THE ELECTROPHYSIOLOGICAL AND NEUROPHARHACOLOGICAL STUDIES OF THE VENTROLATERAL THALAMUS IN THE RAT

BY

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ABSTRACT

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

NEUROPHYSIOLOGY

Doctor of Philosophy

THE ELECTROPHYSIOLOGICAL AND NEUROPHARMACOLOGICAL STUDIES OF THE VENTROLATERAL THALAMUS IN THE RAT.

by Isaac Olukayode Adeoshun

Electrophysiological and pharmacological studies were made on the thalamocortical relay neurones of the ventro-lateral thalamus in the rat. Experiments were performed on urethane anaesthetized male wistar rats of 200 gram weight. The thalamocortical relay cells were identified by two electrophysiological criteria; by stimulating a contralateral hind limb nerve and by the antidromic invasion of the same cell from the somatosensory cortex. Using those two criteria for identification, the cells were then orthodromically activated from the somatosensory cortex. The orthodromically activated principal cells responded to latencies of between 5 and 10 ms with the majority of the cells responding to between 5 to 7.5 ms latency.

The neuropharmacological studies involved microiontophoresis of drugs, their agonists and antagonists. Extracellular recordings were made of neuronal responses by using 6-barrelled iontophoretic electrodes with the recording electrode tip protuding 10-20 μm past the drug barrels.

L-glutamate, L-aspartate, DL-aspartate, DL-homocysteate, N-methl-DL-aspartate, Kainate, Ketokainate and acetylcholine were found to excite most cells studied. Gamma-aminobutyric acid was found to be inhibitory and also depressed L-glutamate excitations. Bicuculline was found to block gamma-amino butyric inhibitions.

Atropine reversibly blocked acetylcholine induced excitation while sparing the amino acids-induced excitations.

The relative potencies of a variety of amino acid analogues were also studied. Kainate was found to be most potent followed by Ketokainate and N-metyl-DL-aspartate relative to L-glutamate and L-aspartate in terms of iontophoresis.

The antagonists, GDEE, DαAA and Atropine were studied on the amino acids induced excitations and acetylcholine. GDEE preferentially depressed L-glutamate, L-aspartate and DL-homocysteate from N-methyl-DL-aspartate induced excitation. In high concentrations GDEE becomes a non-elective antagonist.

DαAA selectively depressed, N-methyl-DL-aspartate, L-aspartate induced excitations and poorly affected L-glutamate and did not affect acetylcholine excitatory responses.

Studies of the antagonists were also made on evoked synaptic excitations both from the stimulation of a hind limb nerve and the orthodromic activation from the somatosensory cortex.

The synaptic excitation of the principal cell at the ventrolateral thalamus from the cortex is more sensitive to the antagonistic effect of DαAA while the orthodromic activation of the same cell from stimulating the hind limb nerve is more sensitive to GDEE. Atropine did not have any antagonistic effect on both orthodromic activations.

In this study it is strongly suggested that L-aspartate is the likely neurotransmitter in the cortico-thalamic pathway.

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ABBREVIATIONS

g	gram
Kg	Kilogram
i.p	interperitoneal
mins	minutes
s	sec
s^{-1}	per second
ms	millisecond
nA	nanoamp
µA	microamp
µm	micrometer
mm	millimetre
mV	millivolt
v	volt
Hz	Hertz
NA	noradrenaline
5HT	5-hydroxytryptamine
ACH	acetylcholine
^{o}C	degree centigrade
mM	millimolar
M	molar
Dα AA	Dα aminoadipate
NMDLA	N-methyl-DL-aspartic acid
Atr	atropine
GDEE	glutamate diethylester
DLH	DL-homocysteate
Glut	glutamate

GABA	gamma-amino butyric acid
HRP	Horseradish peroxidase
P.S.T.H	post stimulus histogram
Asp	aspartate
ARG	Autoradigraphy

GENERAL INTRODUCTION

1.1. CONNECTIONS OF THE VENTRO-LATERAL THALAMUS

The ventro-lateral nucleus of the thalamus (VL) is important among the structures involved in regulation of sensorimotor activities. Angaut (1970)., Angaut and Bowsher (1970)., Kievit and Kuypers (1971)., Mehler, Vernier and Nauta (1958), Mehler and Nauta (1974) have shown from anatomical studies that this nucleus is a common thalamic target for cerebellar outputs. It has been shown that it is also common to pallidal outputs (Grofova and Rinvik, 1974., Kievit and Kuypers, 1971., Kuo and Carpenter, 1973., Mehler and Nauta, 1974., Nauta and Mehler, 1966) and to nigral outputs (Afifi and Kaelber, 1965., Carpenter and Peter, 1972., Cole., Nauta and Mehler, 1964., Mehler and Nauta, 1974., Rinvik, 1975). Moreover, it directly influences the corticospinal pyramidal tract via extensive projections to the motor and premotor cortical areas (Chow and Pribam, 1956., Dennery and Combs, 1961., Smaha., Kaelber and Maharry, 1969, Strick, 1970 and 1976). Other afferent inputs to the ventro-lateral thalamus are the specific sensory fibres arising in the skin, deep tissues, and muscles of the body, which are relayed through the lower brain stem.

There is also a central input from the neocortex. The axons in this pathway arise from cortical pyramidal cells. The axons are termed corticothalamic fibres. These fibres originate in the same areas of the cortex to which the sensory nuclei project. They may thus be regarded as centrifugal fibres feeding back to their input source. Although the anatomical details of this nucleus in relation to the somatosensory cortex is well documented, some of the electrophysiological aspects remain unclear. For this reason, this study was undertaken to electrophysiologically

investigate possible projections from the somatosensory cortex onto the ventro-lateral thalamus and also look at the pharmacology of this pathway.

A vast amount of work has been done on the visual pathway vis a vis from the visual cortex to the dorsal lateral geniculate nucleus in cat (Gilbert and Kelly, 1975; Updyke 1975) and in the rat (Montero and Guillery, 1968; Lund, 1969; Gosavi and Dubey, 1972). Simultaneous application of the HRP and ARG techniques in the rabbit has shown that a very precise reciprocal topography of the geniculocortical and corticogeniculate fibres exist (Colwell, 1975; Karamanlidis and Giolli, 1976). Updyke (1975) has demonstrated by degeneration and ARG techniques in the cat that areas 17 and 18 project to all laminae of the dorsal lateral geniculate nucleus.

Although much work has been done on the corticospinal systems and the pyramidal tract (Gordon and Miller, 1969; Coulter, Ewing and Carter 1976; Cole and Gordon, 1976a), the specific cortico-thalamic interactions seem to have been negleted.

Kemp and Powell 1971b emphasized the central position of the motor cortex as the target and common output of two sets of neural networks. Of these, one originates from most areas of the cortex and converges on the motor cortex by way of basal ganglia and ventro-lateral thalamus in cats. The other network has a similar cortical origin, and converges on the motor cortex by way of pontine nuclei, inferior olive, cerebellum, red nucleus and ventro-lateral thalamus (Eccles et al., 1957).

Jones and Powell, 1968; Grant et al., 1975 have suggested that the inputs arriving in sensorimotor cortex from the ventrobasal (VB) nucleus of the thalamus could be relayed to area 4.
The direct route from thalamus to area 4, that from ventrolateral

nucleus is the other main candidate (Asanuma and Hunsperger, 1975) in cats. Kievit and Kuypers (1975) examined the cells of origin of thalamic afferents to the motor cortex of the monkey by injecting horseradish peroxidase into the precentral gyrus. They studied the distribution of the thalamic cells to which this label had been transported in a retrograde direction from their axon terminals in the cortex. From the results of their thalamocortical mapping it was concluded that area 4 received thalamic input from the ventro-lateral nucleus. Strick, (1976) also reaffirmed this observation. Many other pathways are also available, through the somatosensory receiving cortex involving VL thalamic nuclei (Pandva and Kuypers, 1969 Rosen and Asunuma, 1972; Welt et al, 1967; Asanuma et al, 1968 and Asanuma and Fernadez, 1975).

1.2. MORPHOLOGY AND CYTOARCHITECTURE

The thalamus consists chiefly of gray matter, within which there are a number of recognizable masses. The nuclei of the thalamus have been named from their positions in the subdivisions of the thalamus. There has not been strict adherence to directions referable to the anatomical position of the primate body in these names. The thalamus (and forebrain) is described as having a dorsal or superior surface which is toward the vertex of the head, and ventral or inferior surface opposite; anterior is toward the frontal poles of the cerebrum, posterior toward the occiput: medial and lateral areas.

The nuclei of the thalamus have been classified morphologically into five groups.

(1). The anterior thalamic nuclei occupy the anterior part of the thalamus and are separated from the remainder of it by the diverging limbs of the internal medullary lamina. The anterior group of nuclei receives fibres from the mammilothalamic tract

and sends fibres to the cerebral cortex in the gyrus cinguli. Functionally they are associated with relays involving the olfactory connections.

(2). The nuclei of the midline are clusters of cells situated close to the wall of the third ventricle or in the massa intermedia. Some of these nuclei project to the cortex of the anterior portion of the rhinencephalon and possibly to adjacent gray masses. They are also connected with the hypothalamus and so must be involved in visceral functions.

(3). The medial nuclei of the thalamus lying between the nuclei of the midline and the internal medullary lamina number at least seven. Two of these nuclei are of particular significance. They are (a) nucleus medialis dorsalis which has two parts: a smaller medial portion which receives fibres from the nuclei of the midline and sends fibres to the hypothalamus, but none to the cerebral cortex; a larger lateral portion which receives fibres from other thalamic nuclei and sends fibres to the granular cortex of the frontal lobe, with topographic relation between the parts of the nucleus and parts of the cortex (Pribam et al., 1953);

(b). The nucleus of the centrum medianum, partly embedded in the internal medullary lamina. It appears to have no direct connections with either the afferent paths entering the thalamus or the cerebral cortex. In human material studied after surgical procedures, the centrum medianum was found to have connection with the caudate nucleus and putamen but not with the globus pallidus.

In experimental waork it has been found to have connections with the surrounding main thalamic nuclei, and because of the connections, may have a significant role in the regulatory influence exerted by the thalamus over the entire cortex.

(4). The lateral nuclear mass lies between the internal medullary lamina and the internal capsule. The lateral nucleus is

customarily divided into a larger ventral part and a smaller dorsal part. What has been referred to as the ventral part of the lateral nucleus of the thalamus is best referred to simply as the ventral nucleus.

The most anterior part of the ventral nuclear mass is known as the anterior ventral nucleus or nucleus ventralis anterior. It receives fibres from the globus pallidus and sends other fibres back to the corpus striatum. Behind it lies the intermediate ventral nucleus (lateral ventral) which receives the fibres of the superior cerebellar peduncle and sends fibres to the motor cortex and, in smaller numbers, to the premotor cortex of the frontal lobe. It serves, therefore as a relay station upon the path from the cerebellum to the cerebral motor centres.

The posterior part of the ventral nucleus, nucleus ventralis posterior is the site of termination of the main ascending sensory pathways, i.e, the spinothalamic tracts, medial lemniscus and secondary trigeminal pathways. Two separate nuclear masses receive these tracts, which terminate so that the impulses from the parts of the body are represented from medial to lateral in the order of face, arm and leg. The trigeminothalamic fibres therefore end about cells in the nucleus ventralis posteromedalis. Immediately lateral to this lies the nucleus ventrallis posterolateralis within which terminate the fibres of the spinothalamic tracts and medial lemniscus.

The ventral lateral nucleus (VL), caudal to the ventral anterior nucleus, is composed of small and large neurones that show considerable differences in various parts of the nucleus. This nucleus has been subdivided into three main parts (Olszewski 1952): (1) pars oralis (VLo), (2) pars caudalis (VLc), and (3) pars medialis (VLm). The largest subdivision (VLo) consists of numerous deep staining cells arranged in clusters. The pars

caudalis (VLc) is less cellular but formed of scattered large cells. The pars medialis (VLm) begins ventral to ventral anterior and extends caudally to the subthalamic region. Cerebellar efferent fibres contained in the superior cerebellar peduncle decussate in the mesencephalon and project profusely to the contralateral ventral lateral nucleus, pars oralis (Olszewski 1952). These fibres arise mainly from the dentate nucleus. The pars oralis also receives pallidofugal fibres via the thalamic fasciculus (Nauta and Mehler, 1966).

The ventral lateral nucleus of the thalamus receives a considerable number of fibres from the precentral cortex (Clark, 1932; Levin, 1936 and 1949; Verhaart and Kennard, 1940; Mettler, 1947a). These corticofugal fibres pass to both the pars oralis and pars candalis. Thus corticofugal fibres pass to regions of the nucleus that receive fibres from the cerebellum as well as to regions which do not. Connections of the ventral lateral nucleus with the precentral cortex are reciprocal and topically arranged. Medial parts of the nucleus send fibres to the face area, lateral parts send fibres to the leg area, and fibres from intermediate portions of the nucleus pass to cortical regions representing the arm and trunk (Walker, 1938a& 1949). Most of these fibres pass to area 4, but some may reach area 6. This topical arrangement of thalamocortical fibres passing to the precentral cortex is considered as the anatomical expression of the functional independence of different body parts in the primary motor area. The fact that impulses from the cerebellum are projected to the motor cortex via the ventral lateral nucleus suggests that cerebellar influences upon motor function may be effected primarily at a cortical level. Different regions of the medial part of the ventral lateral nucleus (VLm) receive afferent fibres from the substantia nigra and a smaller number from the globus pallidus.

The sensorimotor cortex, like neopallium in general, sends fibres back to its own thalamic projection nuclei. In addition, it sends fibres to other thalamic nuclei, to parts of the basal ganglia, to midbrain, pontine and bulbar cell clusters and to the spinal cord (Kawana, 1969; Kawana and Kusuma, 1964; Kuypers, 1958a; 1958b, 1960: Kuypers and Brinkman, 1970; Kuypers and Tuerk, 1964; Niimi, Kishi, Miki and Fujita, 1963). However, the physiology of the corticofugal output from that portion of the sensorimotor cortex which is regarded as primarily sensory in character has been largely ignored, more attention having been given to sensation as output than to its corticofugal discharge. Only the pyramidal tract has been extensively studied, and its diffuse and far-flung terminations may account for most of the terminations of fibres emanating from this part of the brain. Wiesendanger, 1969; Towe, 1972 showed that the sphere of influence of the pyramidal tract is very broad. More than half of the corticofugal output from sensorimotor cortex goes by way of pyramidal tract fibres, and among these fibres, a large number terminate in cell clusters that make up the ascending somatosensory system.

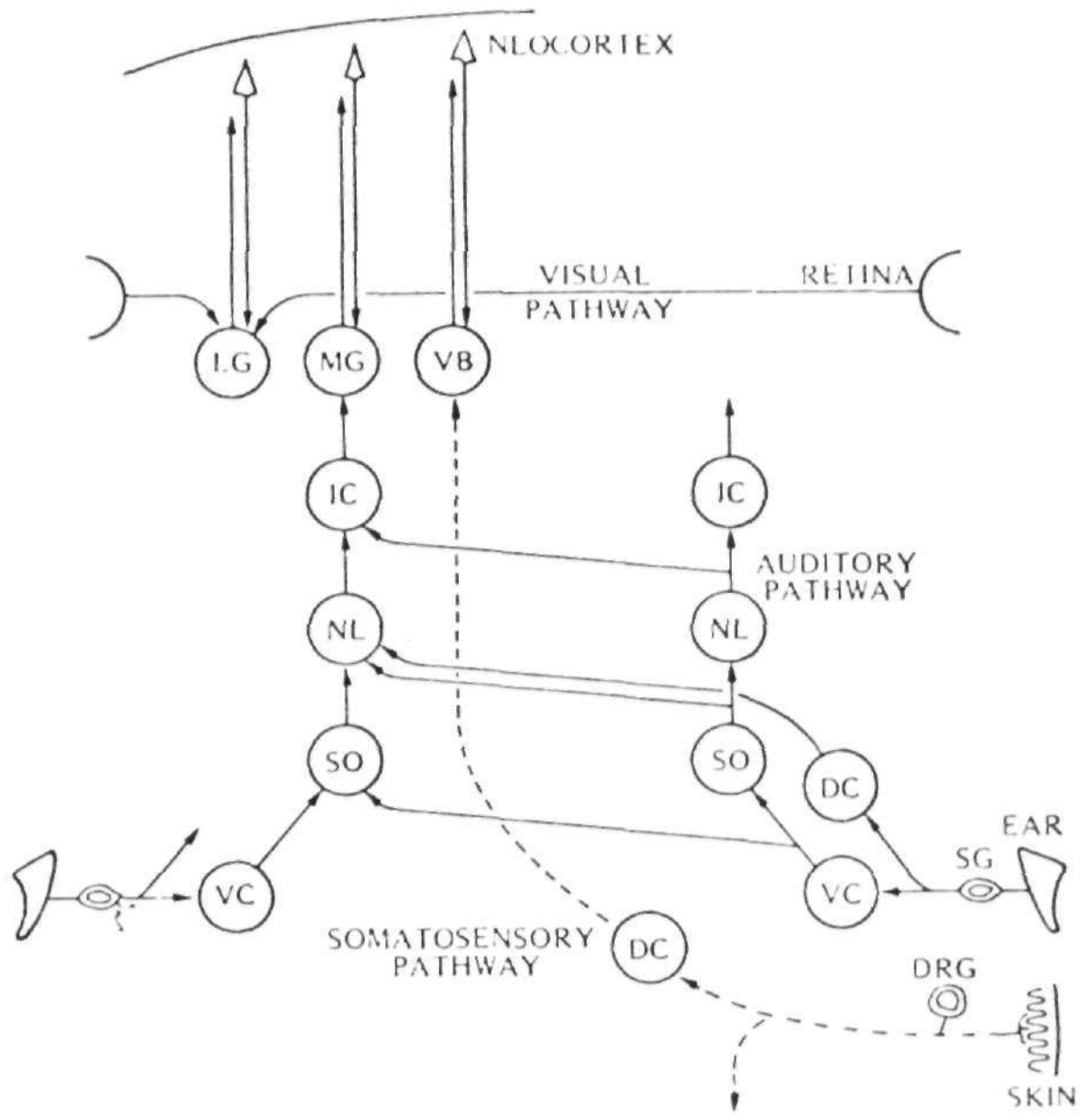
Figure 3: Diagram showing the components involved in electrical stimulations.

S_1: for cortical stimulation.

S_2: for hind limb nerve stimulation.

S_1 and S_2 can be alternated for a particular experiment.

Figure 1: A Schematic diagram of different positions of the thalamic relay nuclei in their respective sensory pathways to the neocortex.


NEOCORTEX
VISUAL PATHWAY
RETINA
LG
MG
VB
IC
IC
AUDITORY PATHWAY
NL
NL
SO
SO
DC
EAR
SG
VC
VC
SOMATOSENSORY PATHWAY
DC
DRG
SKIN

1.3. ELECTROPHYSIOLOGY

In agreement with the anatomical findings, volleys in cutaneous nerves and afferent impulses initiated by tactile and joint stimulation produce potential changes and also evoke the discharge of impulses in a quite sharply demarcated region of the contralateral thalamus, (Mountcastle and Henneman,1949). Extensive investigations of the activation of these cells by various forms of peripheral stimulation have been performed. Rose and Mountcastle (1954) studied with great detail and precision the repetitive spike discharges that are evoked by single volleys in cutaneous nerves. These studies included the effect of variations in volley size on the number and frequency of spikes in the evoked response. They also carried out an analysis of the interspike intervals.

Purpura and Cohen (1962) and Purpura and Shofer (1963) very successfully employed intracellular recording of an adjacent thalamic nucleus (the ventroanterior nucleus) in order to analyse the responses evoked by stimulation of the midline thalamic nuclear complex. They demonstrated for the first time the large and prolonged inhibitory postsynaptic potentials (IPSP's) of thalamic neurones, and the influence of these inhibitions on spontaneous and evoked discharges. They postulated that this inhibition was the major factor in producing bursting activity that was evoked by stimulation of the centre median. Andersen, Brooks, Eccles and Sears (1964a and 1964b) used both extracellular and intracellular recordings in an attempt to analyse the complex responses evoked in this nucleus by stimulation of different inputs. Input stimuli were provided either by contralateral cutaneous afferent volleys or by stimulation of the ipsilateral somatosensory cortex. The objectives were, to establish criteria for identification of the thalamocortical relay (TCR) cells, which relay medial lemniscal impulses onto the somatosensory cortex, and secondly, to attempt

to define the inhibitory pathways and the constituent interneurons of these pathways in the ventrobasal complex.

They reported that cortical stimulation could evoke a spike response with a latency of 0.5 msec from a neurone that could also be fired rhythmically by stimulation of the ulnar nerve. The short latency established that the neurone was antidromically invaded by an impulse set up by cortical stimulation. These two criteria could adequately be used as identification of a TCR cell. They also observed that an afferent volley in the superficial radial nerve evoked a brief repetitive discharge that was followed by a suppression of the background discharge of thalamic cells for almost 200 msec.

It has been well documented from indirect, direct and synaptic testing of the TCR cells that they have alternate phases of inhibition and excitation. The depressed excitability of TCR cells to direct stimulation provided strong evidence for posynaptic inhibition of these cells. This evidence has been reaffirmed by intracellular recording from TCR cells. The size and long duration (about 100 msec) of the IPSP's closely resembled those observed by Purpura and Cohen (1962) and Purpura and Shofer (1963) in cells of another thalamic nucleus.

1.4. NEUROPHARMACOLOGY

There are only moderate amounts of acetylcholine, acetylcholinesterase, and choline acetyltransferase in the mammalian thalamus, in comparison with basal ganglia and ventral roots (MacIntosh, 1941; Feldberg and Vogt, 1948; Burgen and Chipman, 1951 & 1952; Koelle, 1954; Zettler and Schlosser, 1955; Hebb and Silver, 1956; Gerebtzoff, 1959).

Although this may suggest that major afferent sensory pathways to the thalamus are noncholinergic, thalamo-cortical relay neurones were found to be extremely sensitive to acetylcholine, carbamylcholine, nicotine and related cholinomimetics (Andersen and Curtis, 1964a, 1964b).

Atropine and dihydro-β-erythroidine readily abolish acetylcholine excitations.

The excitatory transmitter released on to ventrobasal neurones from fibres which originate in the cerebral cortex is probably not ACH. This conclusion was reached when synaptic excitations from impulses in corticofugal fibres were found to be resistant to antagonists of ACH (McCance, Phillis and Westerman, 1968).

The excitatory effects of glutamic acid in the central nervous system has been widely discussed (Crawford and Curtis, 1964; Curtis, 1965; Krnjevic, 1964 and 1970; Salmoiraghi and Stefanis, 1967). Glutamate has been found to be an excitant in the spinal cord (Curtis., Phillis and Watkins, 1959 and 1960; Curtis and Watkins, 1960) and primary sensory nuclei of the caudal medulla (Galindo., Krnjevic and Schwartz, 1967; Steiner and Meyer, 1966).

Bradley and Wolstencroft, 1965; Curtis and Koizumi, 1961;

and Hosli and Tebecis, 1970 observed the excitatory effects of glutamate on the reticular formation whilst McLennan, et al 1968 observed same effects on the thalamus. Glutamate has also been known to excite the corpus striatum (Fifkova and Van Harreveld, 1970), the caudate nucleus (Bloom., Costa and Salmoiraghi, 1965), the hippocampus (Biscoe and Stranghan, 1966), and cerebral cortex (Boyd and Martin, 1956; Crawford and Curtis, 1964; Fifkova and van Harreveld, 1970; Krnjevic, 1965; Krnjevic and Phillis, 1963; Krnjevic and Whittaker, 1965).

Certain analogues of glutamate have been shown to antagonize both the actions of excitatory amino-acids and the synaptic excitation evoked by stimulating peripheral nerves. These observations provide evidence that glutamate or a related amino acid is the transmitter released from medial lemmiscal fibres on to thalamic neurones (Haldeman et al, 1972). The fibres mediating corticofugal excitation or spontaneous firing of thalamic neurones may well release an excitatory amino-acid as a transmitter, but this remains to be investigated.

Although both glycine and GABA depress the firing of neurones in several thalamic nuclei (Tebecis, 1970;Duggan and McLennan, 1971; Tebecis and diMaria, 1972), pharmacological evidence for release at inhibitory synapses has only been obtained for GABA. In anaesthetized cats (Andersen, Eccles and Sears, 1964; Bishop and Davies, 1960; Fuster, Gentzfeldt and Stracheil, 1965), it has been observed that cells of the ventrobasal nuclei respond to a peripheral afferent volley with cyclic bursts of firing. The period between bursts is associated with an inhibitory hyperpolarization. This pattern of firing was not affected by strychnine (Duggan and McLennan, 1971; Andersen, Eccles, Loyning and Voorhoeve, 1963), but was altered by bicuculline administered either from micropipettes or intravenously (Duggan and McLennan, 1971; Curtis and Tebecis,1972).

There is a good pharmacological evidence that GABA is the inhibitory transmitter released from interneurones onto thalamic relay cells. In addition, bicuculline blocks the inhibition of ventrobasal relay cells following orthodromic activation. 5HT depresses the post synaptic excitability of thalamocortical relay neurones of the ventrobasal complex. This postsynaptic depressant action could be related to an inhibitory transmitter function of 5HT at these sites, as 5HT containing fibres which originate in the brain stem terminate on thalamic neurones. However, although biochemical and histochemical studies are consistent with the concept of 5-Hydroxy-tryptaminergic transmission in the thalamus, direct pharmacological evidence for such pathways has yet to be obtained (Phillis & Tebecis, 1967). NA excites and depresses different proportions of relay cells in the ventrobasal complex. These actions are probably postsynaptic. Both histochemical and biochemical studies suggest that NA is a transmitter (both excitatory and inhibitory) released on to ventrobasal thalamic neurones. However, there is no pharmacological evidence for such pathways (Phillis & Tebecis 1967). DA depresses the large majority of thalamic neurones, including relay cells, but since there is no histochemical evidence for DA- containing nerve fibres in the thalamus, it is not known whether DA serves a synaptic function in this structure (Phillis & Tebecis 1967).

CHAPTER TWO

METHODS

2.1. Preparation of the animals

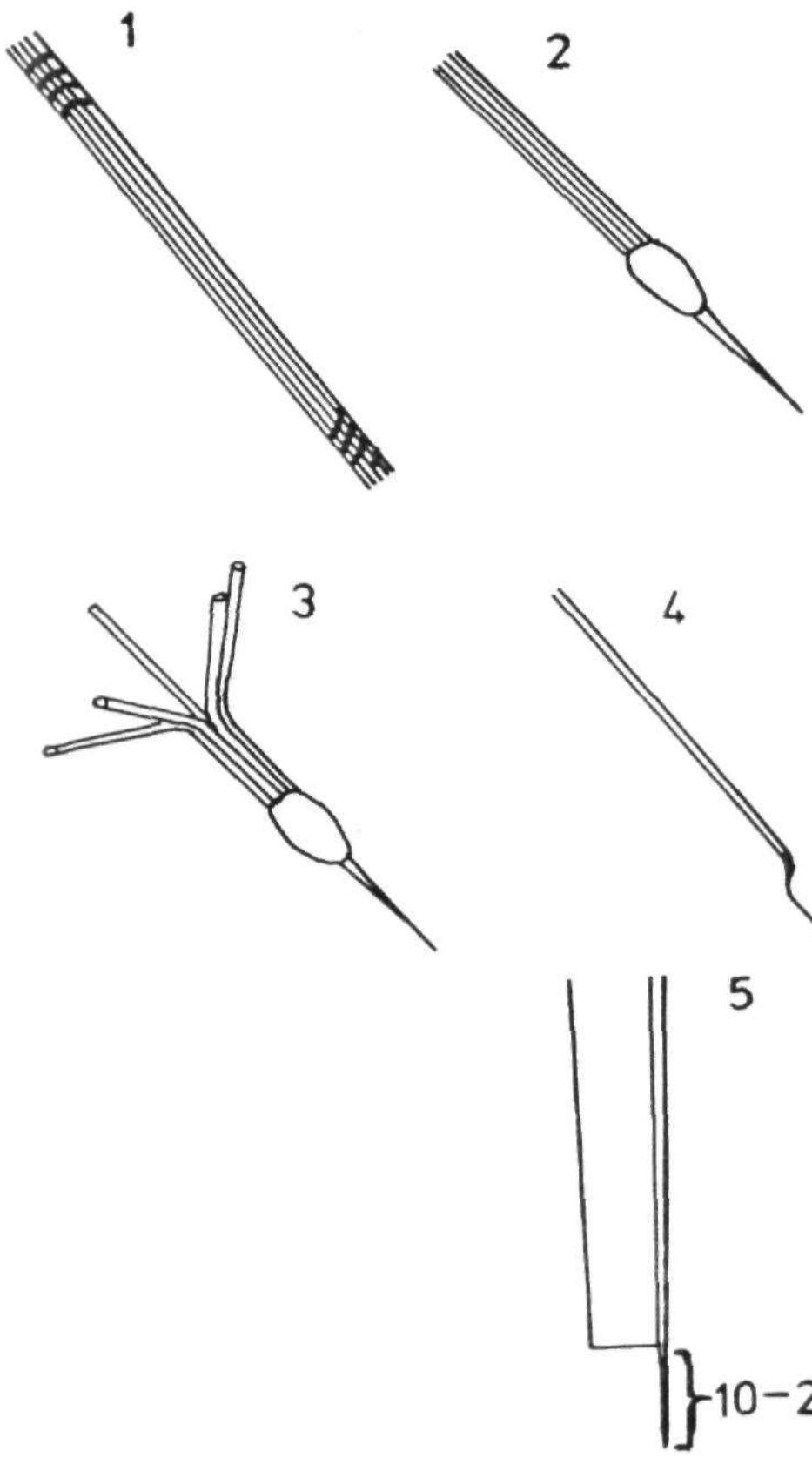
Male wistar rats of 200g weight were used in this study. They were anaesthetized with urethane (1.5g/kg) i.p.

The tibial peroneal nerve was dissected and exposed in a hind limb. The animal was placed in a stereotaxic frame and the exposed nerve suspended on a pair of silver stimulating electrodes in a pool of paraffin oil kept at a temperature of approximately $37^{o}C$. The rat's temperature was maintained between 36.5 and $37.5^{o}C$ with a thermostatic heating pad. The calvarium and duramater was removed to expose the cortex. A concentric bipolar stimulating electrode (Rhodes) was visually introduced in a caudal direction at an approximate angle of 60^{o} to horizontal into the frontal somatosensory areas. The coordinates of the ventrolateral thalamus were taken from the dorsal surface of the cortex, Lambda and the sagittal suture. The anterior posterior co-ordinate was 3.0 to 3.8mm from Lambda and the lateral co-ordinate was between 2.8 to 3.0mm from the midline sagittal suture. These co-ordinates have been modified from the Konig and Klippel stereotaxic atlas (1967) since the animals were positioned in the stereotaxic frame with the horizontal zero plane through Lambda and Bregma.

2.2. Assembly of iontophoretic electrodes

Electrode glass tubing of 7.5cm lengths (1.35-1.6mm O.D., 0.8-1.0mm i.d) were used. The glass was cleaned by boiling in distilled water with a small quantity of hydrochloric acid, washed in acetone and dried in an oven. A finely pulled hair-like glass fibre was passed through the tubes and secured at each end with a

Figure 2: Stage by Stage assembly of 6-barreled iontophoretic electrodes as described in the text.


1
2
3
4
5
10–20 µm

small amount of epoxy resin glue. The fibre enables the finished electrode to be filled rapidly prior to use. This is done by introducing the electrolyte or drug solution into the shaft of each barrel using fine canula tubing attached to a syringe.
The iontophoretic electrode assembly in this study consisted of a conventional five-barrelled iontophoretic electrode with an additional recording barrel attached to the side. The single barrel recording electrode tip protrude past the iontophoretic assembly by 10-20µm.

Five pieces of glass tubing were held in a circular array by means of small metal springs placed at each end of the bundle. These springs hold the five glass tubes together and also protect them from breakage when they are clamped into the chucks of the electrode puller. The five barrel blank was then mounted in a Narishige vertical electrode puller. A clamp was placed 0.5mm to 1mm beneath the bottom chuckto prevent it from dropping. The central portion of the bundle was heated until the glass was soft. The bottom chuck was rotated through 180^{o} with respect to the upper chuck. This process introduced a twist into the electrode blank which gives this part of the electrode more strength and ensures that all five barrels pull to one point. The clamp is removed and the blank was pulled conventionally to give two multibarrel electrodes. The five barrels were permanently bonded by the application of epoxy resin near the base of each shaft. With the aid of a microscope and micromanipulators the electrode was then tipped using the end of a glass rod. The shafts of the five barrels were then bent outwards to accomodate the iontophoretic leads and to prevent current coupling between the barrels. A single barrel electrode was pulled from the same type of glass and two bends placed near the base of the shaft using a small gas flame. The two electrodes

were then placed in micromanipulators and using a microscope the tips were arranged with the single barrel protuding by about 10-20μm past the multibarrel assembly. The electrodes were secured in this position by cautious and gentle application of a small drop of 'Super Epoxy' adhesive (Plastic Padding Ltd) near the tips. After about 15 mins when the epoxy had set, more adhesive was applied around the base of the two shafts to firmly hold them together.

This type of electrode assembly has the advantages of more stable recordings and the units recorded tended to be larger than those obtained using the conventional multibarrel microelectrodes.

The recording electrodes were filled with 5% pontamine sky blue solution in 0.5m Sodium acetate. The typical resistances were between 10 to 40MΩ.

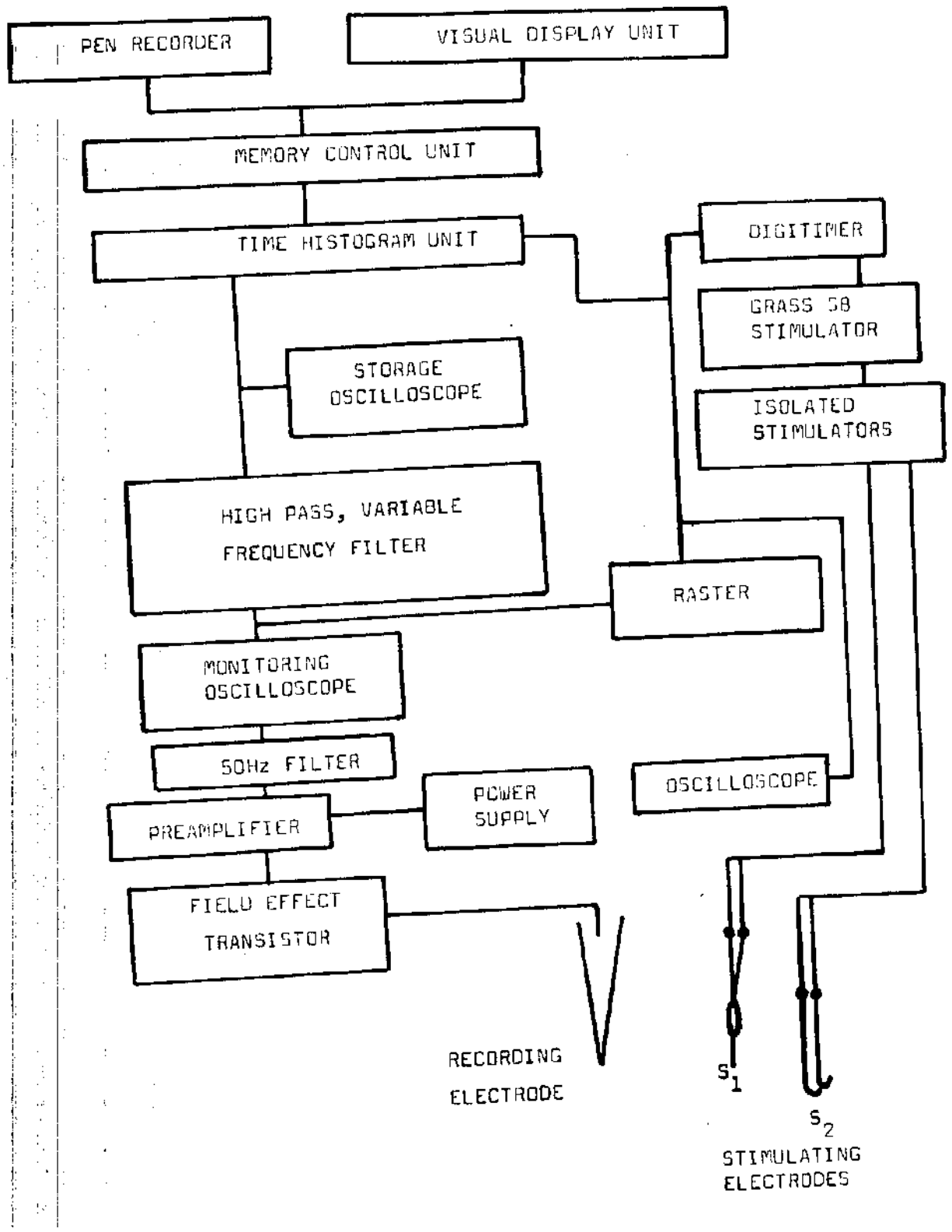
2.3. Stimulation techniques

The hind limb tibial peroneal nerve was stimulated with a square wave pulse of 12-40V intensity, 0.1ms duration and 1Hz frequency. With these parameters, evoked activity from thalamo-cortical relay cells, with latencies of between 8 to 14msec, could be recorded. These relay cells could also be activated antidromically from the somatosensory cortex using a lower stimulus intensity (2-8V).

These two criteria were used for the electrophysiological identification of principal units in the ventro-lateral thalamus.

2.3. (1). Display and analysis of evoked responses.

The recording electrode, filled with pontamine sky blue solution, of resistance 10-40MΩ as earlier mentioned was connected through a probe to a Tektronix preamplifier Type 122. The extracellulary recorded signals were amplified, passed through a 50Hz and a high pass, variable frequency filter.


PEN RECORDER
VISUAL DISPLAY UNIT
MEMORY CONTROL UNIT
TIME HISTOGRAM UNIT
DIGITIMER
GRASS 58 STIMULATOR
ISOLATED STIMULATORS
STORAGE OSCILLOSCOPE
HIGH PASS, VARIABLE FREQUENCY FILTER
RASTER
MONITORING OSCILLOSCOPE
50Hz FILTER
OSCILLOSCOPE
PREAMPLIFIER
POWER SUPPLY
FIELD EFFECT TRANSISTOR
RECORDING ELECTRODE
S1
S2
STIMULATING ELECTRODES

The signal was displayed on an oscilloscope to be photographed on polaroid film.

Action potentials were discriminated using a Digitimer spike processor D130 the output of which could be displayed a raster or recorded as a frequency on a servoscribe recorder. The signals could also be processed using an Ortec 4621 Time Histogram Input and an Ortec 4620 Memory control unit for generation of post stimulus time histograms (PSTH).

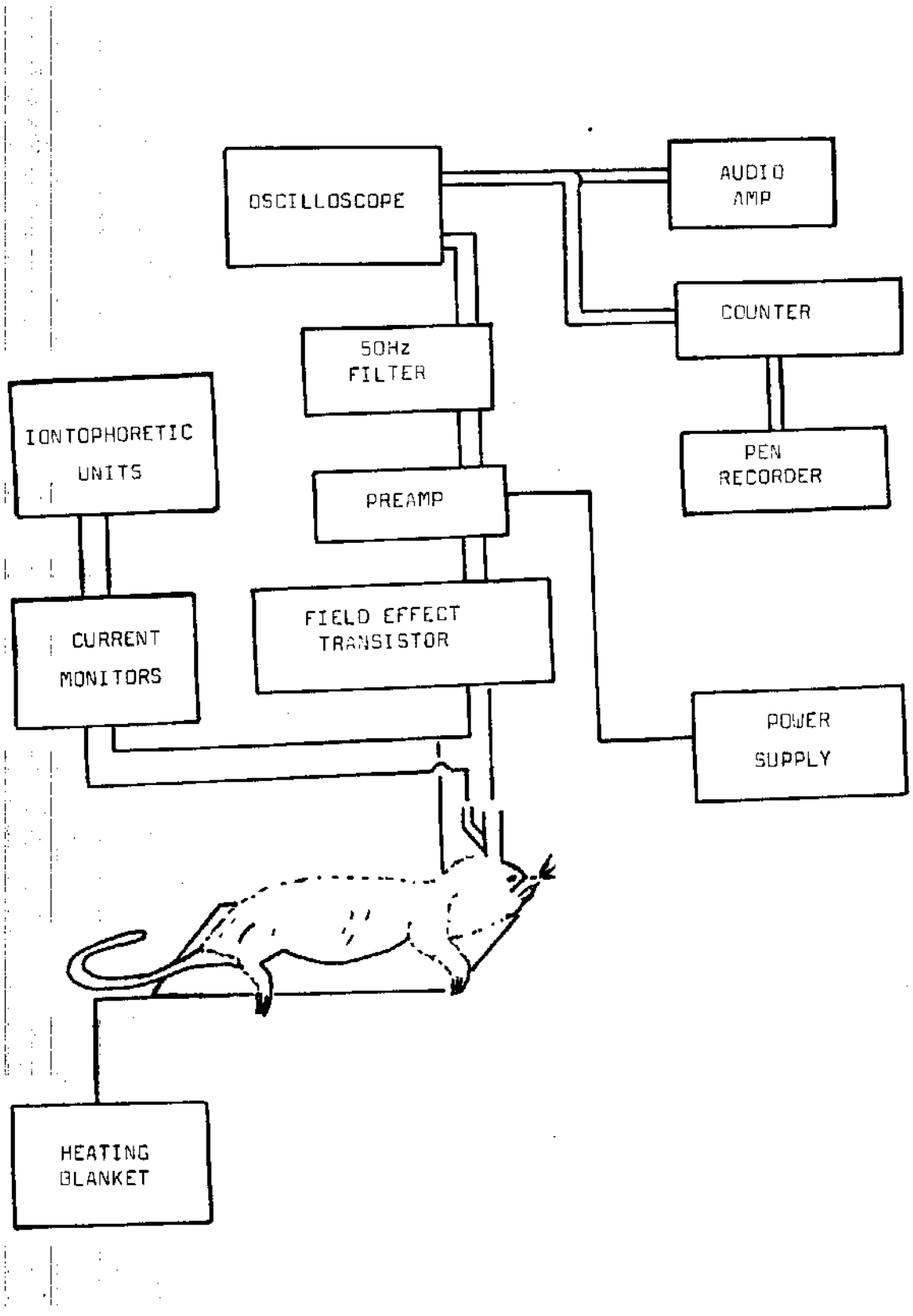
2.4. Iontophoresis

For microiontophoresis, drugs were ionized by adjusting their pH using hydrochloric acid (HCl) and sodium hydroxide (NaOH). The drugs could then be expelled by passing the appropriate cationic or anionic current down each barrel of the iontophoretic micropipettes. Iontophoresis could be carried out whilst simultaneously recording extracellularly changes in cell activity from the protuding barrel.

With this technique, neurotransmitters and substances that are thought to act in a specific fashion on particular synapses can be applied to the area of interest. This allows assessment of their ability to mimic, potentiate or antagonise the actions of the naturally occuring transmitters. Iontophoresis is particularly useful for applying compounds that do not cross the blood brain barrier.

One of the five barrels in the iontophoretic assembly contained 3M NaCl. This was used to balance the net current at the electrode tip to zero (Salmoiraghi and Weight, 1967) or to expel Na^+ or Cl^- in order to evaluate the possible direct effects of iontophoretic currents. The remaining barrels contained the appropriate drugs under investigation. Small currents (5-25nA) of opposite charge to the ejecting current were used to help retain

Figure 4: Block diagram of components involved in iontophoretic studies.


OSCILLOSCOPE
AUDIO AMP
COUNTER
50Hz FILTER
IONTOPHORETIC UNITS
PEN RECORDER
PREAMP
CURRENT MONITORS
FIELD EFFECT TRANSISTOR
POWER SUPPLY
HEATING BLANKET

iontophoretic electrodes. Particular care was used to keep the retaining current to a minimum.

2.5. Confirmation of electrode position.

The site of stimulation in the frontal somatosensory cortex was marked by passing a DC anodal current of 5-15μA for 15-30s through the inner core of the concentric bipolar electrode with reference to the outer lead. The recording electrodes were filled with a 5% pontamine sky blue solution in 0.5M Sodium acetate. At the conclusion of the experiment the animals were perfused with a potassium ferrocyanide-formalin solution. The perfusion technique involved the injection of the fixative into the brain through the heart.

At the completion of the experiment, the animals were given an overdose of urethane. An incision was made through the skin along the left side of the animal's sternum. The skin was peeled back and the rib cage was cut along the sternum through the soft ribs and muscles. A lateral incision was then made along the lower boundary of the rib cage exposing the heart. The vein from the heart was cut to drain the venous blood and the lower tip of the animal's right ventricle is held with forceps then the fixative is quickly injected into the animal's left ventricle. The large hypodermic needle is inserted into the lower part of the left ventricle and extreme caution is taken to prevent the needle tip's puncturing the septum separating the ventricles.

Histological verification of the electrode site was made by locating the deposited blue spot in 50-100um frozen sections cut on a freezing microtome and counterstained with Neutral red.

2.6. DRUGS

		Source
L-glutamate (Sodium Salt)	(0.5M, pH8)	B.D.H
DL-homocysteate "	(0.2M, pH8)	"
L-aspartate "	(0.5M, pH8)	"
Acetylcholine bromide	(0.5M, pH4)	"
Atropine sulphate	(0.2M, pH4)	"
L-glutamic acid diethylester	(0.5M, pH4)	FLUKA
DL- α-Aminoadipate	(0.2M, pH8)	SIGMA
D- α-Aminoadipate	(0.2M, pH8)	Dr J. Watkins
Kainate	(20mM in 165mM MaCL, pH8)	SIGMA
Ketokainate	(20mM in 165mM NaCL, pH8)	Dr R. Sharma
DihydroKainate	(50mM in 165mM NaCL, pH8)	"
N-methyl-DL-aspartate	(50mM in 165mM NaCL, pH8)	SIGMA
DL-aspartate	(0.5M, pH8)	B.D.H
4-amino-n-butyric acid	(0.5M, pH3)	"
Bicuculline	(5mm in 165mM NaCL, pH3)	FLUKA

CHAPTER THREE

ELECTROPHYSIOLOGICAL ANALYSIS OF A PROJECTION FROM THE SENSORIMOTOR CORTEX ONTO THE VENTRO-LATERAL THALAMUS.

3.1. Introduction

The ventro-lateral thalamus occupies a centre in the nervous system where a high order relationship between sensory and motor mechanisms exists. The extensive projections from the thalamus to the motor and premotor cortex allow direct influence on the corticospinal pyramidal tract (Chow and Pribam; 1956., Dennery and Combs; 1961., Smaha., Kaelber and Maharry; 1969., Strick, 1970 & 1976).

Much interest has been shown in the pharmacology of the thalamo-cortical relay cells in the ventro-lateral thalamus. The depolarization of mammalian central neurones by acidic amino acids and acetylcholine have been extensively investigated, (Andersen and Curtis; 1964., Curtis et al; 1972., Hall et al; 1979., McCance et al 1968a., McLennan and Wheal, 1976a). The antagonism of amino acid induced and synaptic excitation of thalamic neurones has also been a subject of considerable interest (Haldeman et al; 1972., McLennan and Hall; 1978., McLennan and Wheal; 1976b). However, most studies have been carried out on the thalamo-cortical relay cells, identified either by peripheral stimulation of a leg nerve or by antidromic invasion from the cortex.

The anatomical details of the ventro-lateral nucleus in relation to the sensorimotor cortex is well documented (Larsen and Asanuma; 1979., Leman and Van der Burg; 1979., Saporta and Kruger., 1979; Strick, 1970 & 1976), but so far the electrophysiology has remained unclear.

Extracellular recording and stimulating techniques can be used for studying the properties and synaptic activities of these cells. This permits detailed examination of the elementary properties of neuronal synchronization. Further clarification of neuro-transmitter(s) involved in these pathways may also be obtained.

The purpose of this study was to investigate possible projections from the sensorimotor cortex to the ventro-lateral thalamus in the rat central nervous system.

3.2. Methods

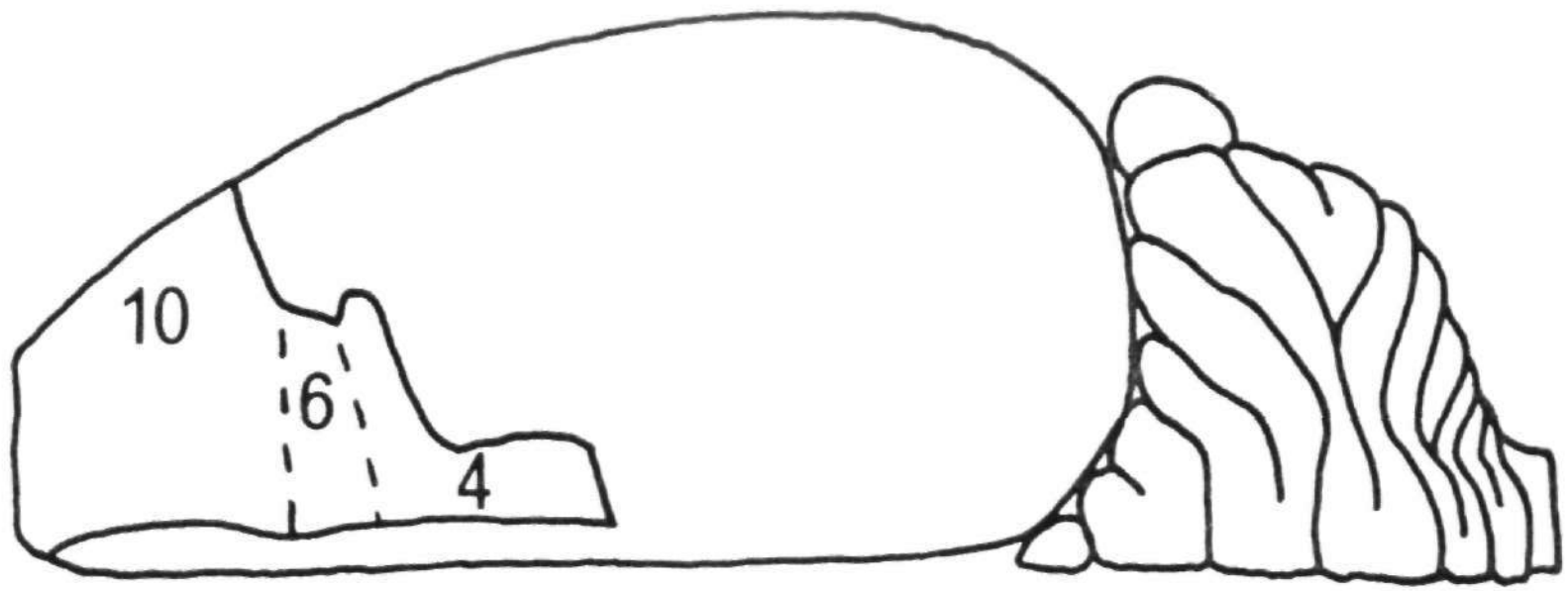
Experiments were performed on 49 male wistar rats of 200g weight. They were anaesthetized with urethane (1.5g/Kg) i.p.

The tibal peroneal nerve was dissected and exposed in a hind limb. The animal was placed in a stereotaxic frame and the exposed nerve suspended on a pair of stimulating electrodes in a pool of paraffin oil kept at a temperature of approximately 37°C. The rat's temperature was maintained between 36.5 and 37.5°C with a thermostatic heating pad.

The calvarium and duramater was removed from an area of about 5mm square, 1mm anterior to Bregma to expose the cortex. A concentric bipolar stimulating electrode was visually introduced in the anterior-posterior direction at an approximate angle of 60° into the frontal somatosensory areas as shown in figure 5. The site of stimulation was marked by passing a DC anodal current of 5-15 μA for 15-30 sec through the inner core of the concentric bipolar electrode with reference to the outer lead. At the conclusion of the experiment the animal was perfused with a potassium ferrocyanide-formalin solution. Histological verification of the electrode site was made by locating the deposited blue spot in 50-100 um frozen sections stained with cresyl violet or neutral red.

A 6- Barrel iontophoretic electrode with a protuding glass

Figure 5: A diagram showing the sensorimotor areas of the cortex.



CORTEX

recording tip 10-20 μm long was stereotaxically placed in the ventro-lateral thalamus. This electrode assembly has been described by Wang and Aghajanian (1977). This system was found to eliminate instability and current artefacts in units recorded as were previously reported Krnjevic and Phillis 1963; Salmoiraghi and Weight 1967, when using conventional multibarrel electrodes.

The recording electrodes were filled with a 5% pontamine sky blue solution in 0.5M Sodium acetate and their resistance was between 10-40MΩ.

The (VL) co-ordinates were taken from the dorsal surface of the cortex,Lambda and the sagittal suture. The anterior posterior co-ordinate was 3.0 to 3.8mm from Lambda and the lateral co-ordinate was between 2.8 to 3.0mm from the midline sagittal suture. These co-ordinates have been modified from the Konig and Klippel (1967) stereotaxic atlas since the animals were positioned horizontally in the stereotaxic frame. The recording electrode was connected through a probe to a Tektronix preamplifier. The extracellularly recorded signals were amplified and displayed on an oscilloscope and photographed on polaroid film. Action potentials were passed through a Digitimer spike processor and a rester, the output of which could be integrated to produce post-stimulus histograms.

The thalamo cortical relay cells were identified by two electrophysiological criteria; by orthodromic activation from stimulation of a contralateral hind limb nerve, and by antidromic activation on stimulating the ipsilateral somatosensory cortex.

Correct positioning of the elctrode was confirmed from the pontamine spot in histological sections.

3.3. Results

3.3. (1). Confirmation of electrode placement at VL

Stimulating the hind nerve with a stimulus intensity of 12-40V, duration 0.1ms and frequency of 1Hz, thalamo-cortical relay cells with latencies of between 8 to 14ms were recorded (see figure 6).

The lower diagrams of figure 6 show a raster display of the evoked spikes from consequtive stimulations preceded by the stimulus artefacts. This demostrates the variable latencies (8 to 14ms) of the orthodromically evoked spikes. Also on stimulating the cortical areas with stimulus intensities ranging from 2 to 8V, duration 0.1ms and frequency 1Hz an antidromic spike was obtained. The antidromic spike was of a fixed short latency ranging from 0.6-2.5ms and followed a repetitive stimulating frequency greater than 80Hz (see figure 7). The raster scan of figure 7 shows the reliability and constant latency of the antidromic spike.

3.3. (2). Orthodromic activation of VL cells

After identifying the ventro-lateral thalamus, it was found that in 45 out of 49 rats used in this study that stimulation of the sensorimotor cortical areas, evoked consistent orthodromic activation of cells in VL. The orthodromic spikes were of 5 to 10ms latency with the majority of cells responding with latencies of 5 to 7.5ms. These spikes could follow stimulating frequencies of up to 10Hz. Some cells responded up to 20 to 30Hz but failed to follow higher frequencies. These excitations were followed by a period of inhibition of 50 to 150ms which was sometimes followed by late excitations (see figure 8). Figure 9 shows a display indicating the reliability of the

orthdromically evoked spikes as well as showing the variability in latency.

In figure 10,some cells have been observed which respond both antidromically and orthodromically to cortical activativation particularly when the stimulus intensity was increased to values between 6 and 12V. Orthodromic activations in figure 9 were obtained with stimulus intensity of 2 to 6V, duration 0.1ms and frequency of 1Hz.

3.3. (3). Collision experiments

In order to investigate the suggestion that the VL cells may be responding to an antidromic activation via a recurrent collateral (TCR neurone), collision experiments were carried out on 15 rats. Two outputs of the Grass S 8 stimulator with different isolation units were used. The experiments were arranged to allow the independent or simultaneous stimulation of the afferent nerve in a contralateral hind limb and the ipsilateral somatosensory cortex. It was possible to vary the delay between alternate stimuli.

No collision was observed between the orthdromic activation produced by stimulating the contralateral hind limb nerve (Andersen etal,1964) and that from stimulating the ipsilateral somatosensory cortex (figure 11c). However, orthodromic activations from the afferent nerve could be made to collide with antidromic activations from the ipsilateral cortex by varying the delay between stimuli.

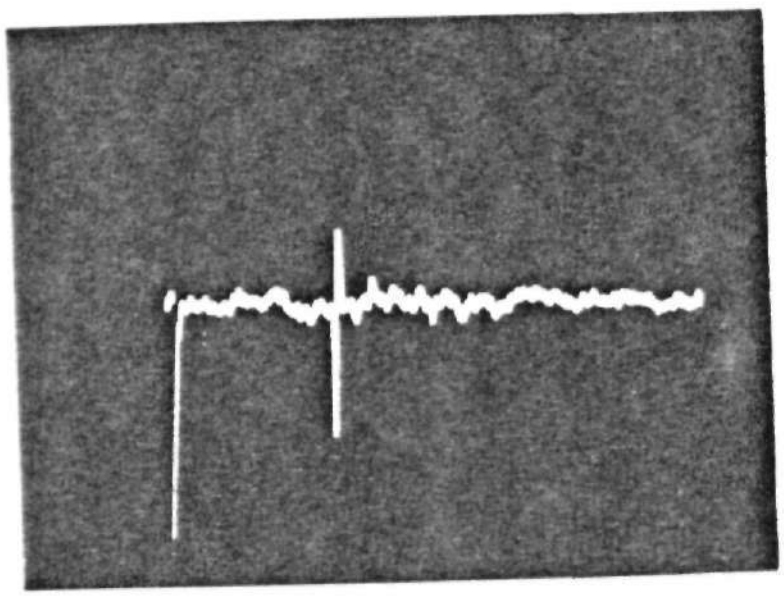
Cortical activations were followed by a period of inhibition (e.g. figure 8).

Figure 6: A) Orthodromic activation of a thalamo-cortical relay (TCR) neurone by hind limb nerve stimulation.

B) A raster display of the variability in latency.

One of the criteria used as identification confirm studies of the ventro-lateral (VL) thalamus.

A



·5mV
5ms

B

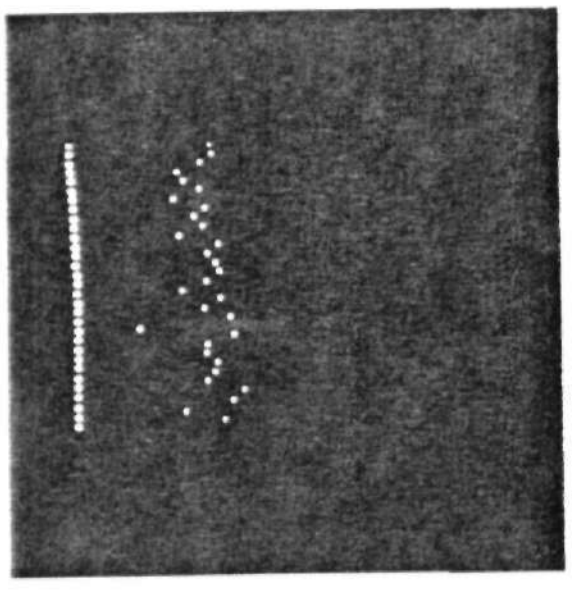
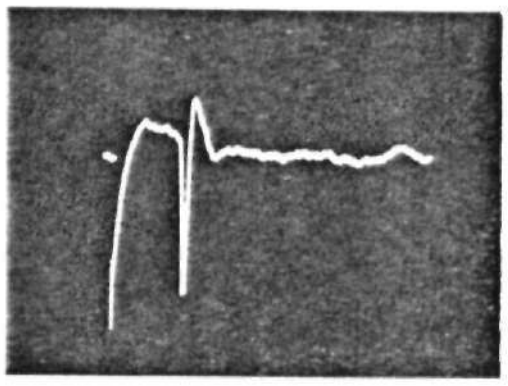


Figure 7: A) Antidromic invasion a thalamocortical relay cell by stimulation of the somatosensory cortex.

B) A raster scan showing the short fixed latency of the antidromic spike.

The second criteria for identifying a (TCR) neurone at (VL) thalamus.

A



1·5mV

2ms

B

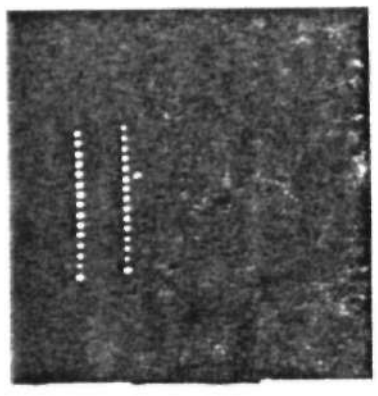
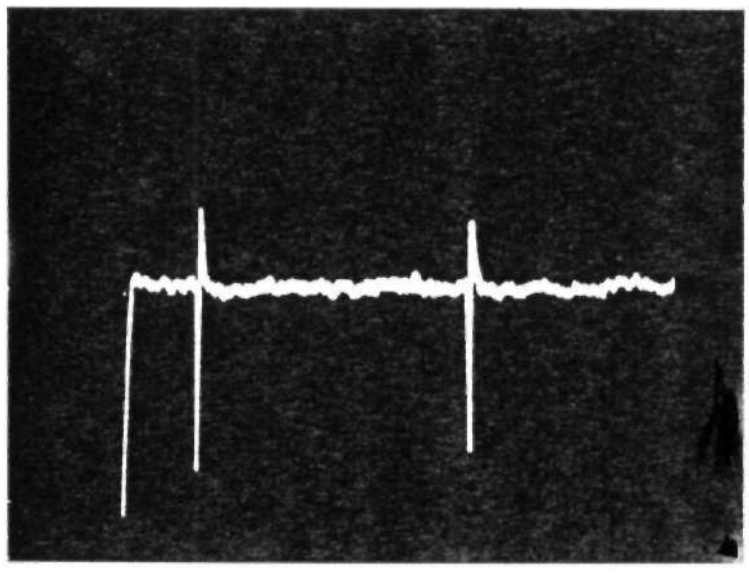


Figure 8: A) Orthodromic cortical activation of principal cells at the ventro-lateral (VL) thalamus.

B) A raster display showing the variation in latency and the consistency of the spikes.

A period of inhibition followed by late excitation is shown.

A



0·5mV
10ms

B

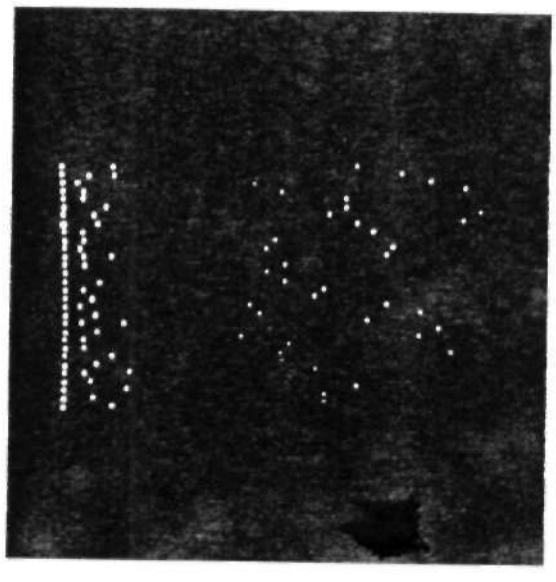
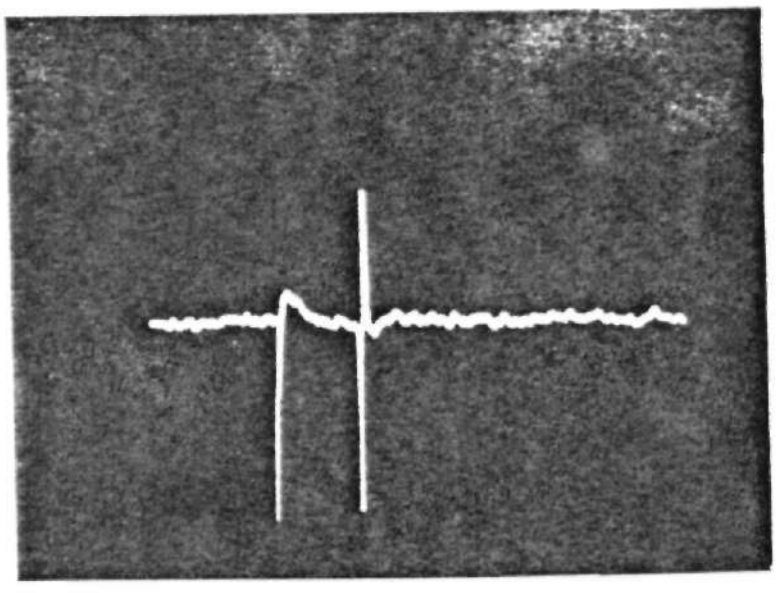


Figure 9: A) Synaptic excitation of a thalamocortical relay neurone by stimulation of the ipsilateral somatosensory cortex.

B) Raster display to show the variability of latency and consistency of the spike.

A



0·5mV
5ms

B

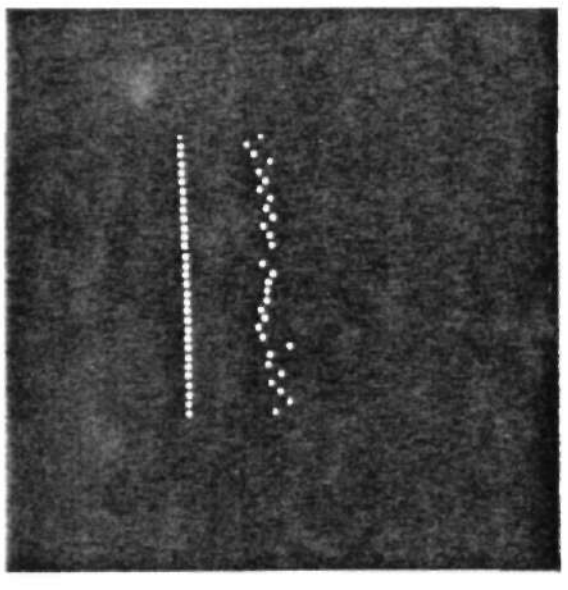
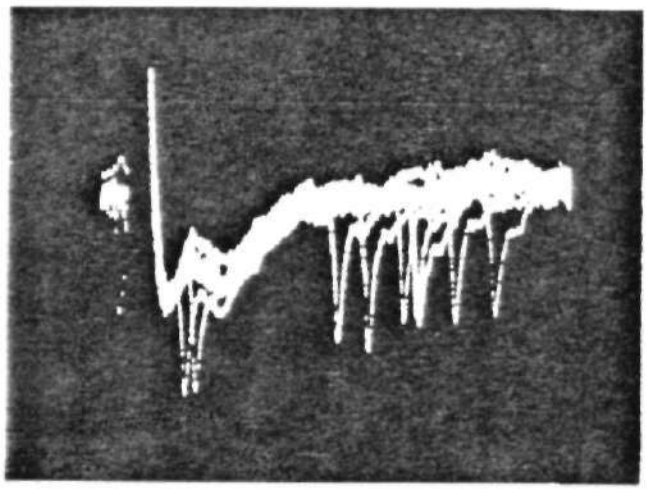


Figure 10: A thalamocortical relay neurone responding to both antidromic and orthodromic cortical activation.

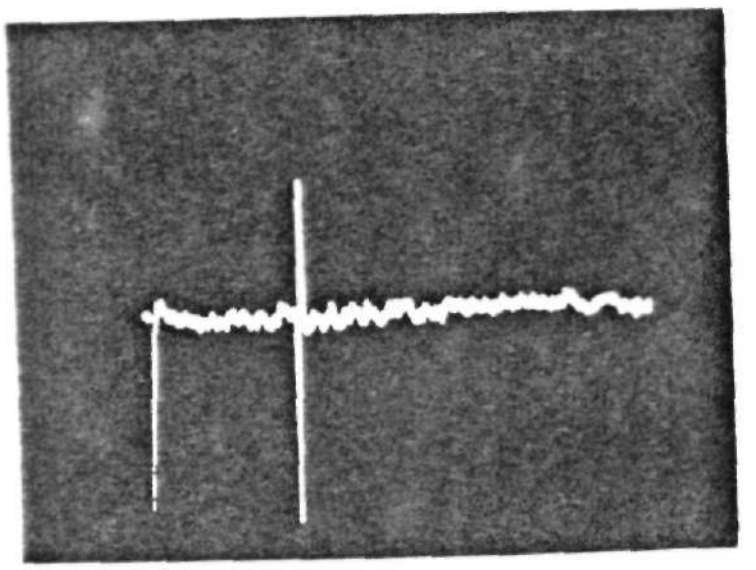


0·5mV

ms

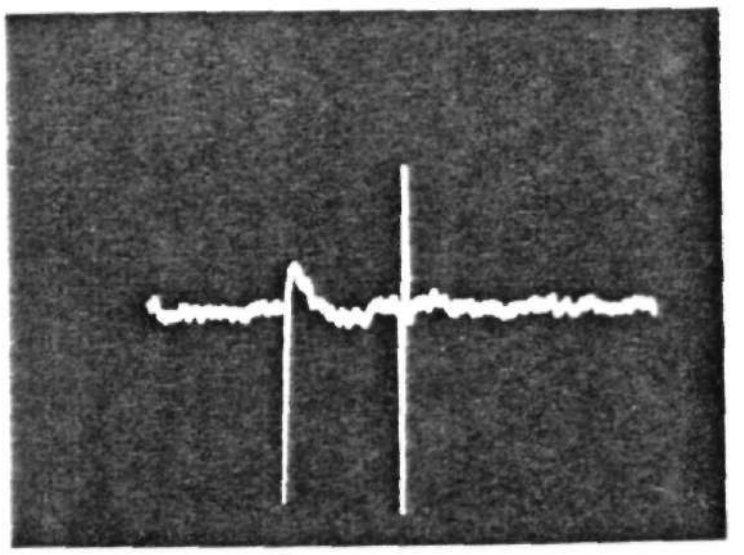
Figure 11: A) Orthodromic activation of a thalamo-cortical relay cell by stimulation of the tibial peroneal nerve of a hind limb. B) Orthodromic activation of the same cell by stimulating the ipsilateral somatosensory cortex. C) Simultaneous stimulations of both the afferent nerve and somatosensory cortex. No collision occurred as a result of both orthodromic activations.

A



S_1

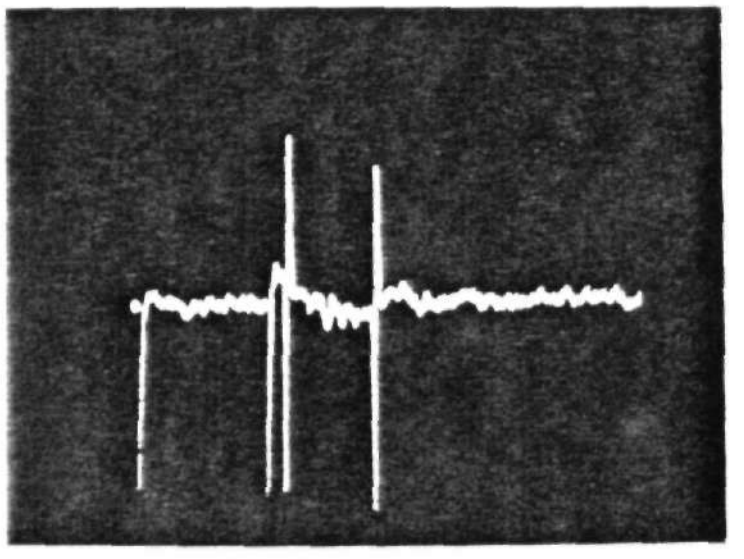
B



0·5mV
5ms

S_2

C



3.4. Discussion

Cortical activations (orthodromic or antidromic) were followed by a period of inhibition reaffirming that the recurrent collateral excites the inhibitory interneurones (Iwama and Yamamoto 1961; Andersen and Eccles 1962, and Angel 1963). The collision experiments suggested that the thalamocortical relay cells were directly monosynaptically activated and not driven through a recurrent collateral of a thalamocortical relay neurone.

In conclusion, thalamocortical relay cells of the ventro-lateral thalamus in the rat can be activated in three ways:- (1). activation by stimulation of a contralateral hind limb nerve, (2). by antidromic cortical invasion from the ipsilateral sensorimotor cortex, (3). by using the previous activations as criteria for identification of relay cells, it has been found in this study that stimulating the ipsilateral somatosensory cortex can produce orthodromic activation of the ventro-lateral thalamus.

The relatively dense projections of fibres from the cortex to the thalamus (Jones and Powell 1968 and 1969b ; Jones, Wise and Coulter 1979 .) provides supporting evidence for the electrophysiological observations made in this study.

CHAPTER FOUR

THE PHARMACOLOGY OF THE ORTHODROMIC CORTICAL INPUT ONTO THE VENTRO-LATERAL THALAMUS IN THE RAT.

4.1. Introduction

The investigations about the nature of the excitatory amino acid receptors in mammalian central nervous system have been concentrated for a long time on studies of the relative responses of a wide range of compounds in comparison with those of the naturally occurring amino acids L-glutamate and L-aspartate Biscoe et al 1975; Curtis and Watkins, 1960 and 1963. L-glutamate and L-aspartate are capable of depolarizing and exciting neurones to which they are administered extracellularly (Curtis, Phillis and Watkins, 1960) and from the report of Zieglgansberger and Puil, 1972, the ionic basis for this depolarization closely resembles that induced by stimulation of excitatory synapses. They are taken up into cells of the nervous system by high affinity mechanisms which are distinct from those used to transport other amino acids (Logan and Snyder, 1972).

Considering these reasons among others there is a general belief that the two compounds probably function as mediators of some synaptic excitations in the central nervous system of mammals (Johnson, 1972; Curtis & Johnston, 1974; McLennan, 1975).

The considerable flexibility of the glutamate and aspartate molecules imposes a problem about the precise conformation of the receptor site, whether or not there exist separate receptors for glutamate and for aspartate (van Gelder, 1971; Buu, Puil and van Gelder, 1976). However, the fact that in some cases a

selective antagonism of the excitatory action of the two agonists has been reported (Haldeman and McLennan, 1972; Biscoe, Davies, Dray, Evans, Francis, Martin & Watkins, 1977a) suggests that separate receptors for the two molecules may exist. The use of conformationally restricted analogues of the molecules and an examination of the excitatory action of certain compounds correlated with the extent of their molecular restraint is promising (Johnston, Curtis, Davies & McCulloch, 1974; Biscoe, Evans, Headley, Martin & Watkins, 1975; McLennan & Wheal, 1978; Hall., Hicks, McLennan, Richardson & Wheal, 1979). Hall et al (1979) observed that D-Glutamate was consistently less active than the naturally occurring L-form but for aspartate, the D stereoisomer was more potent than the naturally occurring L-aspartate.

It has been observed that on rat thalamic neurones the racemic mixture of amino adipates not only fails to excite but depresses both the spontaneous and amino acid induced firing rates, including that due to L-aminoadipate (Hall, McLennan and Wheal, 1977). Further studies had revealed that the D-isomer of α-aminoadipic acid is responsible for the antagonism of excitatory amino acids (Biscoe, Headley, Lodge, Martin & Watkins, 1976; Biscoe et al., 1977a; Biscoe, Evans, Francis, Martin, Watkins, Davies & Dray, 1977b; McLennan & Hall, 1978). DαAA shows a preference for blocking excitation produced by aspartate rather than glutamate (Biscoe et al., 1977a, b).

McLennan and Hall (1978) observed that DαAA produced antagonism of the actions of the excitants, Glutamate, Aspartate and DL-homocysteate but were without effect on the activation elicited by Acetylcholine. The excitatory effect of DLH was nearly always more powerfully reduced by DαAA than was that of Glut in direct contrast to the action of GDEE which preferentially

antagonized Glutamate excitations. They also observed that both Dα AA and GDEE appeared to depress the excitatory responses produced by L-Aspartate and L-Glutamate approximately. However, in some cases Dα AA was more selective in depressing L-Aspartate, while on other neurones GDEE was more specific in depressing L-Glutamate.

The selectivity of action of GDEE was not found by others (Curtis, Duggan, Felix, Johnston, Tebecis & Watkins, 1972; Clarke & Straughan, 1977). This discrepancy might be due to examination of other sites in the nervous system where the properties of the receptors may differ.

Since these two antagonists can distinguish between glutamate and aspartate in different ways an investigation of their effects on evoked excitations may clarify the neurotransmitter (s) involved in the synaptic pathway from the sensorimotor cortex to the ventro-lateral thalamus.

4.2. Methods

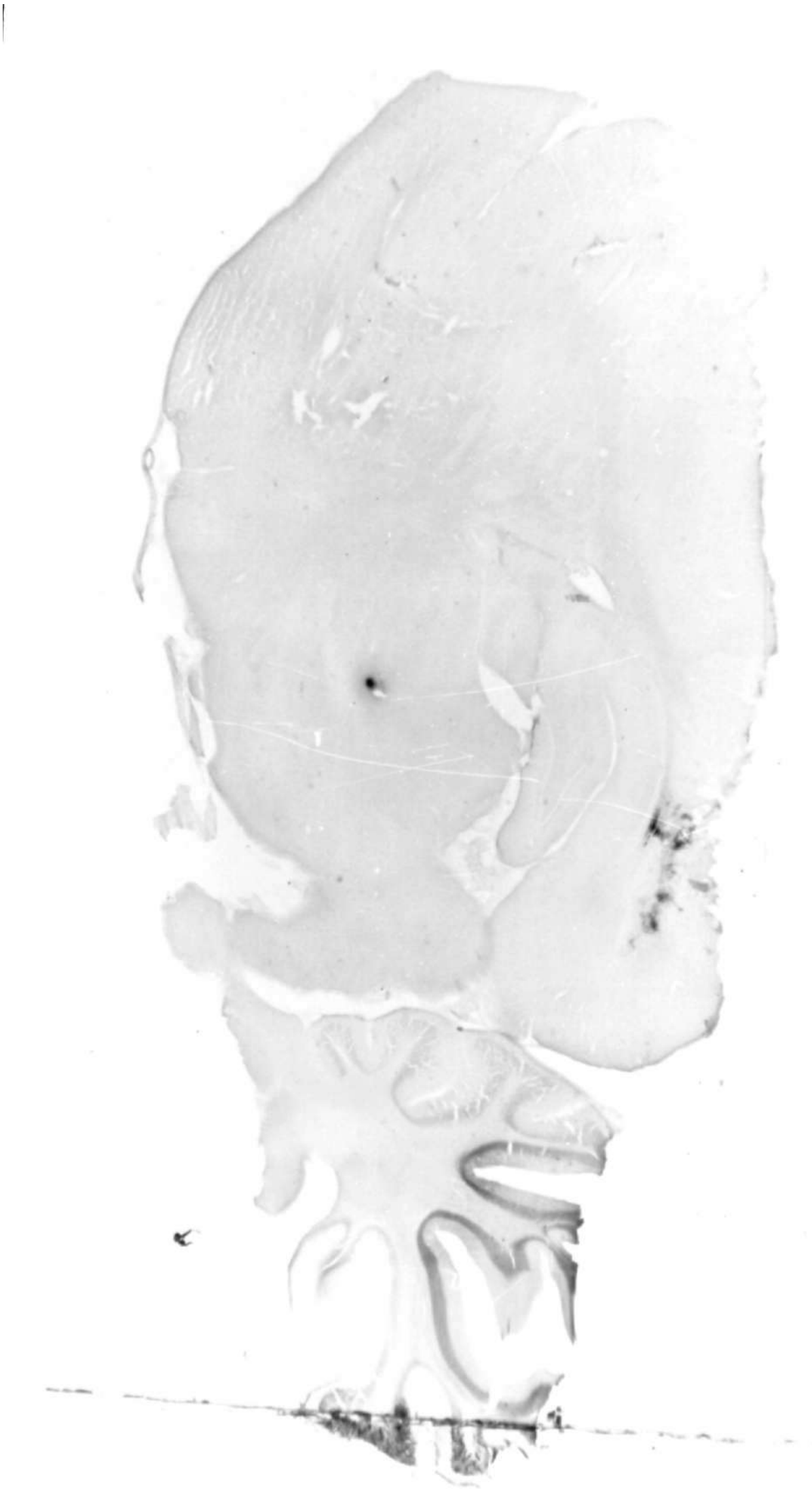
Experiments were performed on 107 male wistar rats of 200g weight. They were anaesthetized with urethane (1.5g/kg) i.p.

The animals were prepared as in previous experiments for the stimulation of tibial peroneal nerve and the somatosensory cortex.

A 6-Barrel ionotophoretic electrode with a protuding glass recording tip 10-20 μ long was stereotaxically placed in the ventro-lateral thalamus as previously described (Adeoshun, Kerkut and Wheal, 1980).

The co-ordinates were taken from the dorsal surface of the cortex, Lambda and the sagittal suture. A histological representation of the ventrol-lateral thalamus viewed from above in a coronal section is illustrated. A sagittal section is also shown in figure 12B.

Figure 12: B) A sagittal section of the rat brain. The ventro-lateral thalamus is also indicated by the blue spot.



Potentials were recorded extracellularly and drugs applied iontophoretically using 6 barrel microelectrodes as previously described (Crossman, Walker and Uoodruff, 1974j Uang and Aghajanian, 1977), The protuding recording barrel was filled with *5%* pontamine sky blue solution in 0.5PI sodium acetate and the indifferent barrel contained sodium chloride solution. The drug barrels contained aqueous solutions of the following drugs: Sodium L-glutamate (0.501, pH8); Sodium Dl-homocysteate (0.21*1, pH8); Sodium L-aspartate (0.51*1, pH8); acetylcholine bromide (0.51*1 pH4); atropine sulphate (0.21*1, pH4); L-glutamic acid diethylester (0.51*1, pH4); DL a Amino Adipate (0.21*1, pH8); D a Aminoadipate (0.21*1, pH8); Kainate (20ml*l in 165ml*l NaCl, pH8) and keto-kainate (20ml*l in 165m(*l NaCl, pH8). The drugs were ejected electrophoretically using appropriate anionic and cationic currents.

The thalamocortical relay cells were identified by two electrophysiological criteria: by orthodromic activation from stimulation of a contralateral hind limb nerv/e, and by antidromic activation by stimulating the ipsilateral somatosensory cortex. Correct positioning of the electrode was confirmed from the pontamine spot in histological sections.

4.3. Results

4.3. (l). Identification of Thalamo Cortical relay (TCR) cells at lL

Stimulation of the tibial peroneal nerve of a contralateral hind limb evoked spikes in the ventro-lateral (VL) thalamus with latencies of 8 to 14 ms (Andersen, Eccles and Sear, 1964). These cells can also have invaded antidromically by stimulating the somatosensory cortex (Adersen et al 1964). The antidromic spike is of a fixed short latency ranging from 0.6 to 2.5ms and follows high repetitive stimulation frequencies (cf PlcCance, Phillis and

Westerman, 1968).

Once the relay cells had been identified it was possible to orthodromically activate them via the corticothalamic projection. As described in the previous chapter these cells responded to stimulation of the somatosensory cortex with a latency of 5-10ms. Most cells were found to have a latency of between 5-7.5ms (figure 10).

4.3. (2). Responses to L-glutamate, L-aspartate, DL-homocysteate, N-methyl-DL-aspartate, kainate ketokainate, Acetylcholine and Gamma-aminobutyric acid.

Iontophoretic application of L-glutamate (30-150nA); L-aspartate (40-150nA); DL-homocysteate (10-60nA); N-methy-DL-aspartate: NMDLA (10-30nA); keto-kainate (10-30nA) and kainate (2-40nA) excited majority of the identified TCR cells. The excitation produced by kainate and keto-kainate was slow in onset and had a relatively prolonged action. In contrast to this slow type of response the application of kainate and keto-kainate (8-18nA) on 7 neurones produced a rapid onset and decay of the excitatory response. These were similar to the fast responses produced by the application of L-glutamate, L-aspartate and DLH except that the decay were somewhat slower. NMDLA excitations were also somewhat slower in onset and offset than those of glutamate and other amino acids (L-aspartate & DLH).

Acetylcholine routinely administered was found to excite 70% of the cells tested. In some cases acetylcholine iniated a very rapid onset of firing and firing again ceased rapidly when the application is terminated while in other cases the onset and offset responses tend to be slow.

10nA of GABA was found to depress the excitations produced

by L-glutamate (figure 13).

4.3. (3). The effects of atropine, GDEE and DαAA

The action of atropine on ACH and the amino acid induced excitation was tested on 52 TCR neurones. Ejection currents ranging from 40 to 80nA and applied for 2 to 4mins were effective in reversibly blocking ACH-induced excitation in 41 neurones (figure 14) with no apparent effect on amino acid induced excitations (figures 15&16).

The application of GDEE (40 to 80nA, 2 to 10mins) was effective in blocking the excitatory response to L-glutamate, L-aspartate and DLH (figures 17&18) on most cells tested. At higher concentrations GDEE becomes less or non-selective and sometimes depress NMDLA and ACH excitations. Kainate and keto-kainate were not affected (figure 19) by GDEE. The action of GDEE persisted for a period of 2 to 15mins following its termination before complete recovery was observed. These observations are illustrated in table 1A.

The administration of DαAA (20-40nA), for 2 to 5mins was effective in blocking the excitatory responses to NMDLA and L-aspartate in most cells tested. The excitations produced by L-glutamate and DLH were poorly affected and those of kainate and keto-kainate were not affected (figures 20,21,22,23,24&25). Table 1B further clarifies these observations. Complete recovery of NMDLA and L-aspartate excitations was obtained 3 to 10mins after the DαAA ejecting current was turned off.

4.3. (4). Effects of atropine, GDEE and DαAA on Cortico-thalamic synaptic excitation.

Atropine (40-100nA, 3 to 9mins) did not affect the synaptically induced responses of TCR cells orthodromically activated from the sensorimotor areas.

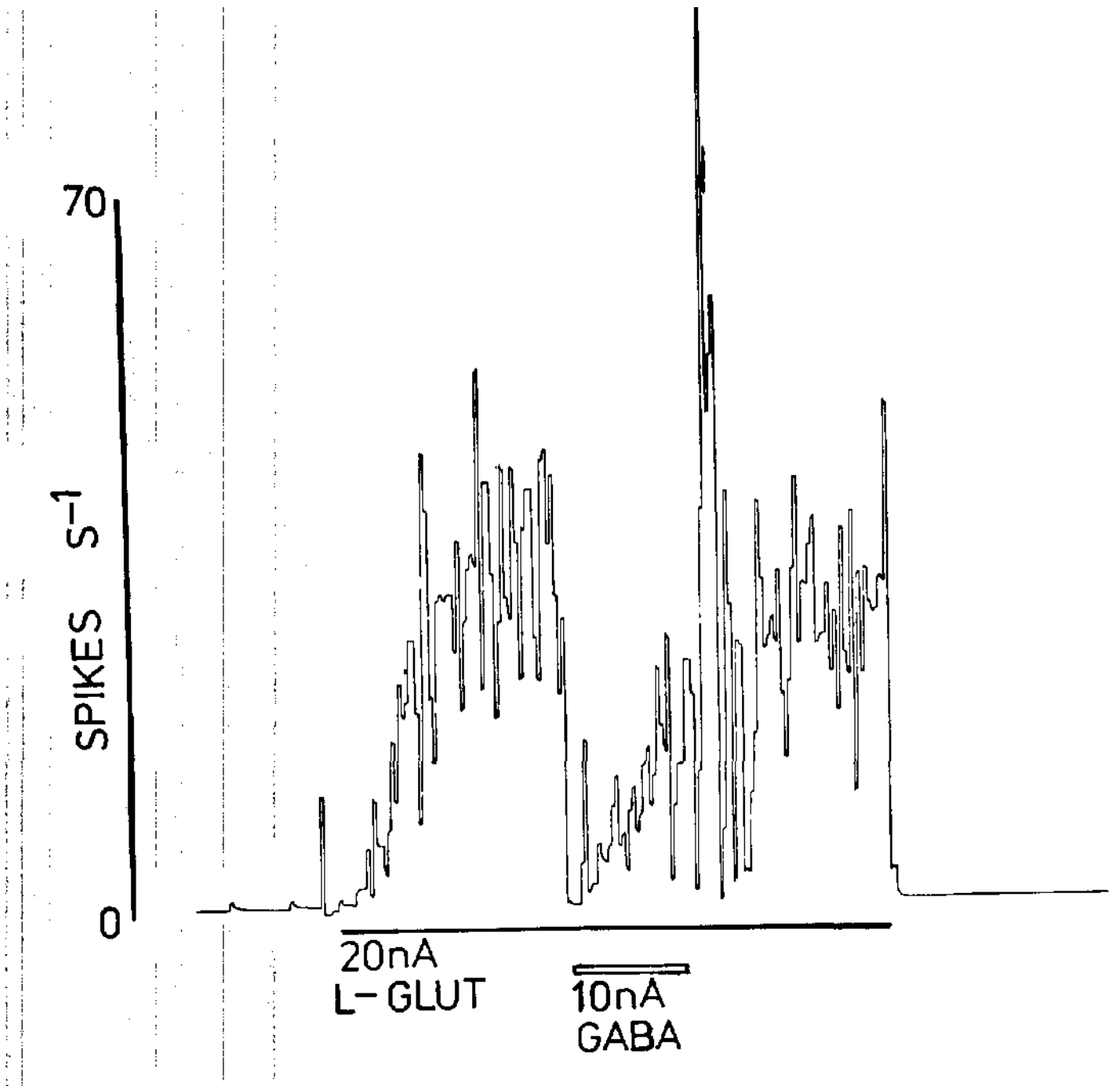
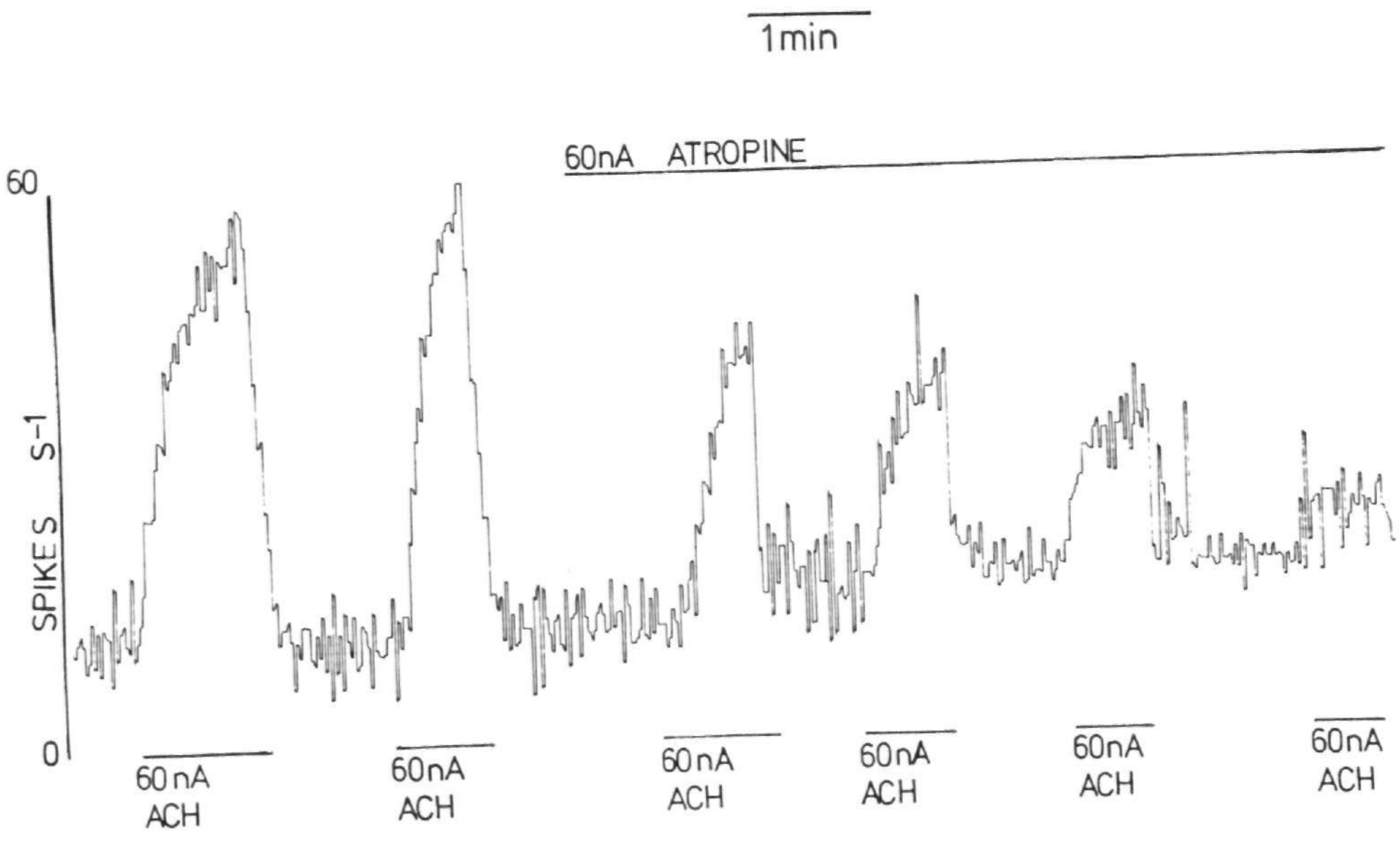

70
SPIKES S⁻¹
0
20nA
L-GLUT
10nA
GABA

Figure 14: A) The antagonistic effect of atropine on Acetylcholine.

B) The recovery of ACH excitations after the current ejecting atropine had been turned off.


1min
60nA ATROPINE
60
SPIKES S-1
0
60 nA ACH
60nA ACH
60 nA ACH
60nA ACH
60nA ACH
60nA ACH

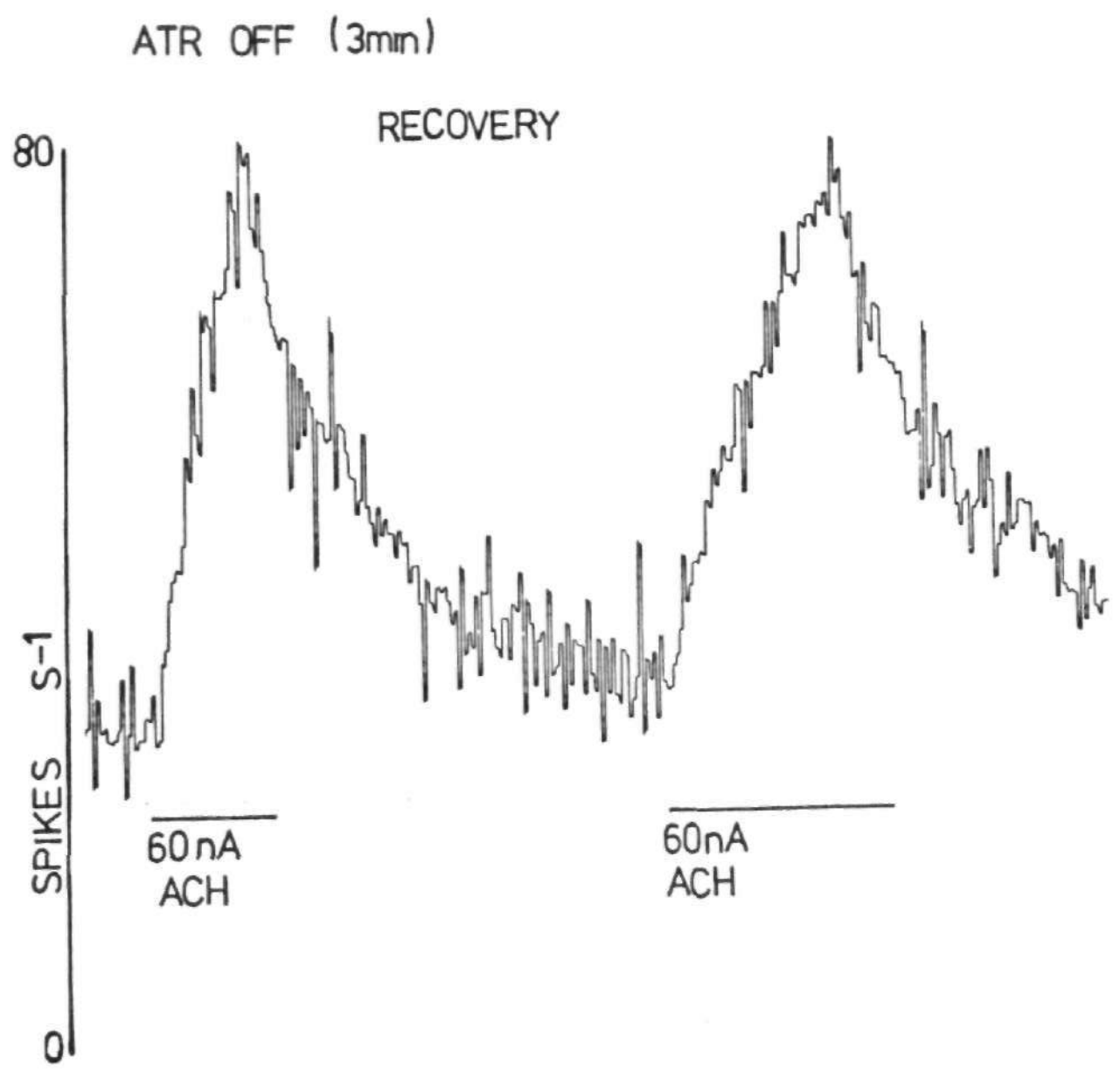
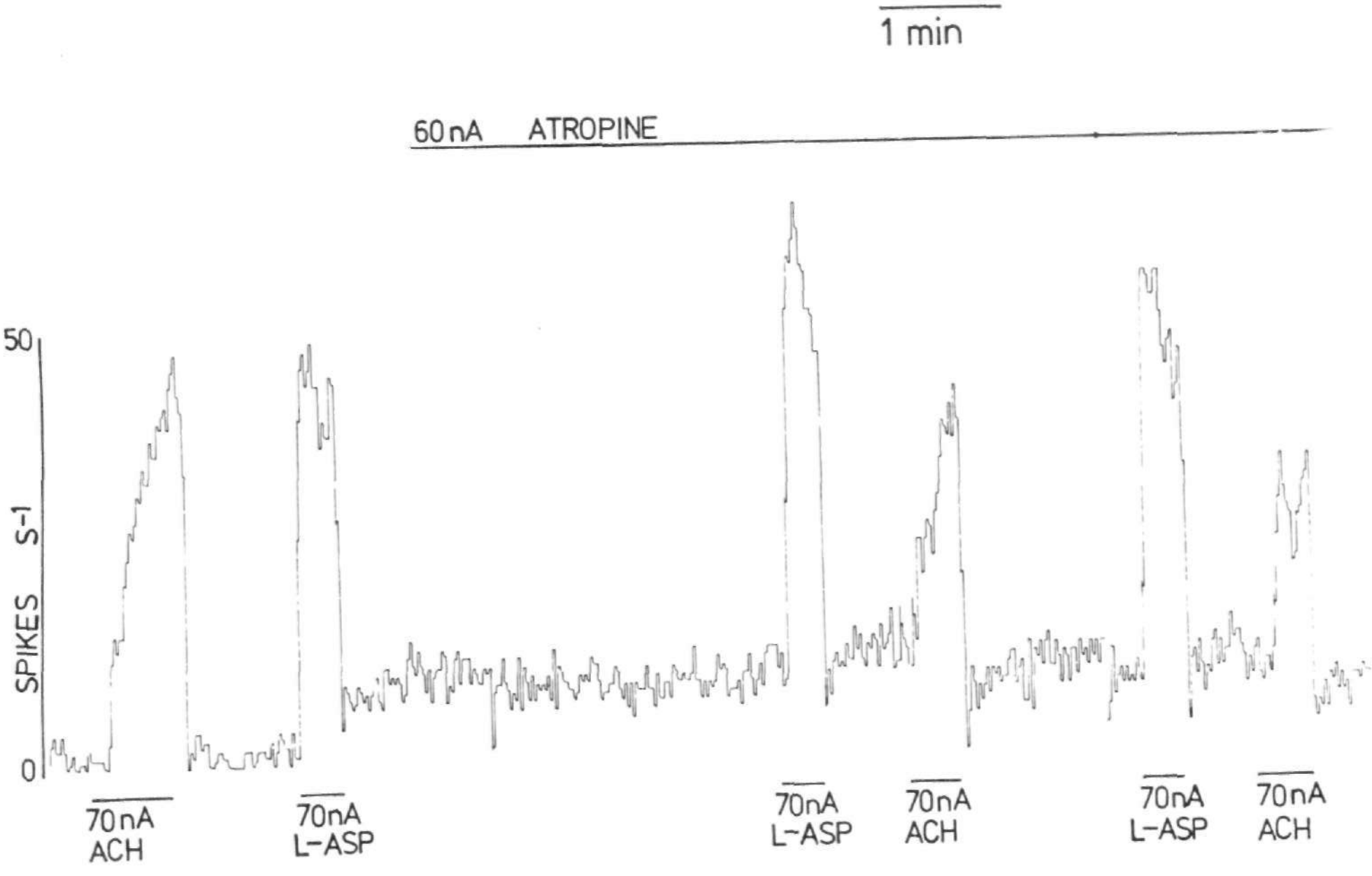

ATR OFF (3min)
RECOVERY
80
SPIKES S-1
0
60nA
ACH
60nA
ACH

Figure 15: The differential antagonism of Atropine on acetylcholine and L-aspartate.
The recovery phase is shown on the rear page.


1 min
60 nA ATROPINE
50
SPIKES S-1
0
70nA ACH
70nA L-ASP
70nA L-ASP
70nA ACH
70nA L-ASP
70nA ACH

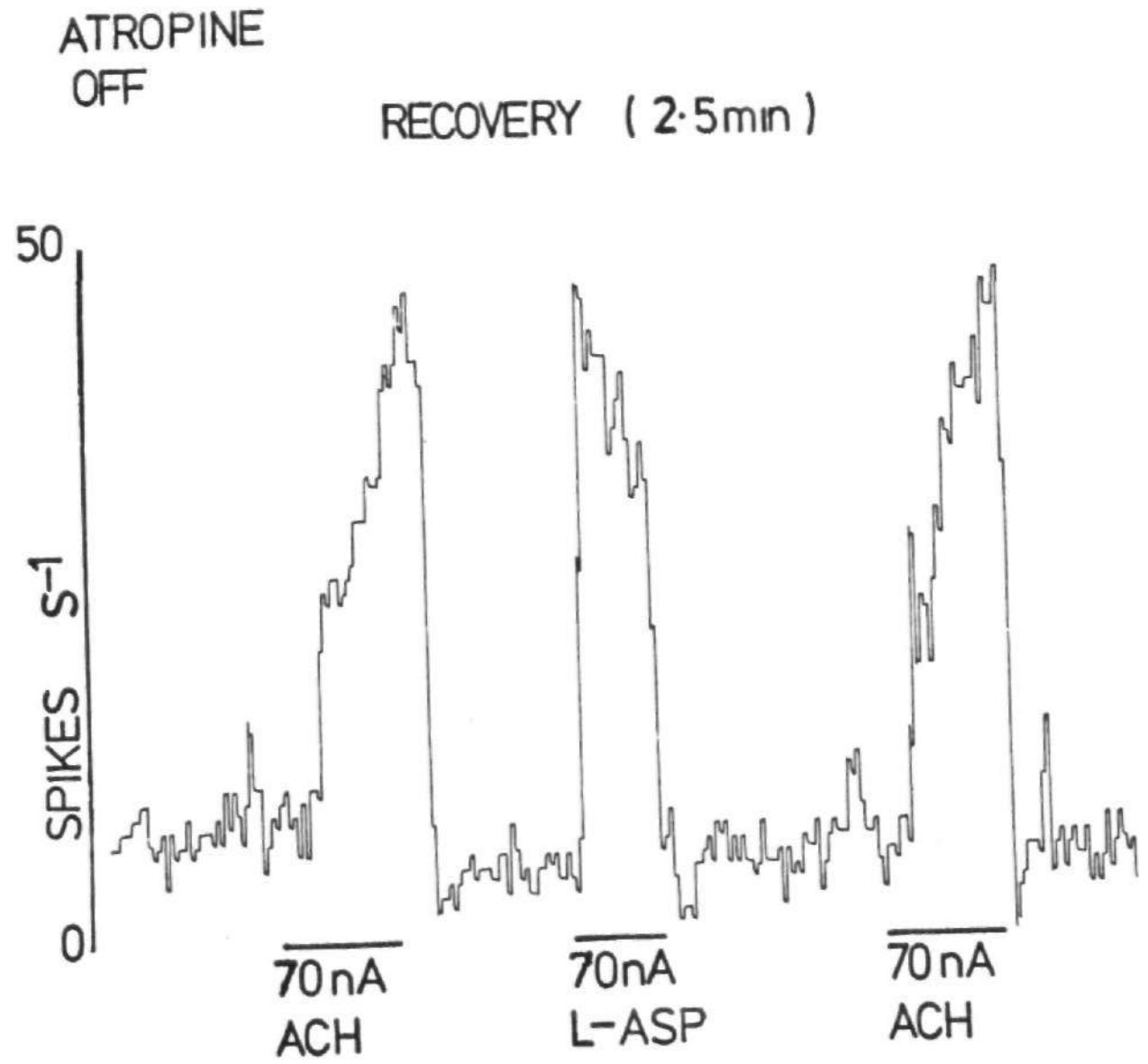
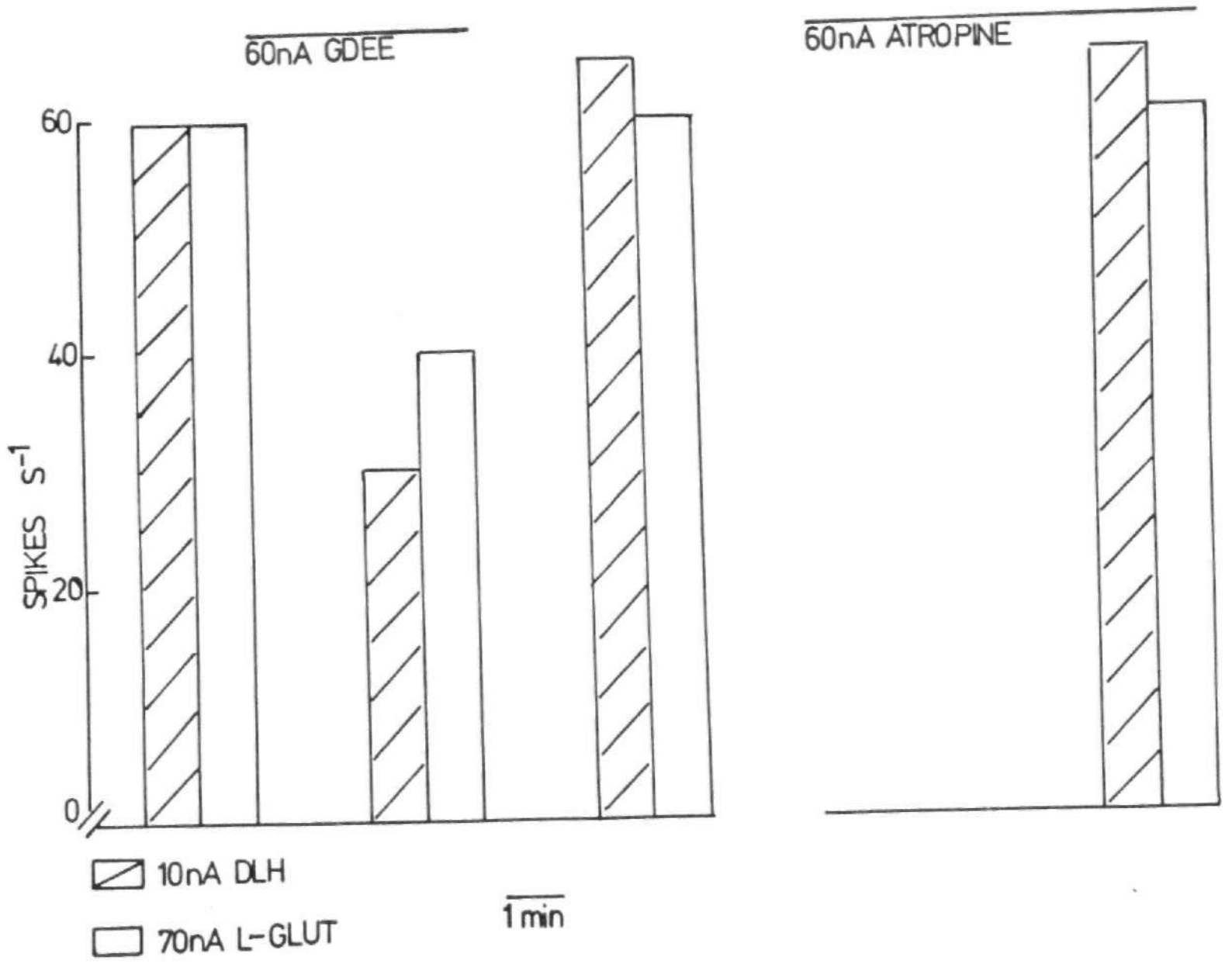

ATROPINE
OFF
RECOVERY (2·5min)
50
SPIKES S-1
0
70nA
ACH
70nA
L-ASP
70nA
ACH

Figure 16: The effect of GDEE and Atropine on DLH and L-glutamate induced excitations of a (TCR) neurone.

Figure 17: The effect of the application of GDEE on L-glutamate and L-aspartate excitations on a thalamocortical relay neurone. Also shown is the recovery period after the GDEE ejecting current had been turned off.


60nA GDEE
60nA ATROPINE
60
40
20
0
SPIKES S-1
10nA DLH
70nA L-GLUT
1 min

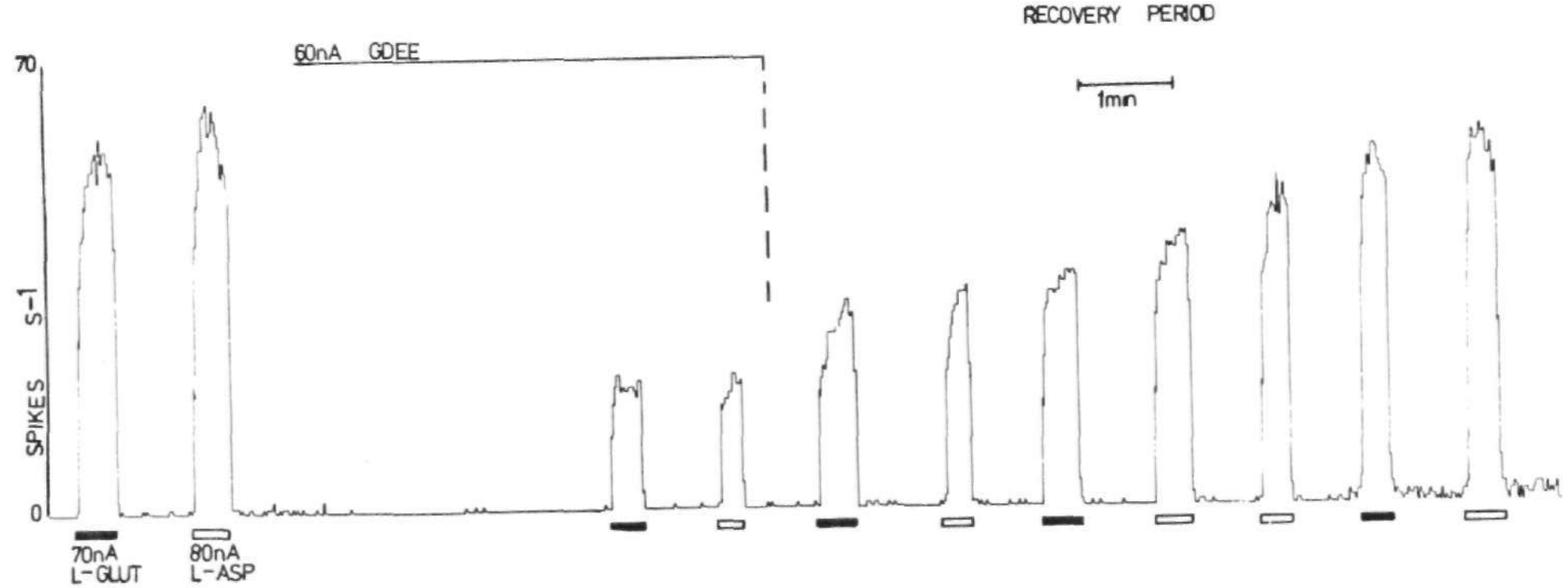
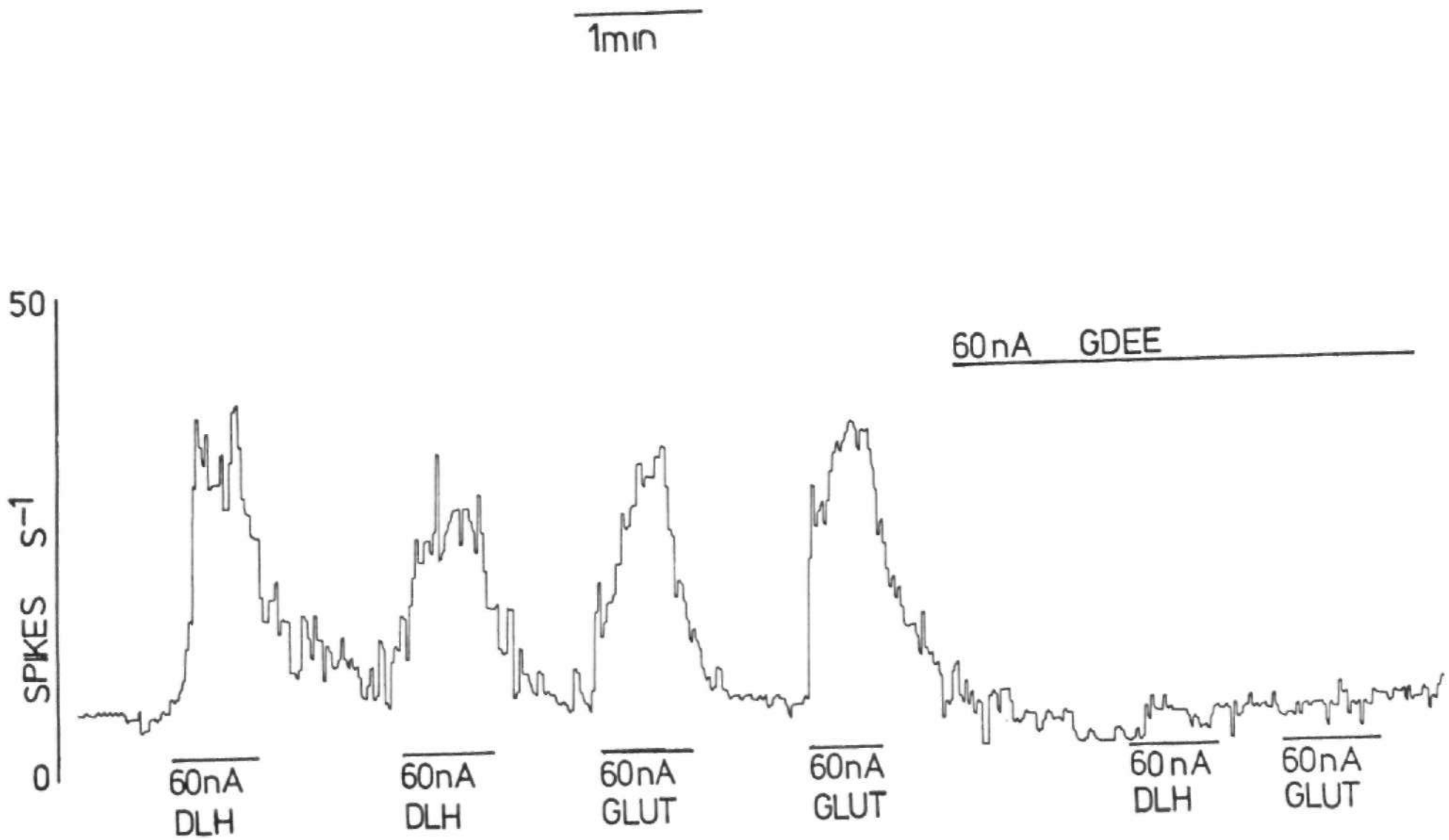

RECOVERY PERIOD
60nA GDEE
1min
70
SPIKES S-1
0
70nA
L-GLUT
80nA
L-ASP

Figure 18: A) Effect of Glutamate diethylester on DL-homocysteate and L-glutamate excitations. The recovery is shown on the next page.


1min
50
SPIKES S⁻¹
0
60nA GDEE
60nA DLH
60nA DLH
60nA GLUT
60nA GLUT
60nA DLH
60nA GLUT

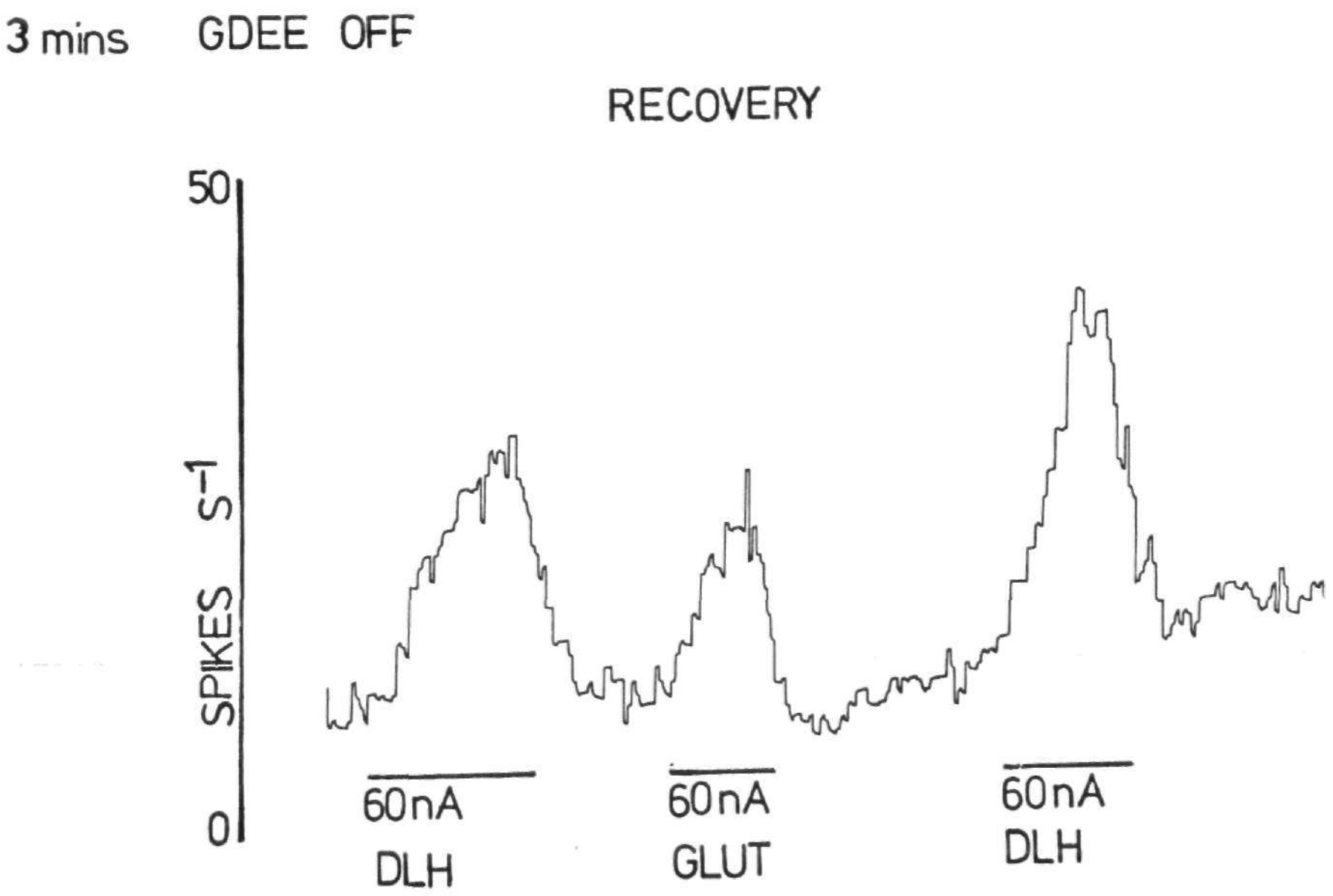
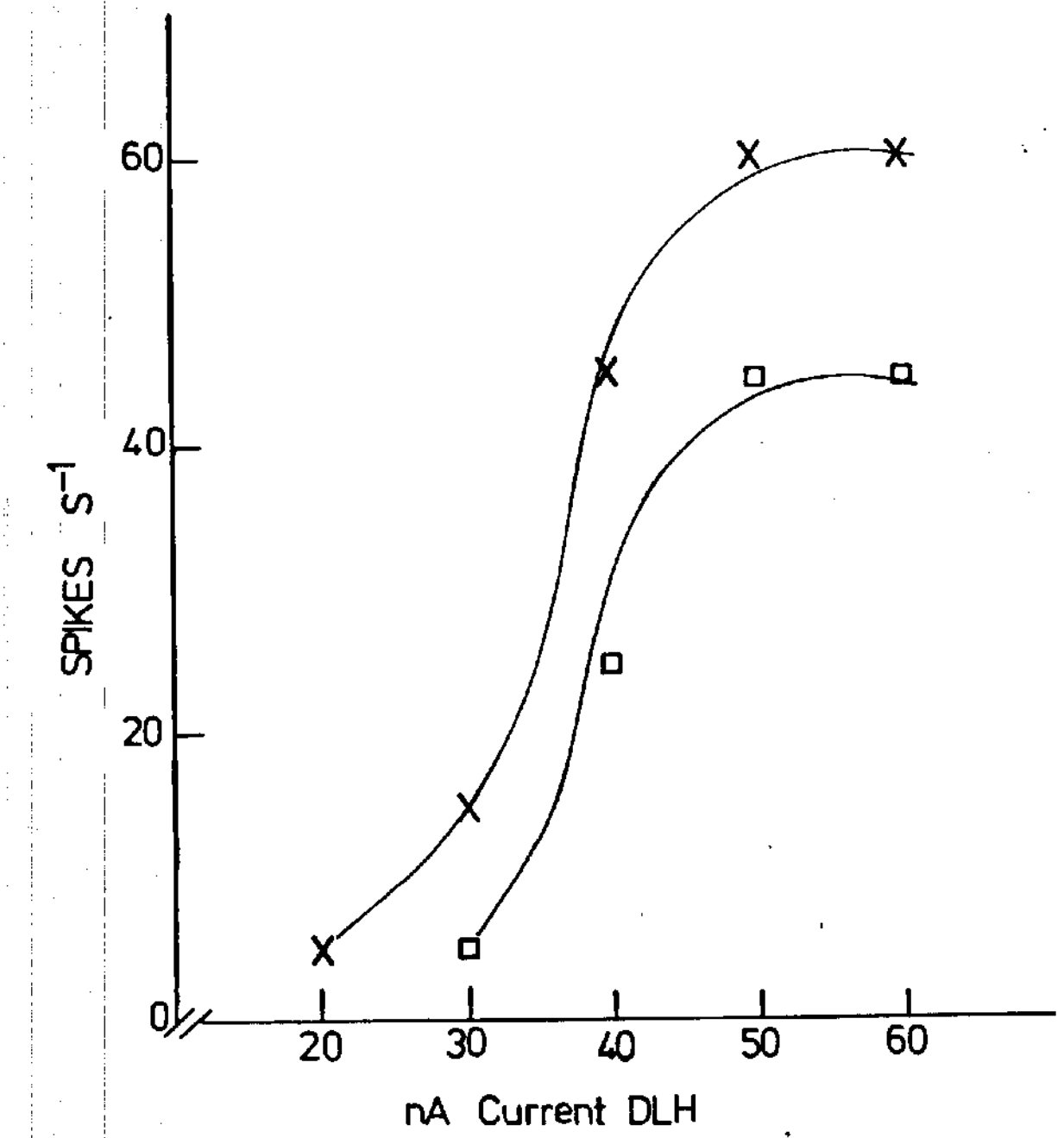

3 mins GDEE OFF
RECOVERY
50
0
SPIKES S⁻¹
60nA
DLH
60nA
GLUT
60nA
DLH

Figure 18: B) A dose response curve of DLH before and after the application of GDEE. A shift to the right is indicated.



X CONTROL

□ AFTER GDEE 40nA[90sec]

Figure 19: The failure GDEE to antagonise the excitatory activity of Keto-Kainate.

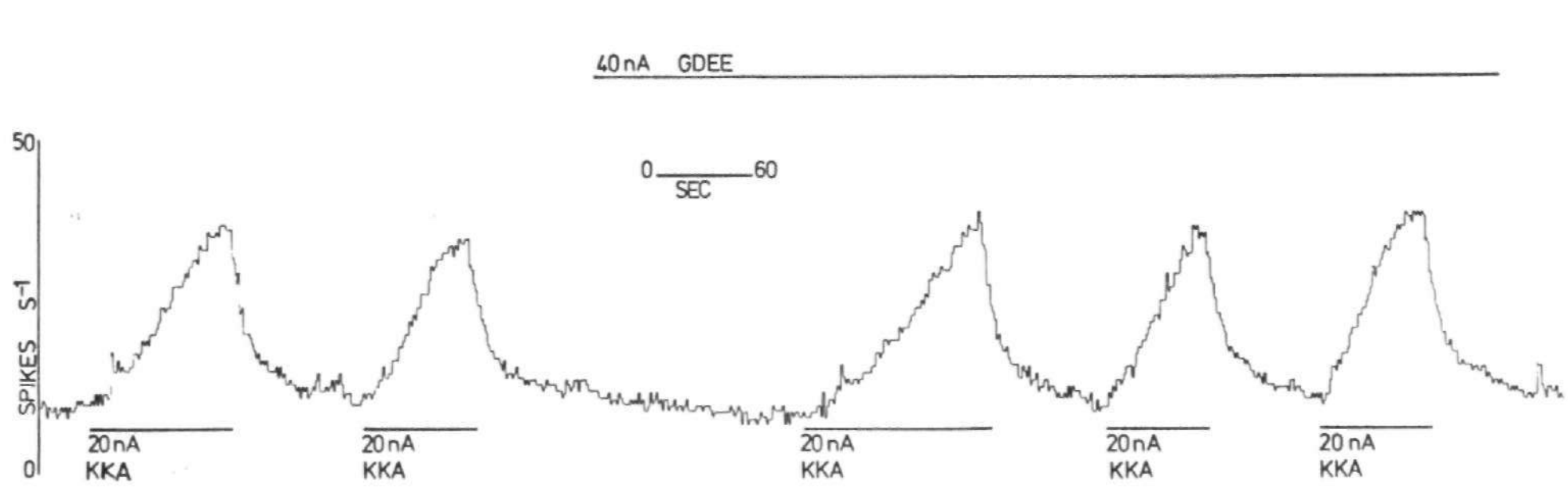
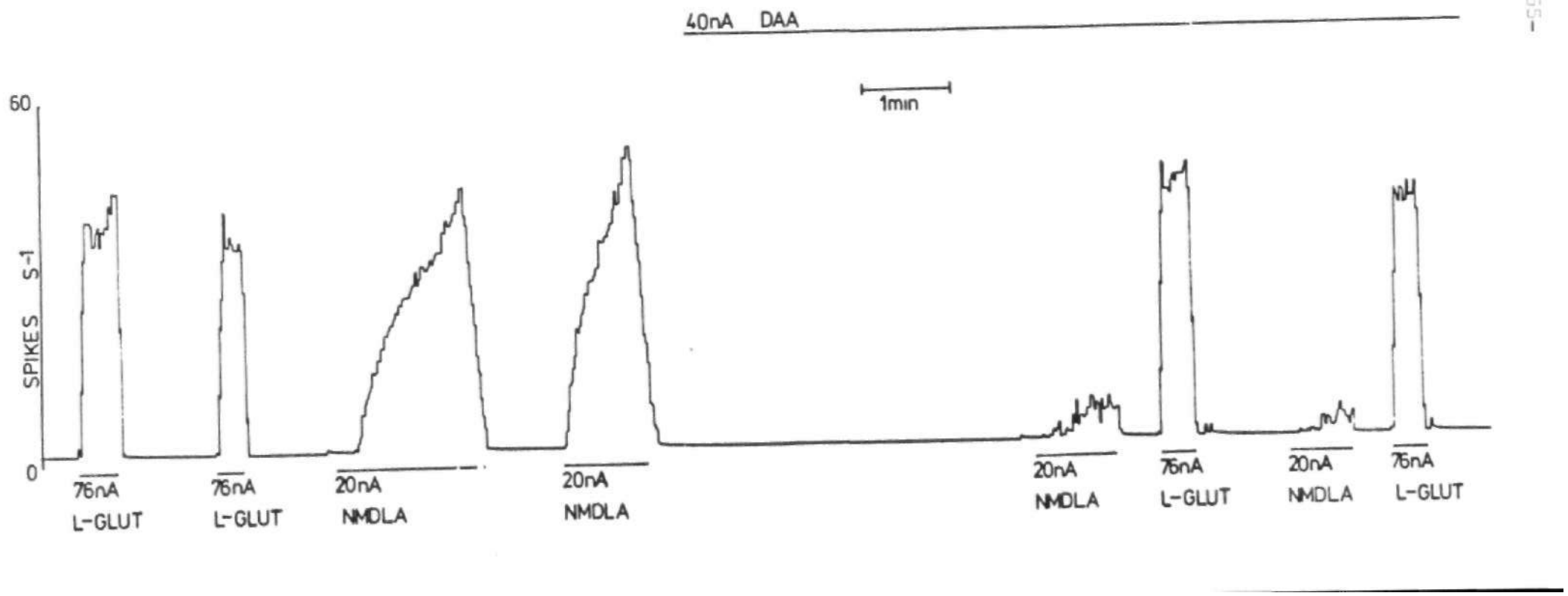

40 nA GDEE
50
SPIKES S-1
0
0
60
SEC
20 nA
KKA
20 nA
KKA
20 nA
KKA
20 nA
KKA
20 nA
KKA

Figure 20: The differential antagonism of D αAA on NMDLA and L-glutamate excitations.

The next page shows the recovery phase of NMDLA induced excitation.


40nA DAA
1min
60
SPIKES S-1
0
75nA
L-GLUT
75nA
L-GLUT
20nA
NMDLA
20nA
NMDLA
20nA
NMDLA
75nA
L-GLUT
20nA
NMDLA
75nA
L-GLUT

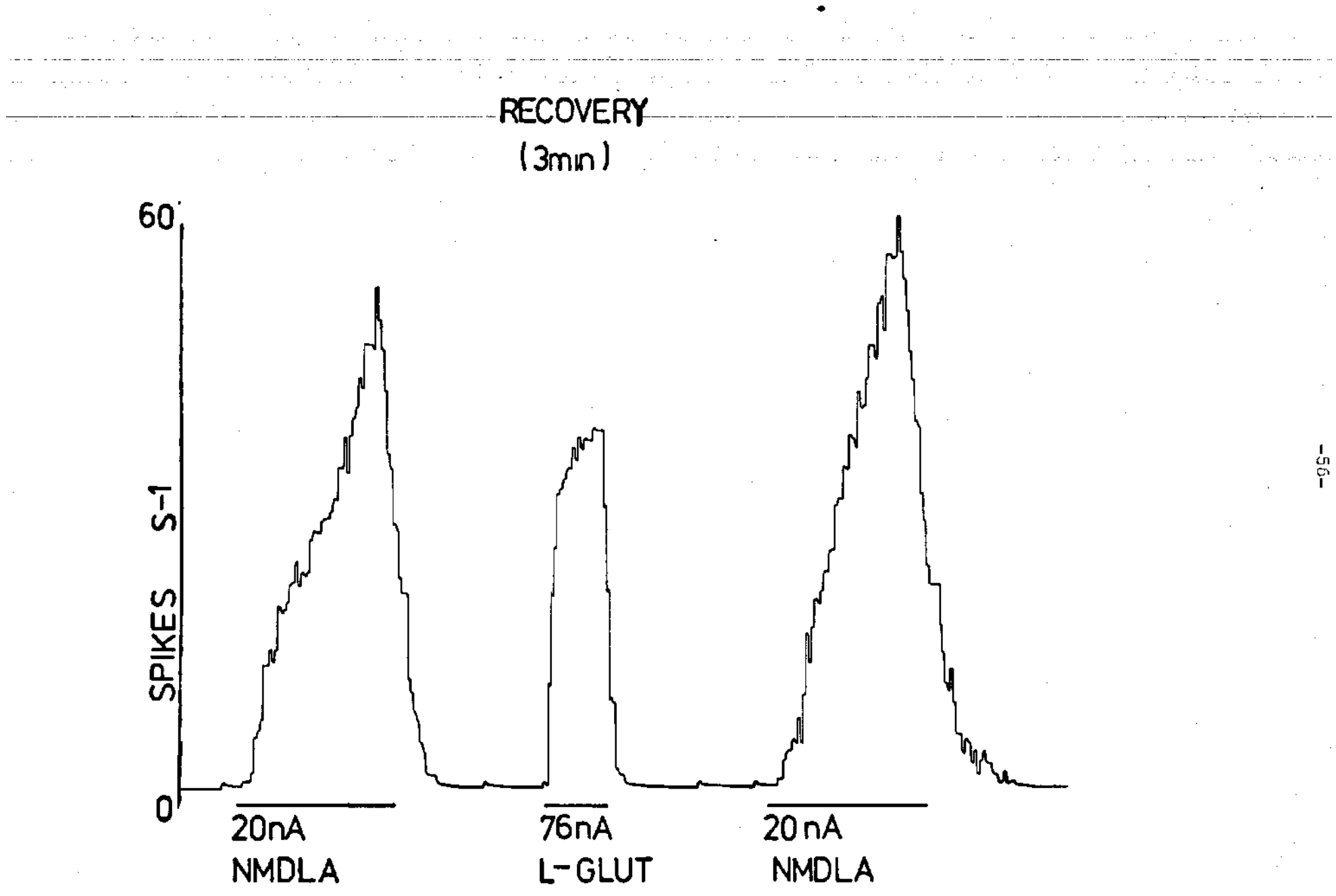
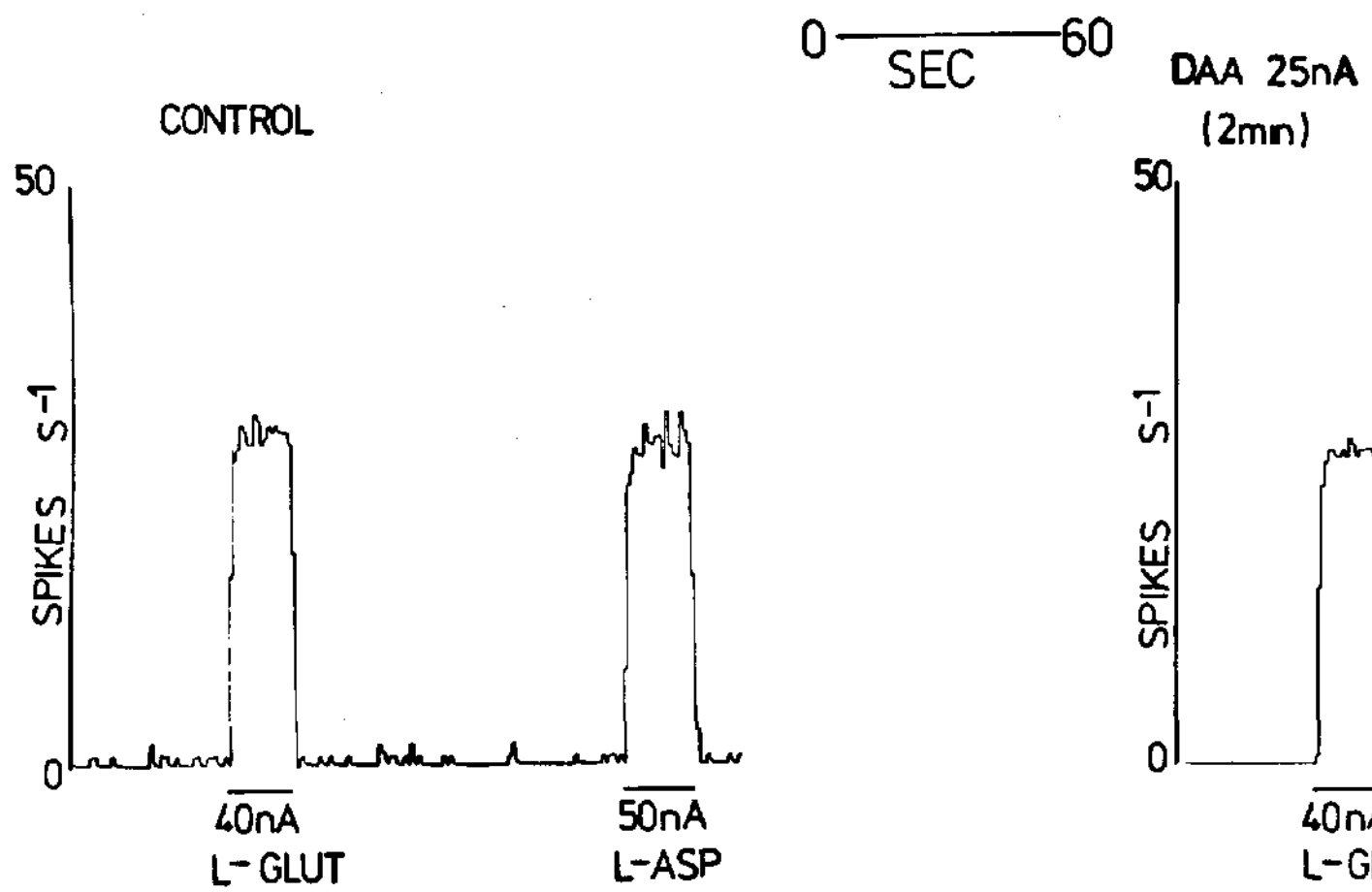

RECOVERY
(3min)
60
SPIKES S-1
0
20nA
NMDLA
76nA
L-GLUT
20nA
NMDLA

Figure 21: The differential antagonistic effect of DαAA on L-glutamate and L-aspartate induced excitations.
The recovery phase is shown
on the rear page.


0
SEC
60
CONTROL
50
SPIKES S-1
0
40nA
L-GLUT
50nA
L-ASP
DAA 25nA
(2mn)
50
SPIKES S-1
0
40nA
L-GLUT
50nA
L-ASP
40nA
L-GLUT

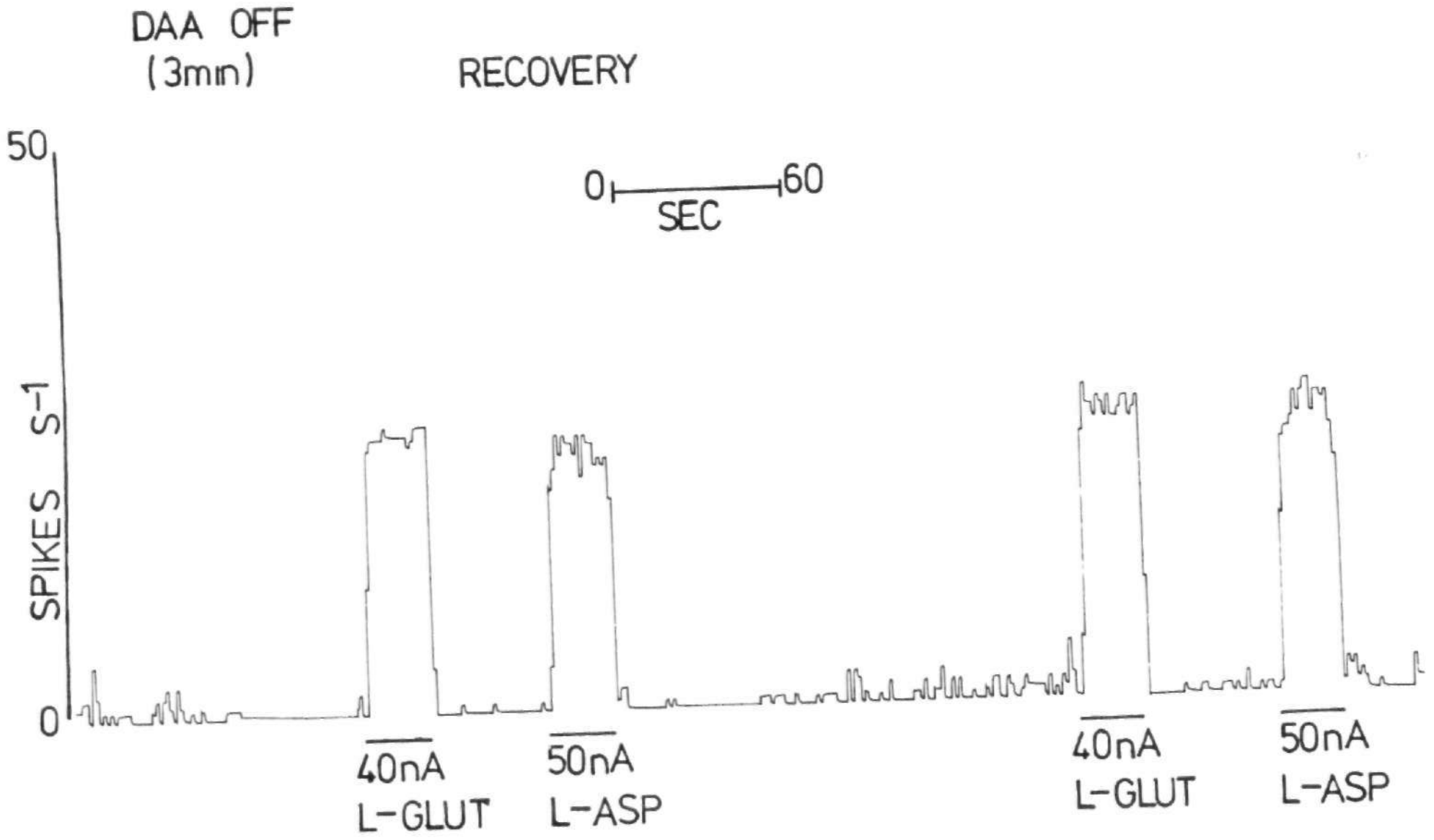

DAA OFF
(3min)
RECOVERY
50
0
60
SEC
SPIKES S-1
0
40nA
L-GLUT
50nA
L-ASP
40nA
L-GLUT
50nA
L-ASP

Figure 22: The apparent lack of effect of DαAA on DLH and L-glutamate excitations.

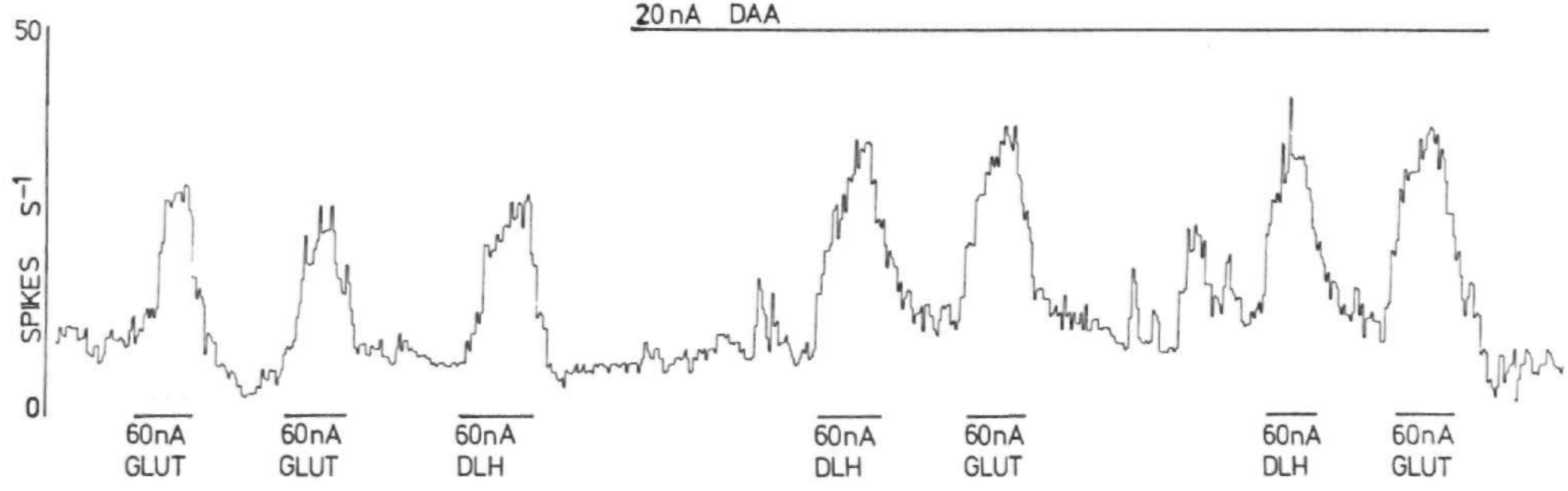
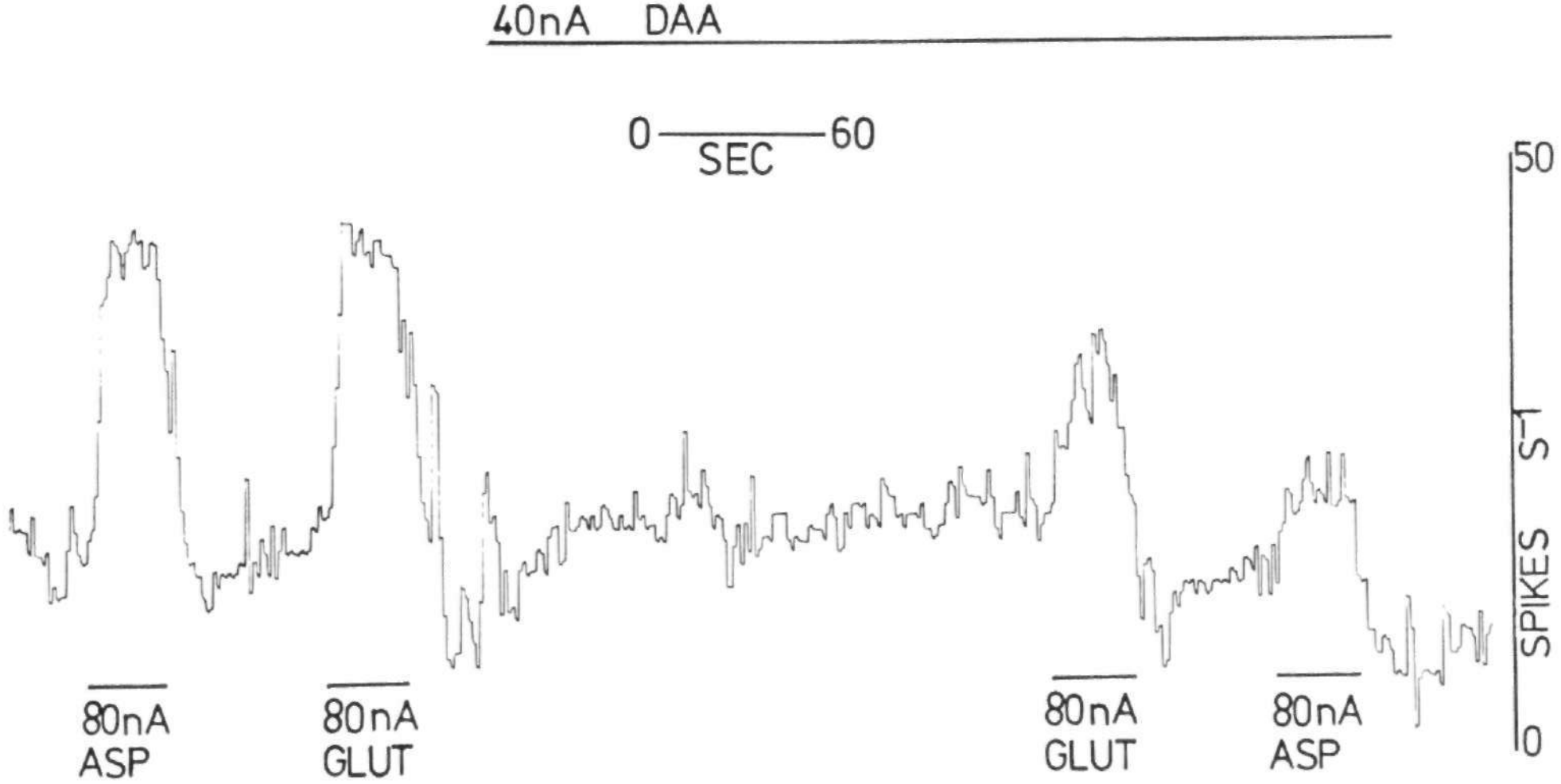

20 nA DAA
50
SPIKES S⁻¹
0
60nA GLUT
60nA GLUT
60nA DLH
60nA DLH
60nA GLUT
60nA DLH
60nA GLUT

Figure 23: A differential antagonism of L-glutamate and L-aspartate induced excitations by D αAA.

The recovery of these excitations are shown on the rear page.


40nA DAA
0
SEC
60
50
SPIKES S⁻¹
0
80nA
ASP
80nA
GLUT
80nA
GLUT
80nA
ASP

2mn DAA OFF

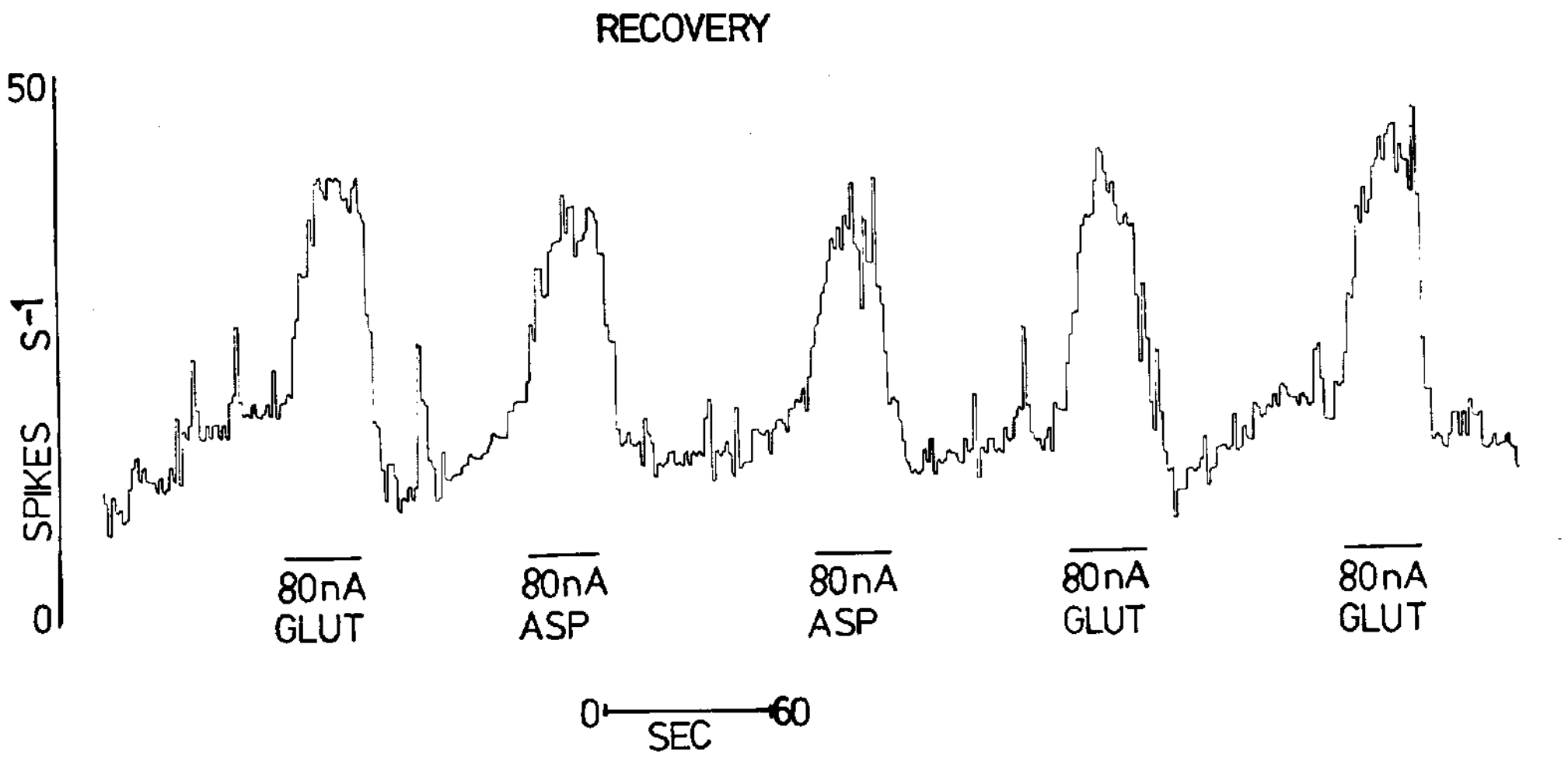

RECOVERY
50
SPIKES S-1
0
80nA
GLUT
80nA
ASP
80nA
ASP
80nA
GLUT
80nA
GLUT
0
60
SEC

Figure 24: The failure of Dα AA to antagonise the excitatory actions of Keto-kainate.

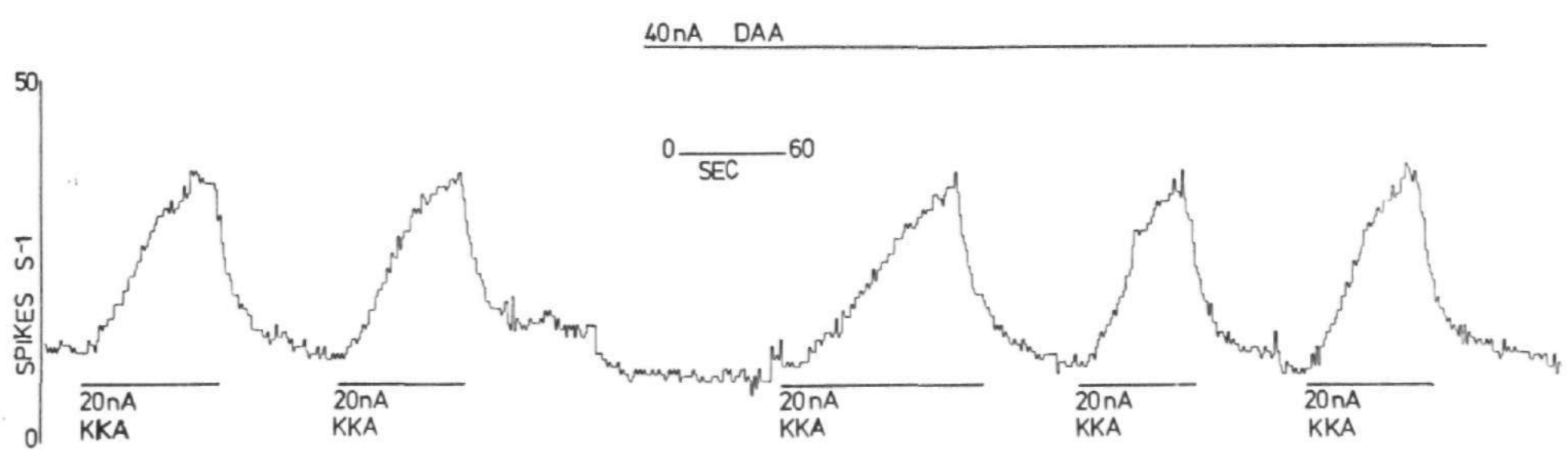
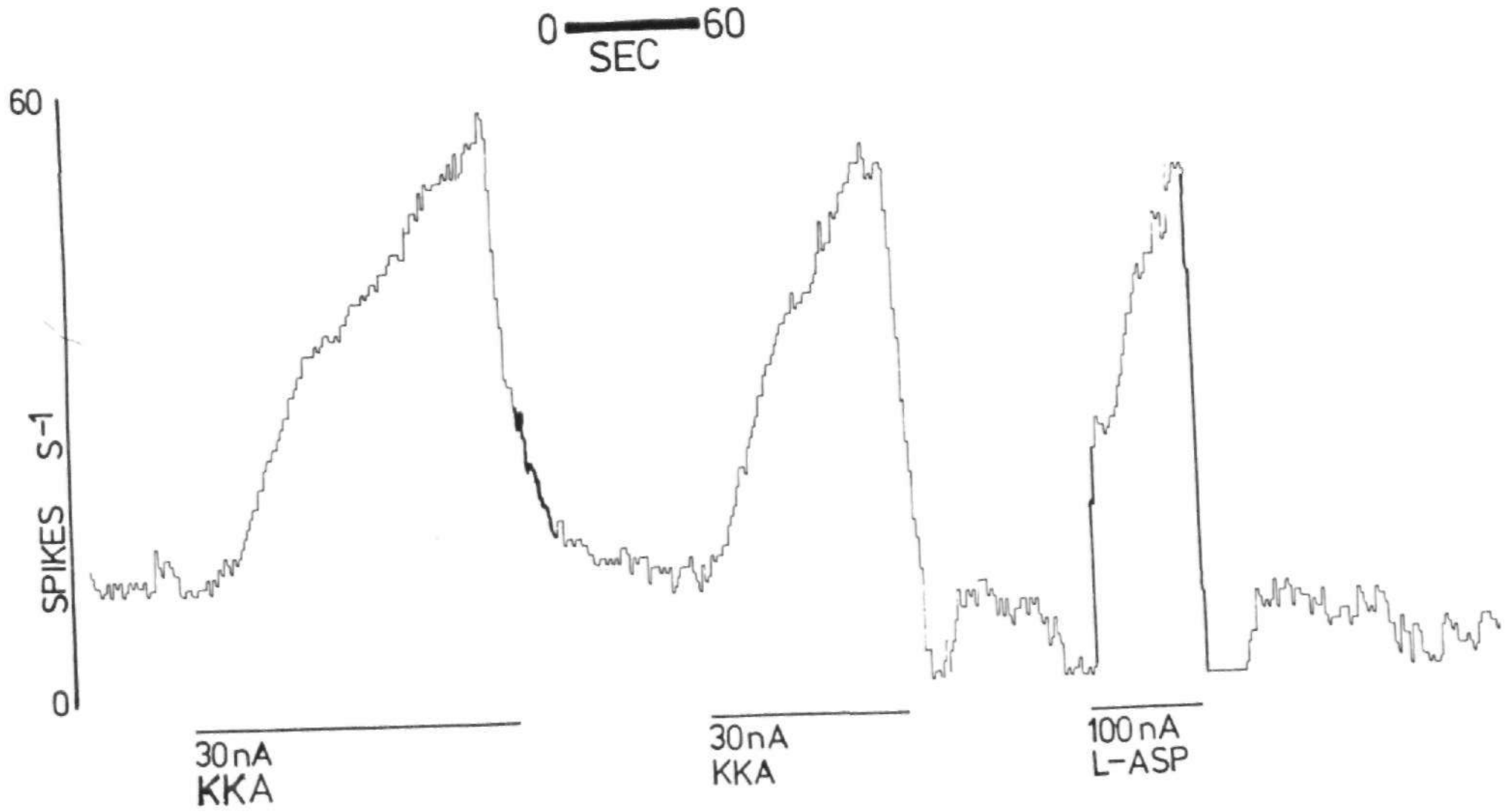
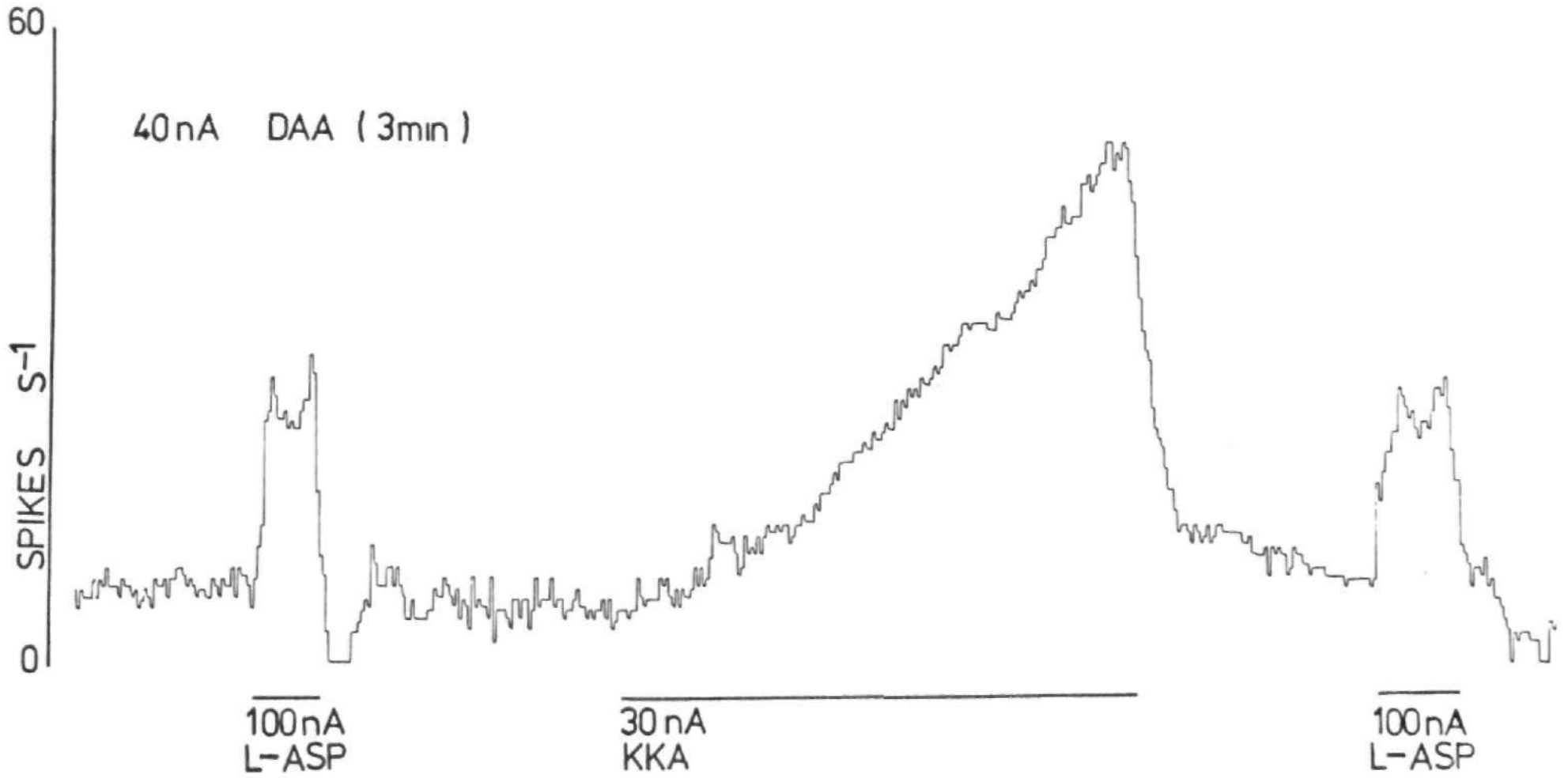
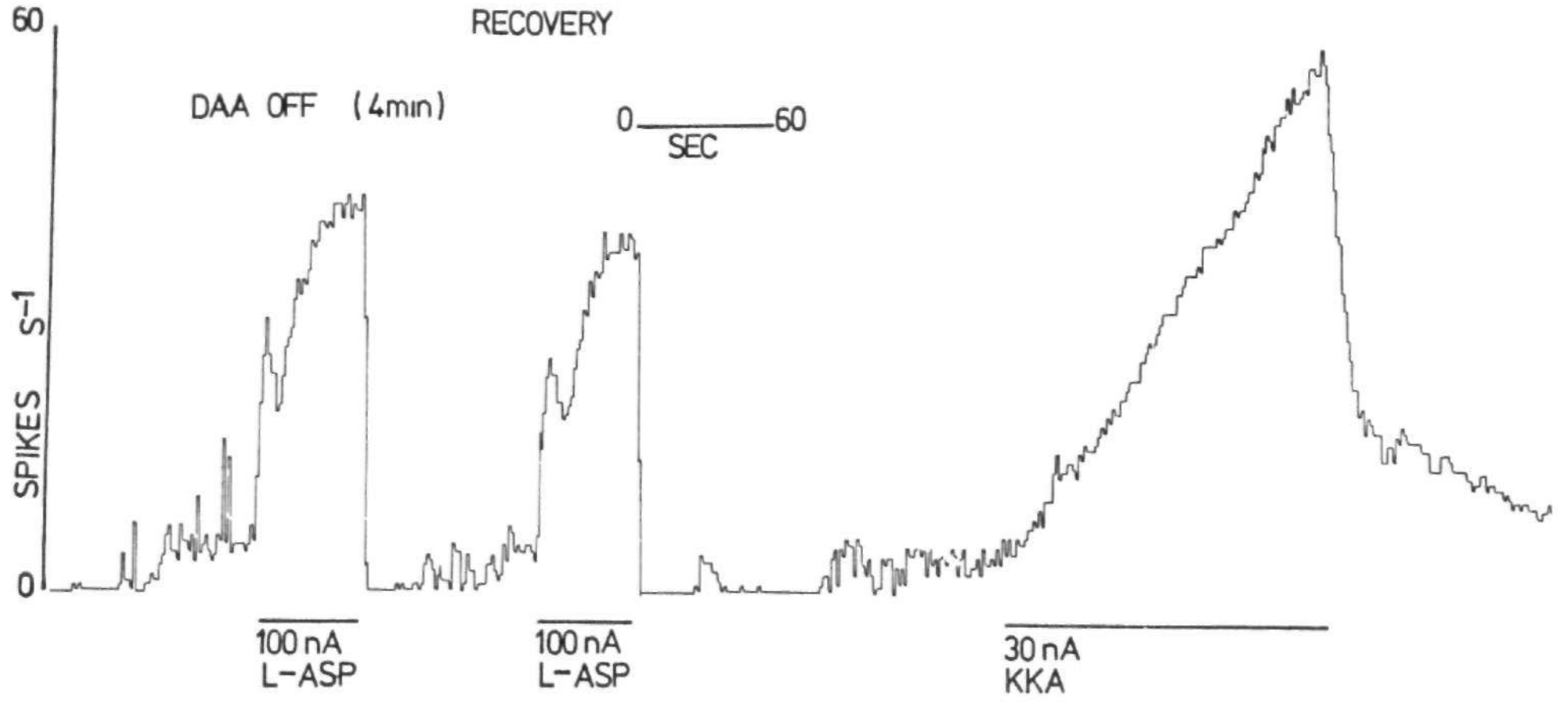

40nA DAA
50
SPIKES S-1
0
0
60
SEC
20nA
KKA
20nA
KKA
20nA
KKA
20nA
KKA
20nA
KKA

Figure 25: The selective effect of D α AA on keto-kainate and L-aspartate induced excitations. A recovery of L-aspartate excitations occurred after the current ejecting the D α AA had been turned off. The slow onset of keto-kainate excitations in contrast to the fast onset of L-aspartate excitations is clearly shown in these traces.


0
60
SEC
60
SPIKES S-1
0
30nA
KKA
30nA
KKA
100nA
L-ASP


60
40 nA DAA (3min)
SPIKES S-1
0
100 nA
L-ASP
30 nA
KKA
100 nA
L-ASP


60
RECOVERY
DAA OFF (4min)
0
60
SEC
SPIKES S⁻¹
0
100 nA
L-ASP
100 nA
L-ASP
30 nA
KKA

The administration of GDEE (40-80nA) for 2 to 10mins blocked the synaptic excitation in 6 out 21 cells studied particularly at high concentrations. Majority of these cells (72%) were poorly or not affected by GDEE (e.g. figure 26).

In 37 out of 43 cells D-α-AA blocked or significantly reduced corticothalamic synaptic excitation on TCR neurones. Ejection currents ranging from 20 to 40nA and applied for 2 to 5mins were effective in producing these antagonistic effects of D α AA. The remaining cells were lost or failed to show satisfactory recovery 10 to 30 mins after the D α AA ejection current had been turned off.

The post stimulus histograms of figures 27A, & B illustrate the reversible blockade of corticothalamic synaptic excitations by D α AA.

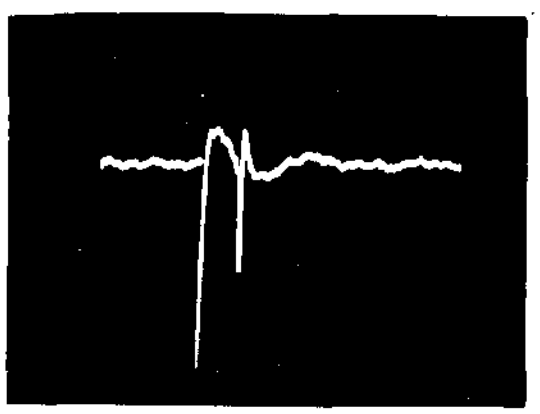
4.3. (5). <u>Effects of GDEE and DAA on the synaptic excitation of thalamocortical relay neurone by stimulation of a leg nerve</u>.

Thalamocortical relay (TCR) cells orthodromically activated by the stimulation of a contralateral hind limb nerve were more sensitive to iontophoretically applied L-glutamate than to L-aspartate and were considerably more sensitive to kainate than to NMDLA. The actions of GDEE and D α AA on these amino acids induced excitatory responses were similar to those described earlier. D α AA reduced the excitations of NMDLA and L-aspartate more than those produced by L-glutamate.

On the other hand L-glutamate excitations were more susceptible to depression than NMDLA induced excitations by the application of GDEE. D α AA (60 to 80nA) ejected for 10 to 15 mins had no effect on the synaptic excitations of 7 out of 9 TCR cells studied following hind limb nerve stimulation (figure 28), whereas NMDLA responses were considerably depressed within 2 mins of commencing the D α AA ejection.

Figure 26: A) orthodromic cortico-thalamic spike.
B) A raster display of the variability in latency and consistency of the spike.
C) GDEE had no effect on the cortico-thalamic synaptically evoked spike.

A

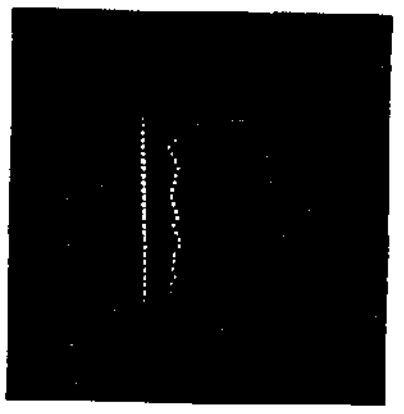


0·5mV
5ms

CONTROL

60nA GDEE
(10min)

B



C

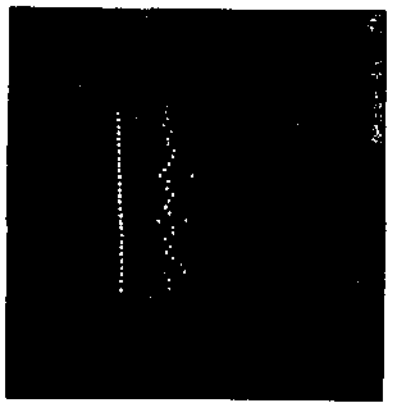
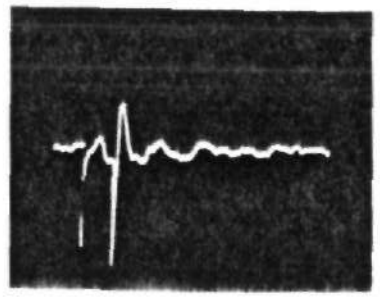


Figure 27: A) The antagonistic action of Dα AA on the orthodromic activation of a thalamo-cortical relay neurone following the stimulation of the ipsilateral somatosensory cortex.

Post stimulus histograms computed from the raster.

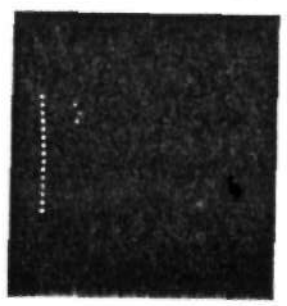
CORTICO-THALAMIC EXCITATION



0·5mV
5ms



20nA DAA (5min)



RECOVERY (6·5min)



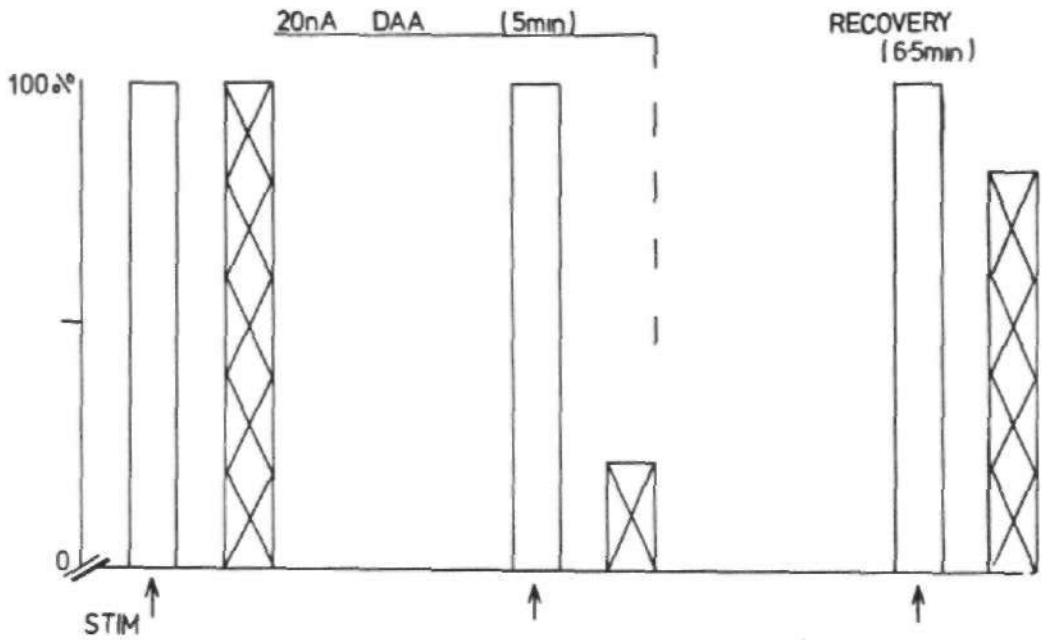
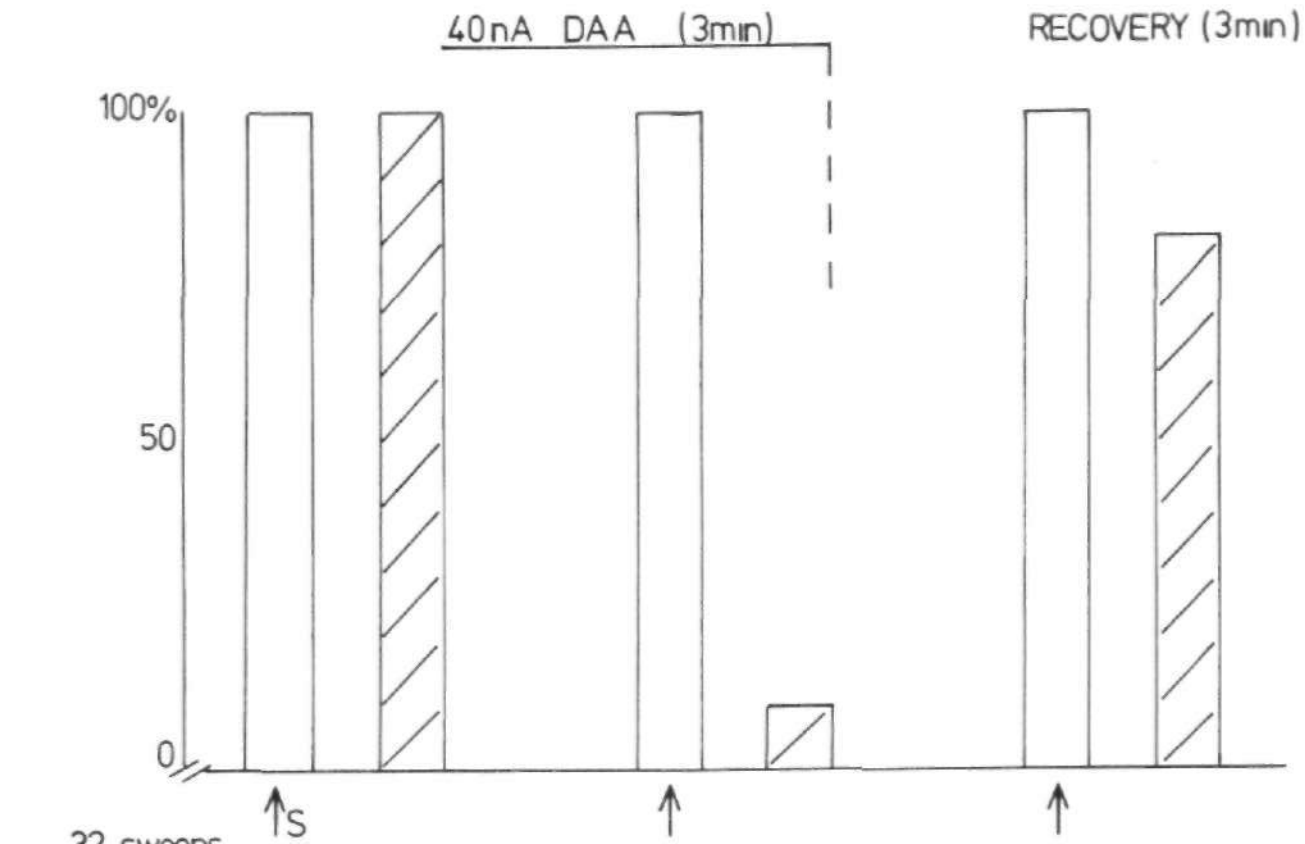


Figure 27: a) The antagonistic effect of Dα AA on the orthodromic activation of thalamocortical relay neurone following stimulation of the ipsilateral somatosensory cortex. The current of Dα AA was larger and for a shorter time.



= 32 sweeps
100% SYNAPTIC EXCITATION

↑ STIMULUS ARTEFACT

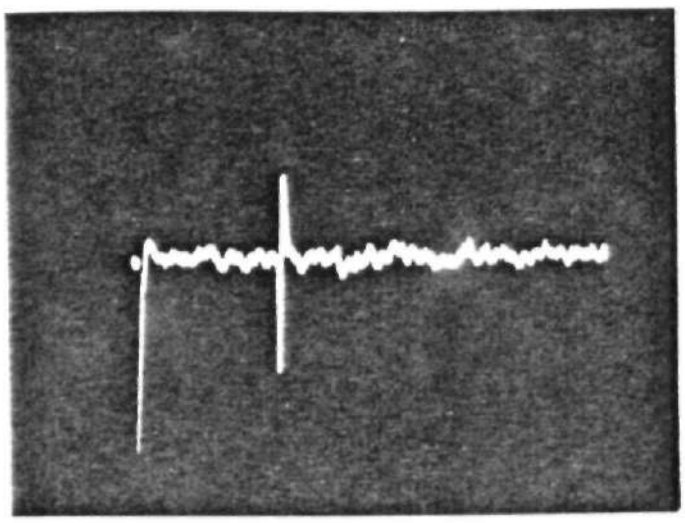
▨ CORTICO-THALAMIC EVOKED EXCITATION

Figure 28: A) Orthodromic activation of a TCR neurone by stimulation of a hind limb nerve.

B) A raster display of the variability and consistency of the spike.

C) DαAA had no effect on this synaptic excitation.

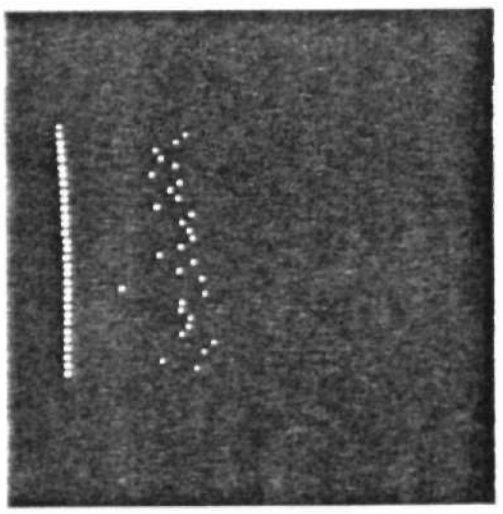
A



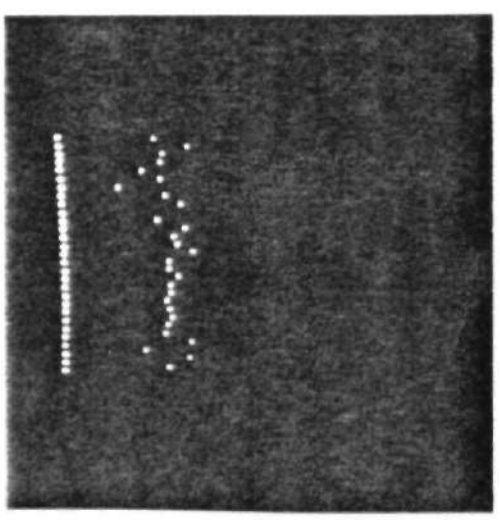
CONTROL

0·5mV
5ms

B



C



60nA DAA
(5min)

In 21/30 TCR cells at VL, GDEE significantly reduced the synaptic excitations produced by stimulating a contralateral hind limb nerve and responses to L-glutamate at doses that did not influence the excitatory responses to NMDLA.

Figure 29,shows a typical effect of GDEE on the synaptic excitation of a thalamocortical relay cell following hind limb nerve stimulation.

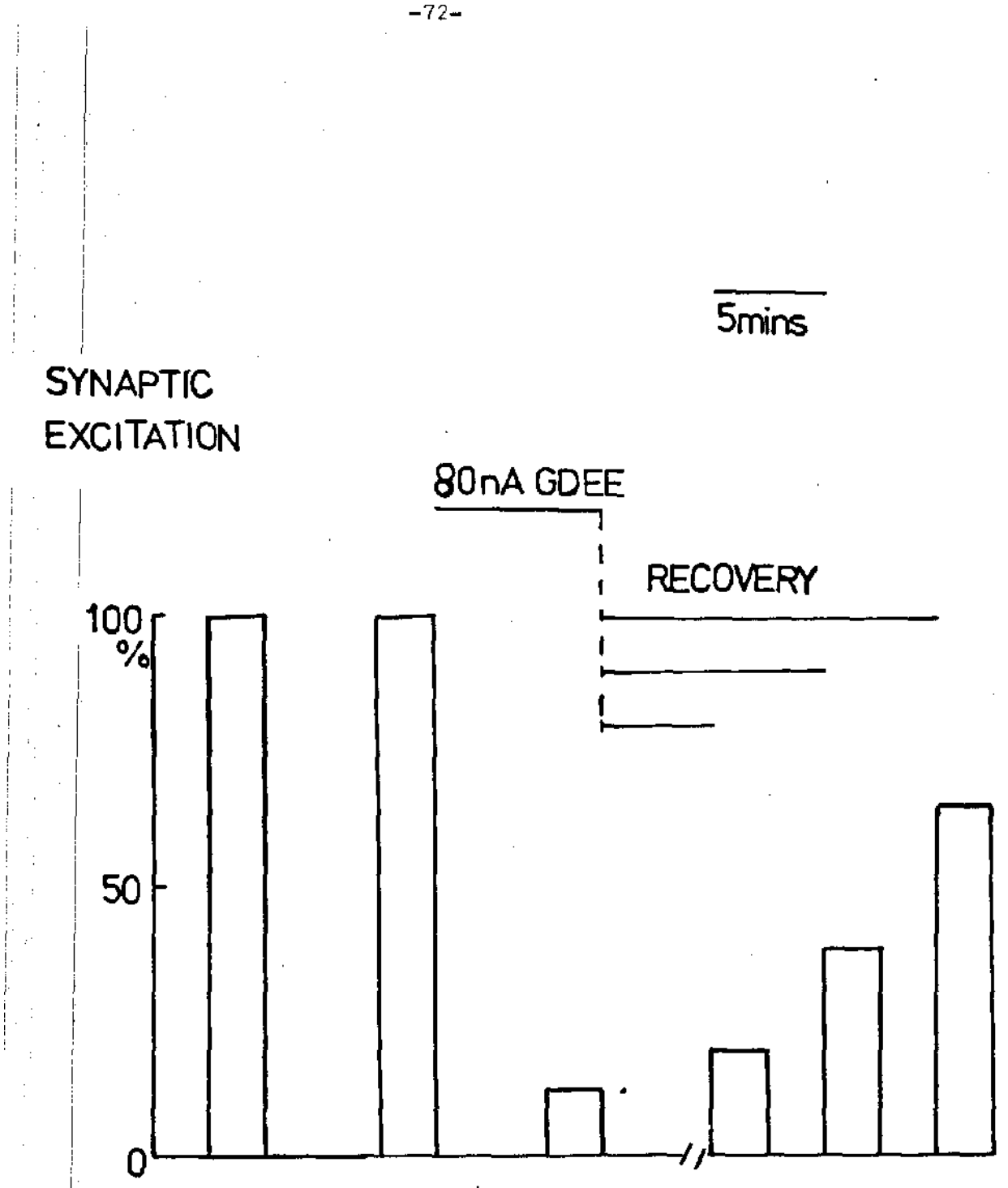
4.3. (6). The relative potencies of excitatory amino acid analogues on thalamocortical relay cells.

The potencies of kainate, ketokainate, N-methl-DL-aspartate, L-aspartate, DL-homocysteate, DL-aspartate, L-glutamate and Dihydro-kainate were tested with submaximal currents to give equivalent or approximate equal number of spikes between 20-60 spikes/sec. Table 1C illustrates the range of currents, potencies and the number of cells tested. Figures 30,31,32 show some typical traces of the observations.

4.3. (7). Dose response curves of L-glutamate, L-aspartate, DL-homocysteate and Acetylcholine.

Since an essentially linear relationship exists between the applied current and the quantity of material ejected (Zieglgansberger, Herz & Teschemacher, 1969), by varying the intensity of the current a 'dose-response' curve for the neurone under study could be developed. Figures 33,34,35,36 show 'dose-response' curves of L-glutamate, L-aspartate, DLH and Acetylcholine respectively, on a thalamocortical relay neurone evoked by orthodromic activation from the somatosensory cortex. The majority of the cells were silent in the absence of amino acids or acetylcholine application.

Figure 29: The antagonistic effect of GDEE on synaptic excitation of a thalamo-cortical relay neurone following stimulation of a hind limb nerve.
Post stimulus histogram computed from the raster.



PERIPHERAL NERVE STIMULATION
32 SWEEPS = 100 %

Figure 30: Potencies of DL-homocysteate, L-glutamate and kainate on a (TCR) neurone.

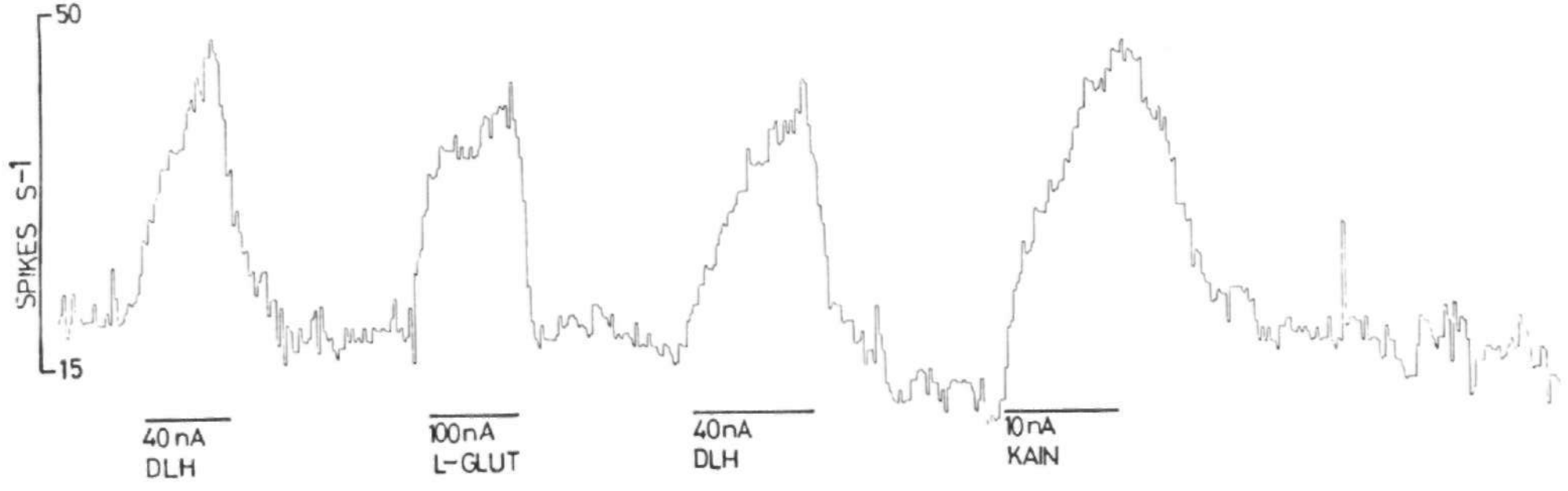

50
SPIKES S-1
15
40 nA
DLH
100nA
L-GLUT
40nA
DLH
10nA
KAIN

Figure 31: Potencies of L-glutamate, Kainate and L-aspartate on a thalamic neurone.

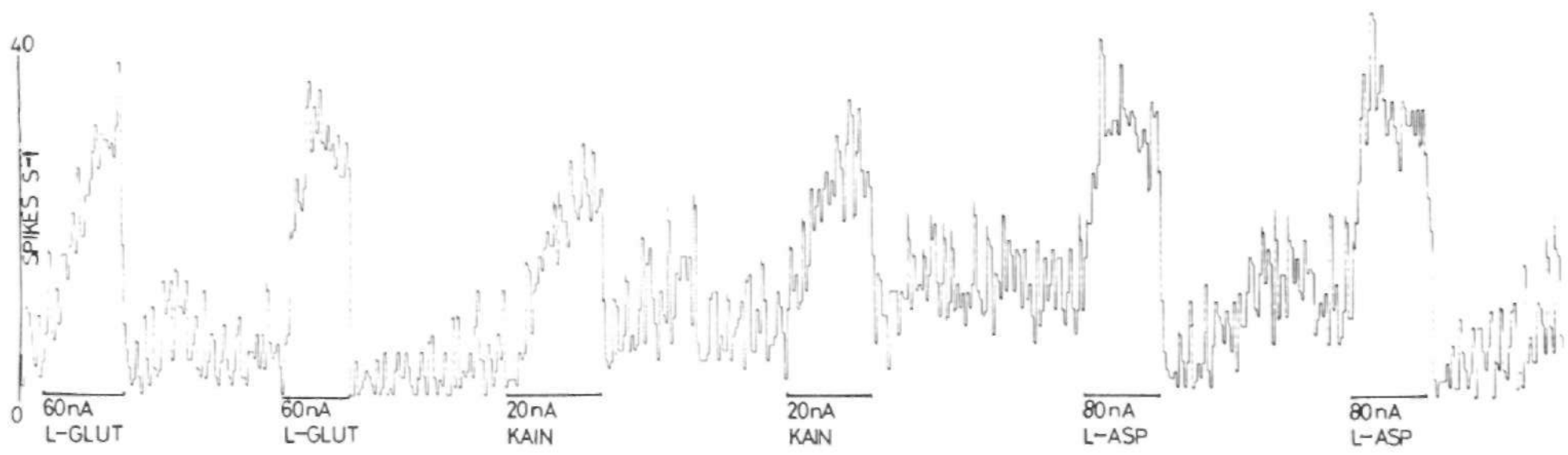

40
SPIKES S-1
0
60nA
L-GLUT
60nA
L-GLUT
20nA
KAIN
20nA
KAIN
80nA
L-ASP
80nA
L-ASP

Figure 32: Relative potencies of L-glutamate, Kainate and Dihydro-kainic acid on a thalamo-cortical relay neurone.

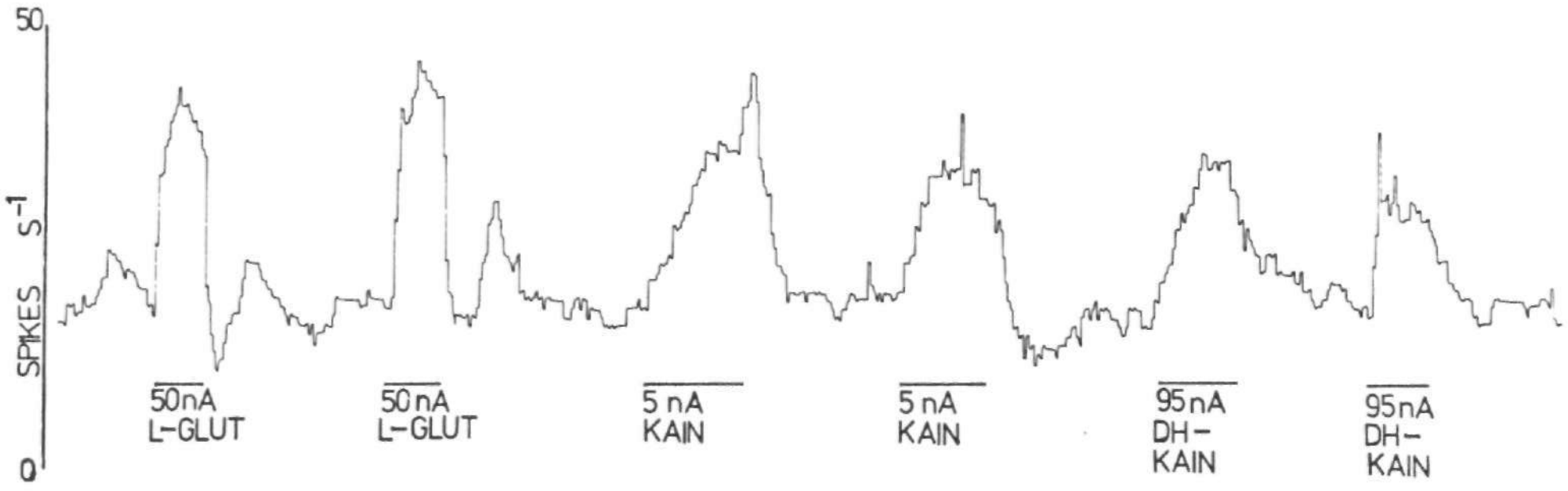

50
SPIKES S⁻¹
0
50nA
L-GLUT
50nA
L-GLUT
5 nA
KAIN
5 nA
KAIN
95nA
DH-
KAIN
95nA
DH-
KAIN

Figure 33: A 'dose response' curve to iontophoretically applied L-glutamate.

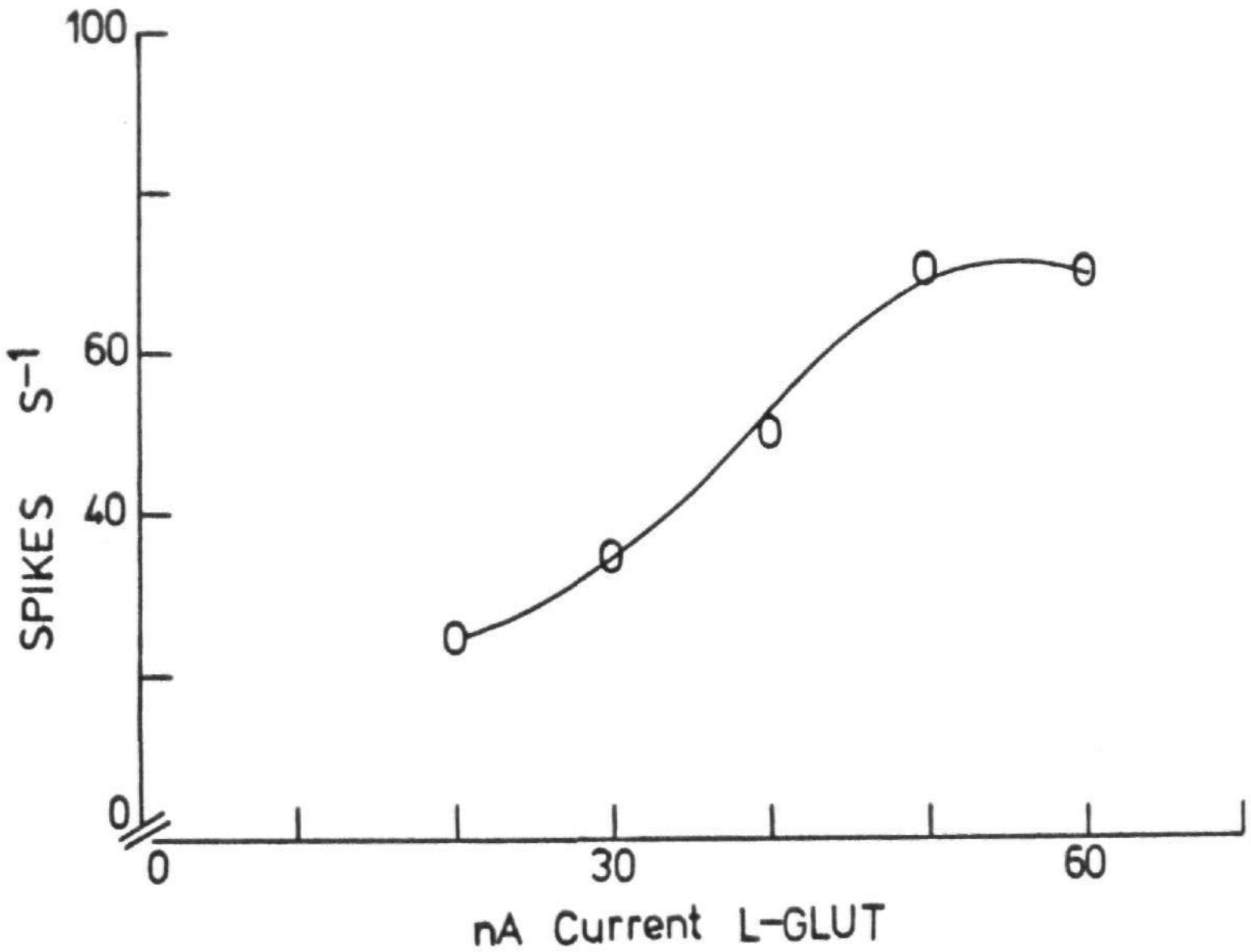

100
60
40
0
SPIKES
S-1
0
30
60
nA Current L-GLUT

Figure 34: A 'dose response' curve to iontophoretically applied L-aspartate.

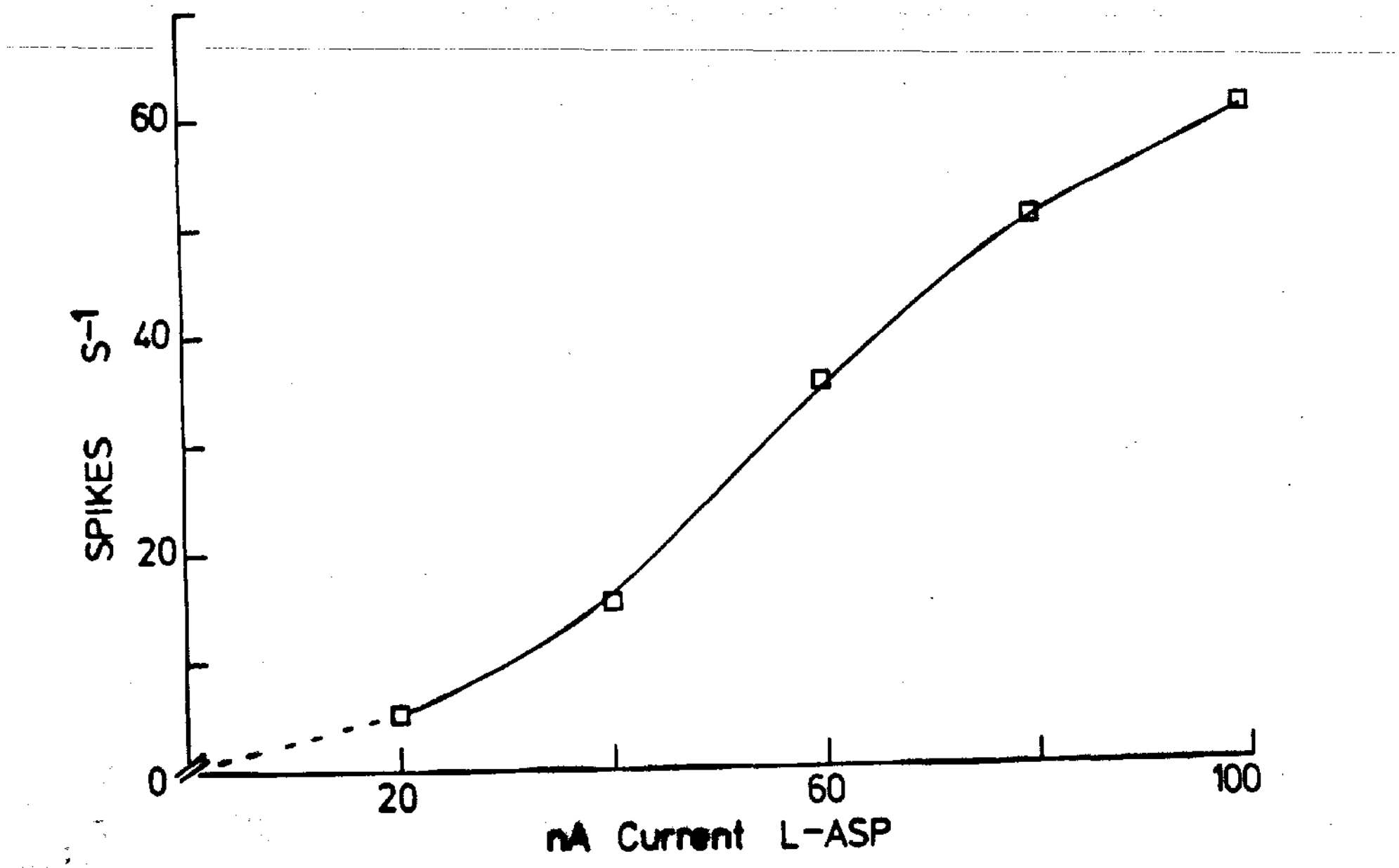
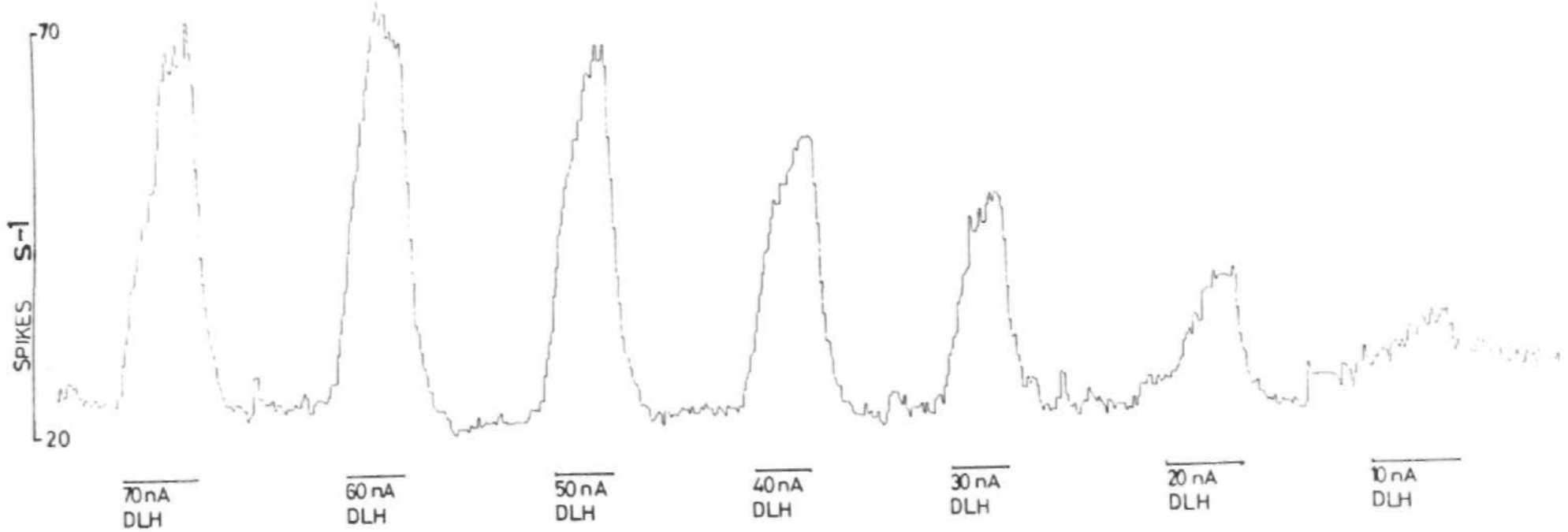

60
40
20
0
SPIKES
20
60
100
nA Current L-ASP

Figure 35: Increasing iontophoretic currents of DLH causing an increase in neuronal firing rate of a TCR neurone.


70
SPIKES S-1
20
70 nA DLH
60 nA DLH
50 nA DLH
40 nA DLH
30 nA DLH
20 nA DLH
10 nA DLH

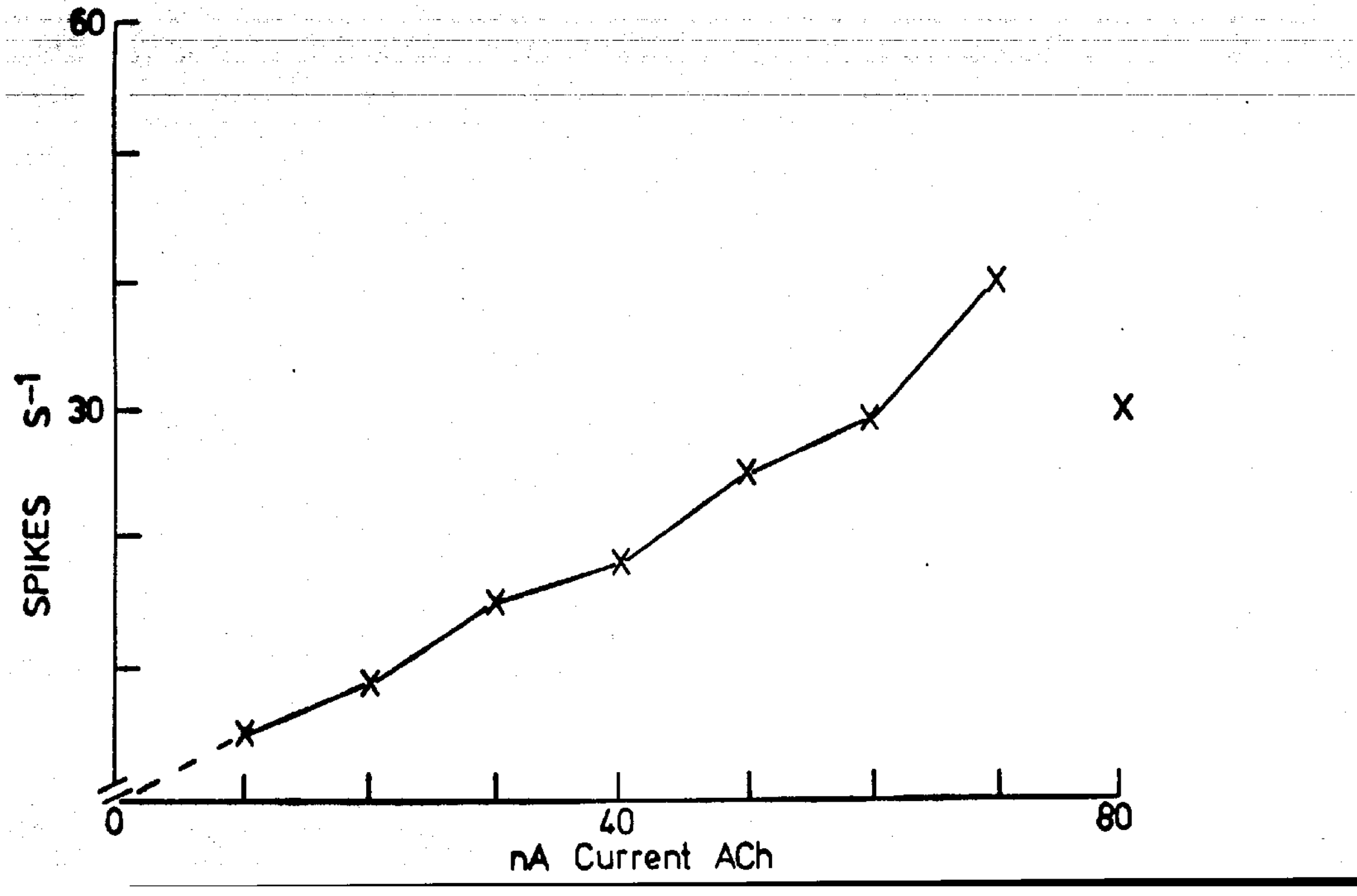
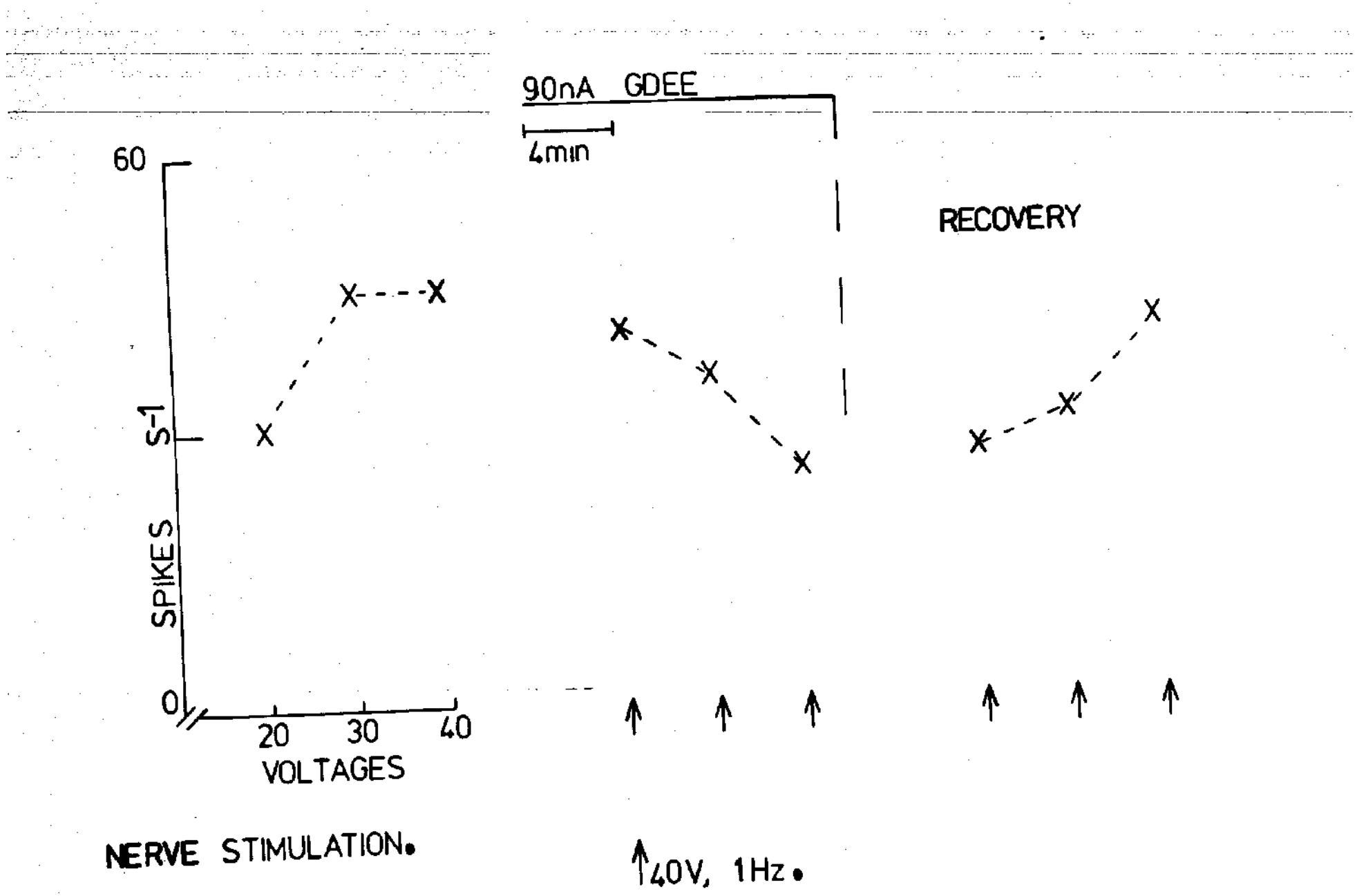

60
30
SPIKES S⁻¹
0
40
80
nA Current ACh

Figure 37: The depressant effect of GDEE on the neuronal firing rate of a principal cell following electrical stimulation of a hind limb nerve.
The duration of stimulus was 0.1ms.


90nA GDEE
4min
RECOVERY
60
S-1
SPIKES
0
20
30
40
VOLTAGES
NERVE STIMULATION.
40V, 1Hz.

4.3. (8). Effect of GDEE on electrical stimulation of a hind limb nerve in relation to the firing frequency of principal cells.

In 9 cases it was observed that silent principal cells were activated by electrical stimulation of an afferent nerve. Increasing the stimulus intensity increases the firing rate of these cells. As the stimulus intensity is further increased a maximal firing rate could be achieved. The application of GDEE (40-80nA) was found to have a depressant effect on the neuronal firing rate. The firing rates recovered satisfactorily when the GDEE was turned off. Figure 37 illustrates this observation.

4.4. Discussion

Studies on synaptic activation of VBC neurones by stimulation of the somatosensory cortex confirmed an earlier report that the cortico-thalamic pathway is non-cholinergic (McCance, Phillis and Westerman, 1968). Studies by Andersen & Curtis, 1964 a.b; McCance et al, 1968 showed that acetylcholine excited neurones in the feline thalamus. This is consistent with the presence of acetylcholine (MacIntosh, 1941), choline acetyltransferase (Hebb and Silver, 1956) and acetylcholinesterase (Phillis, Tebecis and York, 1967) in the thalamus.

The synaptic excitation of VL thalamic neurones by stimulating the sensorimotor cortex was unaffected by iontophoretically administered atropine which readily reduced the sensitivity of thalamic neurones to acetylcholine. This is in agreement with the studies of McCance et al 1968 on the relationship of acetylcholine to synaptic transmission in the thalamus in cats. They reported that both iontophoretic and intravenous injections of atropine and intravenous injections of atropine or Dihydro-β-erythroidine (DHE) failed to block cortically evoked synaptic

responses even though excitations produced by acetylcholine were abolished.

Andersen and Curtis (1964b) had observed that the synaptic excitation of thalamic neurones by afferent volleys in cutaneous nerves was also unaffected by iontophoretically or intravenously administered atropine or DHE indicating that the medial lemniscal pathway to the thalamus is non-cholinergic. From the results described in this study with those from other studies, it is unlikely that acetylcholine could be the transmitter in the monosynaptic pathway from the cerebral cortex to the VL thalamus.

L-glutamate and L-aspartate are dicarboxylic amino acids which occur naturally in the mammalian central nervous system. They are capable of depolarizing and exciting neurones to which they are administered extracellularly (Curtis, Phillis and Watkins, 1960) and mimic the depolarization induced by stimulation of excitatory synapses (Zieglgansberger and Puil, 1972). L-glutamate and L-aspartate are taken up into cells of the nervous system by high affinity mechanisms which are distinct from those used to transport other amino acids (Logan and Snyder, 1972). The role of these amino acids as excitatory synaptic transmitters in the mammalian central nervous system had gained considerable support (Johnson, 1972; Curtis and Johnston, 1974; McLennan, 1975). The similar excitatory potencies of the two amino acids on most neurones, and the absence of selective antagonists of either, have prevented precise determinations of the particular pathways in which they are involved (Curtis and Johnston, 1974).

Lately several reports have indicated that D-α-aminoadipate DαAA is an antagonist of the excitatory amino acids (Hall, McLennan and Wheal (1977)., McLennan and Hall (1978)), and appears to have a greater effect against the action of L-aspartate than

of L-glutamate (Biscoe, Davies, Dray, Evans, Francis, Martin and Watkins 1977; Biscoe; Davies, Dray, Evans, Martin and Watkins 1978; Lodge, Headley and Curtis 1978. The excitatory action of N-methyl-D-aspartate, a potent analogue of L-aspartate (Curtis and Watkins, 1963), is particularly sensitive to the effect of D αAA (Biscoe et al 1978; Lodge et al 1978). A series of amino acids have been ranked by the sensitivity of the excitation of rat thalamic neurones to D αAA and L-glutamic acid diethylester (GDEE) Hicks, Hall and McLennan, 1978. Excitation by N-methyl-DL-aspartate was the most and that by kainate the least sensitive to antagonism by D αAA. On the other hand with GDEE, L-glutamate excitations was the most sensitive (Haldeman and McLennan, 1972) and excitations by kainate and N-methyl-DL-aspartate were the least affected. Curtis, Duggan, Felix, Johnston, Tebecis and Watkins (1972) observed that the excitation by L-glutamate, L-aspartate and DL-homocysteate could not be differentiated by GDEE on cat spinal neurones.

In this study the specificity of D aminoadipate and GDEE as antagonists of the amino acid excitations of rat thalamic relay (TCR) neurones on which glutamate and or aspartate may act as synaptic transmitters have been investigated.

In the light of the results obtained in this study the transmitter of the monosynaptic pathway from the somatosensory cortex to the ventro-lateral thalamus is sensitive to D αAA and poorly affected by GDEE. A tentative interpretation will be that L-glutamate in a partially folded conformation resembling L-aspartate (Johnston et al, (1974); McCulloch et al, (1974); McLennan and Wheal, (1978).

This study also provides further evidence that the transmitter involved in the medial lemniscal pathway is likely to be L-glutamate

in an extended conformation Haldeman, Huffman, Marshall and McLennan.

The relative potencies of the drugs as shown in table 1C is in broad agreement with those that have been reported by others (Curtis and Watkins, 1960, 1963; Johnston et al, 1974; Biscoe et al 1976 and Hall et al, 1979). However, the potencies of ketokainate and the dihydro derivation of kainic acid had not been reported on rat thalamocortical relay cells, although Johnson et al (1974) observed that dihydrokainic acid was about 100 times less potent than kainic acid as an excitant of cat spinal interneurones. Ketokainate is almost as potent as kainate whilst Dihydrokainate is a very weak excitant in the rat thalamus. Saturation of the side chain double bond reduces the excitatory effects of kainic acid. No cellular uptake process exists for kainate and NMDA (Cox, Headley & Watkins, 1977). The absence of uptake systems may also contribute to the apparently high potency of these compounds.

The relative potencies can also be affected by the transport numbers of the substances at the tips of the electrodes (Zieglgansberger, Herz & Teschemacher, 1969; Gent, Morgan & Wolstencroft, 1974). Although 'transport numbers' measurements were not made in this study, Hall et al (1979) using radio-actively labelled solutions of amino acids to determine transport numbers reported no substantial difference between the values for the amino acids tested. Thus variations in transport number would appear not to contribute significantly to changes in the apparent potency of the agonists.

In this study, a number of micropipettes were used and also more than one neurone was tested in an experiment. In this way variations of ejection efficiency with time or between pipettes should not affect the overall pattern of results (Duggan, 1974;

McCulloch, Johnston, Game & Curtis, 1974; Discoe et al., 1976; Hutchinson, McLennan & Wheal, 1978). However, with these safeguards uncontrolled leakage can not be ruled out (Obata et al, 1970, Miledi, 1967).

Table 1A: The arrows indicate the degree of relative depression of GDEE on the neuronal responses to the agonists.

Antagonism of some amino acid induced excitations by GDEE (40-80 nA) for (2-10 mins).

Drugs	Sensitivity	Number of cells tested	Mean (ejecting +SEM current nA)
KAINATE	0	(13)	15.8 ± 2.4
KETOKAINATE	0	(15)	20.1 ± 3.2
NMDLA	↓	(38)	23.5 ± 4.3
DLH	↓↓↓	(43) ↓ (7)	41.8 ± 5.6
L-GLUT	↓↓↓	(101)	76.4 ± 7.3
L-ASP	↓↓↓	(87) ↓ (10)	84.1 ± 8.2

TABLE 1A

Table 1B: Relative depression of the amino acid induced excitations to the antagonistic effect of D α AA.

Antagonism of some amino acid induced excitations by D-α-AA (20-40 nA) for (2-5mins).

Drugs	Sensitivity	Number of cells tested	Mean (nA ejecting) +SEM current
KAINATE	0	(11)	17.5 ± 3.1
KETOKAINATE	0	(16)	23.1 ± 5.2
NMDLA	↓↓↓	(49)	29.7 ± 3.8
DLH	0	(21) ↓ (2)	40.5 ± 6.1
L-GLUT	0	(60) ↓ (3)	72.8 ± 7.9
L-ASP	↓↓	(75)	78.9 ± 9.2

TABLE 18

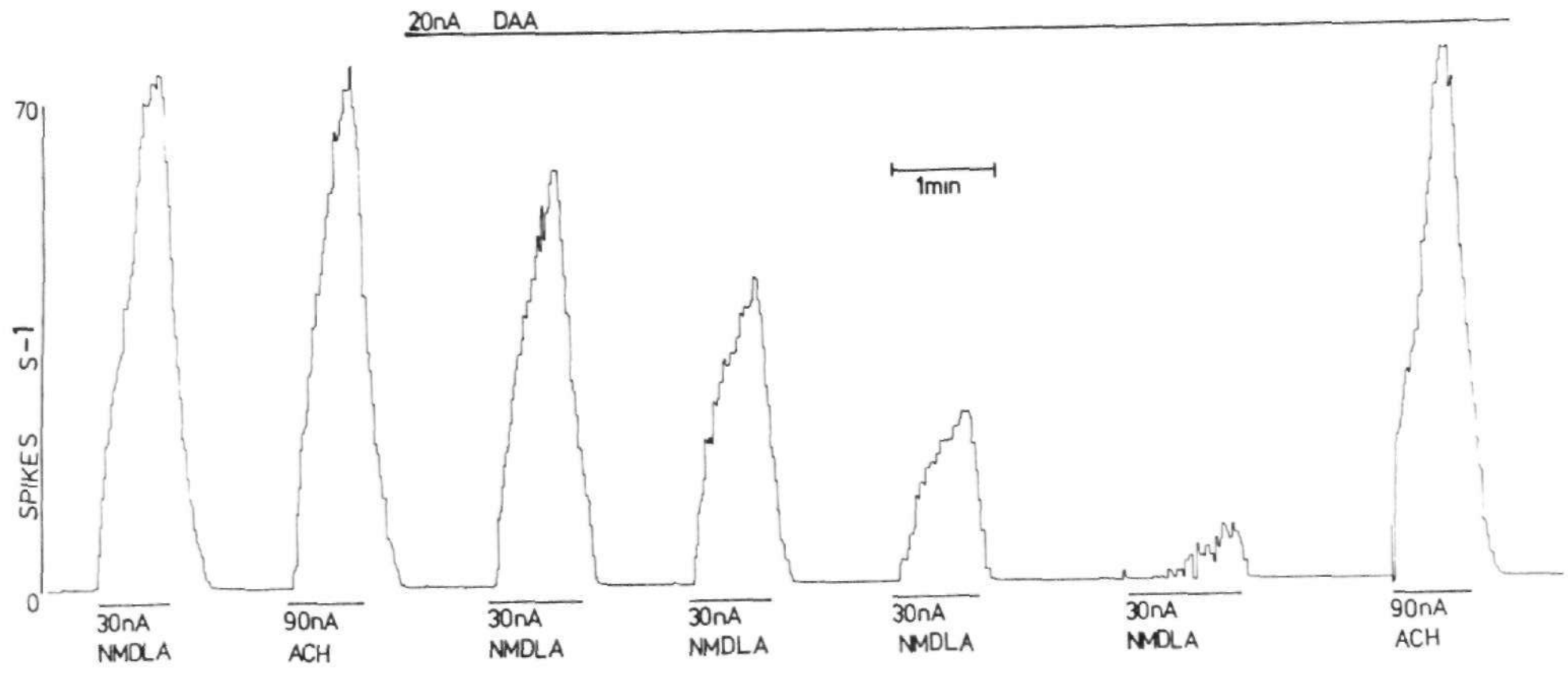
Table 1C: The relative potencies of some amino acid analogues on thalamocortical relay neurones.

P O T E N C I E S

	DRUGS	nA Ejecting Current (Range)	Mean ± SEM	Number of cells tested in parentheses
1.	KAINATE	(2-20nA)	10.6 ± 0.9	(34)
2.	KETOKAINATE	(10-30nA)	17.2 ± 2.0	(9)
3.	N-METHYL-DL-ASPARTATE	(10-30nA)	17.5 ± 1.7	(17)
4.	DL-HOMOCYSTEATE	(10-60nA)	31.7 ± 3.5	(18)
5.	L-GLUTAMATE	(30-100nA)	70.6 ± 3.2	(46)
6.	DL-ASPARTATE	(42-170nA)	77.1 ± 4.5	(22)
7.	L-ASPARTATE	(40-120nA)	78.2 ± 4.7	(28)
8.	DIHYDROKAINATE	(90-200nA)	116.9 ± 10.4	(16)

TABLE 1C.

Figure 38: The selective antagonism of D α AA on NMDLA and ACH induced excitations. The recovery of NMDLA excitations is shown on the rear page.


20nA DAA
70
SPIKES S-1
0
1min
30nA NMDLA
90nA ACH
30nA NMDLA
30nA NMDLA
30nA NMDLA
30nA NMDLA
90nA ACH

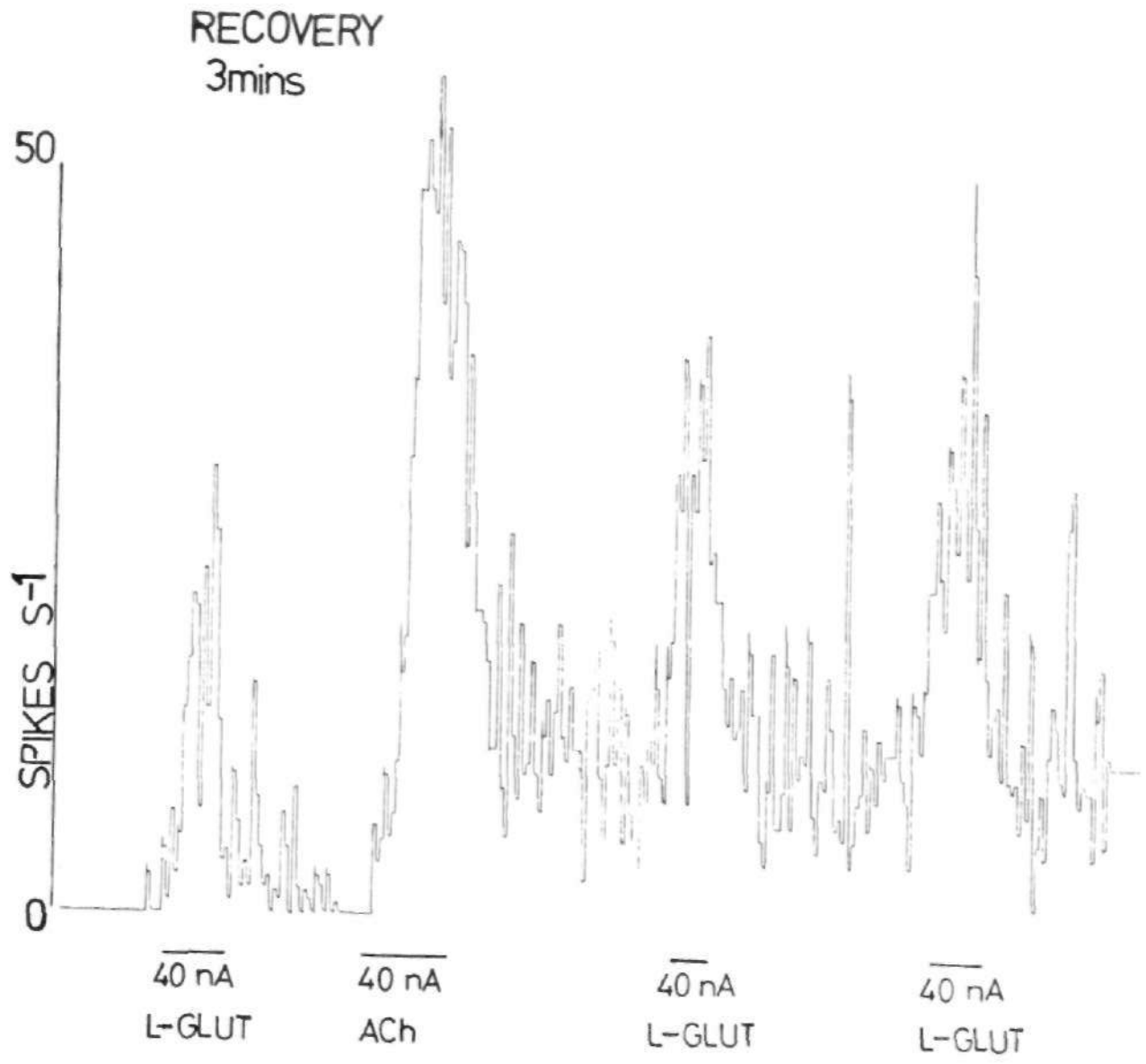
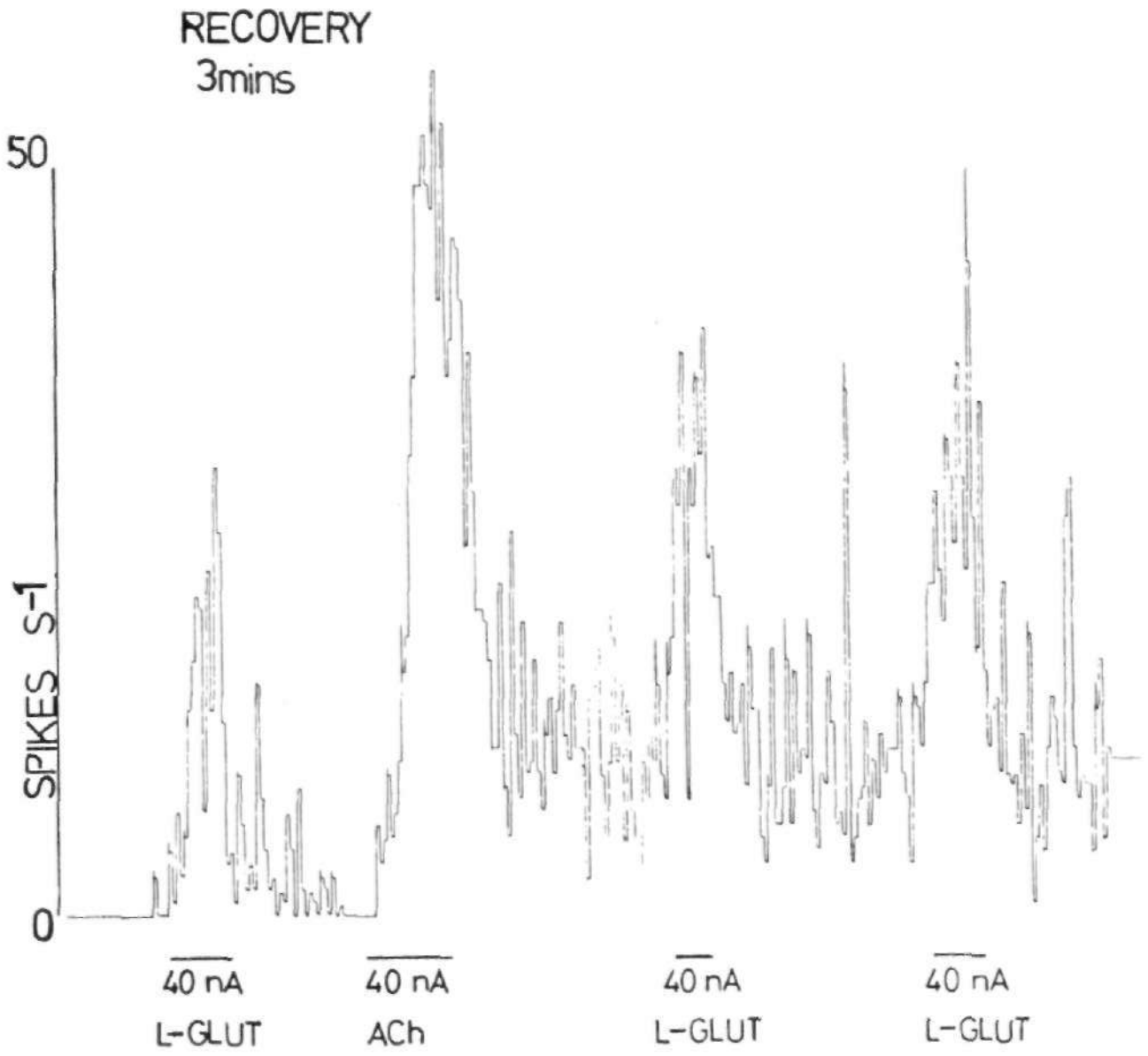

RECOVERY
3mins
50
SPIKES S-1
0
40 nA
L-GLUT
40 nA
ACh
40 nA
L-GLUT
40 nA
L-GLUT

Figure 39: The non-differential effect of GDEE on L-glutamate and acetylcholine excitation.


RECOVERY
3mins
50
SPIKES S-1
0
40 nA
L-GLUT
40 nA
ACh
40 nA
L-GLUT
40 nA
L-GLUT

CHAPTER FIVE

GENERAL CONCLUSION

In this study, stimulation of some sensorimotor areas of the cortex, evoked consistent orthodromic activation of the principal cells of the ventro-lateral thalamus is recorded extracellularly. The cells respond to latensies of between 5 to 10ms while majority of them respond to between 5 and 7.5ms latency.

The pharmacology of this pathway has been investigated and the neurotransmitter likely to be mediated in this pathway is sensitive to Dαaminoadipate (DαAA) and poorly sensitive to Glutamate diethylester (GDEE). This suggests that the transmitter is an amino acid which probably resembles aspartate.

Aspartate meets the criteria generally accepted as necessary for identification of a neurotransmitter: (a) the substance must be present in those neurones from which it is released;
(b) the neurones must possess the necessary enzymic mechanisms for the manufacture of the transmitter and for its release;
(c) the presence of the various precursors and intermediates in the synthetic pathway should be demonstrable; (d) during stimulation the substance may be detectable in extracellular fluid collected from the region of the activated synnapses; (e) when applied to the post-synaptic structure, the substance should mimic the action of the synaptically released transmitter; (f) there may be systems for the inactivation of the transmitter. These could include an enzyme system for the inactivation of the transmitter and a specific uptake mechanism for the reabsorption of the transmitter into pre-or post-synaptic structure; (g) pharmacological agents which interact with the synaptically released transmitter should interact with the suspected transmitter in an identical manner,

(see review, Phillis, 1966). L-Glutamic and aspartic acids are the most abundant of the free amino acids of the mammalian brain, there being respectively 186 and 144mg/100g wet tissue, compared with only 24mg/100g wet tissue of both GABA and glycine as measured in cat brain (Tallan et al. 1954). However, L-glutamate and aspartate are not localized uniquely in brain tissue as is GABA, and even that which is localized to the CNS probably consists of several pools.

The metabolism of these two dicarboxylic amino acids is not as inextricably linked with neural transmission, and the metabolism of glutamate and aspartate in the brain have not been so intensively studied as that of GABA.

Cerebral glutamate can be synthesized directly from α-ketoglutarate and NH_3 by the enzyme glutamic dehydrogenase. While this reaction is reversible the role of glutamic dehydrogenase is normally synthetic rather than catabolic (Roberts et al. 1978). Glutamate is the immediate precursor of GABA and the catabolism of GABA, involving transamination with α-ketoglutaric acid,produces more glutamate as well as succinic acid. However, ^{14}C labelling experiments have shown that the principal source of glutamate in the brain is de novo synthesis from glucose.

Aspartate is also synthesized from glucose. The final part of the pathway is presumably via transamination of oxaloacetic acid and glutamic acid by aspartate amino transferase. Both glutamate and aspartate are formed rapidly by cerebral cortex slices in vitro (Beloff-Chain et al. 1955). At least under experimental conditions, part of this synthesis would appear to occur in nerve terminals rather than cell bodies. Bradford and Thomas (1969) isolated nerve terminals from cortex slices as a synaptosome fraction and incubated them in a medium containing 10uC of ($U^{14}C$)

glucose for sixty minutes. Whole cortex slices were incubated under similar conditions. Both synaptosomes and cortex slices were incubated under similar conditions. Both synaptosomes and cortex slices showed linear respiration and incorporated radioactivity into CO_2, lactate, aspartate, glutamate, alanine and GABA. The cortex slices produced similar amounts of aspartate as the synaptosomes, but three times as much glutamate, eight times as much as glutamine and twice as much GABA. With glutamate in the incubating medium rather than glucose, both synaptosomes and cortex slices produced aspartate, alanine and GABA. Thus it appears that aspartate as well as glutamate, is synthesized, at least in part, in nerve terminals and may therefore be associated with neural transmission.

Brain tissue has a high capacity for removing glutamate and aspartate from the extracellular medium (Blasberg and Lajtha, 1966). Bradford, 1970b observed that following electrical stimulation, a Ca^{2+} dependent increase in aspartate efflux from synaptosomes was obtained. This observation was also made by Osborne et al, 1973 by raising the external K^+ concentration. A Ca^{2+} dependent release of L-(^{14}C)- labelled aspartate has also been demonstrated following electrical stimulation of preloaded, hemisected frog spinal cord (Roberts and Mitchell, 1972). Other substances such as ^{14}C-urea and mannitol and L-(^{3}H)- lencine did not show an increased efflux, suggesting the aspartate release is more specific than a general metabolic response to stimulation. This is in general support of the possibility that aspartate might be a neurotransmitter, released following stimulation.

There have been numerous studies reporting the powerful excitant action of kainic acid on neurones in rat brain (Shinozaki & Konishi, 1970), cat, frog and rat spinal cord (Johnston et al., 1974; Biscoe et al, 1976). Kainic acid is an analogue of L-glutamic acid in which

the pyrrolidine ring restricts the number of conformations available for that part of the kainic acid molecule analogous to L-glutamic acid. The powerful neuronal excitant action of kainic acid appears to result from activation of certain membrane receptors for L-glutamic acid and this had been investigated in vitro via binding of radioactive kainic acid to brain membranes (Simon et al., 1976). Like L-glutamic acid, kainic acid is a neurotoxin which destroys neurones after systemic administration (Olney et al, 1974). Kainic acid is a week inhibitor of the 'high affinity' uptake of L-glutamic acid into rat brain slices (Johnston et al., 1973), rat brain synaptosomes (Lakshmanan & Padmanaban, 1974) and rat neostriatal synaptosomes (McGeer et al., 1978). As part of a continuing interest in the possible roles of uptake systems in the functions of amino acid synaptic transmitters, Johnston et al 1978 investigated the effects of kainic acid on L-glutamic acid in more detail and also went on to investigate Dihydrokainic acid a much weaker neuronal excitant than kainic acid (Johnston et al 1974) and which had also been observed in this study but proved to have similar effects to kainic acid on L-glutamic acid uptake.

Both kainic acid and dihydrokainic acid inhibited the 'high affinity' uptake of L-glutamic acid by rat brain slices (Johnston et al, 1978). They also observed that kainic acid was not taken up by rat brain slices under conditions where exogenous L-glutamic acid reached tissue: medium ratios of 30:1 and exogenous L-aspartic acid reached tissue: medium ratios in excess of 300:1. Thus, kainic acid was not inhibiting L-glutamic acid uptake by acting as a substrate for the 'high affinity' acidic amino acid uptake system, but appeared to be acting as a true competitive inhibitor with the interaction of L-glutamic acid the transport carrier. The extent of inhibition of L-glutamic acid uptake by kainic acid was not influenced

by preincubation of the brain slices with inhibitor before the addition of substrate. This contrasts the inhibition of L-glutamic acid uptake by 3-hydroxy-DL-aspartic acid which increases on preincubation: it seems likly that this linear competive inhibitor (Balcar & Johnston, 1972) is a substrate for the acidic amino acid transport system. Kainic acid was also examined as an inhibitor of the 'high affinity' uptake of L-aspartic acid. It was found that there was no significant difference to that observed with kainic acid inhibition of L-glutamic acid uptake. Kainic acid also inhibited the uptake of D-aspartic acid to a similar extent. These results were consistent with L-glutamic, D-and L-aspartic acids being taken up by the same 'high affinity' acidic amino acid transport system (Balcar & Johnston, 1972; Davis & Johnston, 1976).

Johnston, Kennedy and Twitchen (1978) from their investigations concluded that Dihydrokainic acid is approximately twice as potent as kainic acid as an inhibitor of 'high affinity' L-glutamic acid uptake but is some 500 times less potent as an inhibitor of kainic acid binding and at least 1000 times less potent as a convulsant of immature rats on intraperitoneal injection. Also that Dihydrokainic acid might be useful as a 'control uptake inhibitor' for the effects of kainic acid on 'high affinity' L-glutamic acid uptake since it appears to have little action on excitatory receptors. N-methyl-D-aspartic aid is a potent convulsant of immature rats but does not inhibit kainic acid uptake. So, N-methyl-D-aspartic acid might be useful as a 'control excitant' that activates different excitatory receptors. McLennan and Lodge, (1979); Davies and Watkins, (1979), observed that when iontophoretically administered near cat spinal neurones, L-glutamic acid diethylester had no effect on kainate or NMDA - induced excitations but had a moderate antagonistic

effect on quisqalate-induced responses. Coupled with an antagonist such as DαAA, the pattern of effects suggest that both L-aspartate and L-glutamate have mixed actions on different receptors, but that L-glutamate acts more effectively than L-aspartate on quisqualate receptors, while L-aspartate acts more effectively than L-glutamate on NMDA receptors.

In the cortico-thalamic pathway, DαAA effectively blocked synaptic activity, the relative potencies in producing this effect paralleling those for their NMDLA antagonizing actions. This suggests that NMDA may activate the same receptor population on ventro-lateral thalamic principal neurones as are activated by the endogenous transmitter released on stimulation of the sensorimotor cortex. In contrast, GDEE had little or no depressant action on synaptic activity of these neurones, suggesting that quisqualate receptors are not major transmitter receptors in the cortico thalamic pathway.

On the other hand, the synaptic activation of the principal cells at the ventro-lateral thalamus activated orthodromically by stimulating the hind limb nerve was found to be affected by GDEE suggesting that in that pathway the quisqualate receptors are more favoured.

In this study neither DαAA nor GDEE was found to have any effect on either kainate or keto-kainate. DαAA had no effect on iontophoretically applied ACH, (see figure 38). GDEE had a moderately depressant effect on ACH at high doses (see figure 39).

DαAA has also been observed to effectively depress the synaptic activations of the retina input to the cat lateral geniculate body (Kemp & Sillito, 1979); the cochlear nucleus neurones evoked by auditory stimuli Martin & Adams, 1980 and Martin, 1980 and the commissural projection to the granule cells in the dentate gyrus

(Hicks & McLennan, 1979).

From this study together with other observations, it is clearly indicated that actions of the NMDLA antagonist, DαAA, are highly selective with respect to both synaptic and chemically-evoked excitation. From this selectivity, it can be strongly suggested that these actions are mediated at specific transmitter receptors sensitive to an excitatory amino acid transmitter. The cumulative evidence supports the observation that L-aspartate is the neurotransmitter involved in the cortico-thalamic pathway.

Figure 40 summarises the possible ways by which the principal cells of the ventrolateral thalamus can be activated. The details have been included in the text.

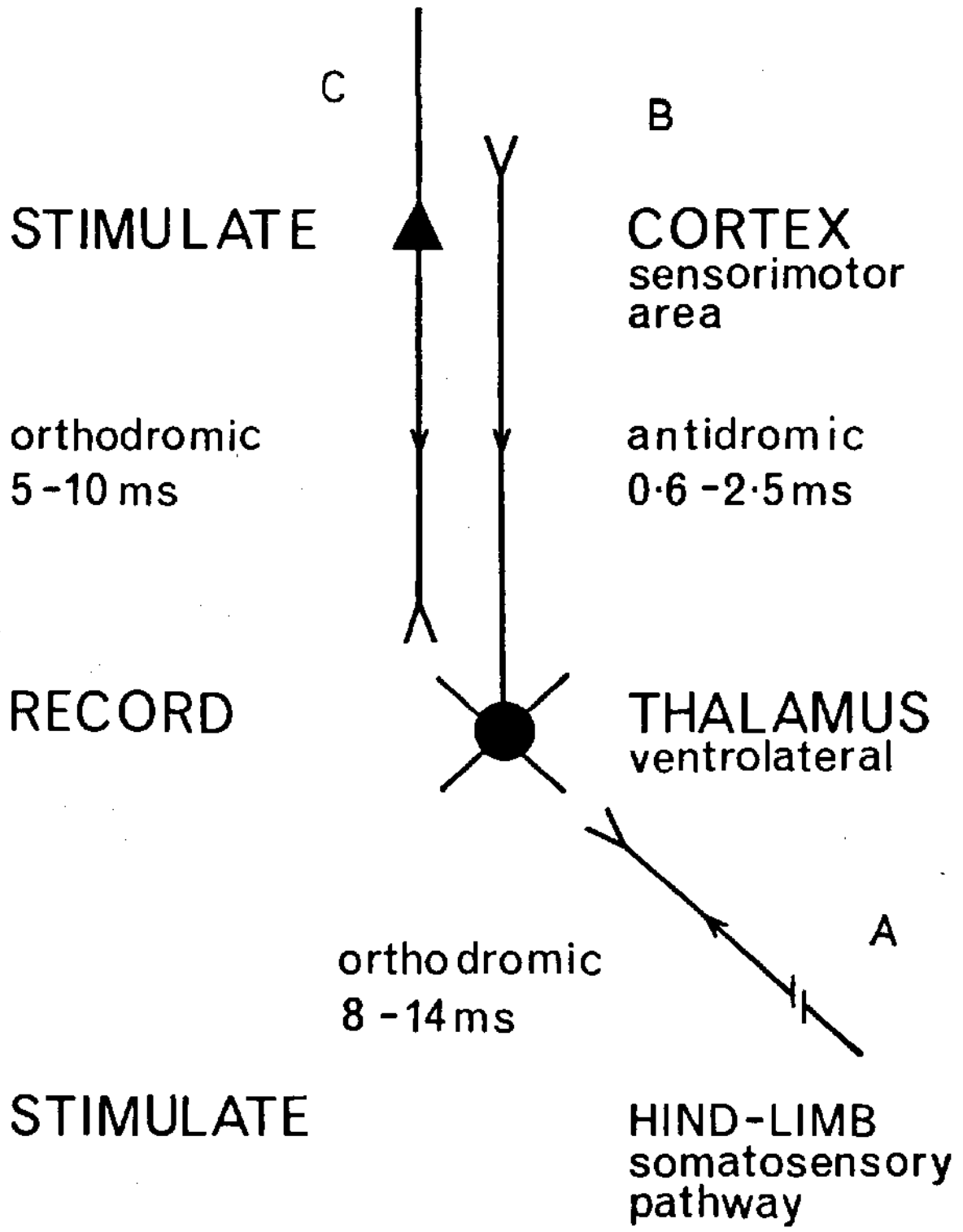
Figure 40: A summary of the various ways of activating a thalamo-cortical relay cell:

A) Orthodromic activation by stimulating the hind limb nerve.

B) Antidromic activation from the sensorimotor cortex.

Using both activations as criteria for identification;

C) A consistent orthodromic activation of the principal cells at the ventro-lateral thalamus is recorded extracellularly by stimulating the ipsilateral sensorimotor cortex.


C
B
STIMULATE
CORTEX
sensorimotor
area
orthodromic
5-10 ms
antidromic
0·6-2·5 ms
RECORD
THALAMUS
ventrolateral
A
orthodromic
8-14 ms
STIMULATE
HIND-LIMB
somatosensory
pathway

SUMMARY

1. Male wistar rats of 200g weight were used in the experiments. They were anaesthetized with urethane (1.5g/Kg) intraperitoneally.

2. The animals were placed in a stereotaxic frame. The calvarium and duramater is cleared from an area of approximately 5mm square, 1mm anterior to Bregma to allow access to the somatosensory cortex.

3. A concentric bipolar stimulating electrode was introduced at an approximate angle of 60^{o} caudally into the somatosensory cortex.

4. 6-barrelled iontophoretic electrodes with the recording electrode protuding 10-20μm past the drug barrels were used in this study.

5. The 6-barrelled iontophoretic electrode was stereotaxically introduced into the ventro-lateral thalamus. The co-ordinates have been modified from the Konig and Klippel, Rat brain stereotaxic atlas since the animals were placed horizontally in the stereotaxic frame.

6. The recording electrode was filled with 5% pontamine sky blue in 0.5M Sodium acetate solution. The resistance of the recording electrode was between 10-40MΩ.

7. A tibial peroneal nerve of a contralateral hind limb is stimulated to synaptically activate a thalamocortical relay cell. The cell can also be invaded antidromically by stimulating the ipsilateral somatosensory cortex. These activations were used as criteria for the identification of a thalamocortical relay cell

and also for confirmation of the electrode placement at the ventro-lateral thalamus.

8. The drugs and compounds were ejected with appropriate cationic or anionic currents. Particular care was taken to keep the retaining current to a minimum.

9. Studies on relative potencies of the amino acid analogues revealed that kainic acid and the keto derivative of kainic acid were potent agonists while dihydrokainic acid was found to be a weak excitant on thalamocortical relay neurones.

N-methyl-DL-aspartate was also found to be a more potent excitant relative to L-glutamate and L-aspartate.

10. The antagonistic effect of Glutamate diethylester (GDEE) was found to be non-differential for L-glutamate, L-aspartate and DL-homocysteate. At high concentrations, GDEE depresses Acetyl-choline and N-methyl-DL-aspartate induced excitations.

11. Atropine reversibly blocks acetylcholine induced excitations while sparing the excitations produced by the amino-acids.

12. Dα aminoadipate is selective in depressing N-methyl-DL-aspartate and L-aspartate induced excitations relative to that produced by L-glutamate, DL-homocysteate and acetylcholine.

13. Neither GDEE nor DαAA had any antagonistic effect on kainate or keto-kainate induced excitations.

14. GDEE preferentially antagonized the synaptic excitation of the

thalamo-cortical relay cell following the stimulation of a contra-lateral hind limb nerve in concentrations that do not affect acetylcholine or NMDLA induced excitations.

15. D αAA selectively blocks the synaptic excitation of the thalamocortical relay cell produced by stimulating the ipsilateral somatosensory cortex in concentrations that affect NMDLA and L-aspartate induced excitations whilst sparing those of L-glutamate and acetylcholine.

16. The orthodromic activation of the thalamocortical relay cell produced by stimulating the hind limb nerve is sensitive to GDEE antagonism and poorly affected by D αAA while the synaptic excitation produced by stimulating the somatosensory cortex is sensitive to D αAA antagonistic effect and least affected by GDEE.

17. Atropine did not have any depressant or antagonistic effect on the orthodromic activations produced by stimulating the contra-lateral hind limb nerve or on the synaptic activation following cortical stimulation.

18. Cumulative evidence suggests that L-aspartate is very likely to be the neurotransmitter in the cortico-thalamic pathway.

FUTURE WORK

SUGGESTIONS

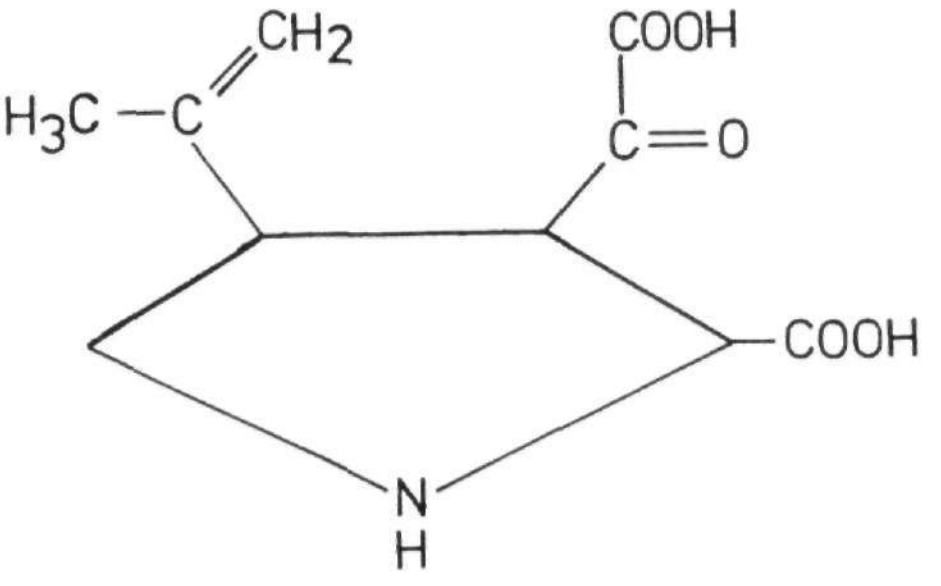
1. The neutransmitter in the optic pathway to the dorsal lateral genuculate thalamus is still debatable while the transmitter in the cortico-thalamic pathway vis-a-vis from the visual cortex to this nucleus is not yet identified.

2. The neurotransmitter involved in the medial lateral geniculate thalamus from the auditory pathway thought to be acetylcholine is in doubt (Tebecis 1972c). The nature of the transmitter involved in this pathway is still not clear. The observations that some polysynaptic responses of medial geniculate neurone following stimulation of the auditory cortex could be blocked by atropine (Tebecis, 1970b and 1972c) also needs further clarification.

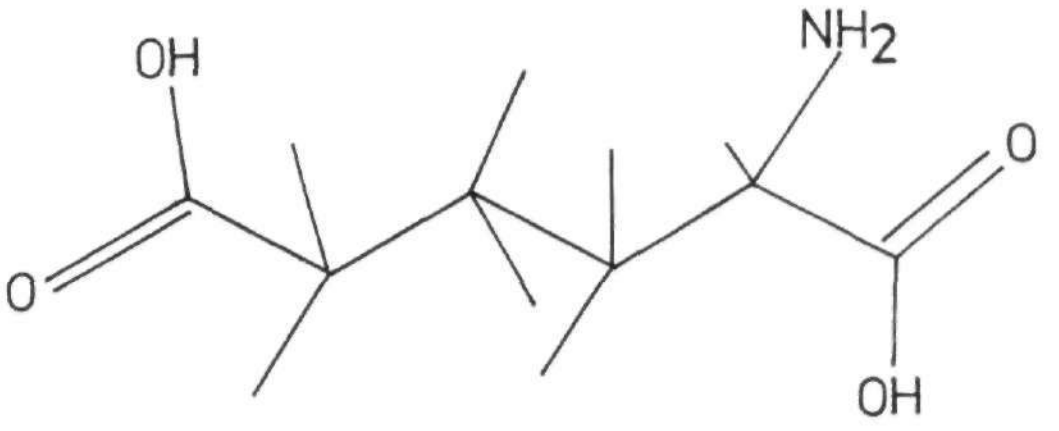
With these investigations in mind, attempts could be made to study the possibilities of identifying and further clarifying the neurotransmitters in these pathways.

APPENDIX

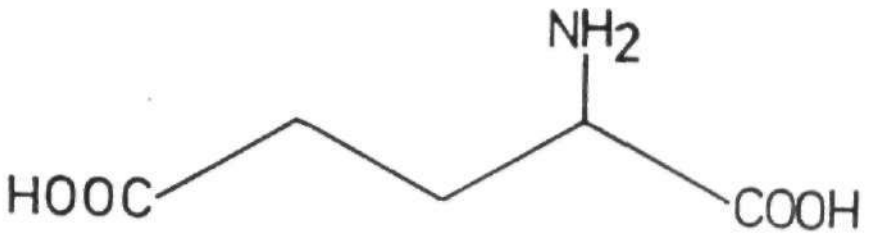
STRUCTURAL FORMULAE OF DRUGS.



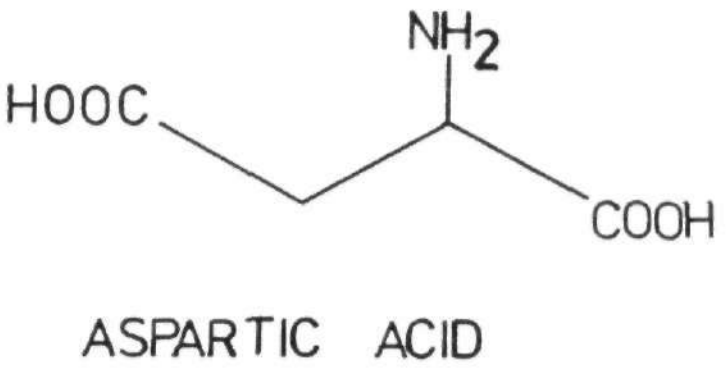
KETO-KAINIC ACID



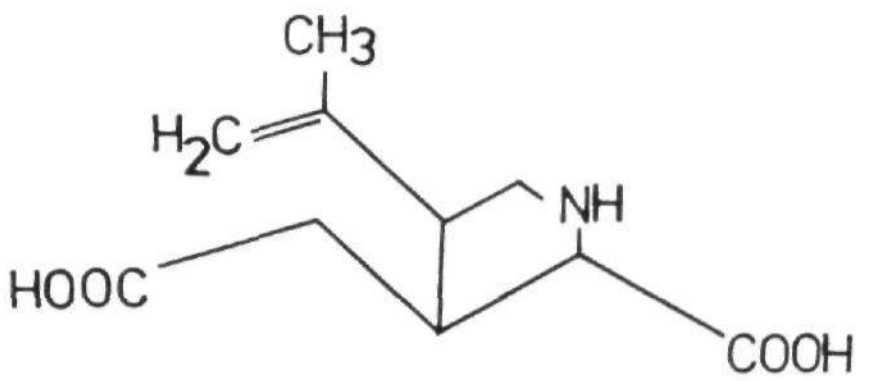
α AMINO-ADIPIC ACID



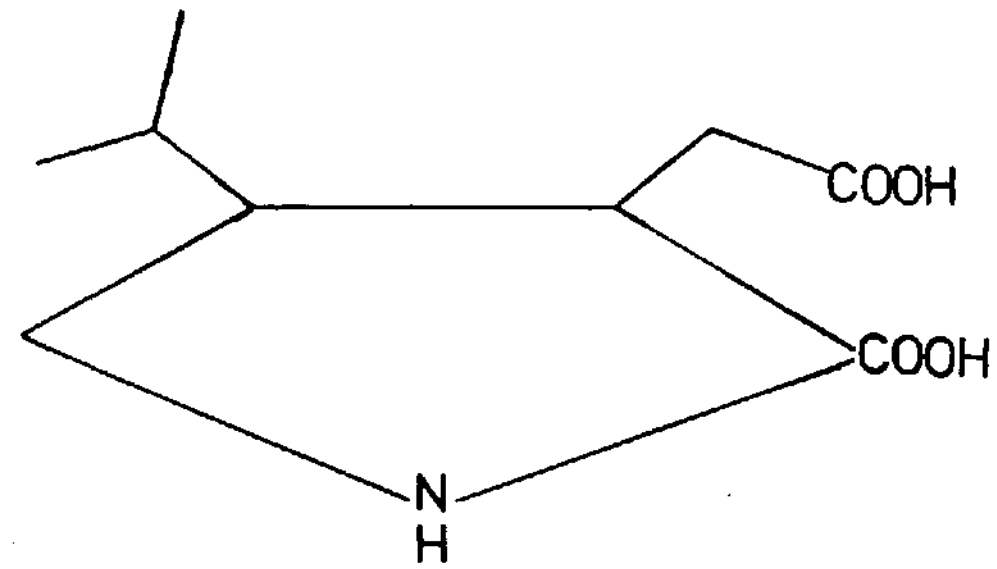
GLUTAMIC ACID



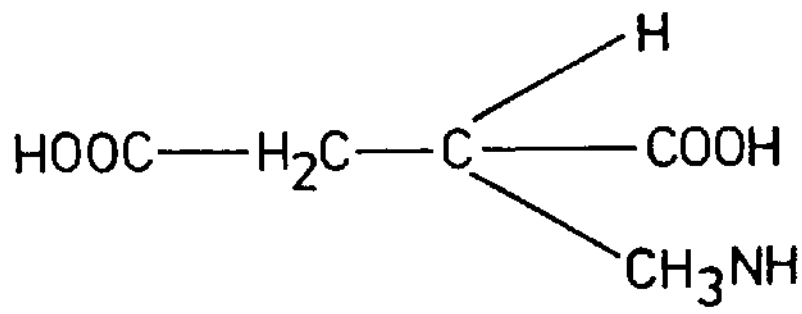
ASPARTIC ACID



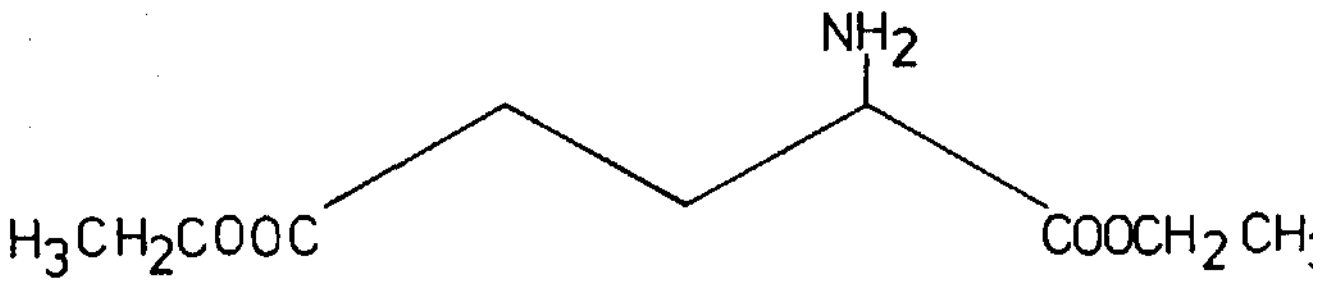
KAINIC ACID



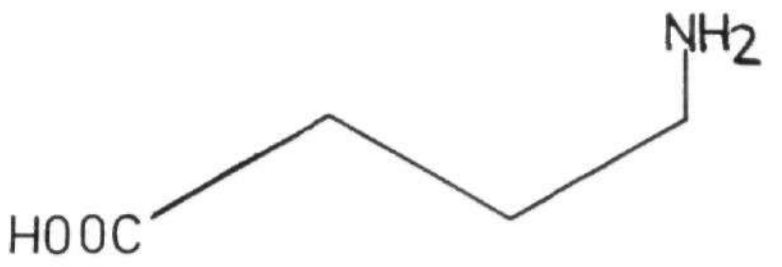
DIHYDROKAINIC ACID



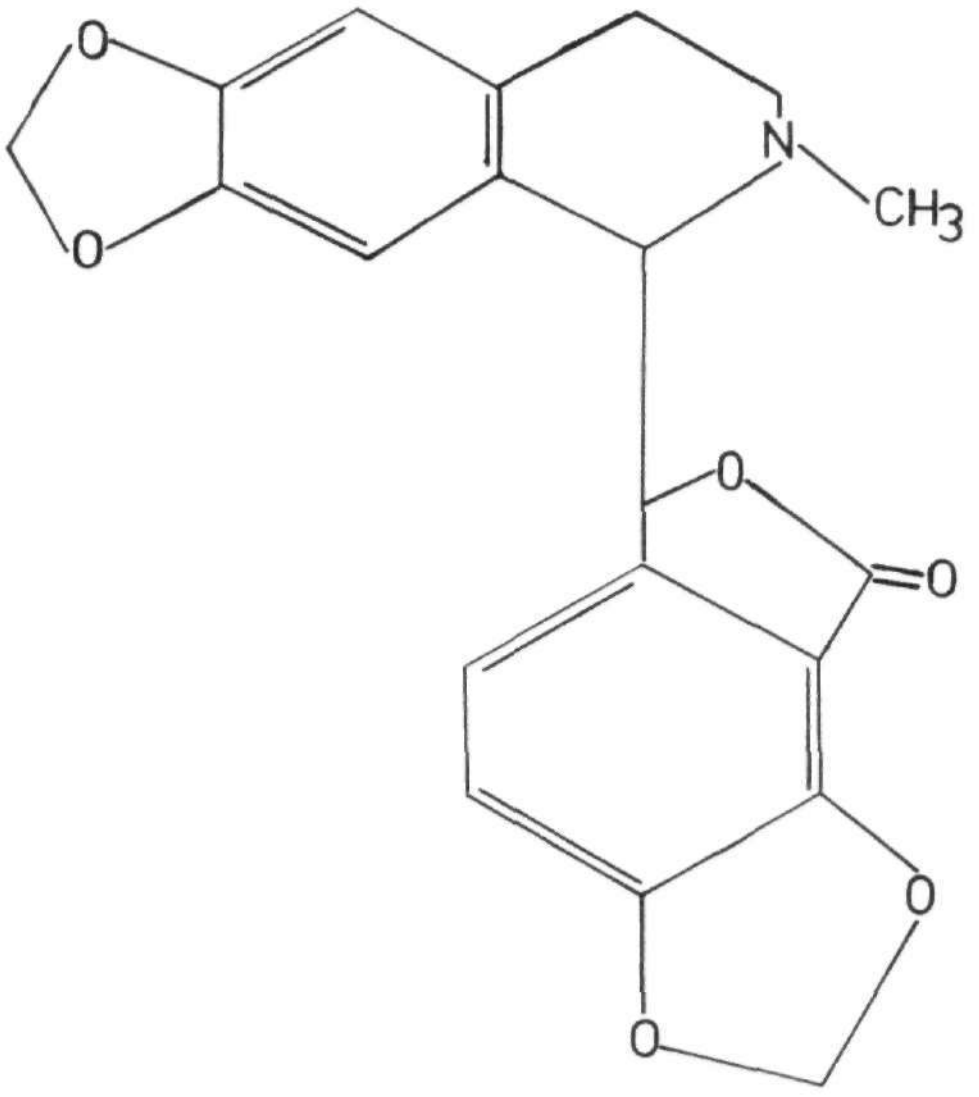
N-METHYL-D-ASPARTIC ACID



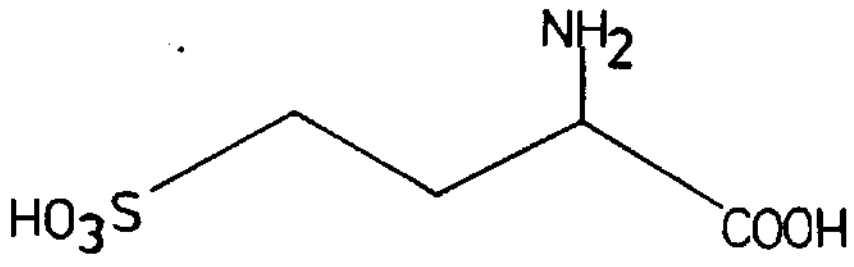
GLUTAMATE DIETHYL ESTER



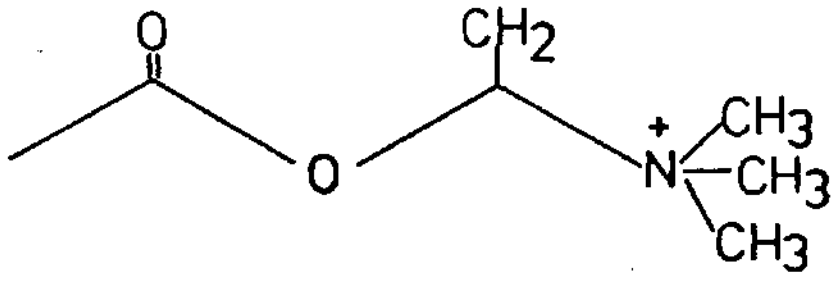
GAMMA AMINOBUTYRIC ACID



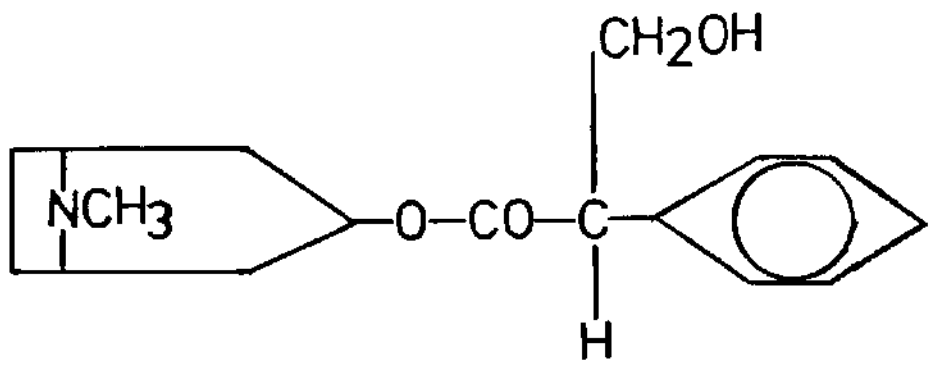
BICUCULLINE



DL-HOMOCYSTEIC ACID



ACETYLCHOLINE



ATROPINE

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