Fig 5,6) which corresponds in time to the initial EPSP reported by Buchwald[1]. This could indicate the presence of a tonic inhibitory drive on the striatal cells which is not directly responsive to afferent input, and which may be GABA mediated.

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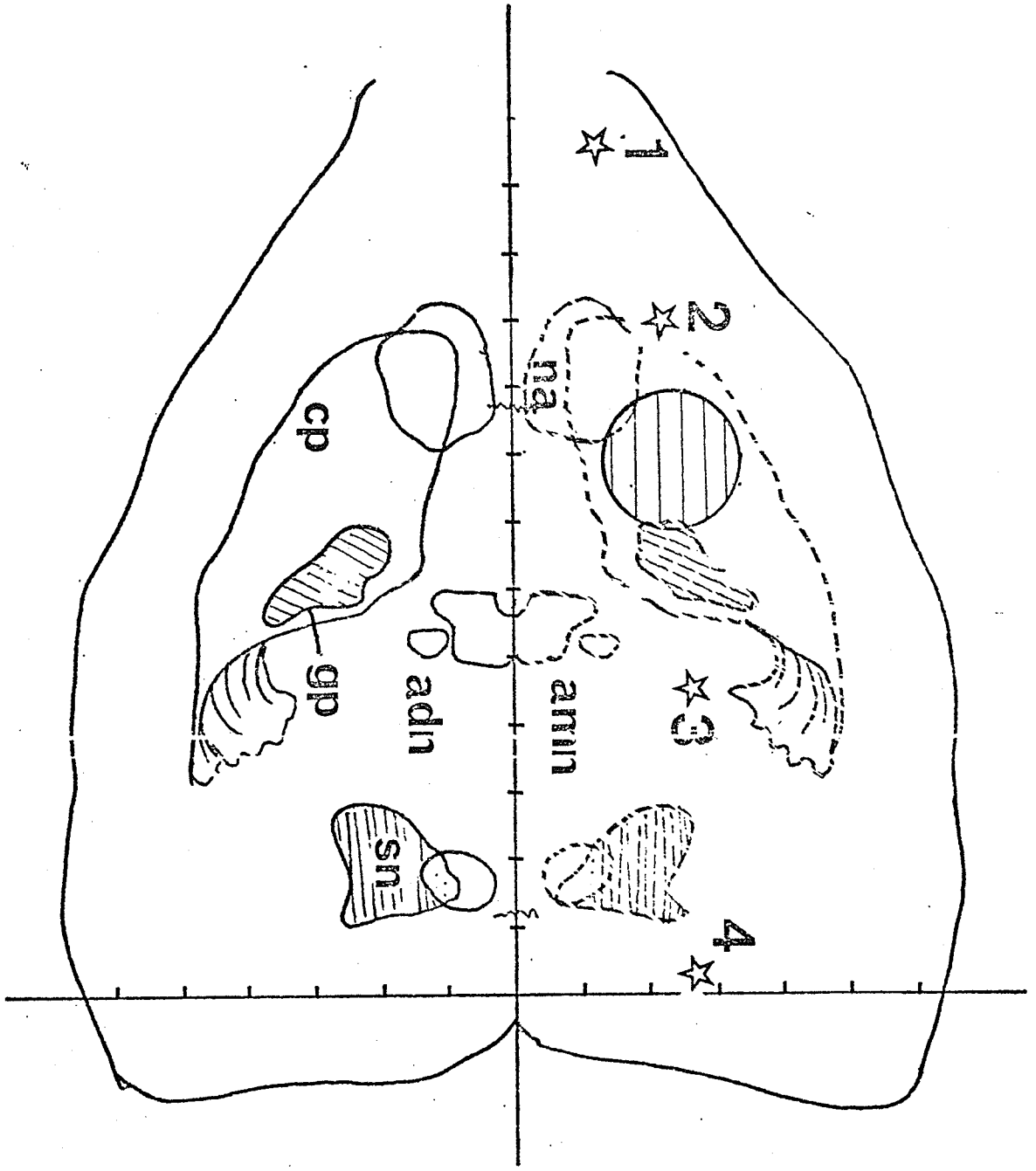


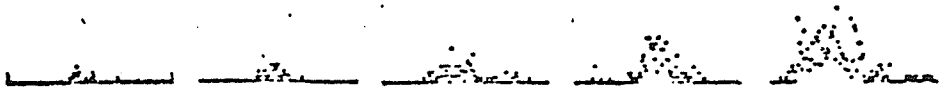
FIGURE 1

Diagrammatic dorsal view of the rat brain indicating the position of the various extrapyramidal structures, the location of the cortical stimulating sites (stars) and the recording site (shaded circle). The axes are those used in Konig and Klippel's atlas[24], and are in millimteres. Abbreviations used; cp - caudate-putamen (corpus striatum); na - nucleus accumbens; gp - globus pallidus; sn - substantia nigra; amn - nucleus antero-medialis (thalamus); adn - nucleus antero-dorsalis (thalamus).

	SITE			
	1	2	3	4
Latency (ms)				
$\bar{x}$	14.8	12.7	6.8	12.0
s	2.3	3.2	2.5	1.9
n	11	18	9	7

TABLE 1

The latencies between cortical stimulation and the initiation of striatal cell firing, as determined from PSTH records. $\bar{x}$, average latency; s, standard deviation, and n, the number of cells responding to each of the stimulus sites.



Control	0	8	22	11	2
GDEE	20	13	7	2	1
Recovery	0	4	17	12	3

TABLE 2

The effect of GDEE application on the intensity of the striatal cell responses to cortical stimulation as compared to that during current control.

The PSTHs which were generated in response to cortical stimulation have been classified into 5 intensity classes, since the area of the histogram provides an index of the intensity of the neuronal response. Each class (1 - 5 illustrated at the top of the table) is approximately twice the area of the preceeding class. The number of responses obtained during the control, GDEE application and recovery periods, of each magnitude have been tabulated above. It is evident that there is a pronounced reduction in the intensity of the neuronal response during GDEE application compared to the current controls (see text). A Chi-square analysis indicated that the difference between the control and GDEE responses was highly significant ($p \ll 0.01$), while there was no significant difference between the recovery and control periods.

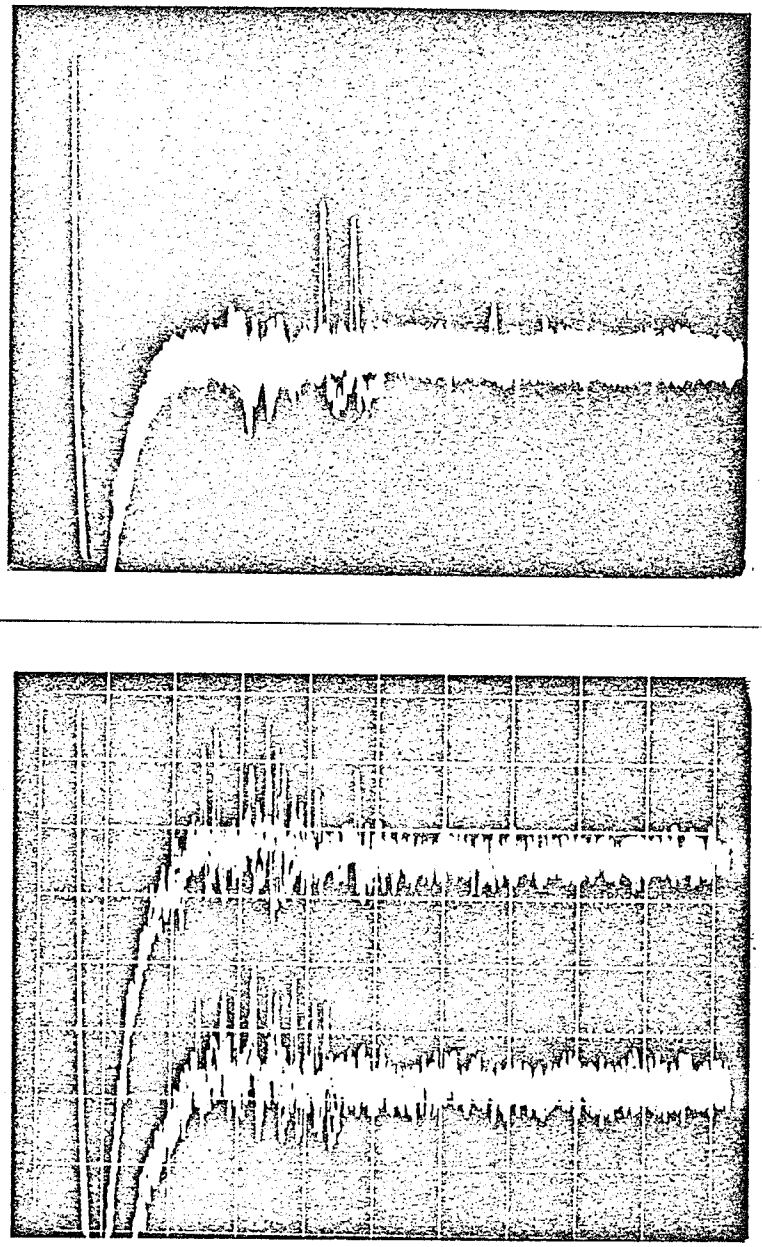


FIGURE 2

Oscilloscope records of two typical responses of striatal cells to cortical stimulation. Top picture is of a cell which fired only sporadically in response to cortical stimulation (at 3/second) This trace represents approximately 10 responses. Lower picture is of a cell which responded with multiple spikes to cortical stimulus (3/second), each trace represents 3-4 responses. Graticule calibration; horizontal- 5 ms per division, vertical; 100 uV per division.

FIGURE 3

Post stimulus time interval histograms of the effects of GDEE on the responses of striatal cells to cortical stimulation of the sites listed at the left hand side. The first column is the control response, i = +100nA, the second column is the response during GDEE ejection (+100 nA) and the third column is the recovery response. The instant of stimulation is indicated by the arrows in the top trace, and by the corresponding marks on the lower traces. The vertical marks above the arrow heads represent 10 counts, all histograms are to the same sacle. The bottom two series of responses are from the same cell but in response to two different stimulation sites. Each trace, with the exception of the first, represent the accumulation of 64 responses; 2750 (top) 128 responses. The numbers at the side of the traces represent the depth of the cell below the cortical surface in microns.

It can be seen that GDEE causes a marked depression in the intensity of the excitatory response to cortical stimulation.

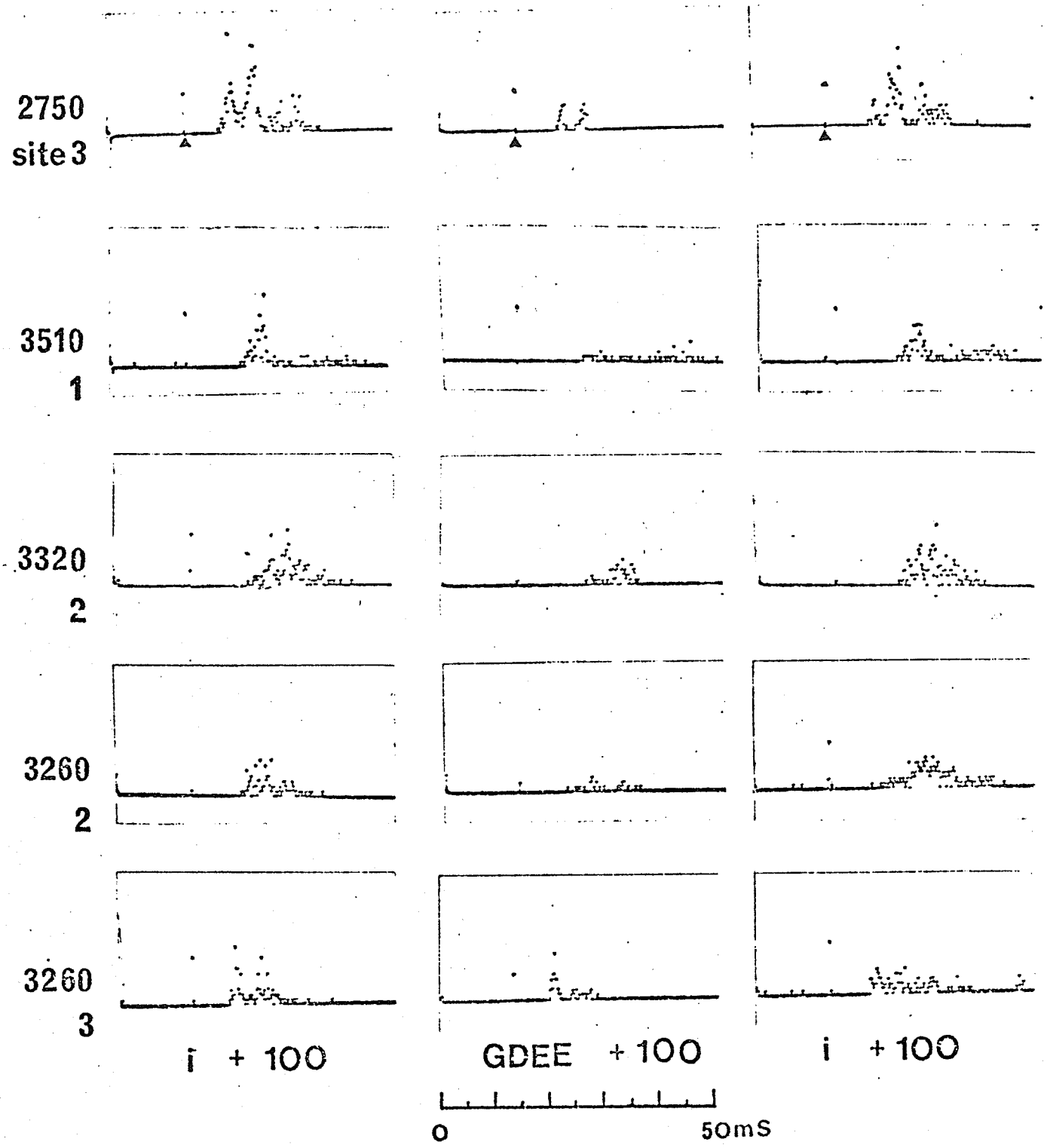

2750
site 3
3510
1
3320
2
3260
2
3260
3
i + 100
GDEE + 100
i + 100
0
50mS

FIGURE 4

Four epochal ratemeter records of striatal neuronal firing in response to iontophoretic application of excitatory amino acids, showing the depression of amino-acid induced firing by GDEE. Glutamate (G), d-l homocysteic (D), aspartate (A) and GDEE were applied for the durations and currents indicated below the traces.

In cell A, (3300 microns) GDEE caused complete inhibition of the amino acid induced excitation, followed by rapid recovery. In cell B (2990 microns) GDEE caused an enhancement of a bursting type of spontaneous activity which was unrelated to the application ofthe amino-acids. The current controls (i +100nA) can be seen to have no significant effect on the cell firing. In cell C, (3970 microns) GDEE inhibition of glutamate induced firing without inhibition of spontaneous activity is illustrated. Cell D is a cortical cell (1480 microns) in which GDEE (+40nA) depresses glutamate excitation selectively.

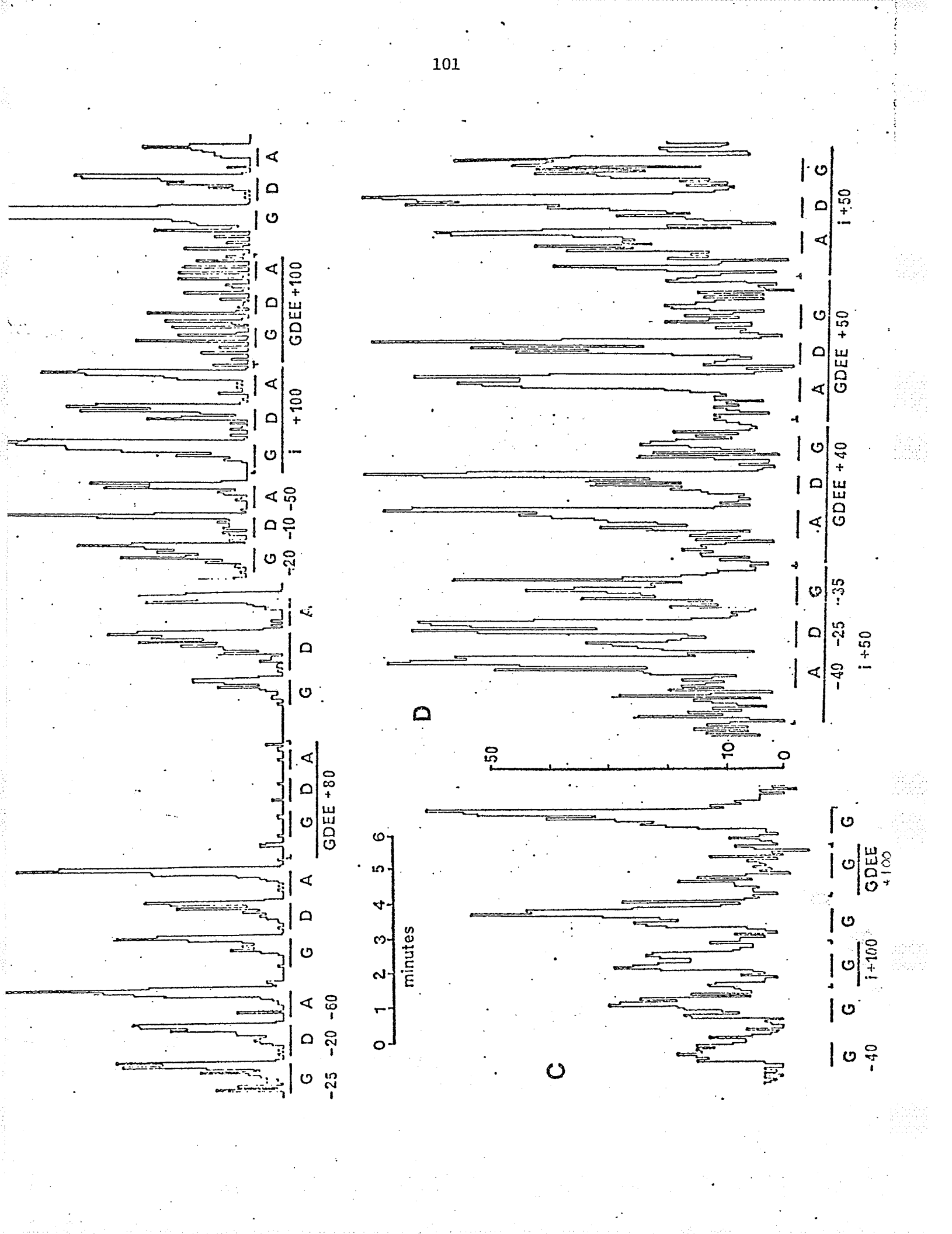

C
D
minutes
0 1 2 3 4 5 6
50
10
0
G D A G D A
-25 -20 -60
GDEE +80
G D A G D A G D A
-20 -10 -50
i +100
GDEE +100
A D G A D G
-40 -25 -35
GDEE +40
i +50
A D G A D G
GDEE +50
i +50
G G G G
-40
i +100
GDEE +100

FIGURE 5

PSTHs illustrating the effect of bicuculline methyl iodide on the responses of a striatal cell to cortical stimulation. In contrast to the effect of glutamic acid di-ethyl-ester (GDEE) which reduces the amplitude of the response, bicuculline (BIC) can be seen to reversibly enhance the magnitude of the response to cortical stimulation as compared to the control (i +100) responses. Each histogram represents the summation of 64 responses and were generated in the order illustrated with approximately 3 minutes interval between each PSTH. Stimulation rate 3/second.

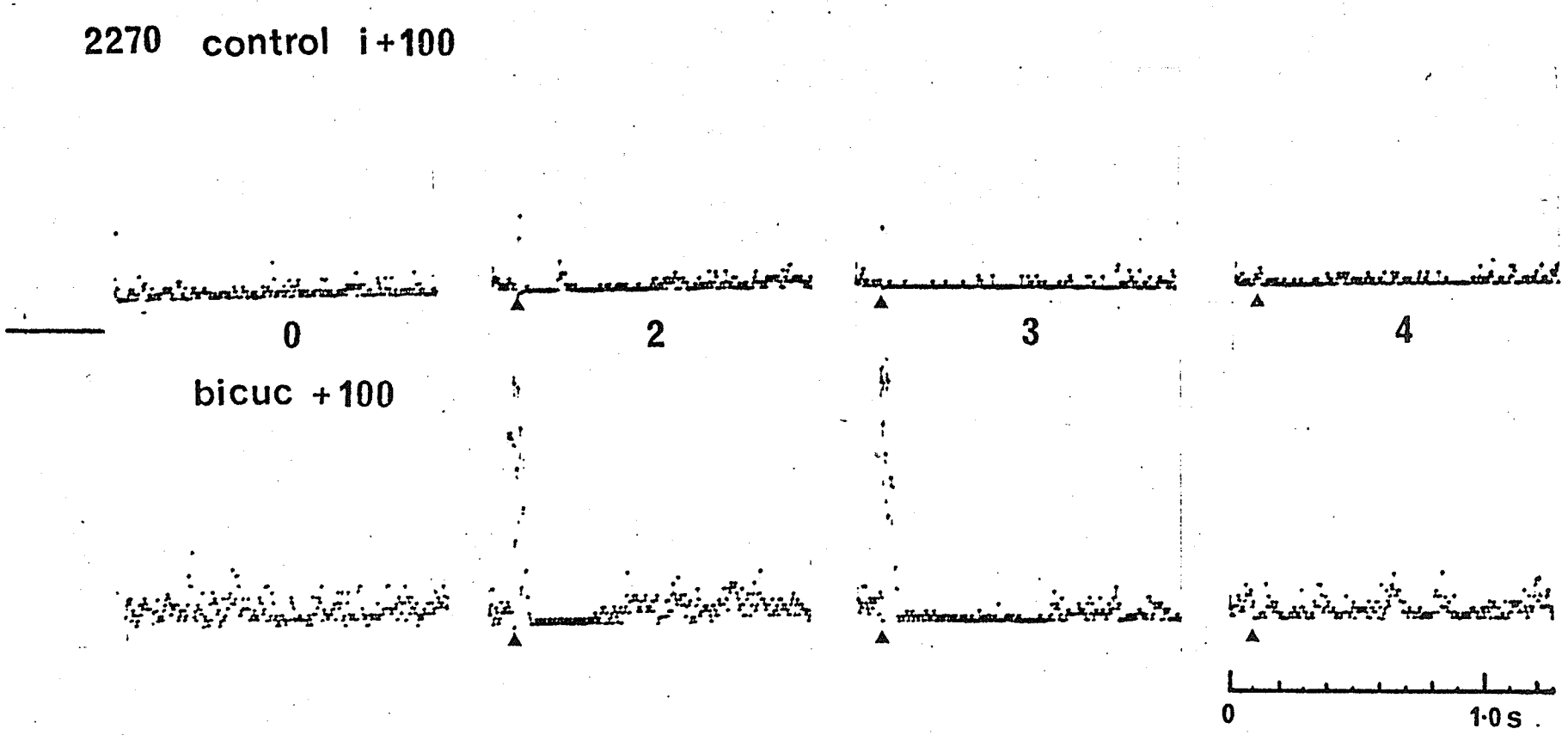


FIGURE 6

Slow time-base PSTH records of the effect of bicuculline methyl iodide (+100nA) on the responses of a striatal neuron to stimulation of three cortical sites. The first PSTH is the unstimulated control period, and the three PSTHs following are in response to stimulation of sites 2,3 and 4 (Fig.1) in that order.

The ' control i+100' group of PSTHs are the control series, in which a +100 nA current was passed continuously during the stimulus. During the 'bicuc +100' series, bicuculline was ejected in a similar manner. The time of stimulation is indicated by the arrow heads.

It is evident that bicuculline application causes a marked increase in the amplitude and duration of the initial excitatory response. Each PSTH represents 64 stimulus cycles.

GENERAL DISCUSSION

Despite the presence of a large concentration of dopamine (DA) and acetylcholine in the striatum, the role of these substances in striatal functioning remains obscure.

Striatal ACh appears to be intrastriatal in origin by analogy with the distribution of acetylcholine esterase and cholineacetylase[21] .Its concentration does not appear to be altered by changes in the concentration of DA resulting from lesions of the nigro-striatal dopaminergic pathway or from the administration of α-methyl-para-tyrosine[19,22]. The putative striatal cholinergic mechanism has been postulated by Costal et al[7,23] to exert effects which are opposite in sign to those produced by the dopaminergic system, on the basis of studies of circling behaviour studies.Unilateral striatal injections of the cholinergic nicotinic agonist arecoline mimics the effect of ipsilateral striatal lesioning, or of ipsilateral antagonism of the DA system by injected haloperidol; while atropine , which presumably causes cholinergic blockade , produces the opposite effect.(turning to the side opposite the lesion). Similarly unilateral injections of DA and DA agonists such as apomorphine, amphetamine and l-DOPA also causes contra-lateral turning [23]. Unfortunately while such studies have been the basis for many of the hypotheses that have been developed to explain the role of ACh and DA in striatal functioning, since the physiological basis for the observed behavioural actions is unknown, such studies are of limited value.

From previous studies, both behavioural and pharmacological[1,13,24,25]. the idea that DA exerts a predominately depressant effect on striatal cells has become generally accepted, and that substances such as amphetamine and APO which appear to mimic DA effects in the striatum do so by either

Leaf blank to correct numbering

releasing DA from nerve terminals (AMPH), or by stimulating DA receptors diectly (APO). Against this may be set the interesting finding that nigro-striatal lesions which cause a lowering of striatal DA concentrations do not cause a concomitant enhancement of striatal cell firing as might be expected[15].

From the data presented in this study iontophoretically ejected DA and ACh appear to induce very much the same spectrum of responses from striatal neurones and there is no evidence for the reciprocal responses to DA and ACh (DA + , ACh - ; DA -, ACh +) which might be expected to occur if Naylor's hypothesis was correct[23].

Iontophoretically applied noradrenaline (NA) also affects striatal neurones in much the same manner as DA and ACh, yet intrastriatal injections of NA are without behavioural effect[5,6].

While the action of the butyrophenone spiroperidol on AMPH effects was not tested, the depressant responses to DA, ACh NA and APO were antagonised by a systemic injection of spiroperidol, with a concommitant increase in the proportion of excitatory responses to DA NA and ACh. This parallel shift in the nature of the responses to DA and ACh indicates that the behavioural effects of spiroperidol, or its related substances haloperidol and spiramide[8], may arise from a direct action on the neuronal membrane, rather than through DA receptor blockade.

The observed distribution of responses to DA obtained in this study favours the hypotheses put forward by Klawans to explain the differential effects of l-DOPA therapy in Huntingtons' Chorea sufferers[19]. He suggests that there are two populations of DA sensitive neurones in the str atum; those excited, the minority, and those depressed. However as evidenced by the responses of striatal cells to NA and ACh, the specificity of striatal

neuronal responses to biogenic amines has not yet been demonstrated.

Iontophoretically applied APO and AMPH, both considered to be DA agonists, in this study have been shown to primarily exert depressant actions on striatal cells, with the responses to APO closely resembling those to NA. AMPH exerted an almost purely depressant effect suggesting that it has a direct effect on the cell, although it is currently accepted as exerting its behavioural effects through the release of DA from nerve terminals. However 6-hydroxydopamine destruction of the nigro - striatal dopaminergic projection has been reported to leave the depression produced by iontophoretically applied AMPH unchanged[9], which supports the possibility that the action of at least locally applied AMPH is direct.

While the specificity of action of 6- hydroxydopamine is open to question[20], the lack of obvious behavioural deficits following demonstrable reduction in brain catecholamines has been remarked on by a number of workers[20,22], and it would appear that whatever role DA and ACh play in the striatum, it can be readily compensated for by other mechanisms. Yet the very high concentrations of DA and ACh suggest the existance of some form of homeostatic balance mechanism operating[7,17]. However the reported lack of change in ACh concentrations, as deduced from acetylcholine esterase and choline acetylase levels, following experimentally induced changes in DA concentrations in the striatum make this assumption questionable.

Alternatively ACh and DA could function as modulators of striatal neuronal excitability through regulation of transmitter synthesis or availability. Both substances could be envisaged as acting independently on the same mechanism, which might be the level of an inhibitory transmitter substance such as GABA. Striatal GABA levels are reported to be

depressed in Parkinsonism sufferers[1], but except for the fact that GABA is selectively taken up by both terminals and neurones in the striatum its normal location in the striatum is unknown[12].

The fine-fibre diffuse dopaminergic nigro-striatal projection from the cell bodies in the substantia nigra pars-compacta is characterised by the existance of a high degree of collateral branching and the estimated presence of some 5×10^5 terminals per fibre[14]. From its anatomy this projection system would not appear to be well suited to the discrete transmission of neuronal information, but would appear to be ideally suited to the intimate and rapid dispersion of a modulator of neuronal excitation.

Alternatively DA and/or ACh could modulate other mechanisms which in turn could regulate neuronal excitability. The latency of 6 to 30 seconds for the onset of behavioural effects following striatal electrical stimulation[6] would suggest such a non-synaptic mechanism of action.

The probability that ACh functions as a classical synaptic transmitter in the striatum does not appear to be very likeley in view of its great lability of action in the presence of sub-anaesthetic doses of barbiturates and related anaesthetics, as demonstrated in this and other studies[2,3,24]. From the foregoing it appear highly possible that the iontophoretic application of DA or ACh either does not mimic the effects of post-synaptic release that would be expected on the basis of conventional wisdom, ie. the production of EPSPs or IPSPs; or these effects may not be manifested as direct changes in neuronal excitability. The effects observed here may in fact be non-specific[13].

Stimulation of cortical, thalamic and nigral striatal afferents has been demonstrated to result in an EPSP - IPSP sequence in striatal neurones, both as recorded intracellularly[4,15] and demonstrated

through the modulation of glutamate driven cell firing in this study. Cortical stimulation in rats results in the production of an excitatory response with a latency of 10 to 20 ms, and this response is frequently followed by inhibition. Such an excitatory response indicates that at least one compound in the striatum functions as a classical excitatory transmitter.

Glutamic acid is present at high levels in the striatum as is its metabolising enzyme glutamic acid decarboxylase (GAD)[16] . Glutamate, in this study, was found to excite virtually all striatal cells, and it was routinely used to raise the normally very slow spontaneous discharge rate sufficiently to allow the effects of iontophoretically applied drugs or the responses to cortical stimulation to be analysed. This glutamate excitation was not affected by the nature or the levels of anaesthesia used.

The reversible blockade by glutamic acid di-ethyl-ester (GDEE), of both the synaptic excitation of striatal neurones following cortical stimulation and the excitation resulting from iontophoretic application of the excitatory amino acids , glutamic, aspartic and d-l homocysteic; strongly suggests that an excitatory amino acid, either glutamic or its immediate precursor aspartic, functions as the cortico-striate transmitter. On the basis of previous studies[10,11] , it is probable that glutamate or aspartate are also involved in the thalamo-striate and non dopaminergic nigro striatal pathways.

Since the striatum is a dense interneuronal plexus[18], it is assumed that the constituent neurones are in a state of tonic inhibition[21] . This inhibition could be mediated through the mechanism of post-synaptic inhibition possibly at the dendritic level, since these neurones are

characterised by having massive dendritic trees[18]. GABA is known to be present in the striatum, although its subcellular localization is unknown, and it is the immediate metabolite of glutamic acid.

Bicuculline ejected at levels adequate to block GABA induced depression caused a marked enhancement of both spontaneous and cortically activated striatal cell firing which suggests that this effect might be due to blockade of a GABA mediated inhibitory drive. Intracellular recording in conjunction with the iontophoretic application of these substances would be necessary to confirm this idea. Bicuculline, however did not affect the longer latency inhibitory component of the responses to cortical stimulation which indicates the presence of yet another inhibitory striatal transmitter.

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A SIMPLE PENTHRANE (METHOXY-FLUORANE) - AIR ANAESTHETIC SYSTEM FOR SMALL MAMMALS

INTRODUCTION

Conventional gas anaesthesia systems for mammals have required the use of nitrous oxide as the induction and analgesic agent; penthrane or halothane as the maintaining anaesthetic and oxygen to maintain respiration. Such a system necessitates the use of bottled gas and elaborate (and expensive) regulators, flowmeters and vapourisers.

By using halothane as an induction agent, penthrane-air anaesthesia has proven to be as effective as the conventional gas mixture system, and very substantially cheaper and more flexible.

One constraint that has complicated the use of penthrane as an anesthetic agent is its low vapour pressure at room temperature, which has necessitated the use of elaborate vapourisers of the 'Copper Kettle' variety. In the system described here, this constraint has been removed by using a gas mixing system wherein a penthrane saturated air stream is metered and mixed with a separate metered mixing air stream, giving complete control over the penthrane levels administered to the animal.

Since low concentrations of penthrane are required both for induction (1-3%) and for maintainence (0.1 -0.4%) of anaesthesia, there is no necessity to administer additional oxygen to prevent anoxia.

This system has been used very sucessfully over several years in this laboratory for neuropharmacological studies on rats.

SYSTEM DESCRIPTION

Air flow is provided by two conventional vibrator aquarium pumps, on of which, pump M, (Fig.1) must be capable of providing a flow of at least 1 litre per minute (the mixing flow). This air flow from pump M passes to a 1 gallon (4-5 litre) bottle which serves as a reservoir to eliminate the pump-produced pressure pulsations which would otherwise cause vibration of the animal. Air flow from the reservoir is connected via a low cost rotameter type flowmeter having an integral needle valve (F.W.Dwyer Mfg.Co. MIchigan city Indiana U.S.A., Type VFA.BV.22; 0.2-1.0 litre per minute), to the halothane vapouriser.In this system an 'Ether' vapouriser (The British Oxygen Co.Ltd.) was used as the halothane vapouriser, but any vapouriser of this type having an integral by-pass valve would be as suitable.

The air flow from pump P, the penthrane air supply, goes directly to a low flow rate flow-meter (50-500 ml/minute). This is a modified Dwyer 'Wind-Meter' anemometer. modified by attaching an input tube to one of the two lower holes and blocking the other, and blocking the small lateral hole in the top tube. A needle valve (Whitey OVM2 or equivalent) is fitted over the top tube to permit fine regulation of the air flow to the penthrane vapouriser. Care should be taken to ensure that all connections are gas tight as this is the 'high-pressure' part of the system. It is essential that this flow meter precedes the penthrane vapouriser, otherwise it would be permanently damaged by the anaesthetic agent.

Any convenient vessel can be used for the penthrane vapouriser - a 1" diameter test-tube permits the most efficient use of the anaesthetic agent. A sintered glass aerator tube is used to generate a stream of fine air bubbles in the penthrane, which means that the air flow becomes completely saturated at room temperature. A small immersion thermometer is put in the test-tube to measure the penthrane temperature.

The tube stopper should be of rubber and be well fitting Once the stopper is inserted it will swell and become impossible to remove. An additional hole in the stopper, plugged with a

length of rod permits filling with a suitable syringe.

The output from the vapouriser must be passed into a spray-trap (Fig.1) before mixing with the output of the halothane vapouriser via a 'Y' tube; from which the mixed gasses are led to the animal. Thin walled PVC aquarium air tube is used for all connections to minimise adsorbtion of penthrane. Between the penthrane vapouriser and mixing tube, the connection should be of gum rubber tubing, since the penthrane causes the PVC tubing to become rigid.

For spontaneously breathing animals a mask can be constructed which obviates the necessity for a tracheostomy, and by using a flow-past arrangement (Fig 2 inset), eliminates any increase in respiratory dead-space. The dividing tongue, which separates the inspiratory and expiratory flows in the mask, can be made from any suitable metal which is sufficiently rigid to wedge into the plastic 'T' tube. It is essential for proper operation that the end of the tongue completely occludes the lumen of the cross piece of the 'T' tube. The mask is constructed from a gum rubber dropper teat.

It is suggested that the excess anaesthetic gas be vented to the outside of the laboratory in view of the sensitising effect of fluorocarbon anaesthetics on the myocardium.

With the mask, it is easy to artificially respirate the animal, should respiration stop, by attaching a valved rubber squeeze-bulb (of the type used in sphygmomanometers) to the outlet tube from the mask. The gas flow should be turned off during this. Since the mouth remains open, removal of mucus and saliva during anaesthesia is easily accomplished using a suitable syringe.

Flow-meter Calibration (Wind-Meter)

A calibration curve for these flow meters is given in Fig.2. Since these flow meters are virtually identical, this calibration should be sufficiently accurate (±2% of reading).

USING THE SYSTEM

The penthrane vapouriser should be filled no more than half full, while the halothane vapouriser should have about 1" of fluid in it.

Induction is carried out in a closed box into which the anaesthetic mixture is piped directly from the mixing Y tube. A plastic mouse cage with a sheet of plexiglas for a top serves admirably for this. After the animal is unconscious (5-7 minutes) the mask is fitted and the anaethesia is continued.

For induction, the penthrane flow is turned on fully, and the bypass valve is put in the 'on' position, with the plunger pulled all the way up (Fig 1). With rats induction under these conditions takes about 5 minutes for the animal to become sufficiently unconscious to permit removal from the box, fitting the mask, and placing in the stereotaxic frame. Following this the anaesthesia is continued at induction levels for another 10 minutes, after which the halothane by-pass valve - is put into the 'on position. Continued high levels of halothane will result in respiratory depression and the animal will require several minutes of artificial respiration before it will resume spontaneous breathing.

After about 15 to 20 minutes the penthrane level should be turned down to about 7 on the flow-meter (350 ml/min), and then steadily reduced to maintain the desired level of anaesthesia. The halothane bypass valve should be in the 'off' position.

For long term anaesthesia (8 - 15 hours), at early stage 3 anaesthesia (corneal reflex just absent), the penthrane level is usually maintained at less than 0.1% (Wind-meter ball just resting on the lower stop).

If a carditachometer is available, the level of anaesthesia should be such as to give a heart rate of approximately 250 beats /minute, with a respiratory rhythm of 5-20 beats/minute. A rising heart rate indicated that the animal is becoming too lightly anaesthetised, a falling heart rate, too deeply anaesthetised.

Since the anaethetised animals lose the capacity to thermoregulate, some form of thermostatically controlled heater is

essential for maintaining body temperature during prolonged anesthesia. Some form of apnea alarm is also valuable, especially when working with rats, as the trachea tends to block readily.

FIGURE 1

Pictorial diagram of the anaesthesia system.

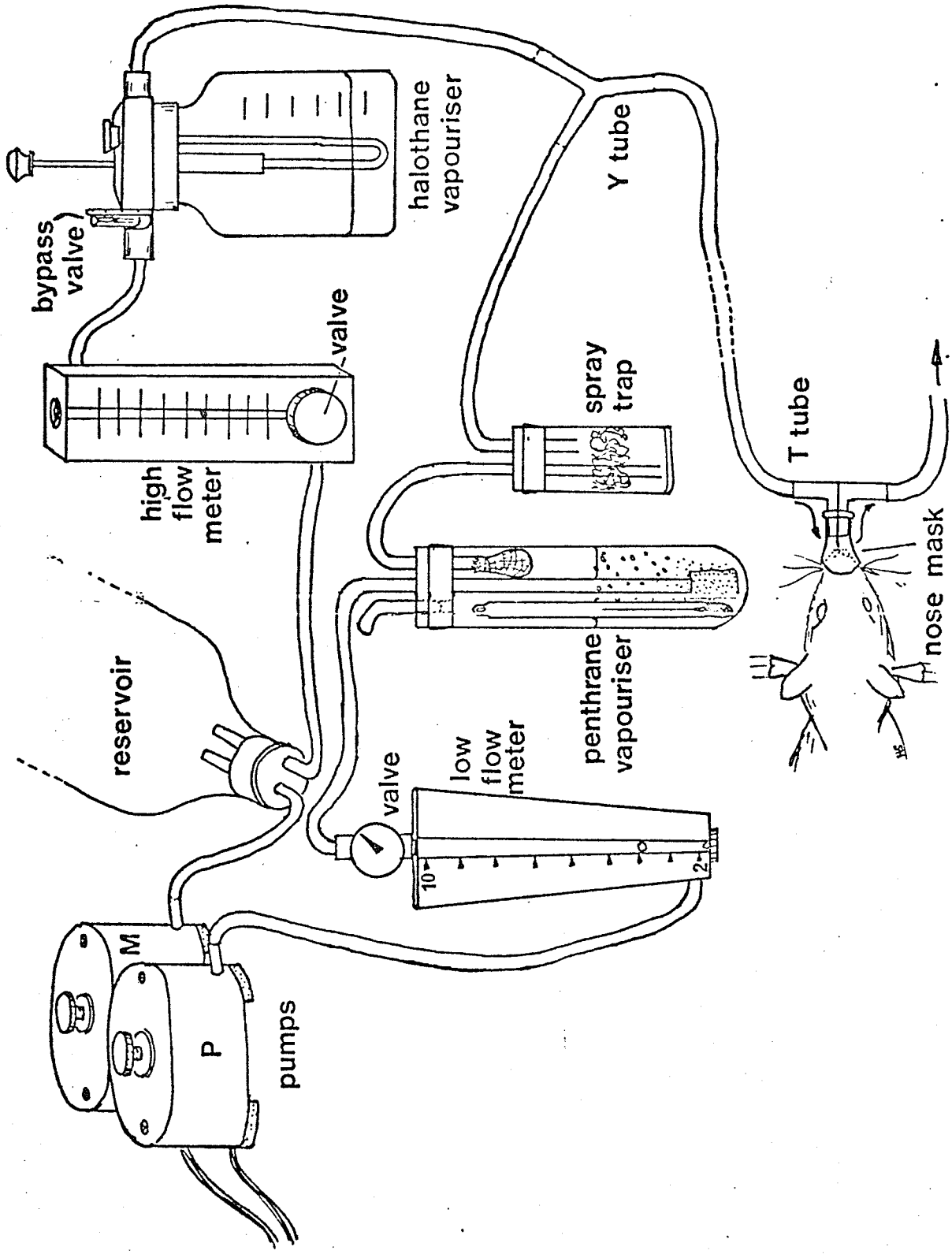

halothane vapouriser
bypass valve
Y tube
valve
spray trap
high flow meter
T tube
nose mask
reservoir
penthrane vapouriser
valve
low flow meter
M
P
pumps

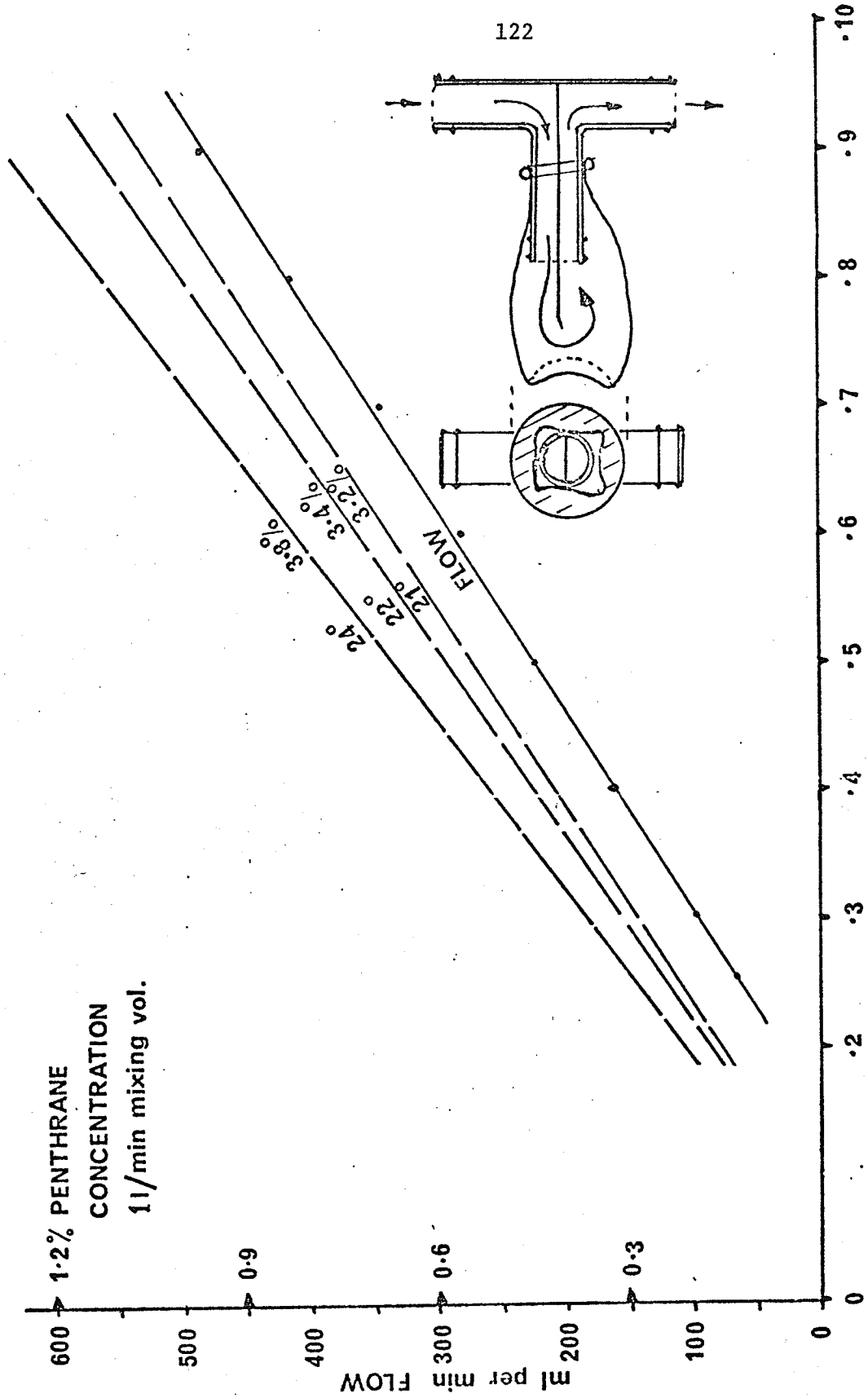
FIGURE 2

Flow calibration curve for the Dwyer 'Wind-Meter', as determined by a bubble flowmeter, and plot of penthrane concentrations versus 'Wind-Meter' reading.

The solid line gives the conversion of "Wind-Meter' reading (left hand scale) to air flow in ml/minute for the flow range of 50 to 500 ml/minute.

The three interrupted lines give the penthrane concentrations for three temperatures, in the air flow delivered to the animal.plotted against the wind meter resding, assuming a mixing air flow of 1 litre/minute. The percentage figures following the temperatures gives the saturated penthrane concentrations at those temperatures.

INSET Details of construction of the T tube and mask construction. The positon of the metal partition in the side tube of the plastic T tube (¼"O-D) can be clearly seen. Arrows indicate the flow path of the gasses.


1·2% PENTHRANE
CONCENTRATION
1l/min mixing vol.
ml per min FLOW
600
500
400
300
200
100
0
0·9
0·6
0·3
·2
·3
·4
·5
·6
·7
·8
·9
·10
WIND METER READING
FLOW
24°
22°
21°
3·8%
3·4%
3·2%

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PROGRAMMABLE NANOAMPERE CONSTANT CURRENT SOURCES FOR IONTOPHORESIS*

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Abstract—A number of constant current sources for microiontophoretic drug application to neurones are described, and the limitations of these discussed.

Some practical constant current circuits are also described which have in addition to constant current generation, capabilities permitting pulsed application of current, as well as programming of the current by both analogue and digital signals, thus allowing closed-loop control of drug application.

INTRODUCTION

IONTOPHORESIS is the injection of drugs or dyes in ionic form into close proximity of neurones or other drug sensitive tissues by the application of a current pulse through a micropipette containing the drug.

The dose of drug ejected from the pipette is proportional to current flow, i.e., approximately $n \times 10^{-14}$ g equiv/nA/s where n is the transport number for the particular drug. Since the electrical resistance of the micropipette is high (10–100 M Ω), and tends to fluctuate during the passage of current, the importance of maintaining a constant injection current can be appreciated if a constant rate of drug release is desired.

When the drug is not being ejected, a holding current is applied to the pipette (negative for cations and positive for anions) which reduces the diffusion of the drug from the tip of the pipette and the subsequent contamination of the experimental area.

TECHNIQUES

Past workers (CURTIS, 1964) have relied on what may be called the 'Brute Force' technique, where a high value current limiting resistor is put in series with the pipette and driving voltage source. To approximate constant current conditions this series resistor must have a resistance of at least 10 times the expected maximum value of the pipette resistance (Fig. 1A).

Thus for a 100 M Ω pipette, a series resistance of at least 1000 M Ω (10^9 Ω) would be required. To pass a current of 100 nA through this, a driving voltage of 110 V would be required and the circuit would give 10 per cent current regulation for a 10–100 MΩ change in electrode resistance. One per cent constant current performance would require a series resistance of 10,000 MΩ (10^{10} Ω), and a driving voltage of 1000 V, not to mention the more stringent insulation requirements to prevent leakage currents.

The 'Brute Force' system has the advantage of simplicity and is probably adequate for non-critical studies.

A constant current system devised by Cox and Oliver, and reported by SALMOIRAGHI and WEIGHT (1967) based on the photon current flowing in a vacuum phototube when illuminated, has an effective output resistance of 10^{10} Ω and uses a lower supply voltage (190 V) (Fig. 1B). However, the apparent simplicity of the system is deceptive, as a formidable array of light intensity controls, batteries, and selected vacuum photocells is required.

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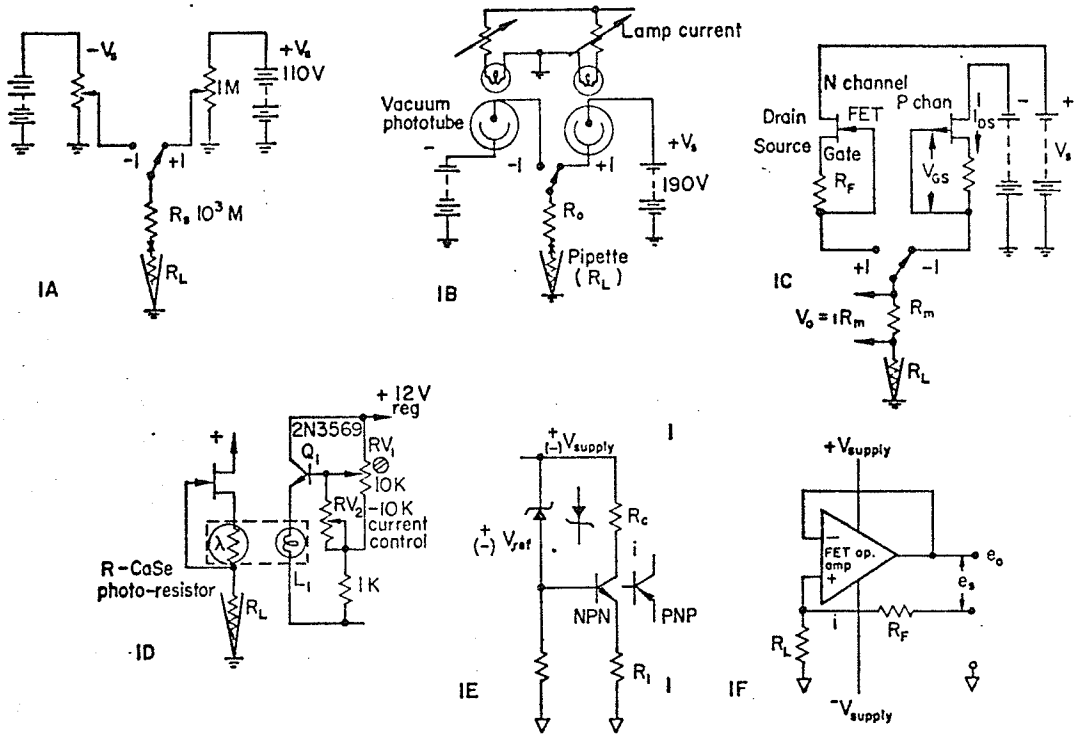

FIG. 1. The six basic constant-current sources described in the text. 1A: The 'Brute Force' circuit: the potentiometers serve to set the driving voltage and hence the current through load. 1B: Simplified diagram of Cox and Oliver's photon current source; the lamp current controls vary the iontophoresis current. 1C: FET feedback constant-current source, Rm is the metering resistance and R_F the feedback resistor. 1D: FET current source with CdSe photoresistor as R_F; the remainder of the circuit constitutes the lamp current control. 1E: Bipolar transistor current source. 1F: FET operational amplifier current source, basic configuration.

BIPOLAR TRANSISTOR CURRENT SOURCES

Bipolar transistors are frequently used as current sources to deliver currents from mA to tens of μA (Fig. 1E). For a given value of *V*ref the value of R_C must be many times the value of R_L for satisfactory performance at low currents, and in addition, the circuit is temperature sensitive. More sophisticated bipolar current sources can be designed (PHILBRICK, 1966). but they become excessively complex compared with the other types to be discussed, and offer no advantages for iontophoresis

FET CURRENT SOURCES

The constant current behaviour of depletion mode field-effect transistors (FET's) is well known and documented (NOLL, 1968). However this constant current behaviour can be augmented very considerably by the insertion of a feedback resistor, R_F, between the source and the gate (Fig. 1), increasing the effective output resistance to the order of 10^{12} Ω (Fig. 1C).

Figure 2 shows the effect of R_F on current regulation when the load resistance (R_L) is varied (curve A) compared with the performance of a FET without R_F (Curve B) and a 'brute force' regulator (Curve C). From this it can be seen that the constant current behaviour of the augmented FET approaches the ideal, provided the voltage developed across the load resistor R_L is less than the supply voltage (V_s) by about 8 V. In addition R_F considerably improves temperature stability. For a given value of R_F an increase in drain-to-source current I_{DS} due to either a change in R_L or V_S results in an increased voltage drop V_{gs} (= I_{DS} . R_F). This results in a compen-

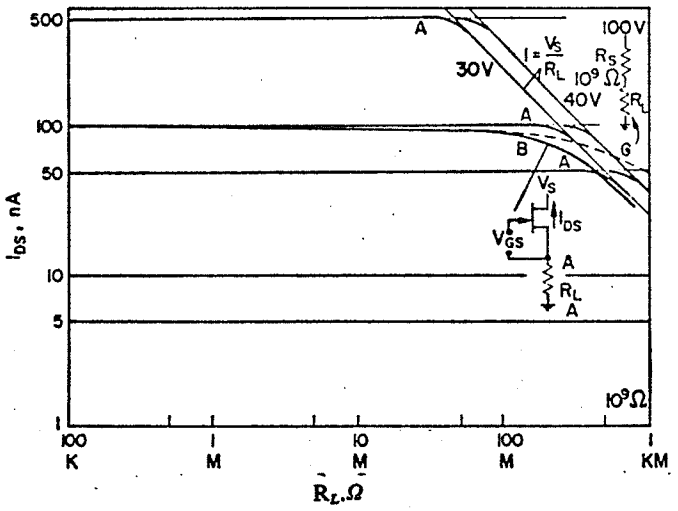

FIG. 2. Plot of current (I_{DS}) v. load resistance (R_L), for 3 different current source configurations. Curves A shows the current regulation attained by the circuit of Fig. 1C. Curve B shows the performance of the lower inset circuit, where the value of V_{GS} is fixed, and the only feedback arises from the voltage developed across the intrinsic gate-source resistance. Curve C (dotted) is the performance of the 'Brute Force' circuit (top right hand corner inset). The curves $I = V_S/R_L$ indicate the limitations imposed by the supply voltage on the maximum current that can be passed for a given R_L.

satory increase in the resistance of the drain-source channel, causing a decrease in I_{DS} and thus maintaining constant current flow through R_L.

The output resistance R_0 can be approximated by the equation

$$R_0 = R_{DS}(1 + g_{fs} R_F) \quad \text{if} \quad R_F > R_{DS}$$

where R_{DS} is the intrinsic drain-source channel-resistance at a given value of V_{gs}; g_{fs} is the transconductance of the FET and decreases with decreasing I_{DS}.

Both *P* and *N*-type depletion mode FET's can be used in this configuration to provide positive and negative current sources. Enhancement mode FET's cannot be used since they require a bias voltage to form the channel which permits drain-source current to flow.

The value of R_F and hence V_{GS} for a given I_{DS} is a function of the particular FET characteristics. Figure 3 shows R_F vs. I_{DS} for a typical FET. Since 1000 MΩ potentiometers are available (Allen Bradley Milwaukee, type J potentiometers—special order), the values for R_F are not unwieldy.

PRACTICAL CONSTANT CURRENT CIRCUITS

Practical iontophoresis circuits require, in addition to the current source, switching and metering circuits to permit alternation from the hold to the injection current polarity, and (with the exception of the operational amplifier systems) to connect the channel use to a current monitoring voltmeter. Where a multi-channel current source, or a current source which must be pulsed, is required, reed relays are normally used to perform the required switching functions, these being activated by remote switches or by a pulse generator (Fig. 4). Such a pulse injection system is frequently used in studies on the mammalian cortex where the pulser is usually permanently connected to the excitatory (glutamate) barrel, while the other channels are operated manually. In dye marking of CNS neurones in invertebrates using Procion Yellow, pulsing of the driving current is essential to prevent disruption of the cell. (KERKUT *et al.*, 1970).

(*i*) *Basic current source*

A 1000 MΩ potentiometer (Fig. 1C, Fig. 4) is used as the current controlling feedback element

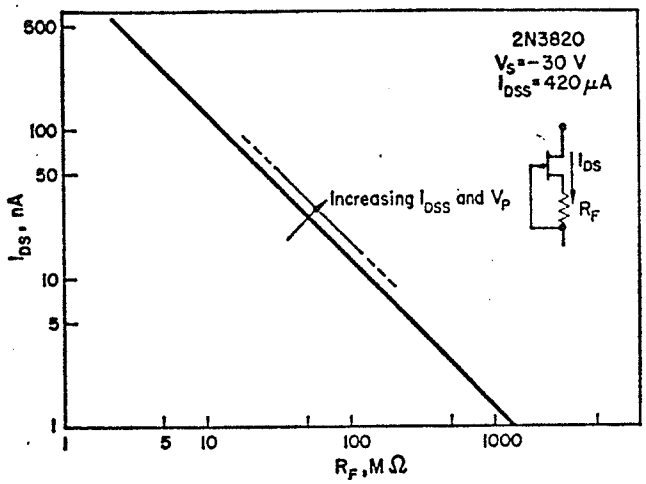

FIG. 3. Plot of feedback resistance, R_F, vs. gate-source current flow for a typical FET. Increasing I_{DSS} (drain to source current with $V_{GS} = 0$ V) or increasing V_p (the value of V_{GS} required to reduce drain-source current flow to 1 μA) results in increasing the value of R_F for a given I_{DS}.

R_F. Since current I_{DS} vs. R_F obeys an acute hyperbolic function (derived from the plot of Fig. 3 $I_{DS} = R_F\, a/c$ where a and c are constants) it helps to use a potentiometer with a negative log taper, if obtainable, to prevent the current adjustment from being crowded at one end of the control.

Positive and negative current is set separately by the + and − potentiometers (R_F). Switch SW2 connects the pipette to either the hold or injection current source. Switch SW1 reverses the polarity of the injection and hold currents when changing from anion to cation iontophoresis.

Current is monitored by the use of a floating high-impedance voltmeter (Fig. 4) or recorder which measures the voltage developed across R_M, the metering resistor. This voltmeter may be switched to any channel by using a 2 pole multi-position switch integral with the channel switch (SW3 Fig. 4), or by using relays (Fig. 4) or even with well insulated metering phone jacks.

Only one pair of batteries is used to supply driving voltage to any number of channels, and these may be mercury or carbon zinc dry batteries. Since the maximum current drain is less than 1 μA (for an 8 channel polarizer) the batteries can be soldered into the circuit as they should last for the duration of their shelf life. The common earth return for the batteries can, if so desired, be isolated from ground and connected to the the preparation in the manner described by SALMOIRHAGI and WEIGHT (1967) via a NaCl-filled pipette to reduce electrotonic disturbance to the preparation.

(ii) *Photo-resistor controlled current source*

Where the 1000 M Ω resistors are unobtainable, cadmium selenide (CdSe) photoconductive cells make satisfactory substitutes (Fig. 10, Fig. 4).

The resistance of these cells falls exponentially with increasing incident light and many types have a dark resistance of the order of 1000–10,000 M Ω, thus suiting them for use as R_F in this circuit. However photoconductive cells have a number of drawbacks, the two most important of which are thermal and voltage dependent changes in resistivity. Cadmium sulphide (CdS) cells suffer badly from voltage dependent effects, and cannot be used in this circuit without taking several precautions, while CdSe cells show less voltage sensitivity. These voltage sensitive changes manifest themselves as erratic shifts in current when changing from hold to inject mode, and can be overcome by shorting the unused current source to ground by additional pair of

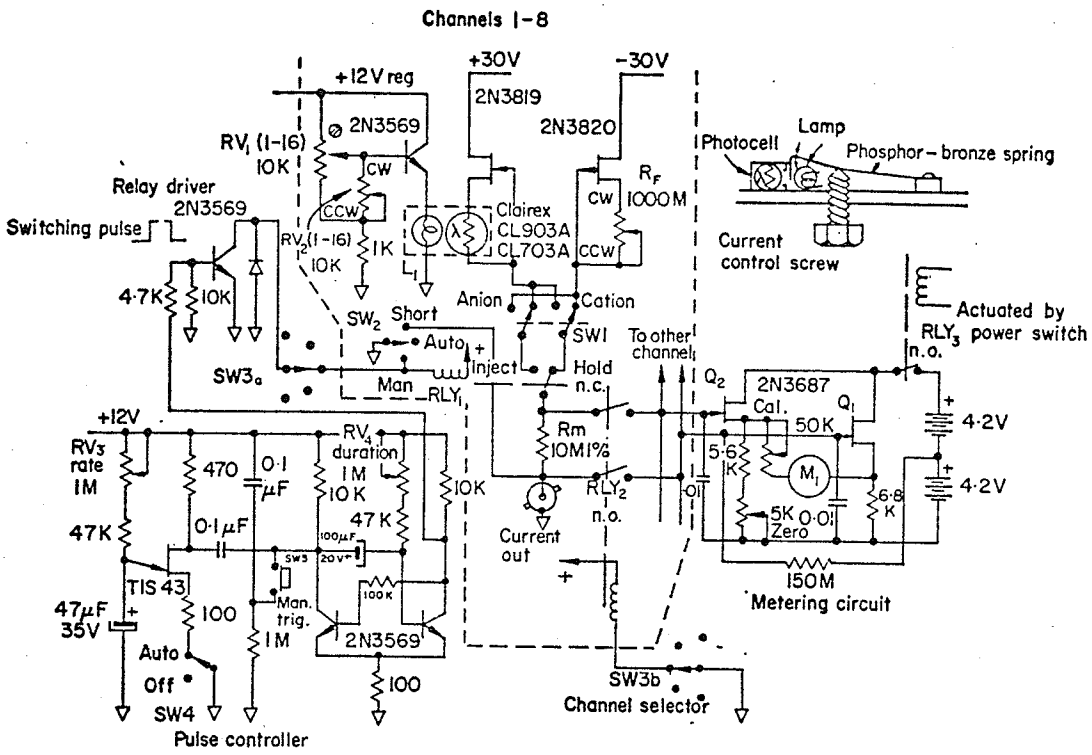

FIG. 4. A multi channel iontophoresis current source (polarizer) using FET current sources. Both types of current source are illustrated and either can be employed. The circuit enclosed in dashed lines is repeated for each polarizer channel required.

The metering circuit is connected to the current source being used by means of a DPST reed relay (RLY_2) actuated either by the channel selector switch or by a separate metering switch. MI is a 100–0–100 or 250–0–250 μA taut band meter. RLY_3, actuated via the external power supply switch, turns off the metering circuit when not in use. As the metering circuit input is floating the circuit must not be grounded and the use of RLY_3 avoids possible leakage path problems.

The lamp L1 is a 12 V, 10 mA type (Chicago lamp type 344), and both the lamp and photocell are mounted axially, in-line, in an opaque metal or plastic mount.

The inset diagram illustrates an alternative photomechanical method of varying injection current. The shutter is a piece of blackened phosphorbronze spring and the photocell is mounted in an opaque housing which has a round window opposite the lamp.

All resistances are ± 5 per cent tolerance ½ W carbon composition types and all timing capacitors are tantalum or low leakage aluminum electrolytes, ± 10 per cent tolerance.

relay contacts activated by R_{LY} 1 to maintain a continuous voltage gradient across the photo cell.

Provided the environment is moderately thermally stable (20–27°C), current changes due to the relatively high temperature coefficient of the photo resistor will not be significant.

The lamp control circuitry consists of a voltage shaping network, which produces a roughly inverse exponential increase in voltage at the base of Q_1, as the current control RV_1 is rotated clockwise. The emitter-follower Q_1 drives the lamp $L1$. Such a voltage vs. rotation characteristic results in a roughly linear iontophoretic current versus rotation of RV_1. The lamp control circuitry must be supplied by a simple stabilized power supply.

As the lamps are operating at a very low radiant temperature (dull red), they require several seconds to reach operating temperature, and the entire current generator should be switched on and stabilized for several minutes before using. The simpler system using a

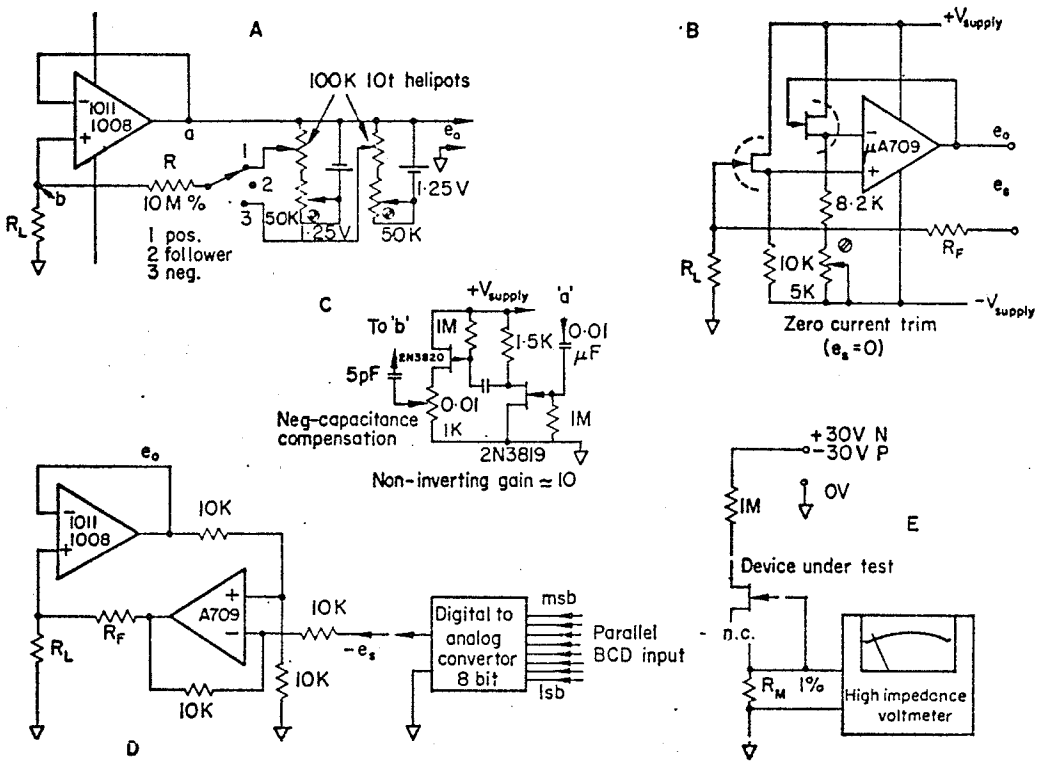

FIG. 5. FET Operational Amplifier Current Sources: A. Basic constant current source with floating reference voltages. Currents are set by the 10 turn helipots. SW1 serves to change polarity or allow the amplifier to operate as a unity gain follower amplifier. This switch can be replaced by a remotely operated DPST reed relay if so desired.

B. Low cost alternative to FET operational amplifier. The FET's may be dual or matched pairs of economy type N channel FET's. The amplifier may be either of the economy I.C. or hybrid variety.

C. Non-inverting low noise, a.c. amplifier with a gain of about 10, for negative capacitance compensation of the amplifier when the amplifier is used in the unity gain follower mode.

D. Use of an additional amplifier to permit the use of earth-referenced control voltages such as those from a stimulator or function generator. A digital-to-analogue converter (Analog Devices DAC 8/H or equivalent) or a simple ladder binary converter permits the current source to be programmed from a digital computer.

E. Test set-up for selection of economy FET's. The metering circuit of Fig. 4 can be used as the voltmeter, or, a high impedance (100 M Ω) VTVM with 15 mV sensitivity can be used; in which case RM will be a 1 M Ω 1 per cent resistor.

1000 M Ω potentiometer is immediately stable and does not suffer from this problem.

An even simpler photo-electric system uses a mechanical shutter instead of the voltage shaping circuit to control photocell illumination. This provides for a finer control of current, but the light supply must still be well regulated. The shutter can be a piece of bent spring bronze (Fig. 4) which is moved across the photocell face by means of a screw. A suitable thread pitch can be chosen to give a 10 turn control for 0–1000 nA current.

Care should be given to the selection of the reed relays used for switching the hold and injection currents. These should be high quality reed relays since many cheap reed relays generate considerable contact bounce and give rise to severe noise artefacts in the recording micro-electrodes.

Selection of FET's. From Fig. 3 it can be seen

that transistors having a high I_{DSS} or V_P (pinch-off voltage; the value of source-to-gate voltage which allows a specified I_{DS}, usually 1 μA, to flow, with a 6 V V_{DS}) will require excessively high values of R_F to pass nanoampere currents. FET's having I_{DSS} of 1 mA or less with V_P in the range 1–2 V, are suitable.

In addition, some FET's exhibit a drain-to-gate leakage current I_{DG} (across the reverse biased junction) in the nano- or microampere range; these are useless for this application. The test circuit in Fig. 5E, can be used to select those types having I_{DG} less than 1 nA; the leakage current can be monitored by the metering circuit of Fig. 4.

The maximum supply voltage (V_S) that can be used is governed by the avalanche breakdown voltage, BV_{DGS}. In the N channel transistors used, this was in the order of 60 V (for the *P* channel types 45 V), and this circuit configuration reduces it by about 20 per cent. V_S should be at least 25 per cent less than this value otherwise the performance will become erratic with increasing temperature.

Economy plastic packaged FET's (Texas Instruments) were used (for current control) in this circuit; about 50 per cent of the *P* channel FET's and 80 per cent of the *N*-channel types were usable. It may be wise to use FET's with more rigidly controlled parameters to avoid the selection procedure—i.e. 2N3687 Siliconix or Texas Instruments. *N* channel and 2N6207 2N2843 Siliconix *P* channel FET's.

(iii) *Metering circuit* (*Fig.* 4)

The voltage developed across the 10 M Ω 1 per cent metering resistor (*Rm* Fig. 4) is read out by a floating differential FET source-follower driving a 100–0–100 or 250–0–250 μA 1 per cent taut-band meter. The gate of one FET, Q_1, is set at 4·2 V by means of a 150 M Ω resistor, and both gates are a.c. bypassed to 0 V by 0·01 μF Mylar capacitors. Reading accuracy is limited solely by the meter accuracy. The follower output can be connected to a potentiometric recorder—provided this has a floating input circuit. The zero potentiometer sets the source voltage at Q_1 to equal that at Q_2 with no current passing through Rm. The CAL potentiometer is used to set FSD with a 1·00 or 2·50 V signal across Rm, the 10 M Ω metering resistor (i.e. FSD reading of 100 or 250 nA). As the current drain of this circuit is low (about 500 μA) the batteries should last for several years with normal use.

OPERATIONAL AMPLIFIER CURRENT SOURCE

The prime advantage of the FET as a current source is the circuit simplicity, stability and low cost per channel. However, due to the nature of the device it is extremely difficult to satisfactorily programme the iontophoretic current remotely, as is sometimes required in experiments where the experimental procedure is part of a closed measurement loop. Such a situation would occur when, say, the glutamate current required to sustain a given level of cell firing is used as an index of cell excitability. For this type of application the operational amplifier current source reigns supreme.

Operational amplifiers have frequently been employed as current pumps, but the majority of circuits are designed to drive a floating load and hence require floating independent power supplied for each current source, if the load is to be earthed. Since operational amplifiers using bipolar transistors normally have input-offset currents that approach or are greater than the current values required for iontophoresis, they will not operate satisfactorily in this role, despite the fact that they are capable of operating extremely well for currents in the milliampere and microampere range.

For the low current applications the FET Operational amplifier is a far more versatile current source, despite the increased cost.

The basic circuit is shown in Fig. 1F. As can be seen the configuration is essentially that of a unity-gain follower amplifier, with the addition of a current resistor R_F and a floating voltage source e_s.

The current output of this circuit can be given by the equation:

$$I_L = \frac{K.e_s}{R_F}$$

where I_L is the output current; R_L is the load resistance (microelectrode resistance); K is a constant which depends on amplifier gain and is < 1; R_F is the current controlling resistor and e_s is the current controlling signal voltage. The voltage e_s can be supplied by a floating mercury cell or battery and a 10 turn voltage divider (Fig. 5A), or from another operational amplifier when e_s can be referred to ground (Fig. 5D). As the polarity of the driving voltage e_s determines that of the output current, the one current serves to apply currents of both polarities.

The maximum current that can be forced through R_L and hence the maximum IR drop across it depends upon the maximum common mode voltage of the amplifier, commonly $\pm$ 10 V for V supply of $\pm$ 12 V, or 100 nA through a R_L of 100 M Ω. Thus for high current flow through high electrode resistances, computing type FET operational amplifiers with $\pm$ 100 V input voltages must be used.

The effective output resistance is normally of the order of 10^{11} Ω and is a direct function of amplifier open-loop gain and input impedance. For applications where current accuracy of $\pm$ 1 per cent for 20 : 1 changes in R_L is adequate and when R_L max. is limited to about 50 M Ω (most applications) a low cost alternative to a FET operational amplifier can be constructed using a matched pair of N channel FET'S and a μA 709C or equivalent operational amplifier (Fig. 5B). The trimmer resistor in the negative input leg of the FET follower serves to set iR_L to zero for $e_s = 0$. All amplifiers may need external compensating capacitors for high frequency stability, especially the integrated circuit types.

Practical circuitry

In the circuit of Fig. 5A, the currents of either polarity can be dialled directly (to an accuracy of $\pm$ 2 per cent by means of the 10 turn potentiometers (Helipots). The trimmer resistors in series with the potentiometers are used to set the full-scale current value with the potentiometers set at maximum voltage position (dial reading 100) while the current is monitored using a high impedance voltmeter to measure the voltage drop across a precision metering resistor (Fig. 5E). As most commercial operational amplifiers have provision for offset adjustment, this adjustment must be used to zero the offset current with $e_s = 0$ (potentiometer dial reading 000) prior to setting the full scale output current.

As the circuit is basically a unity gain follower, open circuiting R_F allows the circuit to be used in this mode. The switch (Fig. 5A) permits selection of +ve, −ve or follower mode of operation, or, for remote control, relays can be used to select the mode of operation, the amplifier being mounted on an electrode-head amplifier. It is also feasible, as shown in Fig. 5C, to add a simple low gain, low-noise, non-inverting a.c. amplifier to permit input capacitance compensation when the amplifier is used in the follower mode. The augmented μA 709 Fig. 5B, is not suitable for use in the follower mode.

Electrode resistance R_L can be measured directly by injecting a known current (say, 10 nA) into the electrode, and measuring the output voltage e_0 which will be exactly equal to the iR_L drop.

Where the current is to be externally controlled, as in the closed-loop situation, or where a computer is used to control or programme the current parameters the circuit of Fig. 5D can be used. To prevent offset current causing spurious responses, the offset currents in both amplifiers should be zeroed. A binary or digital to analogue converter connected to the input of the summing amplifier as shown will permit computer control of the current; current resolution will depend upon the number of binary inputs, 8 bits giving a maximum $\pm$ 0·20 per cent resolution depending on the quality of the converter.

As the summing amplifier shown operates in the inverting mode, the polarity of i_L for a given e_s is inverted.

Pulsed current controller

A monostable circuit triggered by a unijunction transistor (UJT) (Fig. 4) oscillator acts as the injection controller. This permits any selected channel to inject ions for the duration of the monostable on-period, which can be varied from 5 s to 90 s by RV4.

Repetition rate is governed by the UJT oscillator, and RV3 varies this rate from 0·75 to 10 pulses min^{-1}.

Switch SW5 permits manual triggering of the monostable, and SW4 selects the mode of operation either automatic (repetitive) or manual (one-shot) triggering of the monostable.

The output of the controller is used to switch the relays by means of the switching transistors. The selector switch SW3 selects the channel to be pulsed by connecting the relay (RLY1) of that channel as the load of the switching transistor.

Other applications

The FET and operational amplifier circuits can be used to give much higher currents than several hundred nanoamperes. The FET's show constant current behaviour up to several mA, the maximum current being proportional to I_{DSS} of the device. The magnitude of the value of R_F can be extrapolated from Fig. 3. Similarly for the operational amplifier, decreasing R_F for a given value of e_s increases the current. As a result the current sources are valuable for a wide range of applications requiring constant current ramp generators, constant current stimulators, etc. As the FET circuit is insensitive to changes in supply Voltage provided it is below the avalance breakdown voltage BV_{DSS}; it can be placed in series with the output of a conventional stimulator to yield a pulsed constant current stimulator with a maximum output current capacity dependent upon the FET employed.

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NOTES ADDED IN PROOF

Unfortunately while it has been possible to obtain 1000 M Ω potentiometers, it has not been possible to obtain potentiometers with the required logarithmic taper. To circumvent the considerable problems of current adjustment at the high current end when using the circuit of Fig. 1C, it has been necessary to insert a 10 M Ω potentiometer in series with the 1000 M Ω one. This provides a more than adequate measure of control, and for low current settings the 10 M Ω potentiometer acts as a vernier.

In the circuit of Fig. 4, current leakage problems have arisen because of decreases in leakage resistance of the switch SW2 with use. The best approach appears to be to use a separate high quality push-button switch for setting current.

Although high humidity levels are seldom encountered in modern laboratories, we have encountered some leakage problems arising from this source. Careful degreasing of the components involved in the current generators and switching circuits, as well as coating the current generator control boards with a non-hygroscopic lacquer will avoid most problems.

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SOURCES DE COURANT NANOAMPÈRE CONSTANT PROGRAMMABLE POUR IONTOPHORÈSE

Sommaire—On décrit et l'on discute des limitations d'un nombre de sources de courant constant pour l'application de drogue microiontophorétique à des neurones.

On décrit également quelques circuits de courant constant pratiques qui en plus de la génération d'un courant constant sont capables de permettre l'application pulsée de courant, ainsi que la programmation du courant par signaux analogiques et digitaux, permettant ainsi le contrôle par chaine fermée de l'application de drogues.

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TECHNICAL CONTRIBUTION

A LINEARLY READING MICRO-ELECTRODE RESISTANCE METER

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CIRCUIT DESCRIPTION

An N-channel depletion mode junction field effect transistor (FET) wired as a constant current source is used to supply the measuring current. The current source configuration used here is insensitive to changes in both load resistance, supply voltage and temperature, provided the voltage drop across the load resistor (in this case the micro-electrode) is at least 2 V less than the supply voltage. With a metering current of between 10 to 20 nA this condition is fulfilled for maximal electrode resistances of 200 MΩ given a 6 V supply, and 500 MΩ with 12 V.

The magnitude of the current flow is determined by the gate-source cutoff voltage ($V_{gs(cot)}$) of the FETs used and the value of the external gate-source resistor. For the FET types suggested here, the 100 MΩ resistor gives a constant current flow of approximately 10 nA; the exact value of this resistor is unimportant in this application. This resistor provides negative voltage feedback to the gate which enhances the characteristic FET constant current behavior considerably.

A second FET in the source follower configuration is used as a high input resistance voltmeter which measures the voltage developed across the micro-electrode without causing circuit loading. Since FET source followers exhibit an

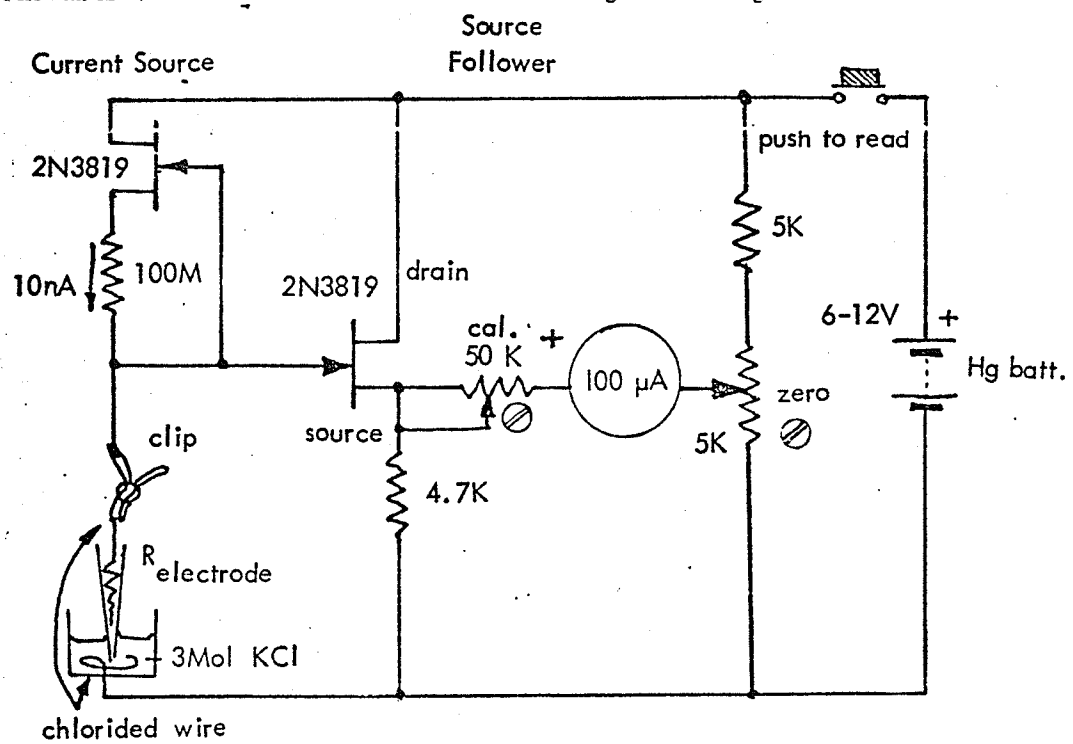

Fig. 1. Circuit diagram of the micro-electrode resistance meter. Resistance values are in ohms and the resistors can be ¼ watt carbon composition types. Although it is advisable to use a high stability resistor as the current controlling resistor, it is not essential. The two trimmer potentiometers are cermet or carbon types. The circuit can be constructed in any suitable manner, but care must be taken to prevent contamination of the input and current source circuitry with saline or KCl solutions, as this would result in erratic and erroneous readings.

output voltage offset, the meter is connected between the follower output and a 5 kΩ zero potentiometer to permit zeroing the meter with the input short circuited (0 Ω). A 50 kΩ calibration potentiometer in series with the 100 μA meter, is used to set the meter to full scale with a 100 MΩ precision resistor across the input. Larger resistors may be used if desired, depending on the resistance range to be covered. Once calibrated initially the instrument should require no further calibration.

Accuracy is limited only by the accuracy of the resistance used to calibrate full scale deflection on the meter, and the meter accuracy. However, it is advisable to use Ag/AgCl electrodes to make connection to the micro-electrode and the KCl bath into which the micro-electrode dips. This will reduce the development of voltage offsets which would sum with the voltage developed across the micro-electrode by the constant current source and so produce erroneous readings. When not in use it is good practice to short the Ag/AgCl electrodes together and leave them in the KCl solution to prevent offset voltages developing.

Any suitable battery may be used to power the circuit, but mercury batteries are recommended for their long shelf life characteristics, as the total current drain of the instrument will be small (4 mA) and intermittent.

SUMMARY

A nanoampere constant current source forces current through the micro-electrode. The voltage so developed is measured by a simple, high input resistance field effect transistor voltmeter and is directly proportional to the micro-electrode resistance.

Since a low (10 nA) current is used, electrode polarization and thermal damage is prevented. Electrodes with resistances in excess of 500 MΩ can be measured.

RESUME

APPAREIL DE MESURE DE RESISTANCE DE MICRO-ELECTRODES EN LECTURE DIRECTE

Une source de courant constant en nanoampères fait passer le courant au travers de la micro-électrode. Le voltage ainsi obtenu est mesuré par un voltmètre simple (transistor à effet de champ) à résistance d'entrée élevée et directement proportionnel à la résistance de la micro-électrode.

Etant donné qu'on utilise un courant faible (10 nA), la polarisation de l'électrode et la détérioration thermique sont évitées. Il est possible de mesurer des électrodes avec des résistances dépassant 500 MΩ.

Reference: SPENCER, H. J. A linearly reading micro-electrode resistance meter. *Electroenceph. clin. Neurophysiol.*, **1971**, *31*: 518–519.

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TECHNICAL CONTRIBUTION

AN EPOCHAL RATEMETER FOR NEUROPHYSIOLOGICAL STUDIES

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In many neurophysiological investigations it is desirable to have continuous records of the changes in nerve firing rates in response to various experimental manoeuvers. This is especially true in studies involving iontophoretic application of drugs to neurones, where the increase or decrease in neuronal firing rates following drug release is the most commonly employed index of the drug's activity.

The simplest and most commonly employed technique for measuring mean firing rates is to use an "integrator" type ratemeter, which is most commonly a diode "pump" ratemeter (Hemmingway 1966) feeding a pen recorder. Unfortunately, although such devices are often found as "extras" on many polygraphs, they suffer from the disadvantage of a fast response to increases in firing rate, and a very much slower response to falling firing rates, an effect which causes considerable temporal distortion in records of firing frequency. In addition, the response is usually markedly non-linear.

Instantaneous ratemeters (Crowe 1965), which provide a beat-by-beat record of firing frequency, yield probably the most information-filled records. For firing rates in excess of 4–5 impulses/sec, however, the high frequency response limitations of most pen recorders prevent them from following the changes in firing frequency, especially if the signal contains pulse pairs and bursts.

In many of the laboratories carrying out iontophoretic research, a digital epochal ratemeter is used (Phillis and Tebecis 1967; Boakes *et al.* 1971). This generally consists of a commercial digital counter coupled to a digital to analogue converter (DAC), together with some form of sample and hold circuit. The neuronal impulses are counted by the digital counter for the duration of the sampling period (epoch), and the binary coded decimal output from the counter is decoded by the DAC and read out on a potentiometric recorder. If the counter has a hold facility, the DAC output for the preceding epoch is displayed for the following epoch. If there is no hold facility, an analogue sample and hold circuit must follow the DAC to achieve the same result.

This method has the advantage of reducing the excess information contained in an instantaneous firing rate record by averaging the firing rate over the epoch period so that a potentiometric pen recorder can follow changes in firing frequentcy. The output voltage is a linear function of the number of impulses counted during the sampling period which permits direct comparison between records.

Unfortunately, however, the conventional apparatus used is very expensive. The circuit described here performs exactly the same function as the digital circuit for a fraction of the cost, by using analogue pulse counting techniques.

Basically the circuit (Fig. 1) consists of a transistor "pump" staircase waveform generator (Hemmingway 1966), which produces output voltage steps of equal height for input pulses of identical amplitude, until the "pump" transistor becomes saturated. In contrast, the successive steps produced by a simple diode "pump" decline in amplitude as the output voltage rises, giving rise to severe non-linearity of response, if used in this application. The transistor pump circuit has an extremely high off resistance ($>10^{10}\Omega$) and does not cause any appreciable decay of the charge on the capacitor C_2 between pulses. After a time interval (epoch) determined by the timing oscillator, the voltage on C_2 is sampled and stored on C_3, following which C_2 is discharged to ground by the switching transistor T_s. The incoming pulses from the charge monostable fed into the transistor "pump" then proceed to recharge C_2 for the duration of the next epoch, when the new voltage on C_2 is again sampled, stored on C_3 and read out via the follower amplifier.

The output voltage with respect to time is thus a stepped histogram (Fig. 2), the height of each step being a linear function of the number of accumulated counts for the preceding epoch.

As previously mentioned, the step height (V_{out}) of the staircase generator is a function of the amplitude of the input pulse height, (V_{in}), *viz.*;

$$V_{out} = \frac{V_{in} \cdot C_1}{C_1 + C_2}.$$

Thus the "span" potentiometer on the charge monostable circuit output and its associated emitter follower, permits variation of V_{in}, and hence the number of accumulated pulses from less than 50 to more than 400, for full scale output.

With the exception of the transistor "pump" the remainder of the circuits in this ratemeter are adaptations of conventional circuits (Fig. 1).

The timing oscillator is a unijunction oscillator and, with the component values given, the sample rate can be varied from between 10 sec to 1 every 10 sec, by means of the "sample interval" control. The ouput pulses from this

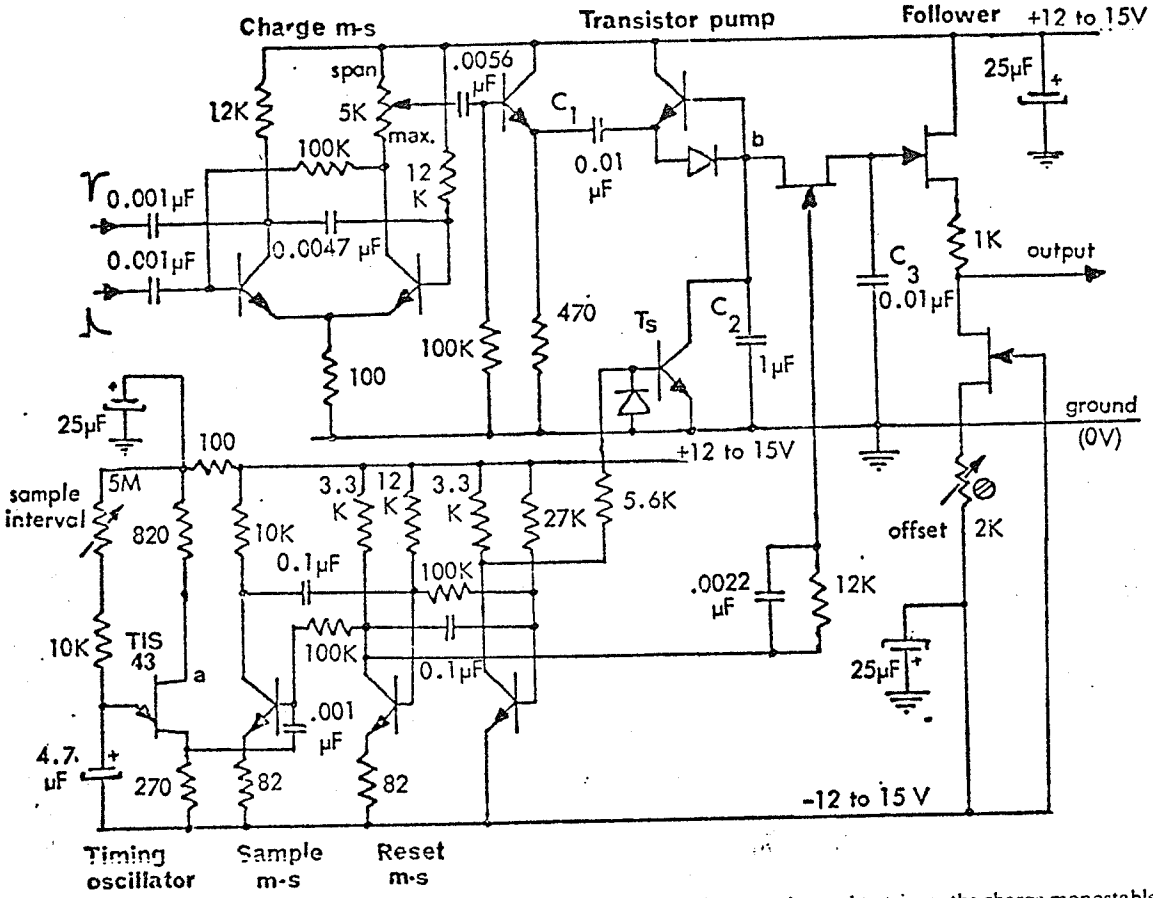

Fig. 1. Circuit of the epochal ratemeter. Either positive or negative going spikes may be used to trigger the charge monostable as indicated. All bipolar transistors excepting T_s are low power general purpose silicon transistors; T_s is a switching transistor 2N 3569 or equivalent. Field-effect transistors are 2N 3819 or equivalent and all diodes are silicon 1N 914 or equivalent. All resistors are 0.5 watt 5% tolerance types and the values are given in ohms. C_2 may be a low leakage tantalum electrolytic capacitor if desired and C_3 should be a polyester or mylar type. Power supply voltages should be equal and well regulated. If desired, the "sample interval" control and the 4.7 μF capacitor can be replaced with switch-selected pre-set values of capacitance and/or resistance to permit rapid changing of the sample interval time.

oscillator trigger the cascaded monostable circuit, the first section of which generates the 500 μsec sampling pulse, followed by the 1 msec reset pulse from the second section. An N channel junction field effect transistor (FET) serves as a sample switch, permitting C_3 to charge to the voltage on C_2 during the sample period. Since C_3 is less than 1% of the value of C_2 there will be a correspondingly small change in the voltage on C_2 as a result of the sampling, and this will be a constant factor for all voltages on C_2.

The current-biased (Spencer 1971) source-follower isolates the charge on C_3 from the output, and permits the output offset voltage to be set at zero by means of the "offset" control.

Drift in the output voltage resulting from decay of the charge on the capacitor C_3 during the sample interval is low. For an output voltage of 5 V, the drift is approximately +0.2 V over 10 min. However, for FETs with significant gate leakage current, this drift could increase.

By connecting the output source-follower directly to C_2 (at point "b") an alternative display, cumulative count record, can be generated. In this display the original instantaneous rate information is retained as the slope of the trace, while the total count between epochs is, as before, the height of the trace. This mode of display may be of value when investigating slowly firing units whose firing behaviour is extremely erratic. In this mode the epochal duration would normally be made much longer (1–20 sec), and as C_2 is 100 times the value of C_3 the output drift with time will be correspondingly 100 times less.

Calibration can be performed most readily with the aid of a calibrated oscilloscope and a calibrated pulse generator (*e.g.*, stimulator). The procedure is as follows: with no pulse input, and the "sample interval" control at minimum resistance, set the output voltage to 0 V with respect to ground by means of the "offset" control. Connect the oscilloscope probe to the point "a" in the circuit diagram and calibrate the "sample interval" control. Set the sample interval to 1.0 sec, and with the "span" control at maximum setting, feed in a

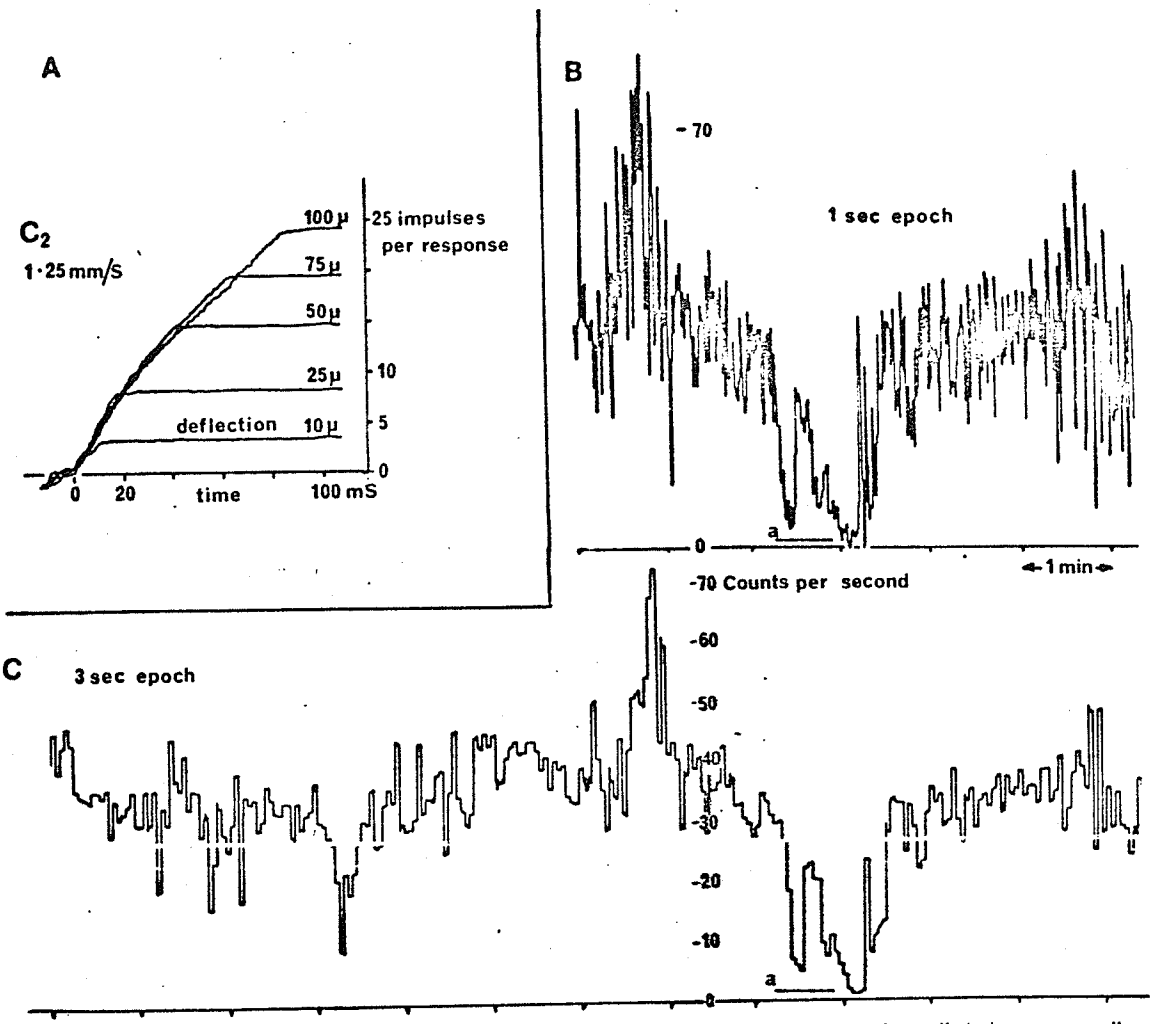

Fig. 2. *A*: Cumulative count curves of the firing of a cockroach (*P. americana*) trochanteral phasic tactile hair receptor cell to stimulation with 1.25 mm/sec ramp mechanical stimuli causing hair tip deflections of 10–100 μ (numbers on the curves). Each curve represents the average of 10 responses for each deflection value; the curves, generated by the circuit described in the text operating in the cumulative count mode, have been averaged with a Computer of Average Transients (CAT) and the resultant curves plotted out with an X–Y recorder (Spencer, unpublished data). *B* and *C*: Part of an epochal rate firing record of a spontaneously firing cat cerebral cortical neurone (700 μ depth). At point "a" iontophoretically applied N. Adr. (40 nA), caused depression of firing. The same firing record, recorded on magnetic tape, was used to generate the two records, *B* and *C*, each having different epochal periods (*B*, 1 sec; *C*, 3 sec) to illustrate the effect of epochal interval on the resolution of the displayed data. The Y axes of both records have been calibrated in terms of counts per second rather than counts per epoch, to make comparison easier, and both records have the same time axis. The portion of lower trace, *C*, preceding that illustrated in *B* has been included to illustrate the general nature of the epochal firing rate record. (Data courtesy of L. Jordan.)

train of pulses with a frequency equal to the desired number of full scale counts (say 100). Adjust the span control until the desired number of steps for full scale output equals 5 V (recommended maximum output voltage), as monitored by the oscilloscope or by a high impedance voltmeter connected to the output. The "span" control, if not to be a pre-set adjustment, may then be calibrated by marking the control positions producing other division ratios (50, 100, 200 full scale counts (FSD), etc.). Changing the sample interval does not alter the full scale counts per epoch calibration.

It is not advisable to connect a load resistance smaller than 20 KΩ to the output, since this can degrade output linearity.

SUMMARY

A simple analogue epochal ratemeter is described, which produces an output voltage linearly proportional to the number of impulses counted during a pre-set sample interval.

This circuit is primarily designed for the analysis of

changes in nerve cell firing rates following micro-iontophoretic application of drugs, and it performs the same function as the more complex and expensive digital equipment normally employed. Nevertheless the circuit is eminently suitable for continuous recording of the average firing frequency of any pulse source. A simple modification is also described which permits the circuit to generate cumulative count curves.

RESUME

UN ANALYSEUR DE FREQUENCE ANALOGIQUE POUR ETUDES NEUROPHYSIOLOGIQUES

L'auteur décrit un appareil simple d'analyse de fréquence analogique, avec une sortie linéairement proportionnelle au nombre d'impulsions comptées au cours d'un intervalle d'échantillonnage pré-établi.

Ce circuit est désigné essentiellement pour l'analyse des taux de décharge de cellules nerveuses après applications micro-iontophorétiques de drogues; il réalise la même performance que les équipements digitaux plus complexes et plus coûteux habituellement utilisés. Néanmoins ce circuit est éminemment adapté à l'enregistrement continu de la fréquence de décharge moyenne de toute source d'impulsions. Une modification simple est également décrite permettant à ce circuit de produire des courbes cumulatives de comptage.

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Physiology and Behavior, Vol. 10, pp. 977–979. Brain Research Publications Inc., 1973. Printed in the U.S.A.

BRIEF COMMUNICATION

Integrated Circuit Animal Heater Control

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SPENCER, H. J. *Integrated circuit animal heater control.* PHYSIOL. BEHAV. 10(5) 977–979, 1973.–A heating pad regulator using a low cost integrated circuit voltage regulator is described. A single control serves both to set and measure the animals' body temperature, as sensed by a rectal thermistor probe. Since the controller is d.c. operated it is suited for use in shielded rooms. A wide variety of heating pads may be used and a method of fabricating these pads is described.

Heating pads Temperature monitoring Thermostat Animal temperature controller Thermistor probe Noise free

MAINTENANCE of constant animal body temperatures during prolonged neurophysiological procedures presents a number of problems stemming chiefly from the importance of preventing a.c. interference to the low-level recording amplifiers. Circulating water baths and d.c. operated heating lamps are two methods commonly used by physiologists, and both tend to be inconveniently bulky and to present problems of temperature control.

The circuit described here, designed to be operated either from storage accumulators which are virtually noise-free high current power sources or, a suitably well filtered d.c. power supply, is eminently suited for this application. A heating pad, upon which the animal's ventral surface rests, can be constructed to form part of the animal support and so not interfere with experimental procedures in any way.

Description

A Fairchild uA 723C integrated circuit voltage regulator (or equivalent) functions as a voltage comparator-switch driver, detecting the error voltage from the temperature sensing bridge circuit and generating a amplified drive voltage to turn the heater current controlling transistor on or off.

Since the gain of the circuit is relatively low and the thermistor response time is in the order of 2–3 sec, no rapid switching transients are produced to cause interference to recording apparatus. The controller dead zone is about ± 0.05° C.

A light emitting diode (LED) or incandescent lamp is used both as a heating indicator and to indicate the output transition point when the controller is used in the temperature monitoring mode.

A high current, high H_{FE}, NPN silicon transistor (2N3055 or equivalent) is employed as the switching transistor and should be mounted on an adequate heatsink (0.5 square in. per W dissipation). A Yellow Springs Instrument (YSI) precision thermistor is used for the rectal probe. These thermistors are supplied with a resistance versus temperature calibration table, permitting the controller to be calibrated by substituting a precision decade resistance box for the thermistor; rather than by using the conventional water bath.

The probe is constructed as illustrated. Use of either heat-shrinkable tubing or chloroform-soaked PVC tubing to protect the junction between the thermistor tube and the cable, is strongly advised. If other types of thermistor beads are used, they can be mounted in a similar fashion to that illustrated.

Heating Pads

Heating pads, if unobtainable, can be fashioned out of resistance wire. A suitable level of dissipation appears to be 1.5 W per square in. of heating pad area. Normally the pad is a flat or slightly curved plate, upon which the animal's abdomen rests. Since the requirements for heating pads vary considerably only general outline of the construction techniques will be given.

Once the area of pad required is determined, the required wattage is calculated. Given a suitable supply voltage (6, 12, 18 or 24 V assuming the use of lead-acid accumulators) the pad resistance required for such a power level is calculated; for example, for 12 W heating power from a 6 V supply, 2 A of current must flow through a heater resistance of 3 Ω.

Choose wire of suitable gauge and resistivity to yield both the required resistance and of sufficient length to

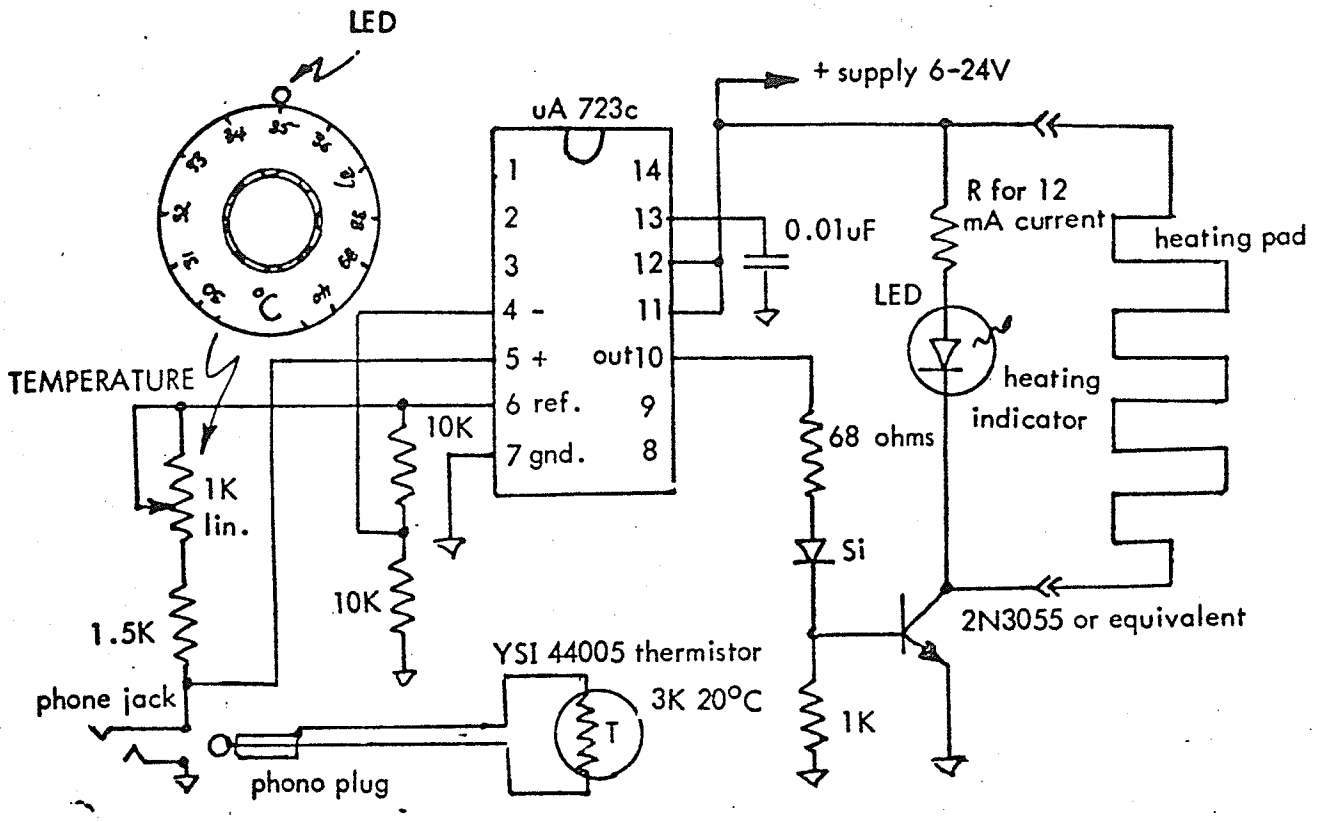

FIG. 1. Controller circuit. All fixed resistors should be 5% tolerance 1/2 W types, and the Temperature potentiometer should be either moulded carbon or wirewound. The resistor in series with the LED (which maybe any suitable variety) should be adjusted to allow the LED to glow at a a suitable brightness level when the heater transistor is in the on condition.

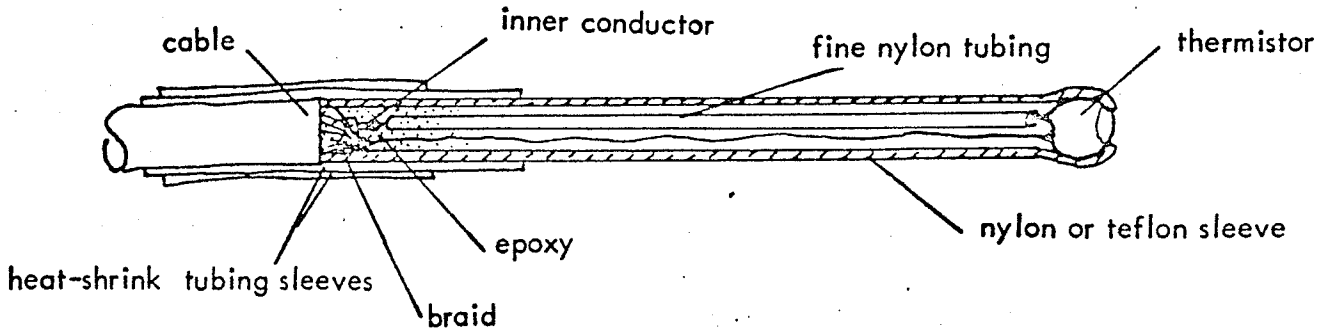

FIG. 2. Construction of the thermistor rectal probe. A YSI thermistor is illustrated. Regardless of the nature of the thermistor bead employed, the junction between the thermistor body and the teflon or nylon sleeve must be watertight – RTV silicone adhesives can be used to seal the junction.

adequately cover the heating pad area (at 1/4–3/8 in. spacing between wires). In this context it may be noted that a single run of heavy wire may not be as satisfactory as several parallel strands of finer wire having equal resistances.

If a flexible pad is required, fine insulated wires (20–27 SWG) of equal resistance connected in parallel should be woven through a piece of heavy fabric, ie hessian. Wires, either insulated or uninsulated can be stretched over nails driven into a piece of timber, and heavy thread woven about them, and this pad is then coated with a thin flexible laquer such as Formvar, and baked dry. Alternately the stretched wires can be sandwiched between cloth using a suitable adhesive (RTV silicone rubber or epoxy).

Calibration

Calibration is performed by substituting a precision

resistance box for the YSI thermistor probe, and with the power supply and heater connected, set the resistance value for the desired temperature and mark the temperature control knob setting at which the light just turns on accordingly. Steps of 1.0°C are adequate. If the YSI thermistors are unavailable, the values of the temperature control potentiometer and the 1.5 K series resistor may have to be altered to allow a temperature span of 30 to 40°C to be covered and a water bath will have to be used to calibrate the unit.

Body temperatures are read by rotating the temperature dial until the light just comes on. If care is taken with calibration, temperatures can be read to ± 0.2°C.

Some form of insulation, such as a folded paper towel should be placed between the pad and the animal to prevent the formation of local hot spots caused by reduction in the peripheral circulation of the animal. The rectal probe should be inserted as far up the rectum (2–3 in.) as possible, and taped to the tail base to prevent its falling out and so causing the animal to become overheated.

Separatum EXPERIENTIA *29*, 1577 (1973)
Birkhäuser Verlag, Basel (Schweiz)

A Microlathe for Constructing Miniature Multibarrel Micropipettes for Iontophoretic Drug Application

Various techniques for the construction of multibarrel micropipettes for the micro-iontophoretic application of drugs to neurones have been published. CURTIS[1] has described a large multibarrel electrode (Figure 1) which must be handmade by an experienced glassworker, a limitation which together with the large drug volume required, reduces its utility. HERZ et al. (in KRNJEVIC[2]) have described a technique using cemented glass tubes which are twisted together just prior to pulling. Unfortunately, while this technique produces excellent small-sized electrodes, considerable time must be devoted to acquiring the technique; the electrodes tend to be very variable in their properties and dimensions and the tip length can seldom be longer than 5 mm.

The microlathe described here permits the construction of electrodes having 3 or more barrels with reproducible characteristics and with small (1.5–2 mm) diameter tip extensions of up to 2 cm or more which permits their use in investigating deep brain nuclei. This microlathe, the design of which is based on the conventional glassworkers lathe, allows simultaneous rotation, heating, fusing, and drawing apart of the softened glass electrode blank. The essentials of the lathe are evident from Figure 1.

An aluminium optical bench (B) forms the bed of the lathe upon which a platform (P) can be moved by means of a rack and pinion (R)[3]. It is imperative that the pair of gears on the head and tailstock should have the same

[1] D. R. CURTIS, *Physical Techniques in Biological Research* (Ed. W. L. NASTUK, Academic Press, New York 1964) Vol. 5, p. 144.
[2] K. KRNJEVIC, *Methods of Neurochemistry* (Ed. R. FRIED, Marcel Dekker, New York 1971), p. 129.
[3] Edmund Scientific Co., Barrington N. J., USA; Catalogue Numbers: Bench 60,573, Platform 40,891.

ratios since both chucks must rotate in synchrony. Any suitable gears can be used, those in the lathe described came from a bombsight computer.

Drawing out the tubing is accomplished by moving the rotating tailstock (T) away from the heater (H) while the glass is still soft, by means of a rack and pinion mechanism (R). A keyway in the lower driving shaft (D) engages with a pin in the lower tailstock gear (L) and couples the rotary motion to the tailstock, allowing it to continue to rotate while the hot tubing is drawn out.

A hollow shaft (S) through which the lower drive shaft slides should extend roughly 25 mm on either side of the lower tailstock gear in order to minimise binding of the drive shaft as the tail stock of rocked forward and back. At the outer end of this hollow shaft is fitted the pin which engages with the keyway.

A geared DC motor (M) powered by a simple variable voltage supply so as to give a chuck rotational speed of 60–120 rpm, is used to drive the lathe. Care excerised in the construction and alignment of the lathe so that it runs freely, will mean that only a small drive motor will be required since the softened glass imposes little load on the mechanism. Three-jaw electric drill chucks are used to hold the glass tubing and the inner surfaces of the jaws should be as smooth as possible to prevent shattering the tubing as the chucks are tightened.

A simple 8 to 9 mm diameter single layer heating coil of 20 g Nichrome wire forms the heating coil (H). Power for the heater is obtained through a step-down transformer (10 V 10A) which has a full wave thyristor domestic lamp dimmer in series with the primary winding. Alternatively, a Variac can be used instead of the dimmer.

Electrode blanks are constructed using 1.3 mm (or smaller) diameter thin walled Pyrex capillary tubing. It is preferable to use tubing which is slightly larger in diameter for the centre tube (roughly + 0.01 mm). If the tubing is purchased in bulk, then grading prior to cutting is easily performed using a pair of vernier calipers.

The electrode glass should be pre-cut and the ends fire polished; the side tubes being half the length of the centre tube. Following this the tubes are washed in chromic acid, followed by distilled water, dry acetone and then air dried.

An assembly jig which can be fabricated from a plastic 1 ml 'Tuberculin' syringe (Figure 2a) is used to aid assembly and minimize hand contact with the glass. A pair of spring wire clips can be constructed to hold the bundle of glass together (Figure 1, C) or alternatively the bundle can be bound with fine wire (Figure 2a).

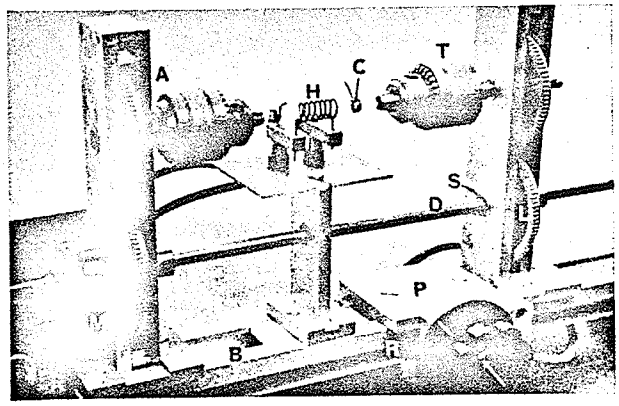

Fig. 1. Overall view of the lathe. The spring clips (C) used to hold the glass bundles together can be clearly seen. The large knob (with arrow) in the lower RHS controls the tail stock movement. To the bottom left of the headstock (A) can be seen the drive motor worm gear and pinion (M). The other parts identified by letters are described in the text.

Heater temperatures and heating time prior to drawing the tubing must be determined empirically. Sufficient heat must be given to fuse the tubes laterally without causing the lumen of the tube to shrink and the rotational rate should be such as to just prevent noticeable sagging of the softened glass bundle, but not enough to cause the softened tubes to fly apart – about 60 rpm appears to be adequate. Some very slight sagging and the resultant rolling action of the softened glass helps the side tubes to fuse to the centre one.

After from 40–60 sec of heating (coil bright yellow) the rotational rate is increased to about 100 rpm while drawing the blank out. It is normally sufficient to move the tail stock back 1 to 1.5 cm to obtain a reasonably long tip length – although if longer electrodes are required the tail stock can be moved as far as desired. The heater should be turned off when the pulling process starts and the electrode blank should be allowed to continue rotating until cool before removing.

The finished blanks (Figure 2a) are then drawn using a conventional vertical or horizontal electrode puller and with as small a heating coil diameter as possible. Both electrodes should be usable.

Centrifugation has proven to be the best method of filling the electrodes since the difficulties of inserting glass fibres into drawn blanks and the vagaries of pulling caused by the fibres makes this method a completely unsatisfactory one for these electrodes. To this end a collar of 22 g copper wire (Figure 2a, C) is slipped over the shank of the electrode (from the tip end) and a small amount of fast-setting epoxy cement is spread carefully around the collar and warmed gently over a small flame. This warming serves to liquefy the epoxy which then flows between the tubes and the collar and then sets rapidly reinforcing this unfused section of the electrode.

A conventional solid centrifuge rotor[4] is used, since swing-out bucket heads produce too much air drag, causing both slow running and overheating of the centrifuge. The rotor is adapted for electrode centrifugation by plugging opposite holes with two identical bored hard rubber stoppers, into which a hard plastic sleeve (against which the wire collar bears) are inserted (Figure 2c). Such sleeves can be constructed from plastic disposable hypodermic syringe needle covers. In order to reduce air drag still further the vacant rotor holes should be taped over on both the top and bottom.

Electrodes are filled using thin (PE 10) catheter tubing, the solutions being filtered through a 0.4 μm 'Millipore' filter before use, and the filling tube rinsed in filtered distilled water before and after inserting into the electrodes. Care must be taken to exclude air bubbles and not to overfill the barrels. Model maker's paints are used to identify the barrels.

Just prior to centrifugation the electrode tips are broken back to give tip diameters of from 3–7 μm, by pushing them, under microscope control, into a polished metal block. This seems to be the most reliable and controllable method of doing this; the electrode is clipped onto the transverse carrier of the mechanical stage, and the block mounted over the condenser lens.

An additional advantage of electrodes constructed in this manner is that a connector can be constructed to connect the drug barrels to the current source (SPENCER[5]), which greatly facilitates the changing of electrodes during an experiment. Such a connector is illustrated in Figure

[4] I. E. C. 800 Series Centrifuge.
[5] H. J. SPENCER, Med. biol. Engng. *9*, 683 (1971).

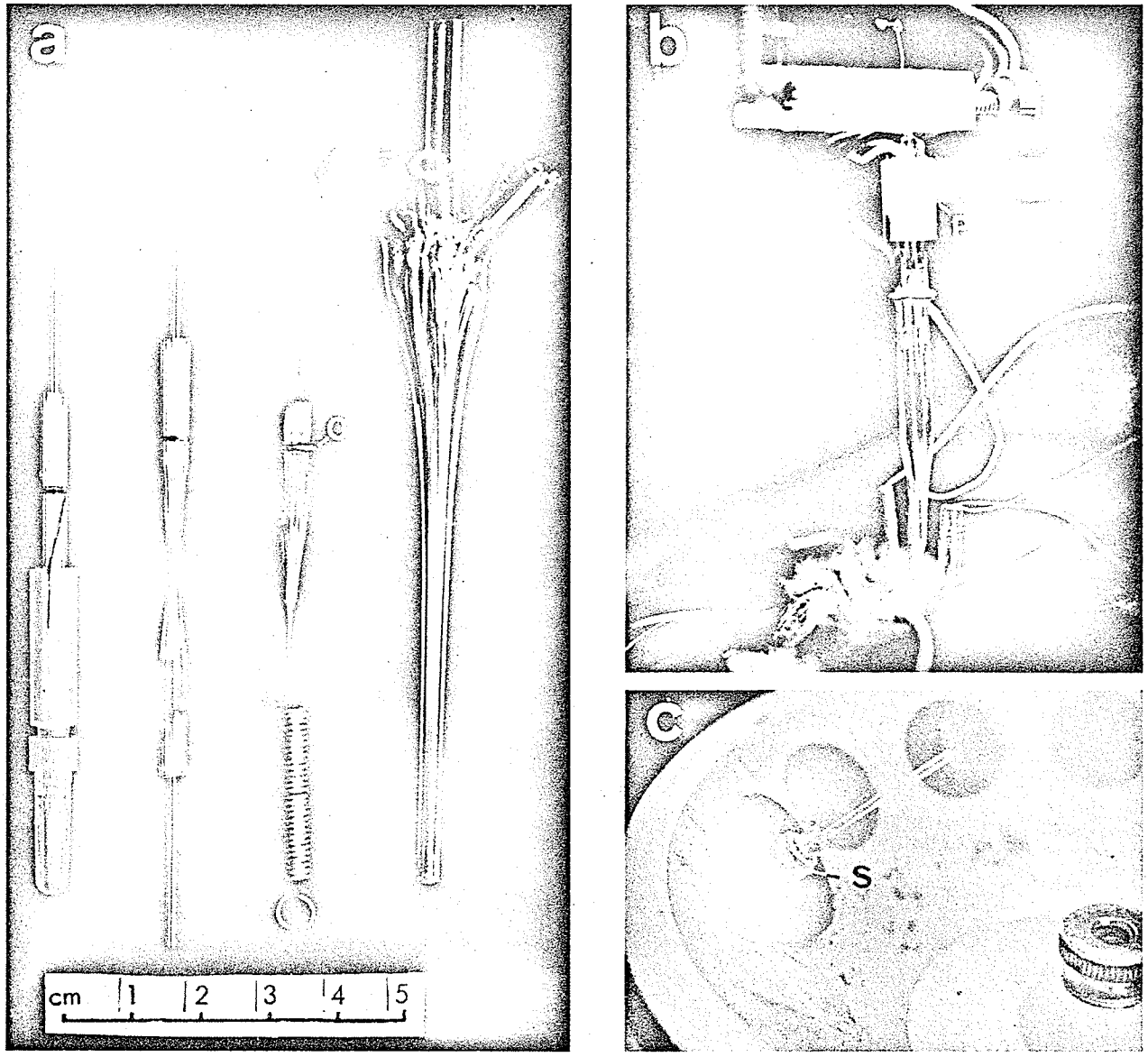

Fig. 2 (a) Stages in the construction of the electrode, together with an electrode of the type employed by CURTIS[1] for comparison. From the left; bundle of glass tubes wired together in the assembly jig; blank electrode assembly following fusion and drawing in the lathe and a finished electrode with the collar (C) fitted after having been pulled in a conventional microelectrode puller and ready for filling. Below the finished electrode is a coil of copper wire from which collar rings (below the coil) can be cut using a pair of diagonal wire cutters. (b) A microelectrode being used to record from the rat caudate nucleus. The connecting plug (P) is constructed from a length of 6 to 7 mm nylon rod (see text). The small vertical rods on the skull are stimulating electrodes. (c) Adapter for filling the electrodes by centrifugation. Since the rotor holes are oblique, the hole in the stopper must be sufficiently large to prevent its narrowing when the stopper distorts under centrifugal force. The plastic support (S) is a push fit into the stopper hold.

2b and can comprise part of the micro-electrode holder. Essentially the connector is constructed from a 6 to 7 mm diameter cylinder of nylon or teflon, perforated with a central hole for the central barrel, with one lateral hole per barrel, into which platinum wire contacts are fitted. The junction between the platinum wire and the cable should be insulated with 'formvar' lacquer or its equivalent and this junction should be within the plastic cylinder to reduce stray leakage paths. The connector should be rinsed after use with distilled water, followed by drying with acetone or absolute alcohol.

Résumé. On décrit un micro-tour pour confectionner de petites microélectrodes à plusieurs pipettes destinées à appliquer des drogues aux voisinage des neurones. Ces microélectrodes ont des tiges droites et leur géométrie et leurs particularités sont très uniformes. On peut faire facilement des électrodes à pointes longes (2 cm) et fines pour les introduire dans les structures profondes du cerveau. On expose aussi une méthode permettant de remplir les électrodes par centrifugation et d'établir un assemblage de connection.

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2 Juli 1973.