# ISOXSUPRINE; A POSSIBLE INTERACTION WITH CHOLINERGIC NEURONES IN THE RAT BRAIN.

by

Stephen Whitworth Davies

1983

A thesis presented for the degree of Doctor of Philosophy
in the Department of Physiology and Pharmacology
of the University of Southampton.

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

Doctor of Philosophy

ISOXSUPRINE; A POSSIBLE INTERACTION WITH CHOLINERGIC NEURONES IN THE RAT BRAIN.

by Stephen Whitworth Davies.

The origins of the cholinergic innervation of the rat neocortex were studied by means of neurotoxic amino acid lesions and neurochemical analysis. A crude topographical pattern of projections from the cholinergic perikarya within the magnocellular nuclei of the basal forebrain to the allocortex were determined. The more rostrally located cells of the medial septal complex project to the hippocampus, whilst the more caudally located neurones of the nucleus basalis project to the ipsilateral parietal cortex and the amygdala. No evidence for a cholinergic projection to the cortex from the medial septum was found. The neurochemical specificity and neuroanatomical selectivity of ibotenic acid lesions of the ventral globus pallidus were studied using both neurochemical and histological techniques. Similarities between the degeneration produced by these lesions and the neurodegenerative disorder Alzheimer's dementia are discussed, in respect of a possible 'animal model' for this disorder.

The effects of isoxsuprine, a drug reported to alleviate the symptoms of senile dementia, on the uptake of oxygen *in vitro*, and [$^3$H] 2-deoxyglucose *in vivo* into discrete areas of rat brain were studied. *In vivo* pretreatment with isoxsuprine was found to stimulate oxygen uptake into rat brain cortex slices. No significant effect on [$^3$H] 2-deoxyglucose uptake could be determined. The effects of isoxsuprine pretreatment on choline acetyltransferase activity and high affinity choline uptake were also studied.

These results are again discussed in terms of the currently available pharmacotherapy for Alzheimer's dementia.

Acknowledgements

I would like to express my sincerest gratitude to Dr N. M. Horn who supervised my research, for his friendship, guidance and encouragement throughout my postgraduate studies. I am also grateful to Dr J. Poat for her advice and support, and to Professor K. A. Munday for providing excellent laboratory facilities.

I should also like to express my thanks to Duphar Laboratories Ltd. for their generous financial support throughout this project, and to Dr L. J. Downey in particular for many helpful suggestions.

# CONTENTS

Page

CHAPTER IV - DISCUSSION

## INDEX OF FIGURES

## INDEX OF TABLES

# CHAPTER 1

# INTRODUCTION

## I.1. Aims of this project

The overall aims of this thesis were twofold. Firstly to develop an animal model of Alzheimer's dementia. This might provide some insight into the nature of the neuroanatomical pathways affected, and might also provide some clues for possible pharmacotherapy. Secondly, to investigate some actions of drugs currently prescribed in dementia, primarily isoxsuprine, in relation to their possible action on central nervous system cholinergic pathways.

The tremendous increase in research into central nervous system cholinergic neurochemistry during the last three years, has to some extent pre-empted some of the results presented in this thesis. For this reason references up to 1980 relating to the development of this project are cited in this section. All results are however discussed in terms of the currently available information.

## I.2. Animal Models of Neurodegenerative Diseases

The immense increase in our knowledge of the basic neurochemical changes occurring in neurodegenerative disorders over the last 5 - 10 years has prompted the development of a variety of 'animal models' which closely reflect these observed changes. The value of these models, which are mostly dependent on the use of specific lesions within the central nervous system (CNS), is that they may provide an insight into the nature of the pathways affected and might also provide clues for possible pharmacotherapy.

### a) Parkinson's Disease.

One of the first neurodegenerative disorders to be characterised in this way was Parkinson's disease (for recent reviews see Marsden, 1980; Schultz, 1982). It has been known for many years that a profound destruction of the substantia nigra (SN) is the basic anatomical substrate of the idiopathic parkinsonian syndrome (Blocq and Marinesco, 1893), but this was only correlated with the later discovery of a drastic reduction of the dopamine (DA) content of the striatum of parkinsonian patients (Hornykiewicz, 1966), when the existence

of a pathway was reported which originated in the SN, terminated in the striatum, and utilised DA as its neurotransmitter (Ungerstedt, 1971).

Parkinson's disease is clinically defined by: rigidity of the limbs, trunk and face tremor; abnormal body posture; and an inability to initiate voluntary motor activity (akinesia). This condition is therefore, believed to be associated with a degeneration of dopaminergic neurones predominantly in the nigrostriatal pathway, and a decrease in the amount of dopamine- and melanin-containing cell bodies in the zona compacta of the substantia nigra (Hornykiewicz, 1971).

In 1968 Ungerstedt (Ungerstedt, 1968) described a technique whereby 6-hydroxydopamine could be stereotoxically injected into the substantia nigra of rats. 6-Hydroxydopamine is a neurotoxin which can cause selective degeneration of catecholamine containing neurones with little effect on other neuronal types (Uretsky and Iversen, 1970; Sachs and Jonsson, 1975; for review see Jonsson, 1980).

Ungerstedt showed that in animals which had received a unilateral injection of 6-hydroxydopamine,(+) amphetamine administration caused the animals to rotate towards the lesioned side (ipsilateral circling). The injection of L-dopa or apomorphine, however, induced contralateral rotation (away from the lesioned side), (Ungerstedt, 1968, 1971). This animal rotational model proved to be an invaluable system for testing drugs of potential use in Parkinson's disease since there exists an obvious similarity between the changes found in this condition - characteristically a degeneration of the nigrostriatal dopaminergic pathway - and that of the rat which had developed a degeneration of the analogous system on one side. Drugs which preferentially activate the denervated dopamine receptors, directly acting agonists, will therefore have potential as therapeutic agents in Parkinsonism (Hejti and Melamed, 1980).

Quite apart from the use of Ungerstedt's model to mimic the main features of human Parkinson's disease and to select promising

new drug therapies, it has also been employed to investigate fundamental phenomena such as the concept of central denervation supersensitivity (Ungerstedt et al., 1975), and the organisation of outflow pathways in the basal ganglia (Marsden, 1980).

Bilateral 6-hydroxydopamine lesions of the nigro-striatal dopamine systems in rodents should produce a syndrome close to Parkinson's disease in man. In fact, such rats rapidly become severely disabled by gross akinesia and more importantly by an inability to drink or feed, such that they die within a few days unless tube fed. When these animals are kept alive, provided there is a near complete destruction of both ascending dopaminergic pathways, they do not exhibit normal feeding or movement and are seriously cataleptic. However, such animals whilst immobile on land, swim if forced under water, a performance reminiscent of the kinesia paradoxica of the human patient with Parkinson's disease.

b) Huntington's Disease

A more recently developed animal model for a long established neurodegenerative disease is that for the inherited neurological disorder, Huntington's chorea (for recent reviews see Marsden, 1980; McGeer and McGeer, 1982; and Spokes, 1981). Huntington's chorea is transmitted as an autosomal dominant with complete penetrance and is a rare disorder occurring in only 1 in 10,000 people. Clinically, the disease is defined by a combination of physical and mental changes which may appear independently or concurrently. The physical symptoms usually include jerky, uncontrollable movements that affect the head, face, trunk and limbs, greatly impeding speech, swallowing, walking, writing and other voluntary motor activities. The other hallmark of Huntington's chorea is dementia which is of a sub-cortical type. This is manifested by emotional and personality changes, impairment of recent memory, defective ability to manipulate acquired knowledge, and slowing of information processing. In contrast to the cortical dementias, the most common form of which is Alzheimer's disease, apraxia, aphasia and agnosias do not develop. Additional psychiatric features include anxiety, depression, mania and schizophrenia-like psychoses (Spokes, 1981).

The most striking pathological changes in Huntington's disease occur in the caudate-putamen where there is marked atrophy and severe loss of neurones accompanied by a patchy loss within the cerebral cortex.

The direct injection of nanomolar amounts of the neurotoxic amino acid,* kainic acid, into the caudate putamen in rats reproduces many of the morphological, biochemical and pharmacological features of Huntington's disease (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). After intrastriatal injections of kainic acid in the rat there is a generalised degeneration of the neuronal cell bodies in the injected region. There is preservation of the bundles of myelinated axons passing through the neostriatum from the cortex. Terminal boutons also survive though many do not remain attached to their sites of synpatic specialisation. Electron microscopic studies have shown that the cell body is involved first, with dendritic elements being affected within 10 hours, smaller axons within 24 - 48 hours and terminal boutons at 72 hours (Hattori and McGeer, 1977). *(see page 149 for discussion of kainate toxicity).

The main biochemical findings in Huntington's disease and the kainic acid model are remarkably similar (Table 1) and can be interpreted in terms of the present understanding of the neuroanatomy of the basal ganglia (Figure 1). In both Huntington's disease and the kainic acid model there is a severe loss of neostriatal neurones which have as their transmitters γ-aminobutyric acid (GABA), acetylcholine (Ach), substance P, and enkephalin. Losses in angiotensin-converting enzyme are also reported in both conditions. However, in both the rat model and in the disease, the myelinated axons of the internal capsule, the dopaminergic neurones of the substantia nigra, and the nerve endings of these dopaminergic neurones seem to be relatively intact. Serotonin levels are also normal, indicating preservation of this system, although serotonin receptors are decreased.

GABA levels and glutamic acid decarboxylase (GAD) activity are decreased in the substantia nigra which is indicative of the loss of

Table 1. Biochemical similarities between Huntington's disease and the kainic acid 'model'. The main neurochemical findings in Huntington's disease and the kainic acid 'model' are remarkably similar and can be interpretted in terms of our current understanding regarding the neuroanatomy of the basal ganglia (see Fig. 1). Table taken from McGeer and McGeer (1982).

TABLE 1.

| Biochemical changes | Huntington's | KA 'model' |
|---|---|---|
| In neostriatum: | | |
| Presynaptic GABA indices[a] | markedly reduced | |
| γ-Hydroxybutyrate levels | increased | |
| GABA transaminase activity | Normal | decreased |
| Presynaptic acetylcholine indices[a] | markedly decreased | |
| Presynaptic dopamine indices[a] | normal or elevated | |
| Presynaptic serotonin indices[a] | normal or elevated | |
| Presyn. noradrenaline indices[a] | normal or elevated | |
| Angiotensin converting enzyme | reduced | |
| Enkephalin levels | decreased | |
| Binding sites 'receptors' for | | |
| Serotonin | decreased | |
| Dopamine | decreased | |
| Acetylcholine (muscarinic) | decreased | |
| Benzodiazepines | decreased[b] | |
| Kainic acid | decreased | |
| GABA | decreased[b] | |
| Noradrenaline (β-adrenergic) | normal | |
| In substantia nigra | | |
| GABA indices | decreased | |
| Substance P levels | decreased | |
| Dopamine indices | normal | |

[a] Including levels, uptake, release, turnover and/or activity of specific synthetic enzymes.

[b] At 1 month following kainic acid injections; no decrease in more acute preparations.

descending (striato-nigral) GABA pathways. Similarly, there is a decrease in substance P in the substantia nigra again signifying the loss of descending pathways following degeneration of the cell bodies in the striatum.

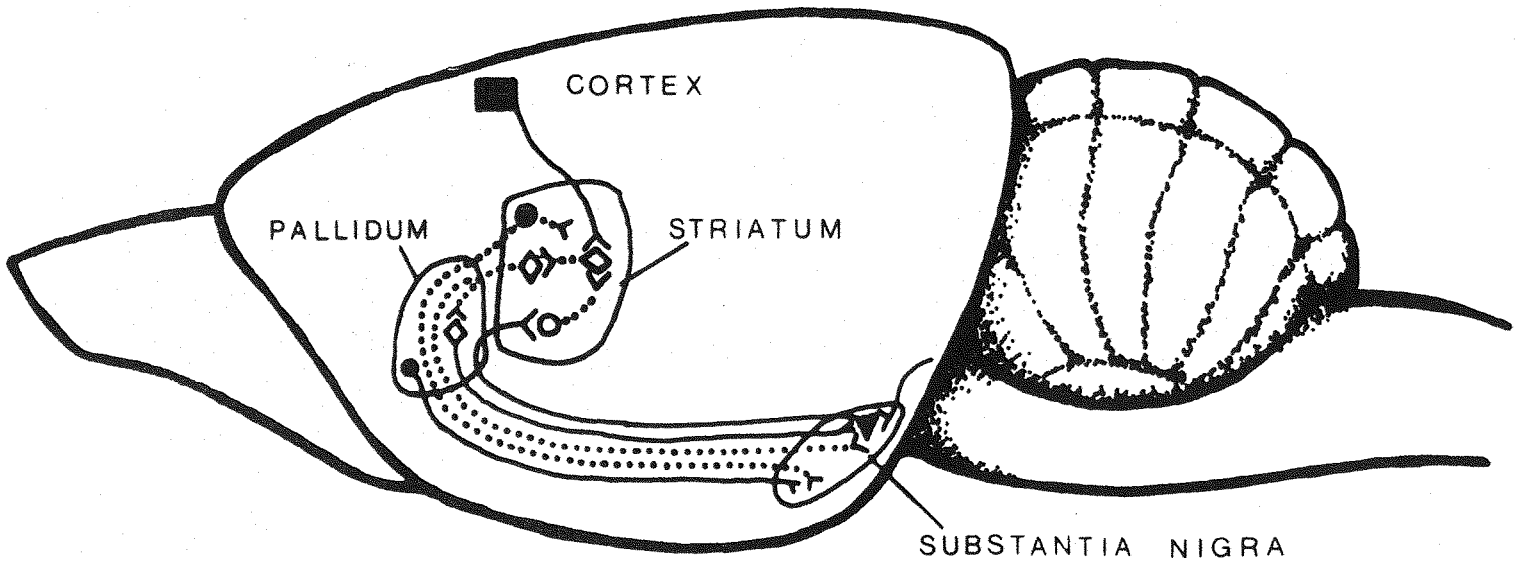
Quantitative data differ somewhat from laboratory to laboratory, not only in kainic acid lesioned animals but in human post-mortem tissue as well (McGeer and McGeer, 1982). Quantitative comparisons are therefore difficult, but qualitatively there would appear to be marked biochemical similarities between Huntington's disease (HD) and the kainic acid 'model'. The biochemical effects observed are in accord with morphological studies showing that neostriatal neurones degenerate whilst afferent systems are spared (Figure 1).

Following bilateral injections of kainic acid into the striatum, rats do not display choreiform movements: they do, however, display a variety of abnormal behavioural symptoms (Mason, 1981). This has been interpreted as the 'rodent' correlates of choreic movements in humans (McGeer and McGeer, 1982; Mason, 1981). The assertion that the kainic acid model provides a good behavioural model of Huntington's chorea (Mason, 1981) is disputed by Pisa (Pisa, 1981, 1982), who suggests that until a more comprehensive description of the human neuropathology is developed identifying rodent behaviour analagous to human abnormalities, occurring in Huntington's disease, remains difficult.

Cholinergic agonists and dopaminergic antagonists appear to alleviate the symptoms of HD, whilst the opposite pharmacotherapy usually intensifies the chorea of Huntington's disease patients (Barbeau, 1973; De Silva, 1977; Klawans and Weiner, 1976).

Investigations using rats with bilateral injections of kainic acid in the striatum (Sanberg, Pisa and Fibiger, 1981) suggest a close analogy between the pharmacological effects of these compounds in both choreic patients and lesioned rats. The kainic acid-induced lesions potentiated the locomotor response to both the dopaminergic agonist d-amphetamine, and the cholinergic antagonist, scopolamine, attenuated the cataleptic response to the dopaminergic

Figure 1. Schematic diagram of some of the neurones projecting to and from, or passing through the neostriatum. Only those neurones depicted with dashed lines would be destroyed by neostriatal injections of kainic acid. Adapted from McGeer and McGeer (1982), and Spokes (1981).




▲ DOPAMINE : ■ GLUTAMATE : O ACETYLCHOLINE : ◇ GABA

● NEUROPEPTIDES

antagonist, haloperidol, and potentiated the cataleptic and convulsive responses to the cholinergic agonist pilocarpine.

Description of the foregoing animal models of neurodegenerative disorders highlighting the similarities between morphological, biochemical and pharmacological factors, has illustrated how the use of selective neurotoxins can further our understanding of both the biochemical pathology and the potential pharmacotherapy of CNS disorders.

In the remaining sections of this introduction I will consider the neurochemical changes found to occur in post-mortem studies on Alzheimer-dementia brain.

c) Alzheimer's dementia

Alzheimer's dementia (AD) is a neurodegenerative disorder characterised by a general deterioration of thought and memory processes. It is quite distinct in both symptomology and pathology, from the sub-cortical dementias such as Huntington's disease (see previous section). AD is characterised neuropathologically by the presence of numerous senile plaques and neurofibrillary tangles in such areas as the cerebral cortex, hippocampus and amygdaloid nucleus (Tomlinson et al., 1970). These pathological features are quantitatively related to the severity of the dementia assessed clinically (Blessed et al., 1968).

Over the past ten to fifteen years there has been a considerable effort devoted to studying neurotransmitter systems in human post-mortem brains, with a view to identifying specific deficits associated with Alzheimer's dementia. In November 1981 there already existed over 200 references to neurochemical studies on post-mortem tissue. These findings have been summarised in several reviews (Terry and Davies, 1980; Davies, 1979; McGeer, 1981; Bowen, 1981). Whilst the number of studies on various transmitter systems is considerable, and the variety of conclusions equally diverse, more recent studies have suggested a far simpler chemical pathology.

Present evidence suggests that certain neurochemical anomalies in AD may not be closely related to the disease process itself. Thus, changes in activities associated with the noradrenergic or GABA systems, for example, do not correlate signficiantly with the extent of plaque formation. Furthermore, abnormalities in the levels of several neuropeptides (e.g. somatostatin, cholecystokinin and vasoactive intestinal polypeptide) are apparently confined to more advanced stages of the disease (Perry and Perry, 1982). The cholinergic abnormality (decreases in all presynaptic cholinergic markers), however, is apparent in earlier stages and correlates significantly with both histological and clinical measurements of the severity of the disease (Perry et al., 1978; McGeer, 1981).

The current concept of Alzheimer's dementia is thus one of a fairly selective, early, involvement of the cholinergic system within specific areas of the CNS (McGeer, 1981; Perry, 1980; Rossor et al., 1982). Whilst several animal models can replicate the neuropathological changes seen in AD, no model as yet displays any neurochemical selectivity for the cholinergic system. This study, therefore, is an attempt to develop an 'animal model' of Alzhiemer's dementia which will exhibit some specificity for the cholinergic system accompanied by a selectivity for defined pathways within the CNS. The methods used are essentially similar to those developed for the previously described neurodegenerative diseases.

## I.3. The Pharmacotherapy of Dementia

It was once thought that senile dementia was the result of impaired cerebral perfusion, which was caused by the narrowing of cerebral arterioles (Branconnier and Cole, 1976). Drugs which dilated these vessels were expected to improve cerebral blood flow with consequent clinical improvement. However, this logic now appears suspect. In Alzheimer's dementia the reduced cerebral blood flow is the consequence rather than the cause of the disease (Branconnier and Cole, 1976) and an increased flow is unlikely to modify primary neuronal degeneration. The rationale for the use of vasodilators in senile dementia has therefore changed from an

attempt to increase cerebral blood flow, to one to improve cerebral metabolism. Thus many drugs have been claimed to arrest intellectual decline and improve the behavioural disturbances associated with senile dementia, and are termed 'cerebral activating'drugs. Despite this classification, their mode of action remains totally unclear. Part of this study is therefore an attempt to examine the possible metabolic properties of* isoxsuprine, previously thought to be a drug with primarily vasodilator properties (Yesavage, 1979) but demonstrating some indications of clinical improvement in demented patients (Yesavage, 1979). This study also attempts to integrate the possible metabolic actions of these compounds with the decreases in cholinergic transmission observed in patients with Alzheimer's dementia. (*see Appendix I for detailed pharmacological properties).

# CHAPTER II

# MATERIALS AND METHODS

## II.1. Materials

The following Radiochemicals were purchased from Amersham International:

[1 - $^{14}$C] Acetyl-coenzyme A, 56.6 mCi/mmol;
[1 - $^{14}$C] Acetylcholine Chloride, 26.5 mCi/mmol;
[Methyl - $^{3}$H] Choline Chloride, 77 Ci/mmol;
L-[G - $^{3}$H] Glutamic Acid, 34 Ci/mmol;
D-[2 - $^{3}$H] Aspartic Acid, 12.5 Ci/mmol;
4-Amino-n-[2,3 - $^{3}$H] Butyric Acid (GABA), 70 Ci/mmol;
l-[7,8 $^{3}$H] Noradrenaline hydrochloride, 17 Ci/mmol; and
2-Deoxy-D-[1 - $^{3}$H] Glucose, 25 Ci/mmol.

Di-isopropyl fluorophosphate (DPF) was from Aldrich Chemical Corporation. The neurotoxic amino acids used in this study were from the following sources, NMDA (Tocris Chemicals), NMDLA (Sigma) and Kainic acid (Sigma). The generous gift of (±) Ibotenic acid by Dr P.J. Roberts and Dr R.J. Walker is gratefully acknowledged.

All remaining chemicals were purchased from either Sigma or BDH.

## II.2. Methods

### II.2.1. Animals and stereotaxic surgery

Male albino Wistar rats weighing 200 - 220 grams at the time of surgery were used throughout this study. All animals were fasted overnight before operation.

Animals were anaesthetised with sodium pentobarbitone (60 mg/Kg i.p.) and placed in a stereotaxic frame (David Kopf). An incision was made along the midline of the rat's head and the skull was cleared of connective tissue. A hole was drilled into the skull for the introduction of a Hamilton 25 gauge (0.5 mm) needle connected to a 5 µl Hamilton syringe mounted in a syringe holder in the stereotaxic frame. A volume of 1 µl of phosphate buffered saline (50 mM) containing a given neurotoxic amino acid

(with pH adjusted to 7.4) was injected at a rate of 0.1 µl/min; the needle being left in place for a further 15 minutes to ensure that all ejected fluid had been absorbed by the brain tissue, thus minimising diffusion of the neurotoxin up the cannula tract to the overlying cortex.

The co-ordinates routinely used for ventro medial globus pallidus lesions were; AP 5.9, ML 2.9, DV -1.8: and for the boundary between the medial septal nucleus and vertical limb of the nucleus of the diagonal band; AP 8.9, ML 0.5, DV -1.0; (co-ordinates from Konig and Klippel, 1963).

The bore hole was carefully sealed with sterile bone wax (Allen and Hanbury) and the animals injected (i.m.) with the antibiotic Ampiclox (ampicillin/cloxacillin, Beechams Research Labs) to protect against infection.

Electrolytic lesions were performed according to the Methods of Mustapha (1982). Bilateral lesions were induced in the globus pallidus using the method of electrolytic coagulation. Male Wistar rats were anaesthetised with sodium pentobarbitone (60 mg/Kg i.p.) and were immobilised in a stereotaxic frame. Lesioning electrodes (Clark Electromedical) 0.35 mm in diameter were used to induce the lesion. The electrodes were insulated to within 0.5 mm of the tip. An anodal D.C. current of 1 mA was passed for 10 - 15 seconds at one of several sites, the stereotaxic frame acting as the cathode. In control animals the electrode was lowered into the brain just dorsal to the pallidum but no current was passed.

### II.2.2. Cortical dissection

In preliminary studies the cortex was divided into three sections (Kelly and Moore, 1978): anterior, middle, and posterior regions (Figure 2). Later studies used a more selective dissection of the middle cortex, the limbic cortex within the longitudinal fissure (fissura longitudinalis cerebri) and the pyriform cortex below the rhinal fissure (fissura rhinalis) were removed, leaving fronto-parietal cortex corresponding to areas 1 - 4, 6 - 8, and 40 of the atlas of Krieg (Krieg, 1946). See Figure 3.

Figure 2. View of the ventral and dorsal surfaces of the rat brain to indicate the positions of the frontal cuts used to dissect the cortex into anterior, middle and posterior regions. From Kelly and Moore (1978).

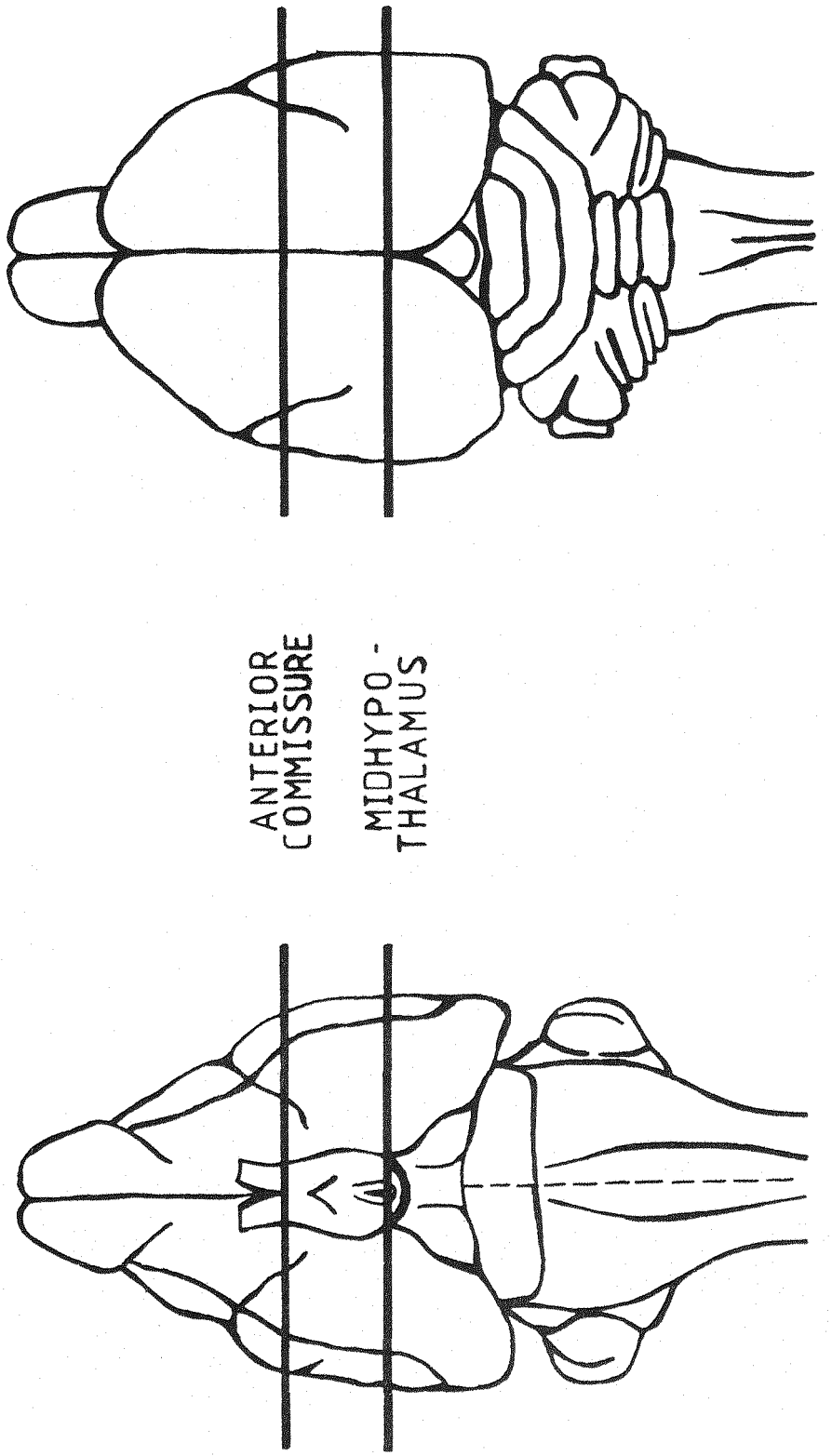
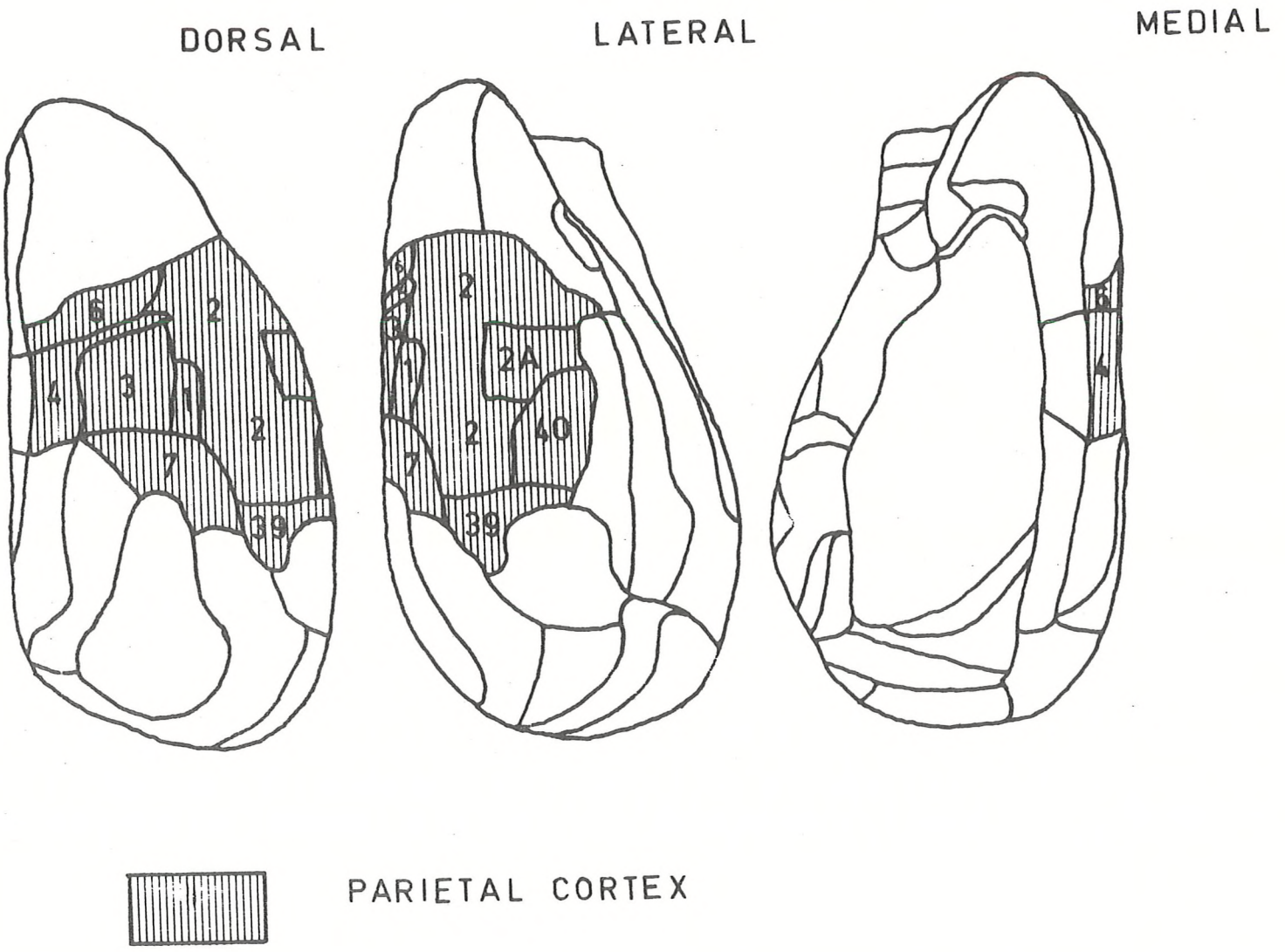

ANTERIOR COMMISSURE
MIDHYPO-THALAMUS

Figure 3. Dorsal, lateral and medial elevations of cerebral cortex of right side showing topography of areas. Shaded area corresponds to parietal areas 1, 2, 3, 7, 39 and 40, and frontal areas 4 and 6. From the Atlas of Krieg (1946).


DORSAL
LATERAL
MEDIAL
5
2
4
3
1
2
7
39
2
2A
40
PARIETAL CORTEX

To measure enzyme activity at the site of direct cortical kainate injection, a circle of cortical tissue of 3 mm radius around the injection site was obtained using a stainless steel punch: the subjacent white matter being cut away. All dissections were performed on a glass plate chilled on ice and were performed immediately after decapitation of the rats.

II.2.3. Enzyme Assays

a) Choline acetyltransferase (CAT EC.2.3.16) was assayed by the method of Fonnum (Fonnun, 1975a, b). The formation of [$^{14}$C]-acetyl choline from labelled acetyl-COA as substrate (Fig. 4) was found to be linear with protein concentration (Fig. 5) and time (Fig. 5); the enzyme exhibiting maximal activity in the presence of 100 - 300 mM $Na^+$, $Cl^-$ ion concentration (Fig. 7b) (Rossier et al., 1977; Hersh, 1980).

b) Acetylcholinesterase (AChE; EC.3.1.1.7) was assayed by a modification of the method of Fonnum (Fonnum, 1975b) and was essentially similar to the method for CAT (Fig. 6). [$^{14}$C] - Acetylcholine is enzymatically hydrolysed to [$^{14}$C] acetate and choline which are then separated by liquid phase cation exhange. A low ionic concentration and inclusion of 0.1 mM ethopropazine minimise spontaneous and butyl-cholinesterase induced hydrolysis respectively. The assay was found to be linear with increasing protein concentration (over a very wide range) and time (Fig. 7a and 8). It was inhibited by both low temperature and the inclusion of 1 mM physostigmine.

c) L-Glutamic Acid Decarboxylase (GAD, EC.4.1.1.15) was assayed by the method of Kanazawa, Iversen and Kelly (Kanazawa et al., 1976). Where a combined CAT and GAD assay was performed the tissue was homogenised in 10 mM EDTA, 0.5% Triton X-100 (pH 7.4). While Triton X-100 has been shown to inhibit non-GAD dependent decarboxylation of glutamate (MacDonnell, 1975) no significant difference between tissue homogenates in this buffer and in de-ionised water were observed (results not shown). Simi-

Figure 4. CHOLINE ACETYLTRANSFERASE ASSAY

HOMOGENISE TISSUE (10% W/V) in 10 mM EDTA (pH 7.4), 0.5% TRITON X-100.

10 µl HOMOGENATE
and 25 µl BUFFER*

↓ INCUBATE FOR 15 MINS AT 37°C

RINSE INTO SCINTILLATION VIALS KEPT ON ICE WITH 2 x 1.25 mls Na PHOSPHATE BUFFER (50 mM, pH 7.4 and 4°C). ADD 2 mls ACETONITRILE (METHYL CYANIDE) CONTAINING 10 mg Na TETRAPHENYL BORON.

↓ SHAKE LIGHTLY FOR 1 MIN.

ADD 10 mls TOLUENE/BUTYL PBD, COUNT FOR [$^{14}$C] WHEN UPPER (ORGANIC) LAYER IS CLEAR.

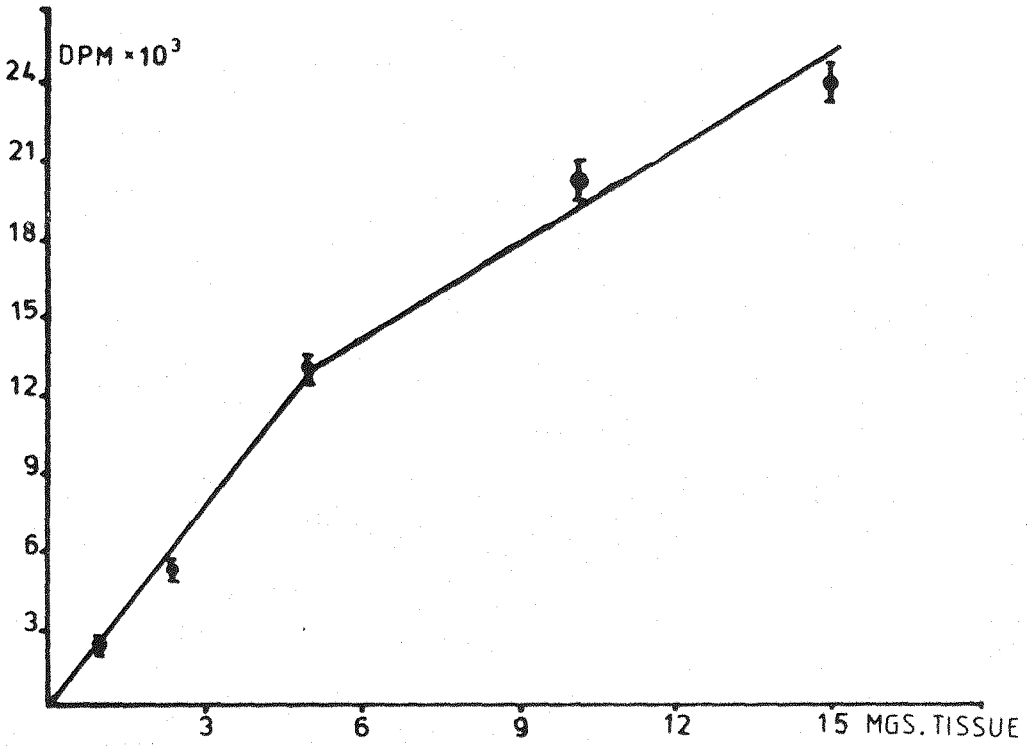
*CHOLINE CHLORIDE (8 mM), EDTA (20 mM), PHYSOSTIGMINE (0.1 mM), SODIUM CHLORIDE (300 mM) and acetyl [1-$^{14}$C] CO-ENZYME A (diluted with non-radioactive CoA from 55.4 to 2.1 mCi/mmol to give 0.26 mM) all dissolved in 50 mM SODIUM PHOSPHATE BUFFER pH 7.4. (All Final Concentrations)

Radioachemical assay for choline acetyltransferase (EC. 2.3.1.6). Labelled acetylcholine is isolated by liquid cation exchange using sodium tetraphenyl boron in methyl cyanide (acetonitrile).

Figure 5a. The correlation between CAT activity and different amounts (mgs tissue) of rat fronto-parietal cortex homogenates. The incubation was carried out in a total volume of 35 µls at 37°C for 15 mins.

Figure 5b. Synthesis of [$^{14}$C] acetylcholine determined for various incubation times. The acetyl-CoA was made up from a mixture of labelled and unlabelled acetyl-CoA at a final concentration of 0.26 mM.

## CHOLINEACETYLTRANSFERASE




## CHOLINEACETYLTRANSFERASE

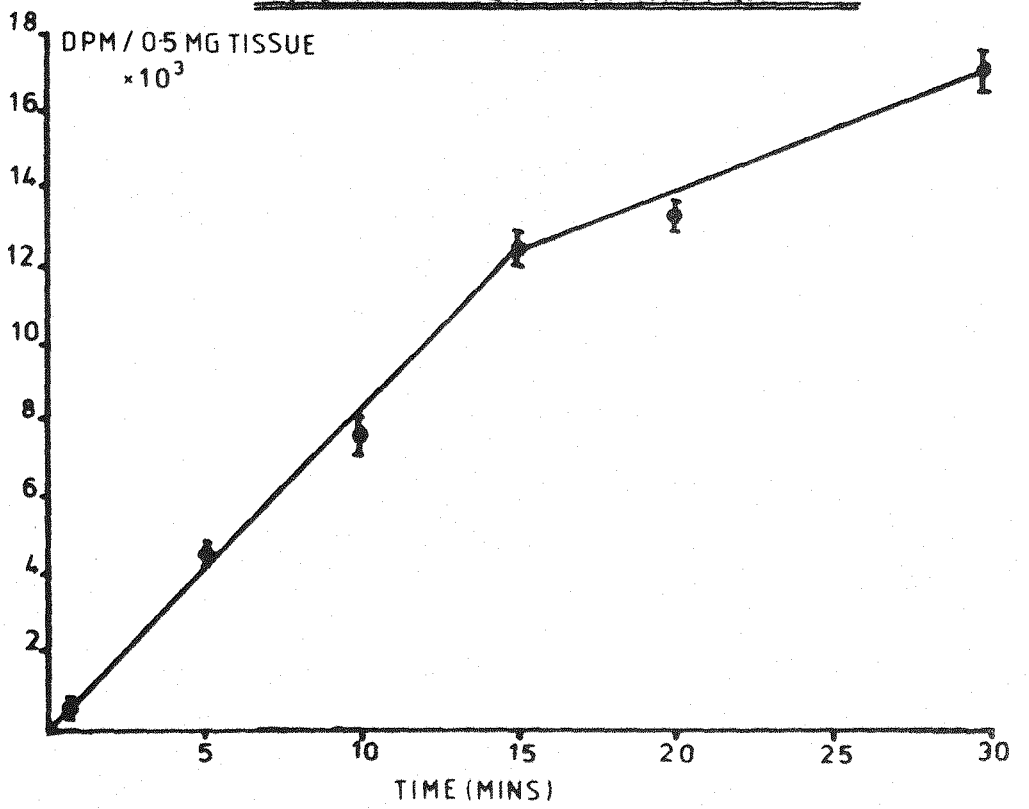


Figure 6. ACETYLCHOLINESTERASE ASSAY

HOMOGENISE TISSUE (1% W/V) IN 10 mM EDTA 0.5% TRITION X-100 (pH 7.4).

10 µl HOMOGENATE
and 25 µl BUFFER*

↓ INCUBATE AT 37°C FOR 15 MINS

RINSE INTO SCINTILLATION VIALS KEPT ON ICE WITH 2 x 1.25 mls ICE COLD SODIUM PHOSPHATE BUFFER (20 mM pH 7.4)
ADD 2 mls ACETONITRILE (METHYL CYANIDE) CONTAINING 10 mg SODIUM TETRAPHENYL BORON.

↓ SHAKE LIGHTLY FOR 1 MIN

ADD 10 mls TOLUENE AND FREEZE AT -18°C FOR 2 HOURS. POUR OFF ORGANIC LAYER AND WASH FROZEN AQUEOUS PHASE WITH 2 mls TOLUENE AT -18°C. ADD 15 mls TRITOSCINT AND COUNT FOR [$^{14}$C].

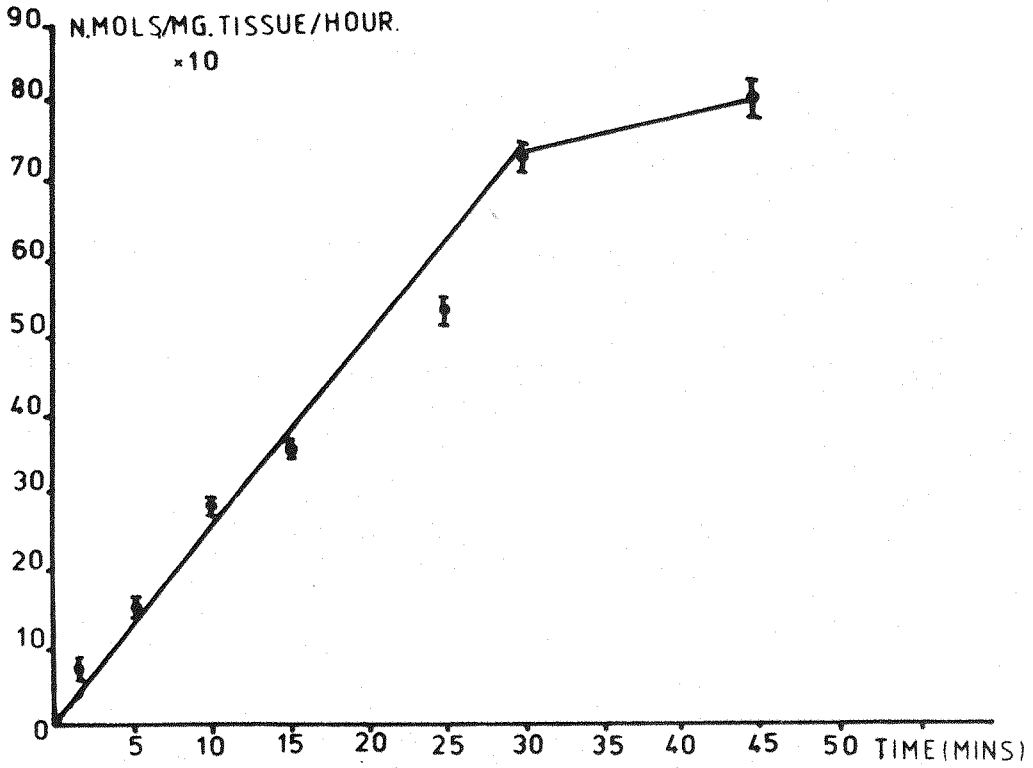
* SODIUM PHOSPHATE (20 mM): ETHOPROPAZINE (0.1 mM): [1-$^{14}$C] ACETYLCHOLINE (0.7 mM and 2.65 mCi/mmol).

Radiochemical assay for acetylcholinesterase (EC. 3.1.1.7). Differentiation between acetylcholinesterase and butylcholinesterase is achieved by inclusion of ethopropazine (0.1 mM), a reversible competitive inhibitor of butylcholinesterase. (Inhibition ratio butylcholinesterase:acetylcholinesterase = 2800:1).

Figure 7a. Synthesis of [$^{14}$C] acetate determined for various incubation times at 37°C. [1 - $^{14}$C] acetylcholine at a final substrate concentration of 0.7 mM.

Figure 7b. Effects of increasing NaCl concentration on choline acetyltransferase activity. Incubation buffer contains: choline chloride (8 mM); EDTA (20 mM); physostigmine (0.1 mM); and acetyl [1 - $^{14}$C] Coenzyme A (diluted with non-radioactive CoA from 55.4 to 2.1 mCi/mmol to give 0.26 mM). All dissolved in 50 mM sodium phosphate buffer (pH 7.4), plus varying concentrations of sodium chloride from 0 - 400 mM.

## ACETYLCHOLINESTERASE




## CHOLINEACETYLTRANSFERASE

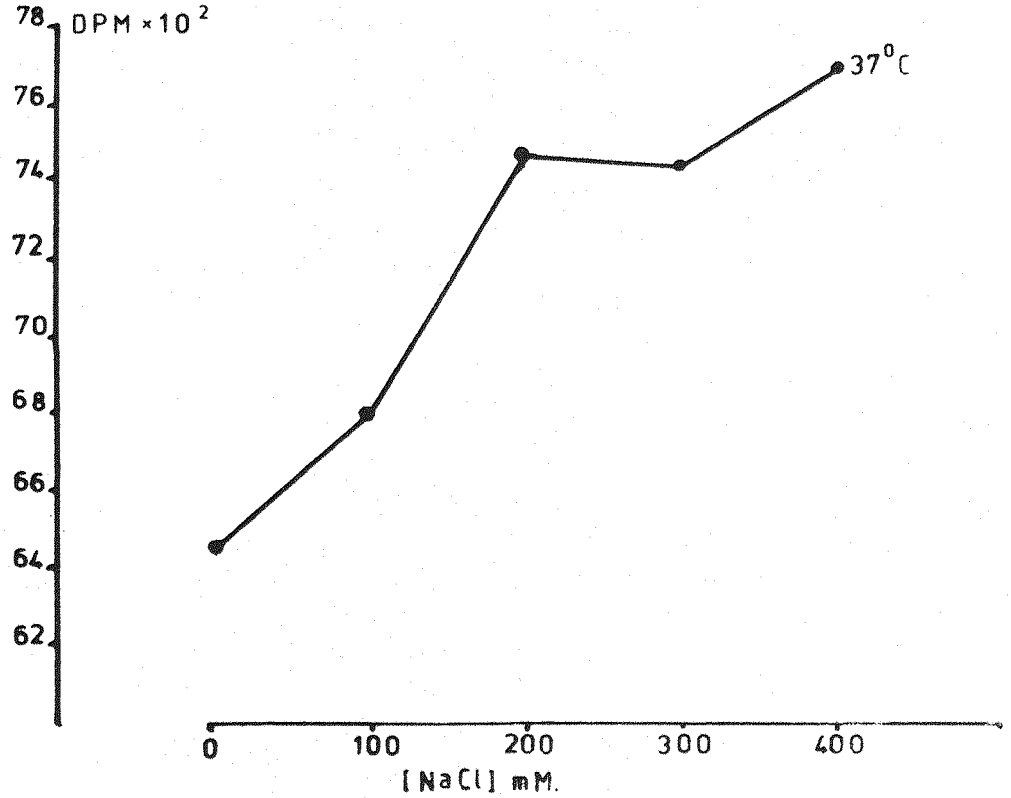
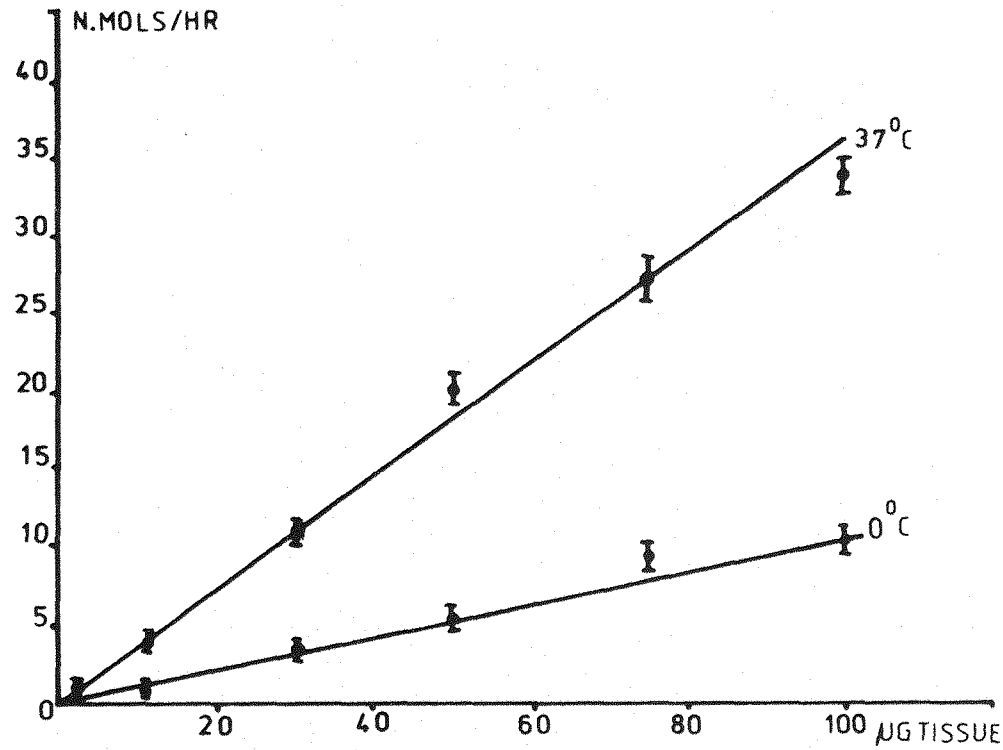
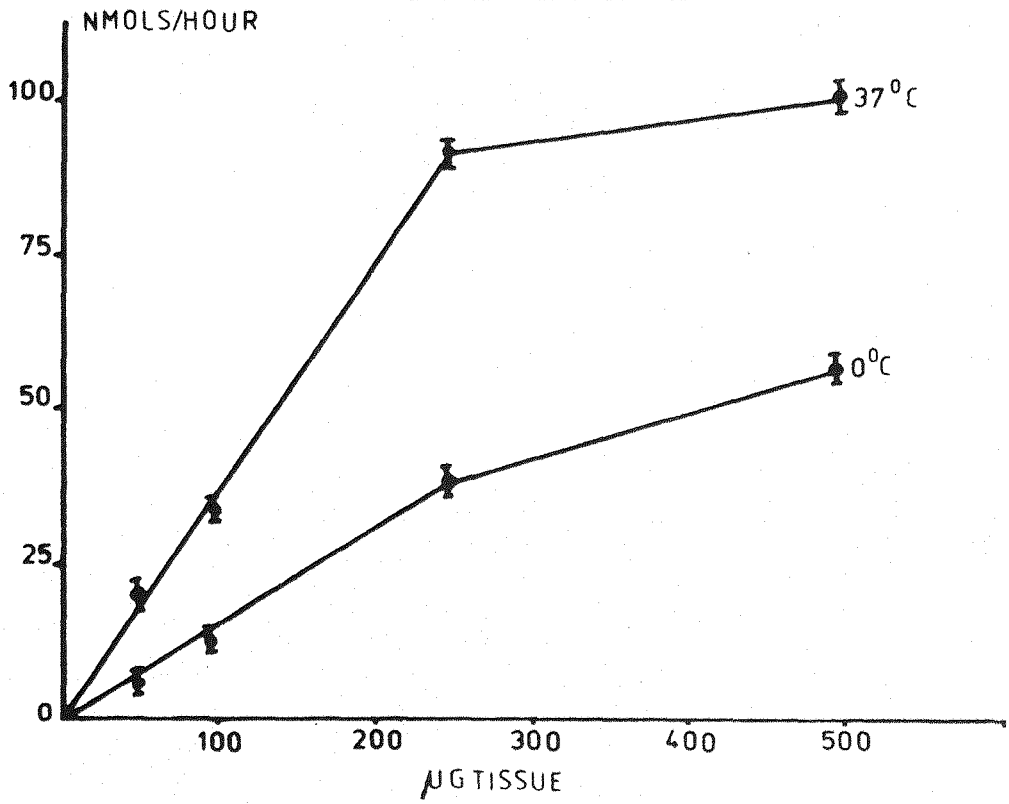


Figure 8. The correlation between acetylcholinesterase activity and different amounts (mgs tissue) of rat cortex homogenate. The incubation was carried out in a total volume of 35 μl at 37°C for 15 mins.

## ACETYLCHOLINESTERASE




## ACETYLCHOLINESTERASE



larly whilst antioxidants have been shown to inhibit GAD (Charington et al., 1981), dithiothrietol at 1 mM has no inhibitory effect (Charington et al., 1981). In this method [$^3$H] -GABA is separated from the substrate [$^3$H]-glutamate by an anion exchange resin column, eluted with de-ionised/distilled water (Fig. 9). The assay was found to be linear with both time and protein concentration (Fig. 10).

II.2.4. High Affinity Neurotransmitter Uptake

a) High affinity choline uptake (HACU).

Although neuronal tissue can accumulate acetylcholine when external concentrations are in the micromolar range this occurs only in the presence of acetylcholinesterase (AChE) inhibitors and is not specific to cholinergic neurones (Kuhar and Simon, 1974). However the existence of a unique high affinity transport system for choline, the immediate precursor of acetylcholine, is well established and found to be associated with acetylcholine formation both in central (Yamamura and Snyder, 1973) and peripheral (Pert and Snyder, 1974) neuronal tissue.

The uptake of choline was measured both into cerebral cortex slices and into synaptosomes prepared from parietal cortex. The methods and results for the synaptosomal preparation will be presented. A crude $P_2$ pellet was obtained by centrifugation in sucrose at 4°C (Fig. 11) and synaptosomal uptake determined as presented in Figure 11. Uptake was determined at 37°C in quadruplicate samples, a parallel series being routinely incubated at 0°C (in quadruplicate) as blanks. Filter blanks were also measured under identical experimental conditions (see Fig. 12).

The sodium-independent uptake of choline into synaptosomes was not determined using this method, because exclusion of sodium (replaced by sucrose) from the incubation and washing buffers significantly increased the blank values for [$^3$H] choline binding to Nitrocellulose filters. Inclusion of Hemicholinium-3 (HC-3, Aldrich Chemical Co.), 1 μM, in the incubation buffer was found to inhibit

Figure 9. L-GLUTAMATE DECARBOXYLASE ASSAY

HOMOGENISE TISSUE (10% W/V) IN DE-IONISED WATER AT 4°C.

5µl HOMOGENATE
5µl BUFFER*

↓ INCUBATE FOR 15 MINS AT 37°C

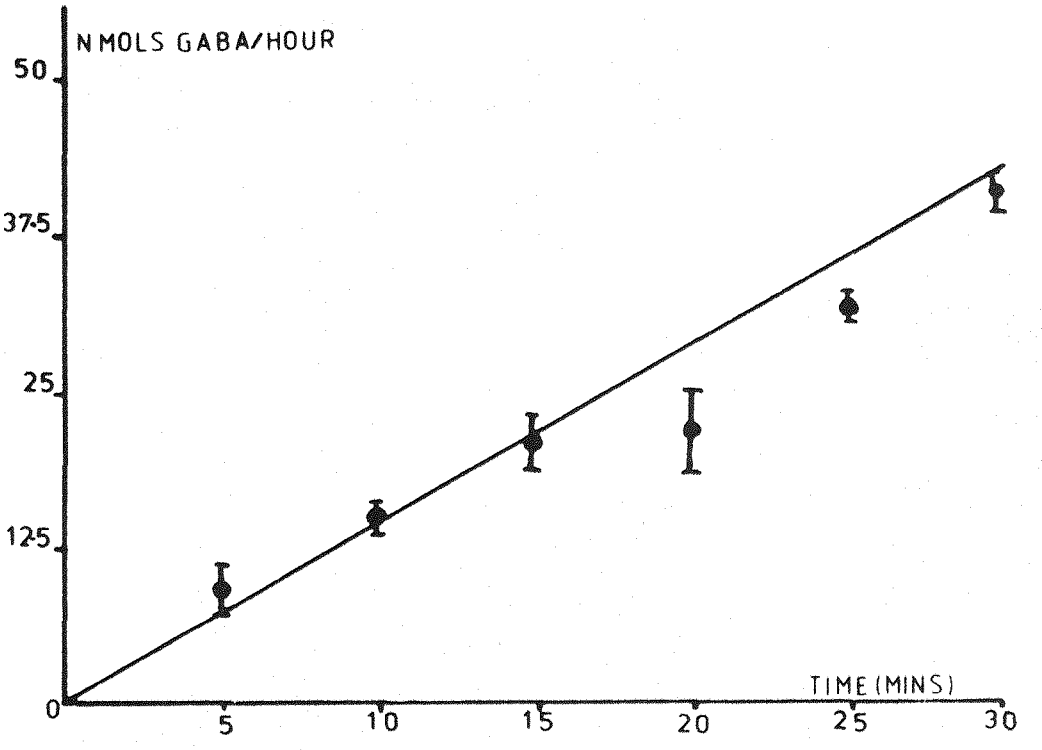
ADD 0.4 mls ICE COLD DE-IONISED WATER. PLACE ON DOWEX 1-X8 RESIN (100-200 MESH) ACETATE FORM. WASH THROUGH WITH 1.2 mls DE-IONISED WATER. ADD 10 mls TRITOSCINT AND COUNT FOR [$^3$H].

* 200 mM POTASSIUM PHOSPHATE BUFFER CONTAINING 1 mM DITHIOTHREITOL, 1mM PYRIDOXAL PHOSPHATE, 50 mM SODIUM GLUTAMATE and 50 µCi/ml [$^3$H] GLUTAMATE (pH 6.4).

L-glutamate decarboxylase assay (EC. 4.1.1.15). Radioactive GABA is separated from radioactive glutamate by means of an anion exchange resin column. 99.5 $\pm$ 0.02% added [$^3$H] L-glutamate retained on column. Blanks 19% of control value.

Figure 10. The correlation of GAD activity with incubation time at 37°C and with differing amounts (μg tissue) of rat cortex homogenates. Incubation was carried out in a total volume of 10 μl at 37°C for 15 mins. The glutamate was made up from a mixture of labelled and unlabelled glutamate at a final concentration of 50 mM.

## L-GLUTAMATE DECARBOXYLASE




## L-GLUTAMATE DECARBOXYLASE

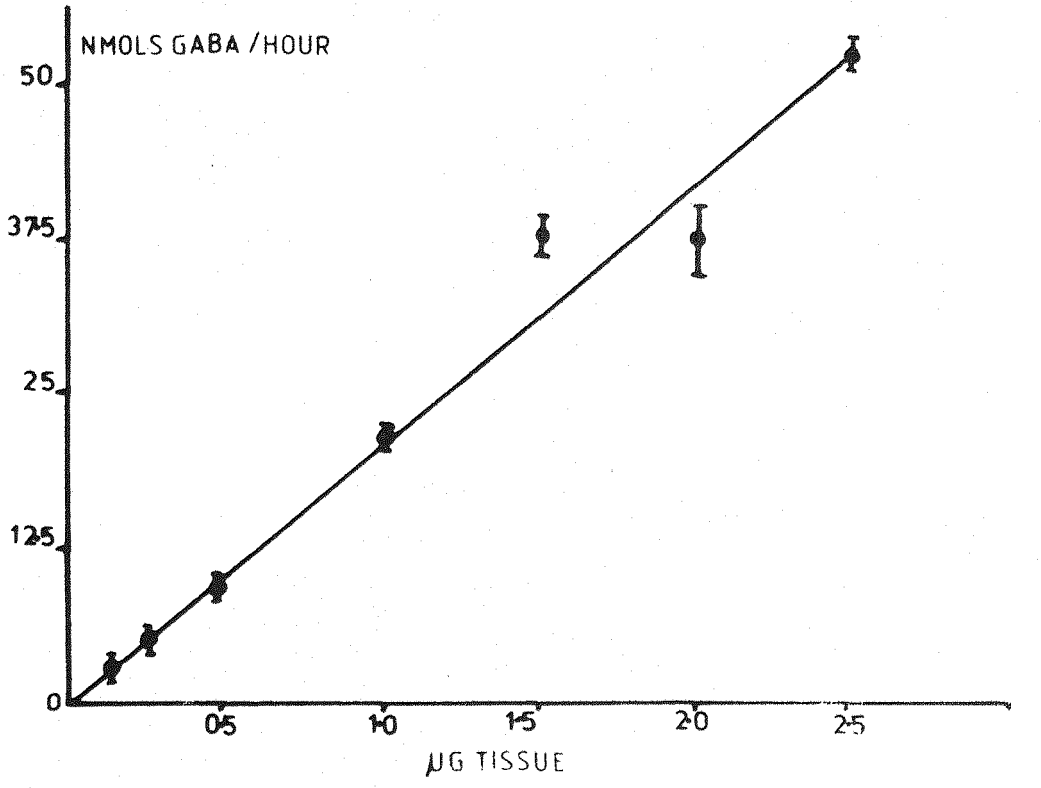


Figure 11. High Affinity Choline Uptake

A $P_2$ synaptosomal preparation and incubation conditions essentially similar to those of Simon et al (1976) were used. [ Methyl-$^3$H] -choline chloride, 0.06 μM final concentration, is 20% $K_m$ for the high affinity uptake reported in rat cerebral cortex ($K_m$ 0.3 μM; $V_{max}$ 25 -mol/ 4 mins/mg protein). Initial studies using sodium free Krebs Ringer phosphate gave greatly increased retention of choline by millipore filters. Blanks were therefore incubated in KRP at 0°C. Filter blanks were routinely 5.4 ± 0.64% control values. Uptake in parietal cortex was 6.48 ± 0.45 pmol/4 mins/mg protein (n = 5).

Figure 11. HIGH AFFINITY CHOLINE UPTAKE

HOMOGENISE TISSUE (LOOSE FITTING TEFLON-GLASS HOMOGENISER, 0.5 mM CLEARANCE) IN 20 VOLUMES OF 0.32 M SUCROSE

↓ CENTRIFUGE AT 1000g FOR 10 MINS

DISCARD $P_1$ PELLET

$S_1$

↓ 17,000g for 20 mins

RESUSPEND $P_2$ PELLET IN ORIGINAL VOLUME SUCROSE

$S_2$

900 µl BUFFER*
and 10 µl [METHYL-$^3$H] CHOLINE CHLORIDE
(0.06 µM and 8.3 Ci/mmol)

↓ PREINCUBATE AT 37°C FOR 5 MINS

100 µl SYNAPTOSOMES

↓ INCUBATE FOR 4 MINS AT 37°C

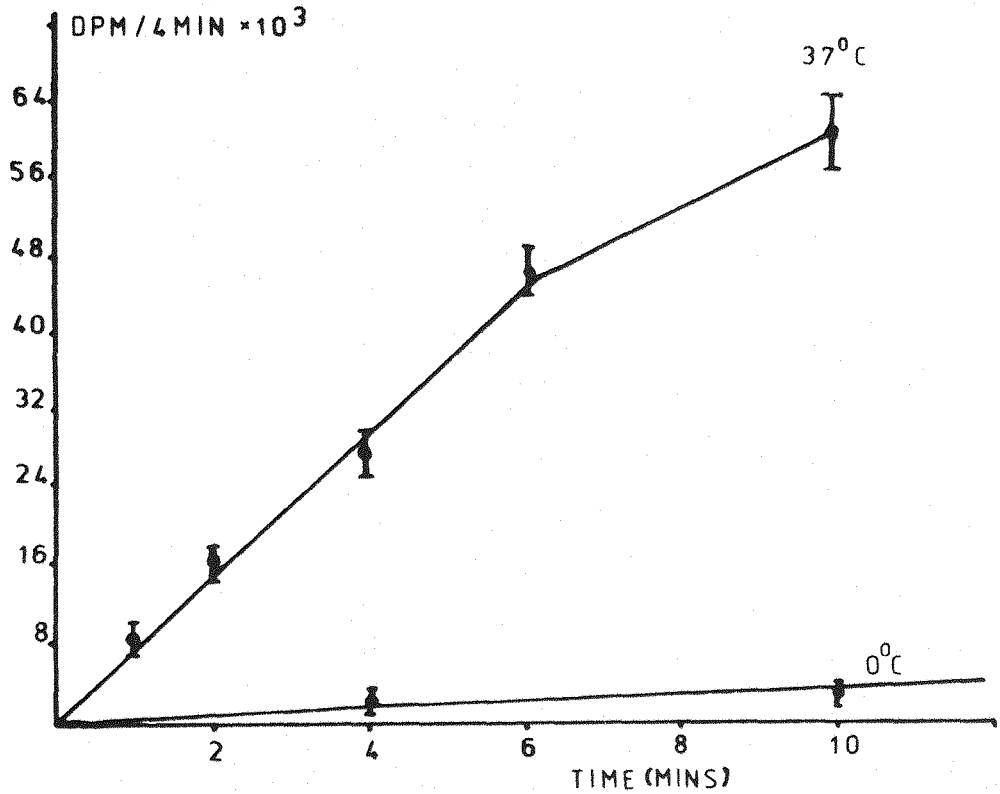
RAPIDLY FILTER ON MILLIPORE FILTERS WASHING WITH 3 x 4 mls BUFFER. ADD 1 ml CELLUSOLVE and 8 mls CELLOSCINT AND COUNT FOR [$^3$H].

*KREBS RINGER PHOSPHATE (pH 7.4)

NaCl (126 mM), KCl (4.75 mM), $CaCl_2$ (1.27 mM), $Na_2HPO_4$ (15.8 mM) $MgCl_2$ (1.42 mM), GLUCOSE (2 mg/ml), PHYSOSTIGMINE (0.5 mM): gassed with 95% $O_2$:5% $CO_2$.

Figure 12. The correlation of high affinity choline uptake with incubation time and with differing amounts of synaptosomes (fronto-parietal cortex). Uptake is linear until 6 mins and up to 300 µls synaptosomes. Uptake at $0^{\circ}C$ is 9% of control values.

## HIGH AFFINITY CHOLINE UPTAKE




## HIGH AFFINITY CHOLINE UPTAKE

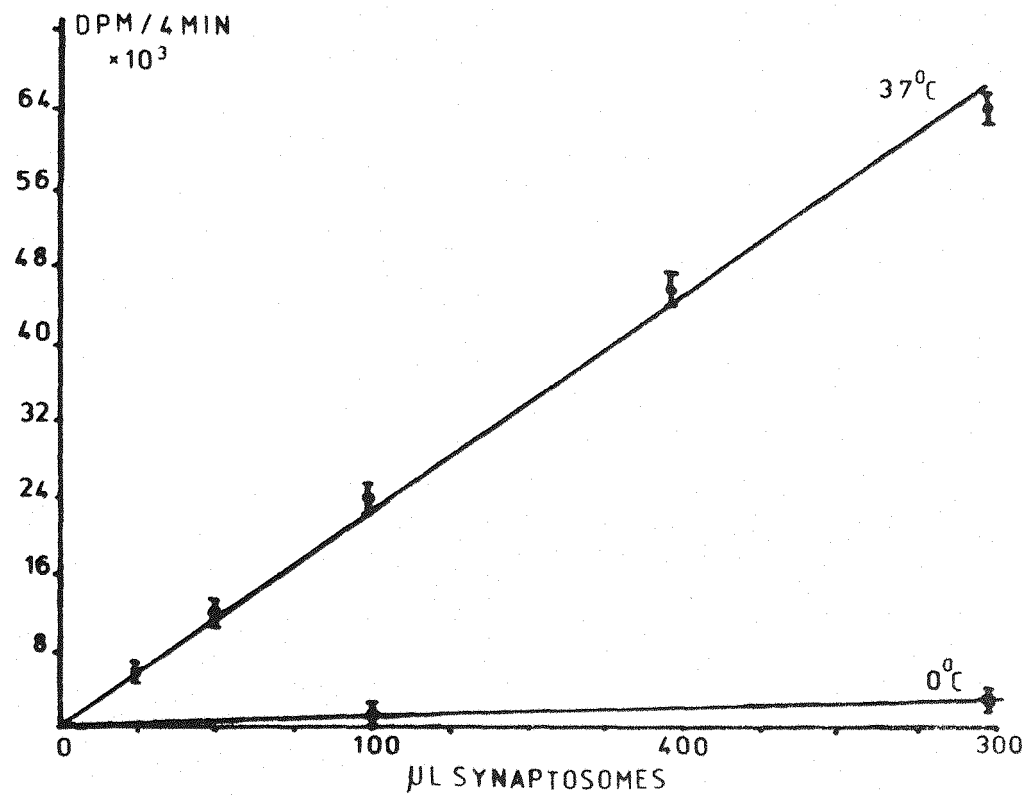
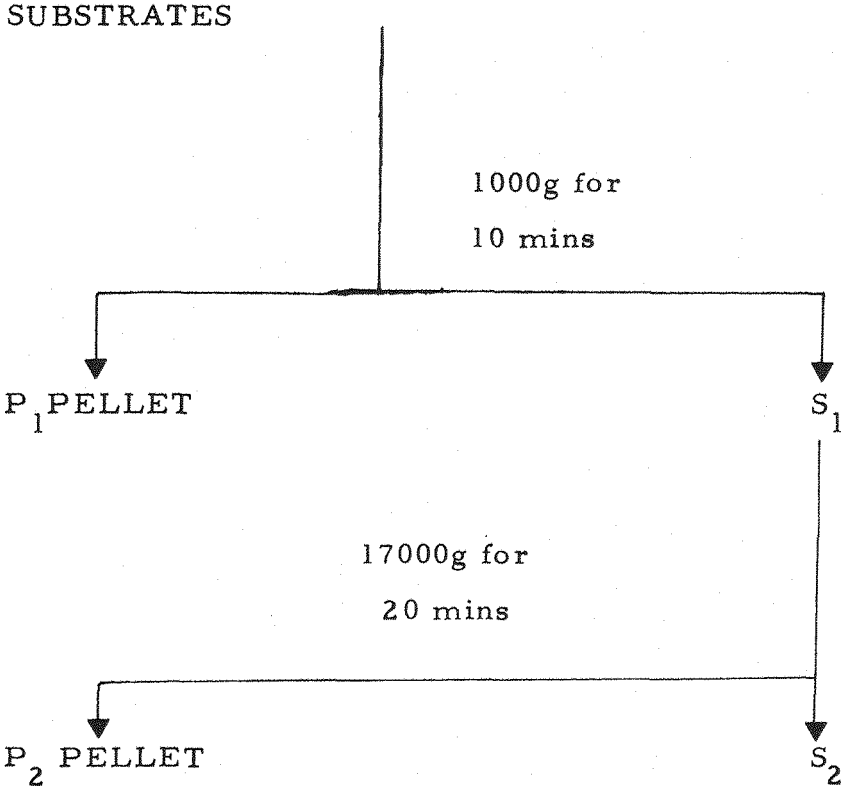


Figure 13. WASHED $P_2$ PREPARATION

HOMOGENISE TISSUE IN 10 VOLS. 0.32 M SUCROSE - ADD FURTHER 50 VOLS. TO DILUTE ENDOGENOUS SUBSTRATES




RESUSPEND $P_2$ PELLET IN SMALL VOLUME 0.32 M SUCROSE.

the uptake of choline by 80 - 85%; this is in accord with published results (Guyenet et al., 1973).

b) γ-amino Butyric Acid (GABA) Uptake

The uptake of [$^3$H]-GABA into a washed $P_2$ preparation of parietal cortex was determined in the presence of 50 μM β-alanine to inhibit glial uptake of GABA (Iversen and Kelly, 1975; Schon and Kelly, 1975). Incubations were performed at 37°C in quadruplicate with both 0°C and filter blanks being measured in parallel (Figs. 13 and 14). The inclusion of 50 μM β-alanine was found to inhibit the uptake of GABA into striatal synaptosomes by 20% (preliminary experiments and Foster, 1983). This compares with the results of Early et al. (1981) who have shown that inclusion of 100 μM β-alanine inhibits [$^3$H]GABA (0.12 μM) uptake into whole rat brain synaptosomes by 17.0 ± 8.5%. The removal of glial uptake of [$^3$H] GABA thus provides a more sensitive assessment of changes in the neuronal uptake of this amino acid.

c) Noradrenaline Uptake

[$^3$H]-L-noradrenaline uptake was determined in a washed $P_2$ preparation of parietal cortex (Fig. 15) in the presence of 1 μM nialamide. Incubation were performed at 37°C in quadruplicate; blanks were determined at 0°C and in the presence of 10 μM imipramine. Filter blanks were also measured under identical conditions. The purity of the 1- 7,8 -[$^3$H] -noradrenaline hydrochloride was assessed by TLC on cellulose Avicel plates (20 x 20 cm) eluted with n-butanol:acetic acid:water (12:3:5). The radioactivity ran as a single peak with an $R_f$ value identical to that of noradrenaline run under identical conditions and observed under ultra violet light.

d) Excitatory Amino Acid uptake

It seems probable that the acidic amino acids aspartate and glutamate (Asp and Glu) are important transmitters in the CNS, particularly in cortical neurones (reviews by Fonnum et al., 1979; Johnson, 1978). Since the two amino acids occupy a central posi-

Figure 14. [$^3$H] AMINO ACID UPTAKE

2 mls *KREBS BICARBONATE BUFFER (pH 7.4) AND 20 µl $P_2$ PREPARATION GASSED WITH 95% $O_2$, 5% $CO_2$.

↓ PREINCUBATE AT 37°C FOR 2 MINS

ADD [$^3$H] AMINO ACID TO GIVE FINAL CONCENTRATION OF 1.0 µM

↓ INCUBATE FOR 3 MINS AT 37°C

RAPIDLY FILTER THROUGH WHATMAN GF/C FILTERS, WASHING WITH 10 mls KREBS BICARBONATE BUFFER. DISSOLVE IN 0.5 mls PROTOSOLVE FOR 3 HOURS. ADD 8 mls TRITOSCINT AND 0.25 mls GLACIAL ACETIC ACID, AND COUNT FOR [$^3$H].

*KREBS RINCER BICARBONATE (pH 7.4)

112.54 mM NaCl: 4.75 mM KCl; 2.57 mM CaCl: 1.19 mM $KH_2PO_4$: 1.90 mM $MgSO_4$ $7H_2O$: 25.0 mM $NaHCO_3$: 11.54 mM GLUCOSE: gassed with 95% $O_2$:5% $CO_2$.

GAMMA AMINO BUTYRIC ACID UPTAKE

GABA UPTAKE IN THE PRESENCE OF 50 µM β-ALANINE.

Figure 15. $[^3H]$ NORADRENALINE UPTAKE

2 mls KREBS RINGER BICARBONATE BUFFER* (pH 7.4) CONTAINING 1 μM NIALAMIDE (and 10 μM IMIPRAMINE for controls) IN 10 ml. CONICAL FLASKS. GASSED WITH 95% $O_2$ and 5% $CO_2$.

+ 20 μl SYNAPTOSOMES (5 mgs ORIGINAL TISSUE)

↓ 2 min PREINCUBATION AT 37°C

10 μCi $[^3H]$ NORADRENALINE (containing 1 mg/ml ASCORBIC ACID) TO GIVE A FINAL CONCENTRATION OF 50 nM.

↓ 4 min INCUBATION AT 37°C

RAPIDLY FILTER ON WHATMAN GF/C FILTERS WASHING WITH 10 mls KRB. DISSOLVE IN 0.5 mls PROTOSOLVE FOR 3 HOURS. ADD 0.25 mls GLACIAL ACETIC ACID AND 8 mls TRITOSCINT AND COUNT FOR $[^3H]$.

*c.f. AMINO ACID UPTAKE.

tion in general metabolism, it has been difficult to separate the transmitter pool from the metabolic pool of these amino acids. Study of the high affinity uptake has therefore proved to be an important tool in this area.

The uptakes of [$^3$H] L-glutamate and of [$^3$H]-D-aspartate were determined in a washed $P_2$ preparation of parietal cortex (Fig. 13). Incubations at 37°C were in quadruplicate with blanks being determined at 0°C. Filter blanks were also measured.

II.2.5. Neurotransmitter Levels

Noradrenaline, dopamine and GABA were isolated using Sephadex G-10 columns according to the method of Earley and Leonard (1978). Noradrenaline and dopamine were subsequently assayed by a fluorometric method (Earley and Leonard, 1978; Welch and Welch, 1969). GABA was assayed by the fluorometric method of Uchida and O'Brien (1965).

II.2.6. Protein Estimation

Protein content was established using the Folin phenol reagents described by Lowry et al. (Lowry et al., 1951). Bovine serum albumen was used as a standard.

II.2.7. [$^3$H]-2-Deoxyglucose uptake

Sokoloff and his colleagues have described a procedure for the measurement of cerebral glucose uptake on a regional basis (Sokoloff et al., 1977). The technique uses 2-deoxyglucose (2DG), an analogue of glucose which is taken up by brain cells at a rate proportional to glucose uptake. It is then phosphorylated in an analogous manner to glucose, but 2DG-6-phosphate is relatively stable metabolically and is sequestered inside the cell for several hours (Sokoloff et al., 1977).

Most previous studies have used [$^{14}$C] 2-deoxyglucose and have determined brain levels of the 2DG-6-phosphate autoradiographically. This technique is appropriate for the gross manipulations

that had been reported, but autoradiography is difficult to quantify, especially without elaborate equipment, and very little quantitative data have been reported. For the more subtle changes we anticipated following long-term drug treatments, we needed a procedure more readily quantifiable. Thus we chose to analyse the [$^3$H] content in brain regions by scintillation counting of hand dissected brain regions. This procedure has been observed to produce results qualitatively similar to those obtained by autoradiography (Delanoy and Dunn, 1978; Meibach et al., 1980).

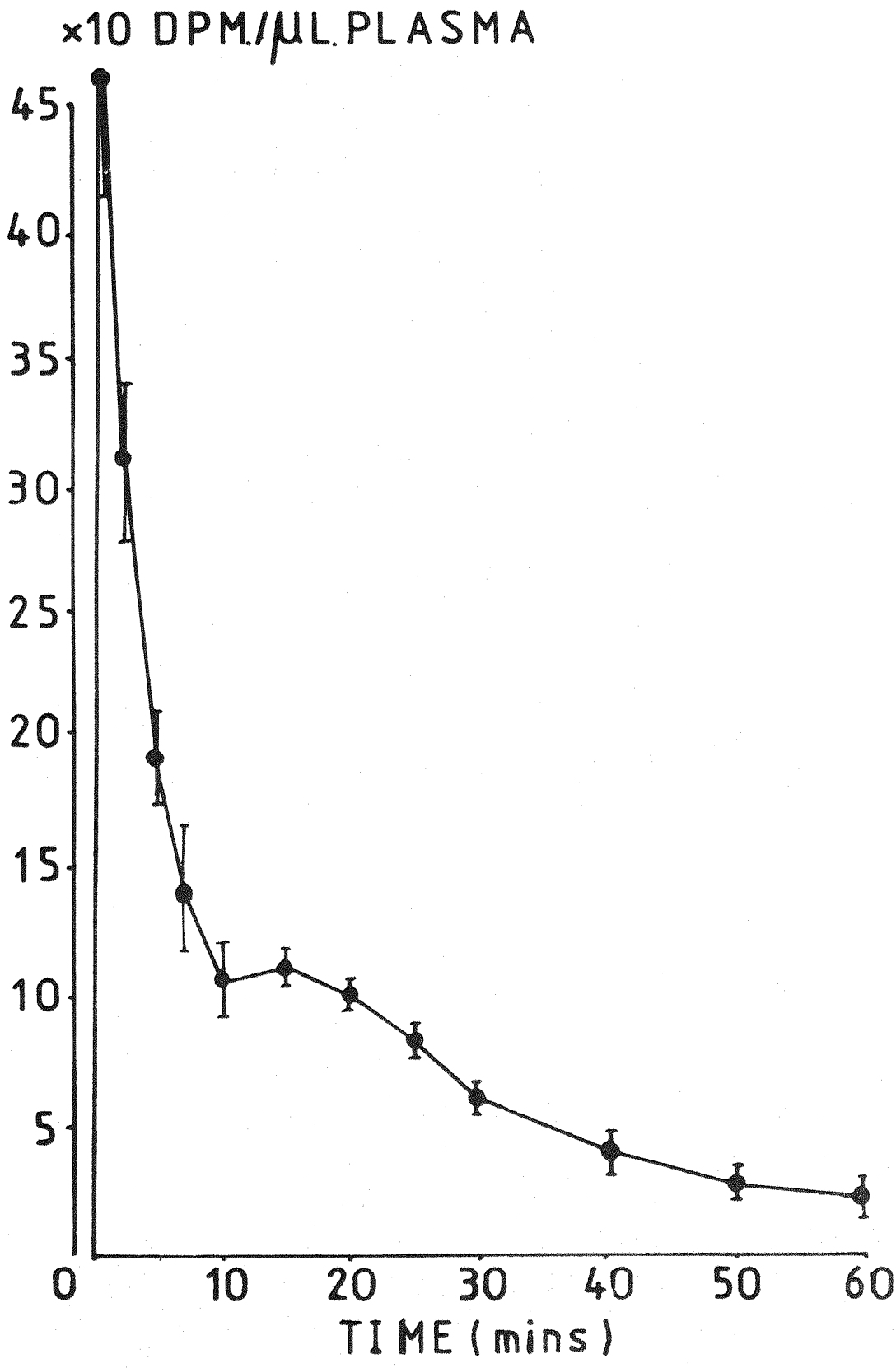
Animals received an injection of 15 μCi (0.15 ml) [$^3$H]-2-deoxyglucose in physiological saline 30 minutes before sacrifice. After 30 minutes, plasma levels of [$^3$H] are negligible (Fig. 16). The animals were decapitated and the brains rapidly removed and dissected on a glass plate. Discrete brain areas were dissected free, blotted dry and weighed. They were then solubilised in 'protosolve' (14% sodium hydroxide in methanol) at 60°C for two hours after which time radioactivity was counted by conventional means.

### II.2.8. Oxygen uptake by hand cut cerebral cortex slices

Oxygen uptake was measured in hand cut slices of fronto-parietal cortex (Krieg, 1946) by methods essentially similar to those reviewed by Bradford (1973).

Slices of cortex (12.64 ± 0.44 mgs. dry weight per flask; mean ± S.E., N = 100) were placed in 3 mls Krebs Ringer phosphate buffer (NaCl, 141 mM; KCl, 4.2 mM; $CaCl_2$, 3.0 mM; $MgSO_4$, 1.41 mM; Sodium phosphate buffer, 18.34 mM, pH 7.4, gassed in 95% $O_2$, 5% $CO_2$) in Warburg Manometer flasks. These single side-arm flasks contained 3 mls KRP in the outer well, 0.2 mls 10% KOH within the centre well and 0.2 mls 200 mM succinate in the side arm. Flasks were placed in a Gilson GR14 differential constant pressure respirometer, and preincubated for 10 mins at 25.0°C, shaking at 80 cpm (amplitude 40 mm). After preincubation the contents of the side arm were added, to give a

Figure 16. Time course for [$^3$H] 2-deoxyglucose in conscious rats (200 - 250 g, ♂). 10 μCi [$^3$H] 2-deoxyglucose was injected via an indwelling right internal jugular vein cannula. Blood is sampled via left indwelling carotid artery (100 μl samples). Results are means ± S.E. of three independent determinations.


×10 DPM./µL. PLASMA
45
40
35
30
25
20
15
10
5
0
10
20
30
40
50
60
TIME (mins)

final concentration of succinate of 12.5 mM, and the flasks incubated for a further 5 mins.

Following this pre-incubation period of 15 minutes, the external valves of the respirometer were closed, and the rate of oxygen uptake measured at 5 minute intervals for a further 60 minutes. The slices were removed from the flasks, blotted and dried overnight in an oven, their dry weights being determined.

II.2.9. Histological procedures

a) Kluver Barrera stain: Animals were perfused transcardially with Heidenhain's 'Susa' solution. The brains were removed, fixed, and wax embedded. 10-20 μm sections were stained by Kluver Barrera stain (luxol fast blue and cresyl fast violet). The extent of the lesion being assessed as the area of distinct degeneration of neuronal perikarya with surrounding glial cell proliferation. For the demonstration of the site of delivery of the neurotoxic amino acids, animals had 0.1 μl of pontamine sky blue stereotaxically injected in an identical manner to that for the amino acids. The animals were anaesthetised, perfused transcardially with neutral buffered formyl saline (pH 7.4), and the brains removed and sectioned on a freezing sledge microtome. Sections (20-40 μm) were stained with Neutral Red according to the method of Schmued et al. (1982).

b) Acetylcholinesterase Stain: The pharmacological procedure of Butcher (1975) was used to visualise acetylcholinesterase positive neuronal perikarya. Rats were pretreated with di-isopropyl phosphorofluoridate (DFP, Aldrich: 1.5 mg/Kg. i.m. in arachis oil) six hours before sacrifice by transcaridal perfusion (Mesulam, 1982). The brains were sectioned and stained for acetylcholinesterase by the method of Karnovsky and Roots (1964; El Badawi and Schenk, 1967). Ethopropazine (0.1 mM) was included in the medium to inhibit pseudocholinesterases (EC.3.1.1.8).

c) Histofluorescence of catecholamine containing neurones: The introduction of the formaldehyde histofluorescence method by Falck and Hillarp twenty years

ago (Falck et al., 1962; Falck, 1962) made possible, for the first time, microscopical studies on monoamine neurotransmitters in their intraneuronal storage sties. In addition to high sensitivity in the detection of mono-amine-containing cell systems, the method has a high specificity. Catecholamines first undergo a cyclisation reaction yielding low fluorescent tetrahydroisoquinolines, which are then transformed to strongly fluorescent dihydroisoquinolines (Björklund et al., 1975). Since the original discovery of Falck and Hillarp continuous methodological development has been carried out to improve the sensitivity of the histofluorescence method and to make it more versatile. Currently the most sensitive of these techniques is the aluminium formaldehyde (ALFA) procedure (Loren et al., 1980; Lindvall et al., 1981).

Under sodium pentabarbitone anaesthesia, animals were perfused transcardially with 250 ml of 2% glyoxylic acid, 0.5% paraformaldehyde, 100% aluminium sulphate in Tyrodes buffer (pH 5.0) at 120 mm Hg pressure. Following decapitation the brains were plunged into isopentane cooled in liquid nitrogen, and left immersed for several minutes. The tissues were then removed to the cold stage (-80°C) of an Edwards-Pearse tissue dryer and freeze-dried for three days, after which time they were exposed to paraformaldehyde vapour at 80°C for one hour and processed for histofluorescence examination according to the methods of Loren et al. (Loren et al., 1976).

# CHAPTER III

# RESULTS

III. 1. Kainic Acid Injections into the Corpus Striatum

The efficacy of the kainic acid (Lot 69C-0010; Sigma Chem. Corp.) was determined by direct unilateral stereotaxic injection into the corpus striatum. Neurochemical marker enzymes for intrinsic striatal neurones, L-glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT), were significantly reduced when determined four days post-lesion (see Table 2 and legend for details). The results of this pilot study therefore established both the neurotoxicity of the kainic acid (a factor of considerable variability, Moore et al., 1982; and see Sanberg et al., 1981) and the sensitivity of the assays for both CAT and GAD.

III. 2. Amino Acid Injections into the Ventral Globus Pallidus

Taken in combination, the original neurochemical findings of Kelly and Moore (1976) and the more recent histochemical investigations of Lehmann et al. (1979, 1980) suggest the existence of a monosynaptic cholinergic pathway originating in the ventral globus pallidus and terminating diffusely within the neocortex. We confirmed these findings of Kelly and Moore, by placing bilateral electrolytic lesions within the globus pallidus ( METHODS PAGE 14 for details) and assaying for choline acetyltransferase activity in the neocortex, and hippocampus after 28 days (Table 3). These results confirm the presence of cholinergic fibres innervating the cortex but not the hippocampus, within the globus pallidus. They do not, however, give any indications as to the origins of this pathway. The suggestion that the cholinergic cell bodies of this pathway, reside within the ventral boundaries of the globus pallidus (Lehmann et al., 1979, 1980), was investigated by direct injection of kainic acid into this area. The injection of 0.5 μg of kainic acid was found to produce a small decrease in CAT activity primarily within the medial cortex, with a smaller decrease in frontal cortical areas (Table 4).

Histological verification of the lesion site (cresyl fast violet/ luxol fast blue) revealed the need for some adjustment of the stereotaxic co-ordinates. We therefore decided to investigate the relative

| CORPUS STRIATUM | | |
|---|---|---|
| CHOLINE ACETYLTRANSFERASE (nmols/mg tissue/hour) | | % |
| IPSILATERAL | CONTRALATERAL | |
| 10.41 ± 0.26*** | 22.54 ± 0.10 | 54 |
| L-GLUTAMATE DECARBOXYLASE (nmols/mg tissue/hour) | | |
| IPSILATERAL | CONTRALATERAL | |
| 17.89 ± 0.34*** | 29.78 ± 1.07 | 40 |

Table 2. Choline acetyltransferase and L-glutamate decarboxylase activities assayed in the corpus striatum of rats unilaterally lesioned 2 days previously with 1 μg/μl kainic acid (AP 7.9; ML 2.6; DV +0.5. From Konig and Klippel, 1963). Results are mean ± S.E. of triplicate determinations from 5 animals.
Analysis was by Student's unpaired t-test, *** $p < 0.001$.

| CORTICAL AREA | LESIONED | CONTROL |
|---|---|---|
| Hippocampus | 4.69 ± 0.19 | 4.89 ± 0.34 |
| Frontal cortex | 2.89 ± 0.32** | 4.86 ± 0.31 |
| Medial cortex | 2.32 ± 0.17** | 4.65 ± 0.23 |
| Posterior cortex | 4.65 ± 0.04 | 4.48 ± 0.37 |

Table 3. Choline acetyltransferase activity within various areas of allocortex following bilateral lesions of the globus pallidus. Results are means ± S.E. of triplicate determinations within 6 animals (controls) and 4 animals (bilaterally lesioned). Analysis was by Student's unpaired t-test, ** $p < 0.01$. CAT activity within the frontal and medial cortex if 59% and 49% of control levels respectively.

| CHOLINE ACETYLTRANSFERASE (nmols/mg tissue/hour) | | | % |
|---|---|---|---|
| | CONTRALATERAL | IPSILATERAL | |
| FRONTAL CORTEX | 4.80 ± 0.26 | 4.43 ± 0.26 | 8 |
| MEDIAL CORTEX | 4.56 ± 0.39 | 3.49 ± 0.16 | 24 |
| POSTERIOR CORTEX | 4.49 ± 0.24 | 4.41 ± 0.24 | 2 |

KAINIC ACID (2.5 nmols.(0.5μg)/μl) LESION AT AP 5.90; ML2.90; DV -1.80.

Table 4. Regional neocortical choline acetyltransferase activity following unilateral kainic acid lesions of the ventral globus pallidus, cortical dissection according to Kelly and Moore (1978); lesion coordinates AP 5.90; ML 2.90; DV -1.80 (Konig and Klippel, 1963) with 2.5 nmols (0.5 μg) kainic acid in 1 μl. Results are mean and S.E. of triplicate determinations from 5 animals.

sensitivity of these cholinergic cells to a variety of neurotoxic amino acids with differing properties. Kainic acid (1.0 μg), ibotenic acid (20 μg), and N-methyl-DL-aspartic acid (NMDLA; 60 μg) were injected into the ventral globus pallidus (modified co-ordinates) in a volume of 1 μl, and the enzymes CAT and GAD assayed in a homogenate of the modified middle cortex dissection (c.f. methods) 8 - 10 days later.

All three amino acids produced a significant decrease in CAT within the parietal cortex (Fig. 17) whilst having no effect on the activity of GAD (see Fig. 18), a marker for intrinsic GABA-containing neurones (Emson and Lindvall, 1979). The neurotoxicity of these three amino acids for cholinergic neurones within the globus pallidus is in general agreement with their potency in the striatum (Schwarcz et al., 1978) but not in the medial septum (Malthe-Sørenssen et al., 1980). Thus kainic acid $\gg$ ibotenic acid > NMDLA in terms of neurotoxicity to cholinergic cells in the ventral globus pallidus.

On initial inspection there appears to be a correlation between the extent of the reduction in CAT, with the cortical levels of GAD. However when the decrease in cortical cholinergic markers (CAT) is compared with either the absolute GAD activity (ipsilateral and contralateral cortex), or the change in GAD activity (ipsilateral versus contralateral side) there is no significant correlation between changes in cortical GABAergic neurones and loss of ascending cholinergic fibres (see Figs. 19 and 20). The histological verification (cresyl violet/luxol fast blue histology from blocks fixed in Heidenhain's susa solution) of the lesion sites within the 18 rats used in the neurochemical studies is presented in Figures 21, 22 and 23.

Having established the neurotoxic potency of ibotenic acid, relative to NMDLA and kainic acid, within this population of cells, further studies concentrated on the neurochemical and neuroanatomical specificity of ibotenic acid lesions, in preference to kainic acid lesions (for reasons, see discussion). For all further studies investigating changes in neurochemical parameters (neurotransmitter levels, related enzymes and synaptosomal uptake) following lesions of the cholinergic nuclei of the basal forebrain, a survival time of 8 - 10

Figure 17. Choline acetyltransferase activity in parietal cortex 8 - 10 days after unilateral lesions of the ventral globus pallidus with i) 1.0 μg kainic acid, ii) 20 μg ibotenic acid, and iii) 60 μg N-methyl-DL-aspartic acid. (See Appendix II for structures).
Results are means ± S.E. of triplicate determinations within groups of 6 animals.
Analysis by Student's un-paired t-test. *** $p < 0.001$, ** $p < 0.01$.

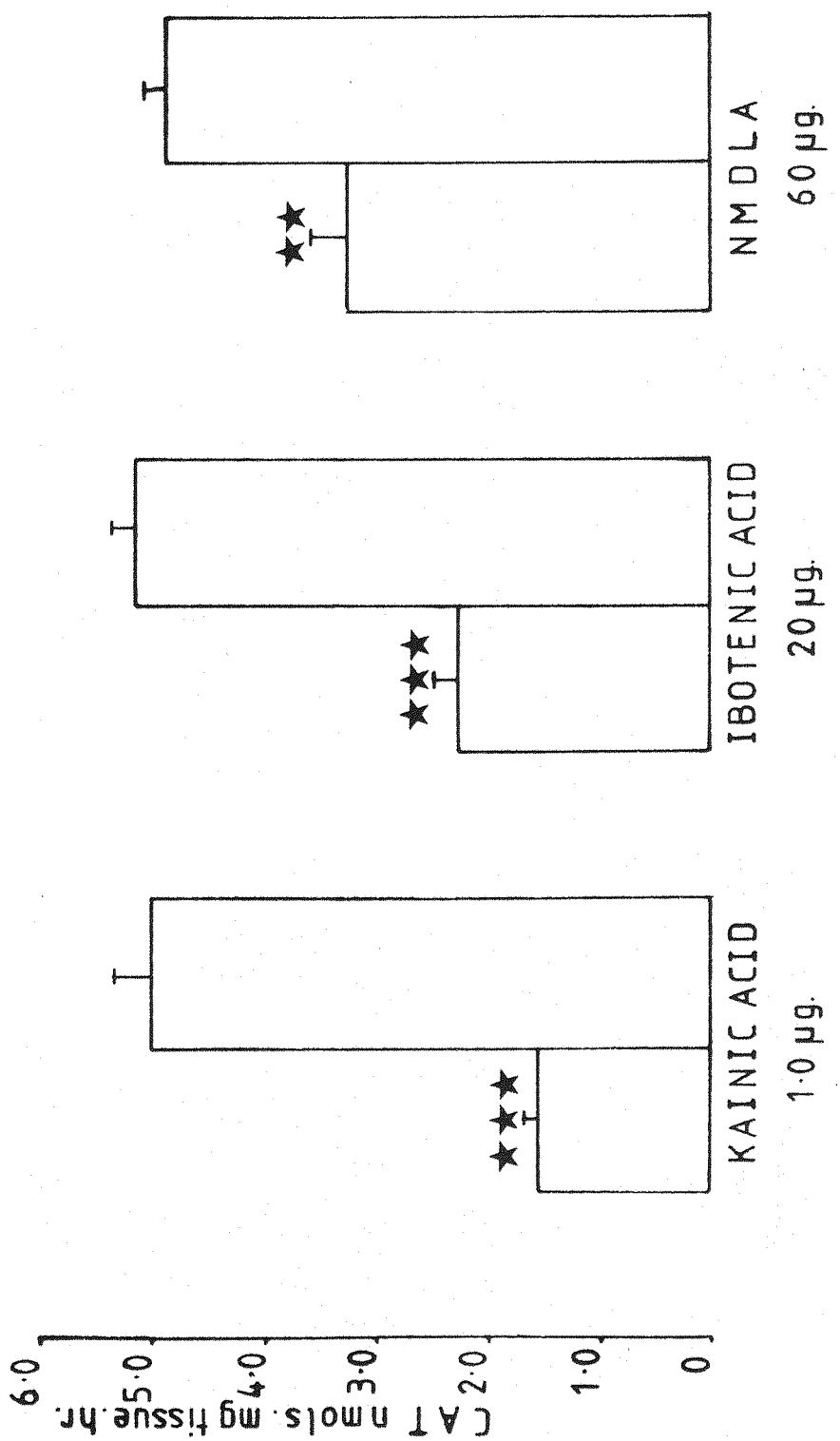
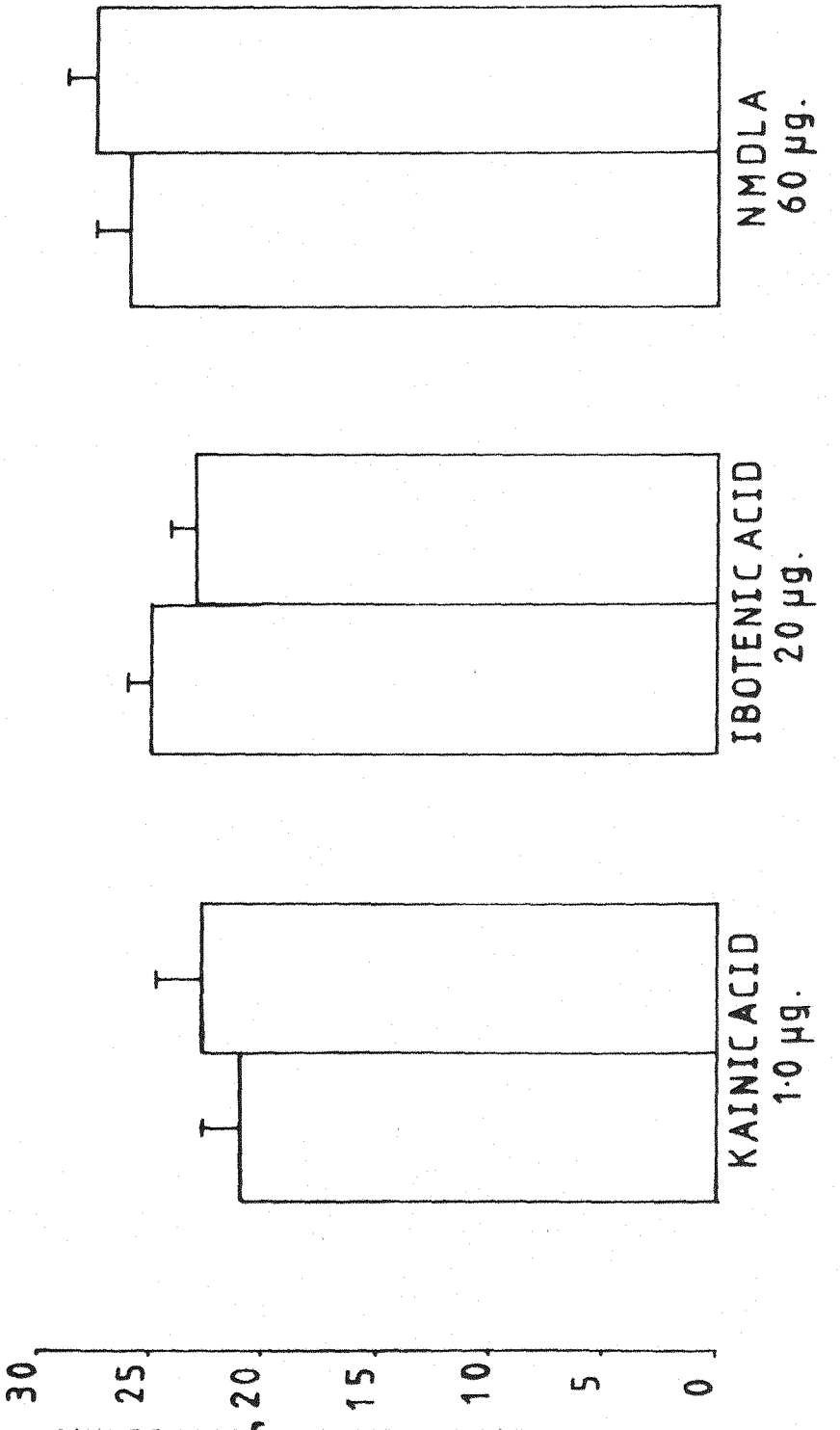

6·0
5·0
4·0
3·0
2·0
1·0
0
C A T n mols. mg tissue. hr.
KAINIC ACID
1·0 µg.
IBOTENIC ACID
20 µg.
NMDLA
60 µg.

Figure 18. L-glutamate decarboxylase activity in parietal cortex 8 - 10 days after unilateral lesions of the ventral globus pallidus with i) kainic acid, ii) ibotenic acid, and iii) N-methyl-DL-aspartic acid. Results are means $\pm$ S.E. of triplicate determinations within groups of 6 animals.


GAD nmols.mg tissue.hr.
30
25
20
15
10
5
0
KAINIC ACID
1·0 µg.
IBOTENIC ACID
20 µg.
NMDLA
60 µg.

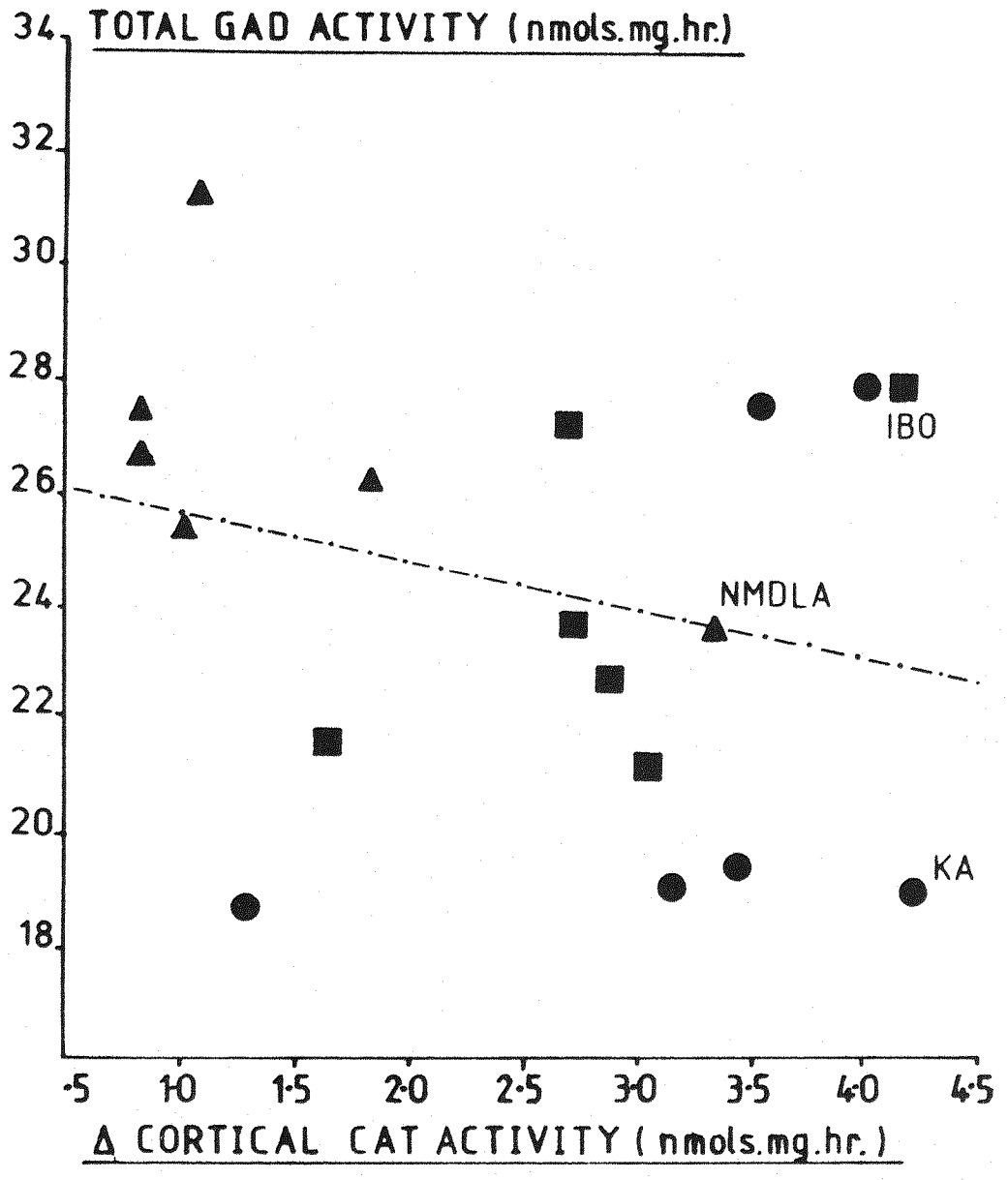



Figure 19. Correlation between total GAD activity (contralateral + ipsilateral, ÷ 2) against decrease in CAT activity (contralateral - ipsilateral) within the parietal cortex of animals with a lesion of the nucleus basalis. Data obtained from figures 17 and 18. Correlation coefficient is -0.293.

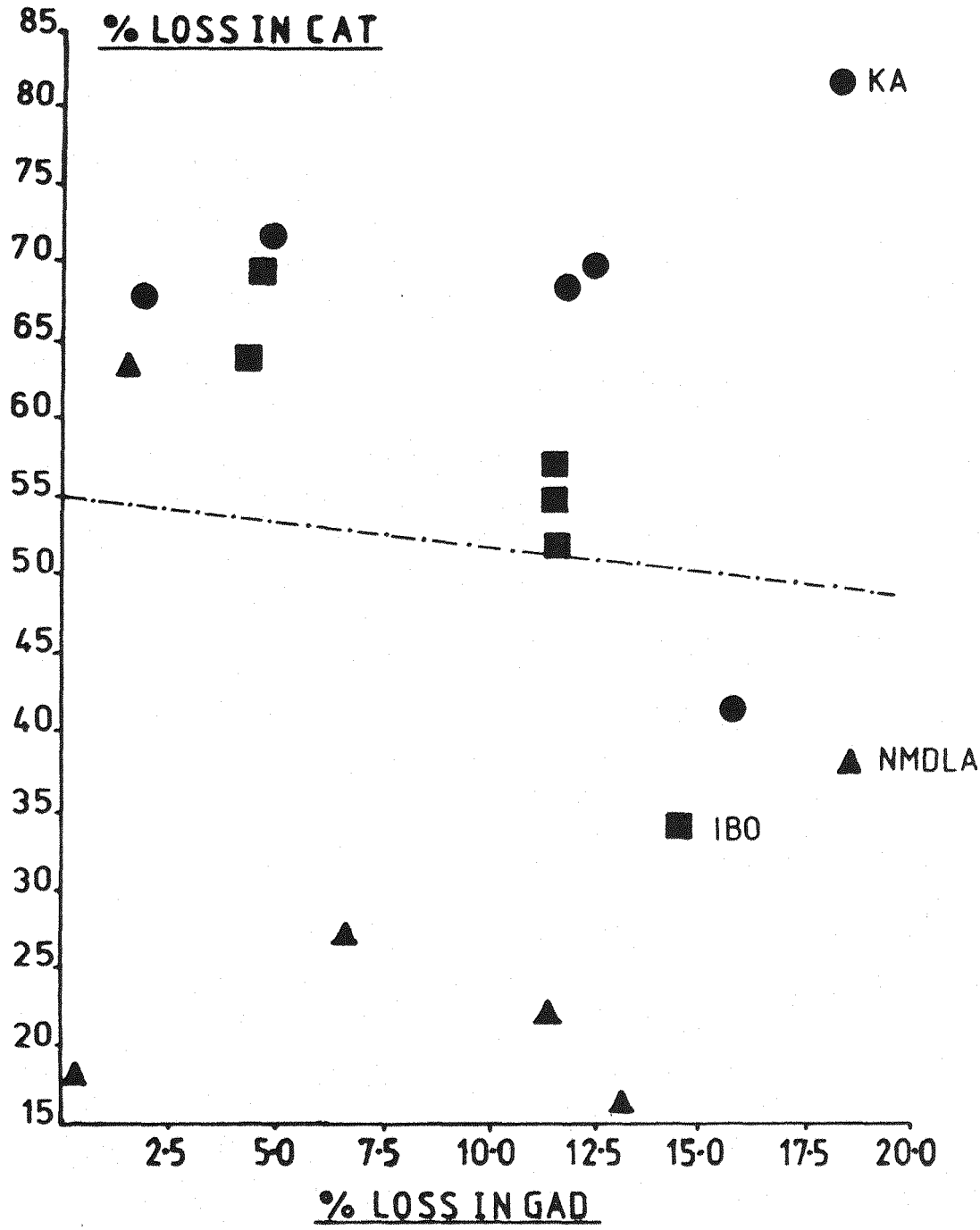



Figure 20. Correlation of % loss in CAT with % loss in GAD within the parietal cortex of animals lesioned within the nucleus basalis. Data obtained from figures 17 and 18. Correlation coefficient is -0.116.

Figures 21, 22 and 23:

Extent of kainic acid (1 μg), ibotenic acid (20 μg) and N-methyl-DL-aspartic acid (60 μg) induced degeneration of the ventral globus pallidus. Shaded areas indicate area of distinct neuronal degeneration with marked glial cell proliferation. Results were determined in cresyl violet/luxol fast blue stained, wax embedded sections, cut from forebrain blocks fixed in Heidenhain's SUSA solution. All groups (n = 6 per group) correspond to the neurochemical results presented in figures 17 - 20 where a 20 - 85% decrease in cortical choline acetyltransferase was demonstrated with no loss of intrinsic cortical GABAergic neurones. Sections correspond to those of König and Klippel (1963).
Scale bar represents 1 mm.

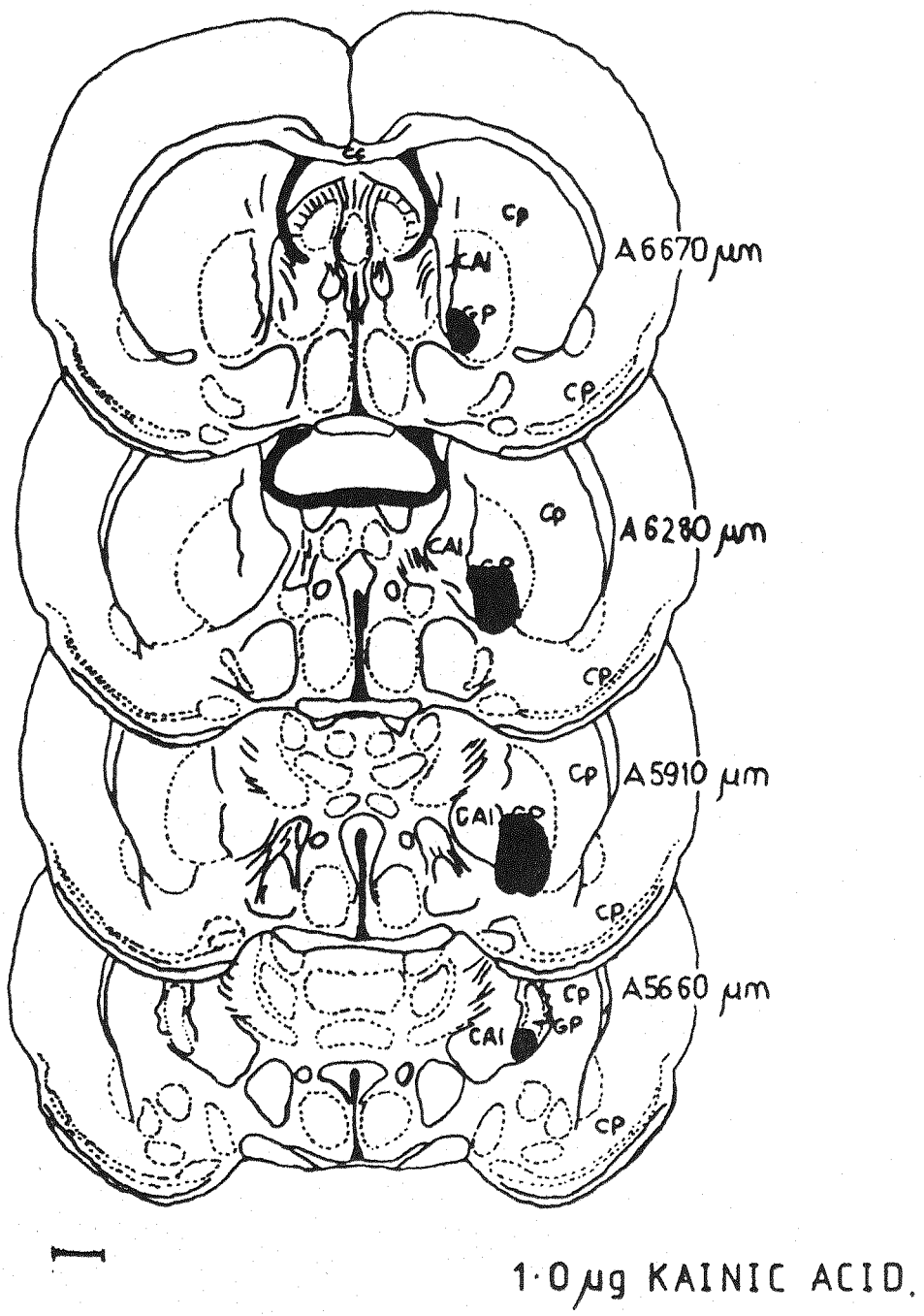

A6670 µm
A6280 µm
A5910 µm
A5660 µm
CP
CN
GP
CAI
1·0 µg KAINIC ACID.

Figure 21.

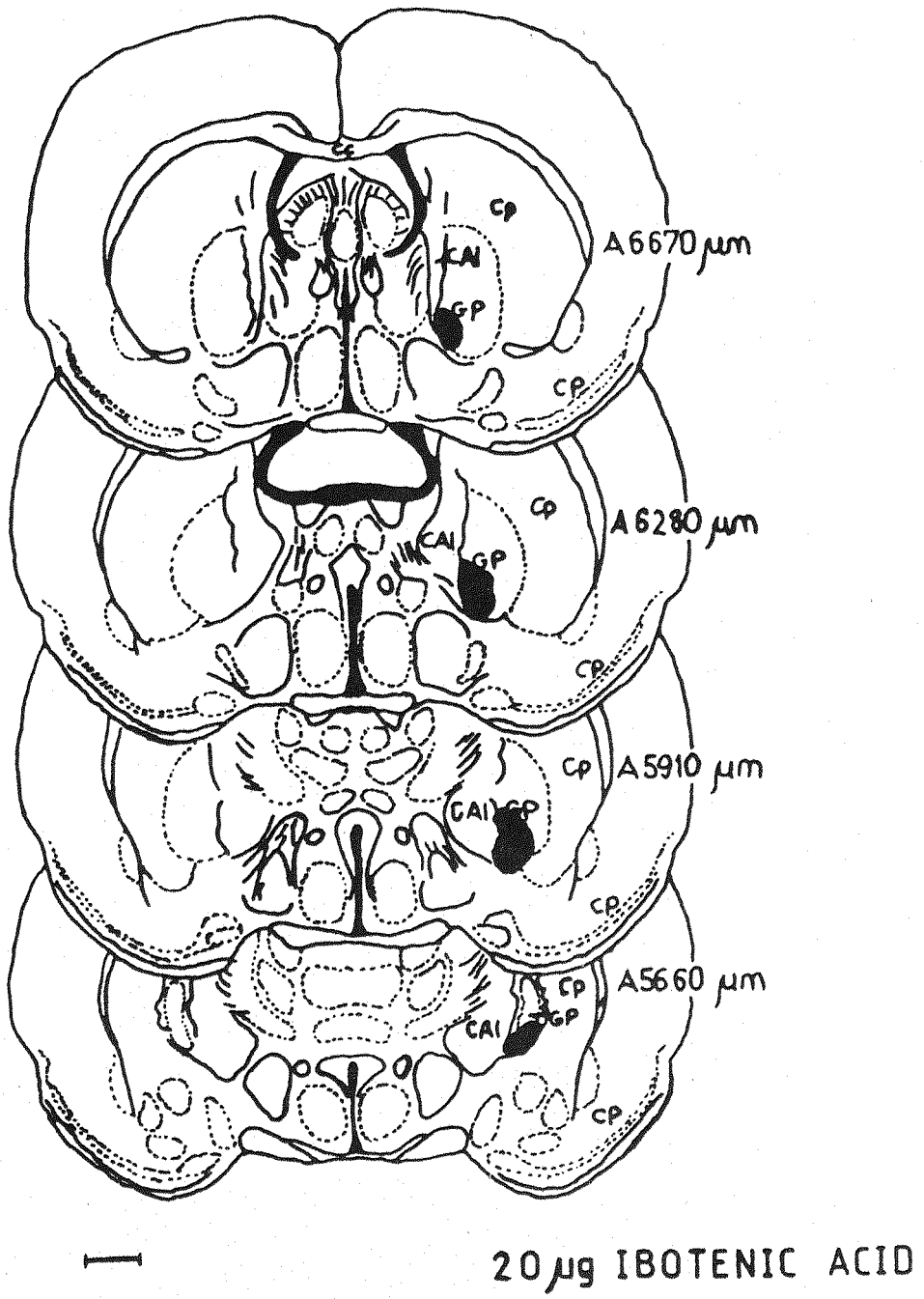

A6670 µm
A6280 µm
A5910 µm
A5660 µm
Cp
CN
GP
CAI
20 µg IBOTENIC ACID

Figure 22.

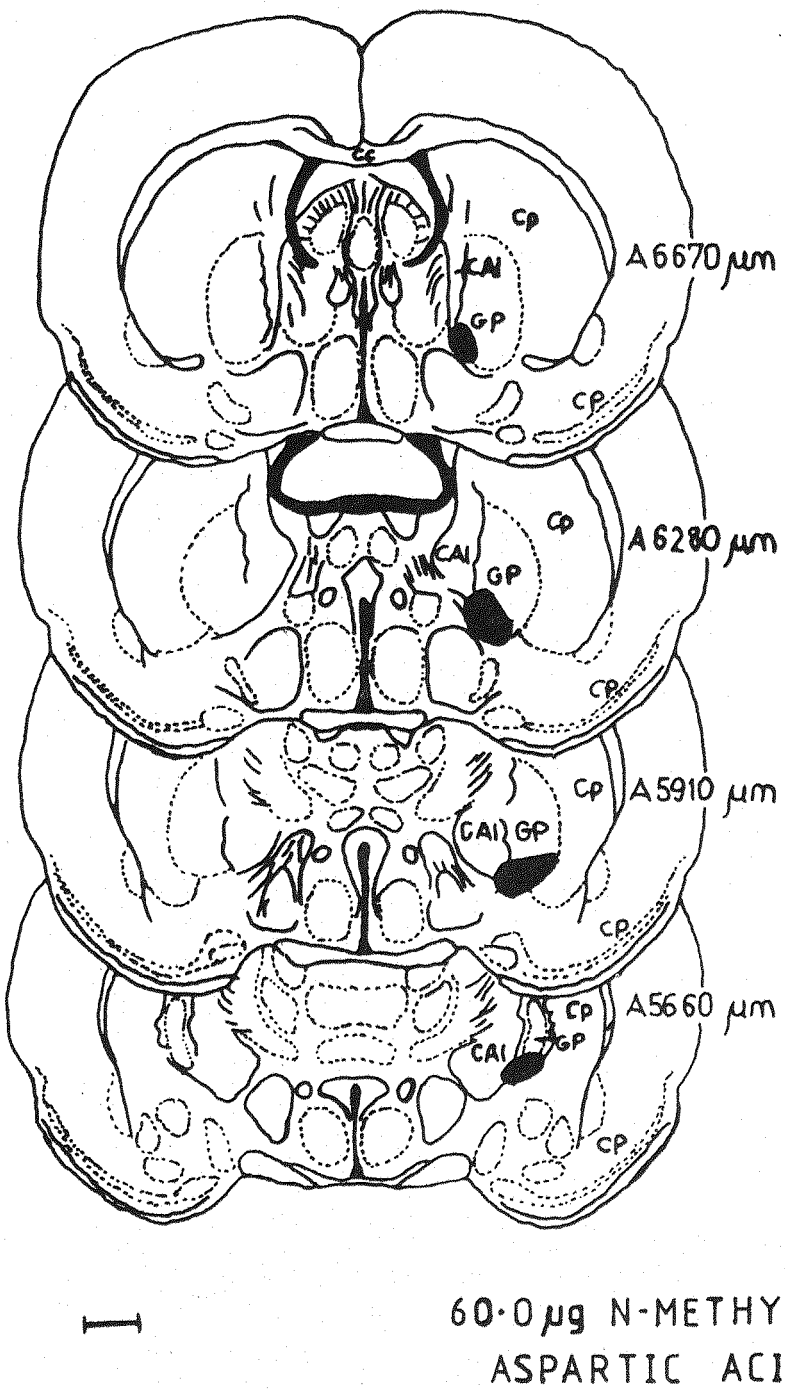

Cc
Cp
CAI
GP
A6670 µm
A6280 µm
A5910 µm
A5660 µm
60·0µg N-METHYL-DL-
ASPARTIC ACID

Figure 23.

days was chosen. The choice of this interval is prompted by:-

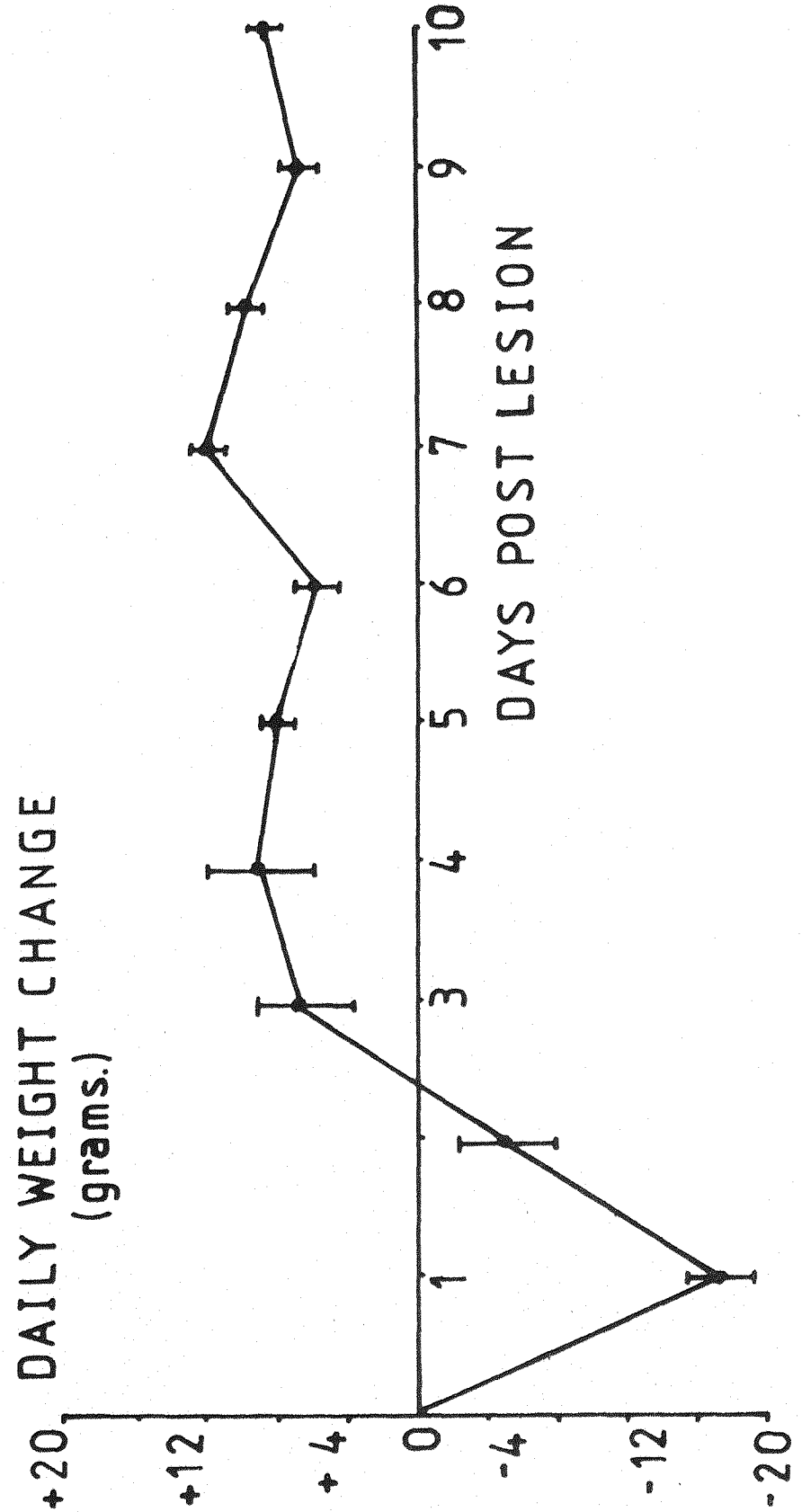
i) The physical state of the animals. Lesions of the globus pallidus have been reported to have profound effects on food intake and forelimb dexterity (Labuszewski et al., 1981) and on appetite (Har ing and Davis, 1983). A recovery time which allowed animals to return to a steady weight increase was therefore chosen (Fig. 24).

ii) The time course for the degeneration of the cholinergic pathway. This was investigated (Table 5) by assaying CAT activity at various time intervals after lesions. The time course was found to be similar to that previously described for dopamine-β-hydroxylase activity after transection of the locus coeruleus - neocortex projection and is compatible with the process of anterograde axonal degeneration (Reis and Ross, 1973). Data presented for neurochemical markers within the neocortex following neurotoxin lesions of the ventral globus pallidus are expressed as 'units activity/mg tissue/unit time'. The justification for this is that these basal forebrain lesions were found not to alter either the wet weight of the parietal cortex slab or the protein content (Table 6). The data from this type of experiment, or from retrograde degeneration studies, are thus not as difficult to quantify as those from areas receiving direct neurotoxin injections e.g. kainic acid lesions of the striatum. The marked oedema and gliosis which take place in areas of brain lesioned with kainic acid can significantly alter wet weight, protein content, and the ratio between these frequently used references (Coyle, 1981). Data presented for such experiments are therefore expressed in both forms.

The specificity of ventral globus pallidus lesions for cortical cholinergic pathways was investigated since there exists some confusion as to the origins of the cholinergic pathways innervating the hippocampus, amygdala and cortex (Burton et al., 1981; Emson et al., 1979).

Ibotenic acid lesions of the ventral globus pallidus were found to produce decreases in CAT both within the parietal cortex and the amygdala (Fig. 25) but not within either the temporal or septal hippocampus. Conversely, ibotenic acid lesions of the medial septum

Figure 24. Changes in body weight following unilateral ibotenic acid (20 μg) lesions of the nucleus basalis. Results are means ± S.E. of 12 animals.


DAILY WEIGHT CHANGE
(grams.)
+20
+12
+4
0
-4
-12
-20
1
3
4
5
6
7
8
9
10
DAYS POST LESION

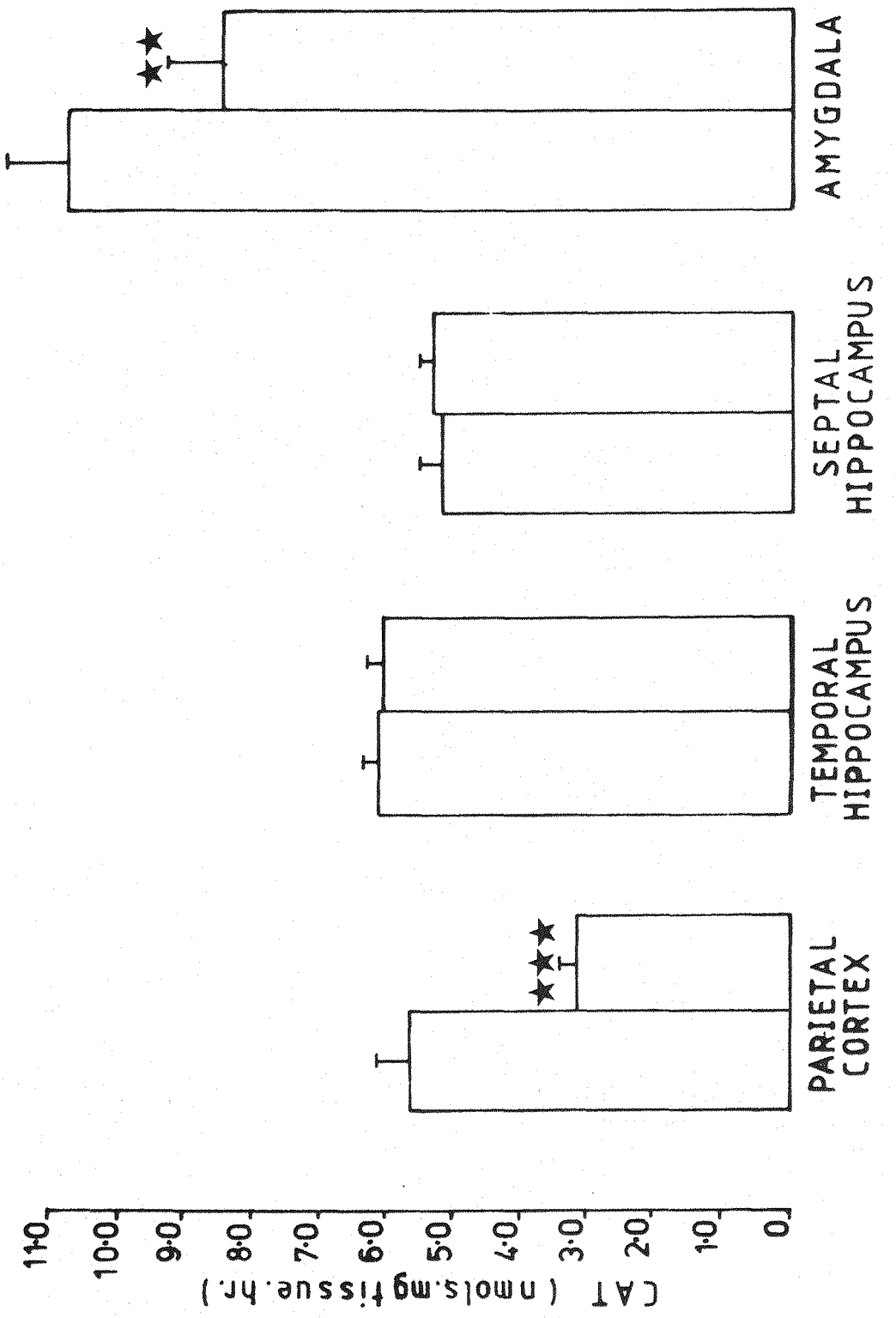
| DAYS POST LESION | CAT nmols. mg tissue. hr. | | % |
|---|---|---|---|
| | LESIONED | CONTRALATERAL | |
| 0 | 5.47 ± 0.23 | 5.36 ± 0.34 | - |
| 1 | 2.78 ± 0.34 | 5.37 ± 0.40 | 48 |
| 5 | 2.61 ± 0.28 | 5.90 ± 0.42 | 56 |
| 10 | 2.26 ± 0.24 | 5.14 ± 0.22 | 56 |
| 20 | 1.75 ± 0.32 | 6.17 ± 0.56 | 72 |

Table 5. Correlation between choline acetyl transferase activity within the ipsilateral and contralateral parietal cortex and time, after unilateral lesion of the ventral globus pallidus (nucleus basalis) with 20 μg ibotenic acid. Results are mean ± S. E. of triplicate determinations in 6 animals.

| FRONTO-PARIETAL CORTEX (Areas 1-4, 6-8 and 40 of Krieg, 1946) | |
|---|---|
| TISSUE WET WEIGHT mgs. | |
| IPSILATERAL | CONTRALATERAL |
| 101.84 ± 2.07 | 104.77 ± 2.52 |
| PROTEIN CONTENT (µg/mg tissue) | |
| IPSILATERAL | CONTRALATERAL |
| 140.07 ± 1.23 | 140.13 ± 0.89 |

Table 6. Effect of unilateral lesions of the ventral globus pallidus (20 µg ibotenic acid) on the wet weight and protein content of the parietal cortex determined 8 - 10 days later. Results are mean ± S.E. from 20 animals. Protein determinations are in triplicate.

Figure 25. Choline acetyltransferase activity within the parietal cortex, hippocampus and amygdala 8 - 10 days following unilateral ibotenic acid (20 μg) lesions of the nucleus basalis. Results are means ± S.E. of triplicate determinations within 6 animals. Analysis is by Student's unpaired t-test, *** $p < 0.001$, ** $p < 0.01$.


ChAT ( nmols.mg tissue.hr.)
11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PARIETAL CORTEX
TEMPORAL HIPPOCAMPUS
SEPTAL HIPPOCAMPUS
AMYGDALA

(including the nucleus of the diagonal band) produced significant decreases in both areas of the hippocampus but not in the cortex or amygdala (Fig. 26). These results clearly differentiate the origins of the cholinergic innervation of these three areas (Davies and Horn, 1982) and are supported by the parallel decreases in acetylcholinesterase in the amygdala and parietal cortex following lesions of the ventral globus pallidus (Fig. 27).

## III. 3. Neurochemical Specificity of Ibotenic Acid Lesions of the Ventral Globus Pallidus

### a) Intrinsic Cortical Neuronal Cells.

Intrinsic cells in the neocortex include neurones that utilise GABA (Emson and Lindvall, 1979) and probably glutamate (Emson and Lindvall, 1979) as neurotransmitters. In order to substantiate the preliminary findings for the stability of GAD activity after ibotenic acid lesions (Fig. 18) and to extend these to glutamatergic neurones, we measured the high affinity uptake of GABA and glutamate as well as endogenous GABA levels in lesioned animals. No significant difference was found for either GABA levels, GAD activity, or GABA uptake between the lesioned and non-lesioned sides (Table 7). Determination of the high affinity uptake of L-glutamate and D-aspartate, however, revealed a significant decrease in the uptake of L-glutamate on the lesioned side. The uptake of D-aspartate, a non-metabolised glutamate analogue transported by the same uptake process, remained unchanged (Fig. 28). We therefore measured the uptake of both D-aspartate and L-glutamate into the same synaptosomal preparation for a group of five lesioned rats (Table 8). In these experiments no differences between lesioned and control sides were found for the uptake of either amino acid (Table 8). Indeed the ratio between aspartate to glutamate uptake was unchanged and of a similar magnitude to that reported by Storm-Mathisen (Storm-Mathisen and Woxen Opsahl, 1978). No reason for the discrepancy between the two sets of results can be found. However, since no histological analysis of the lesion site was performed on these groups of animals it is possible that a more extensive lesion was produced in the former

Figure 26. Choline acetyltransferase activity in parietal cortex, hippocampus and amygdala following unilateral ibotenic acid lesions (20 μg) of the medial septum/vertical limb of the nucleus of the diagonal band (of Broca). Results are means ± S.E. of triplicate determinations in 6 animals. Analysis by Student's t-test, ** $p < 0.01$.

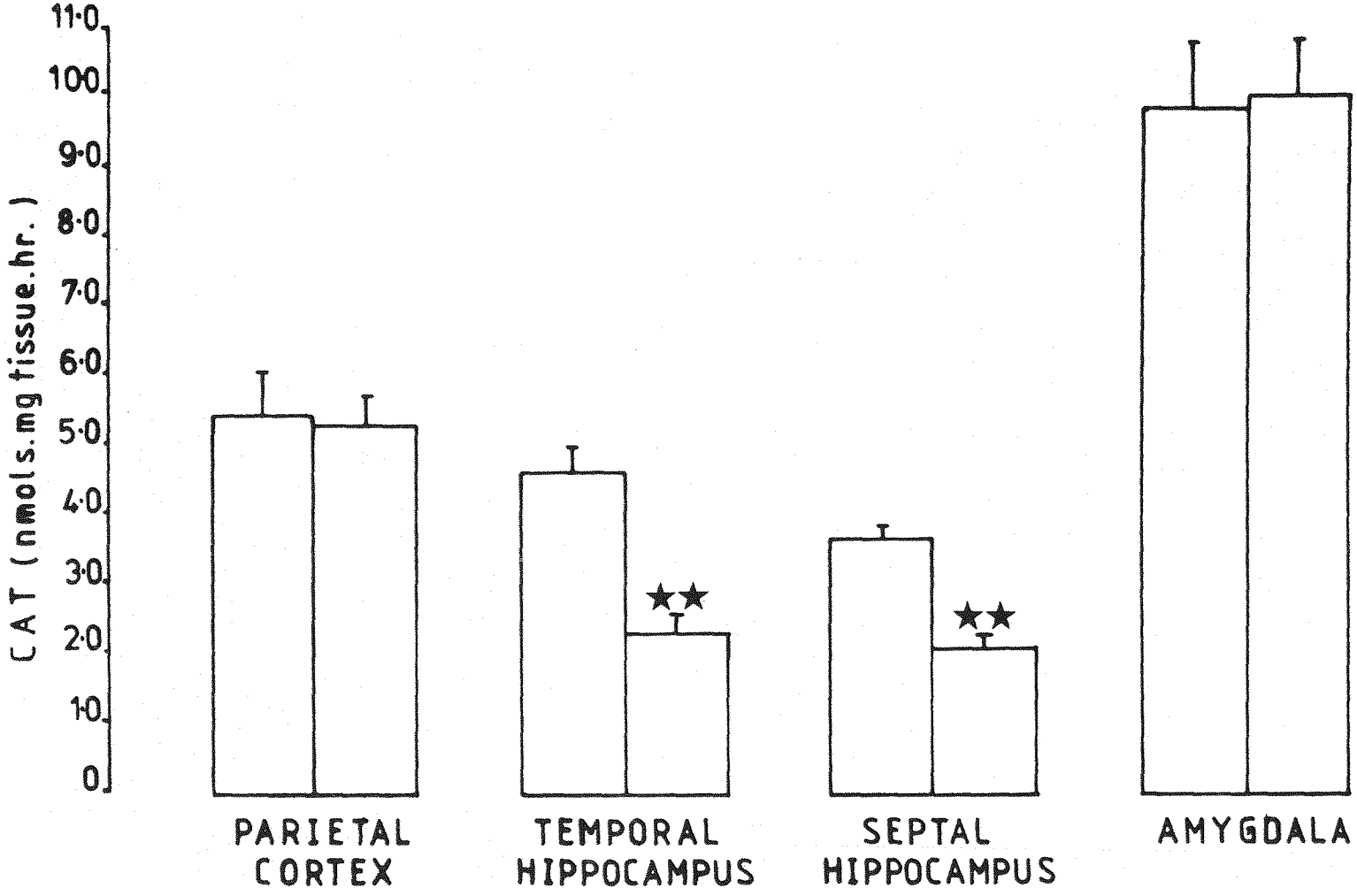
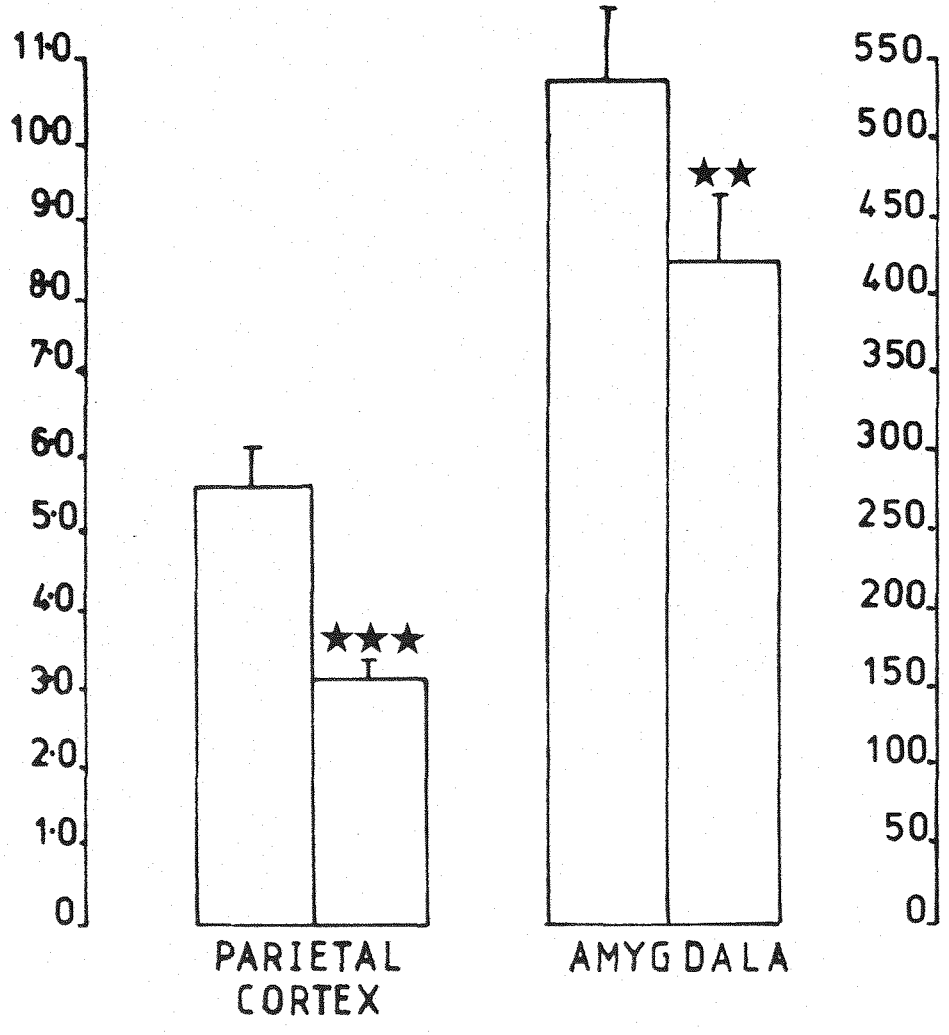
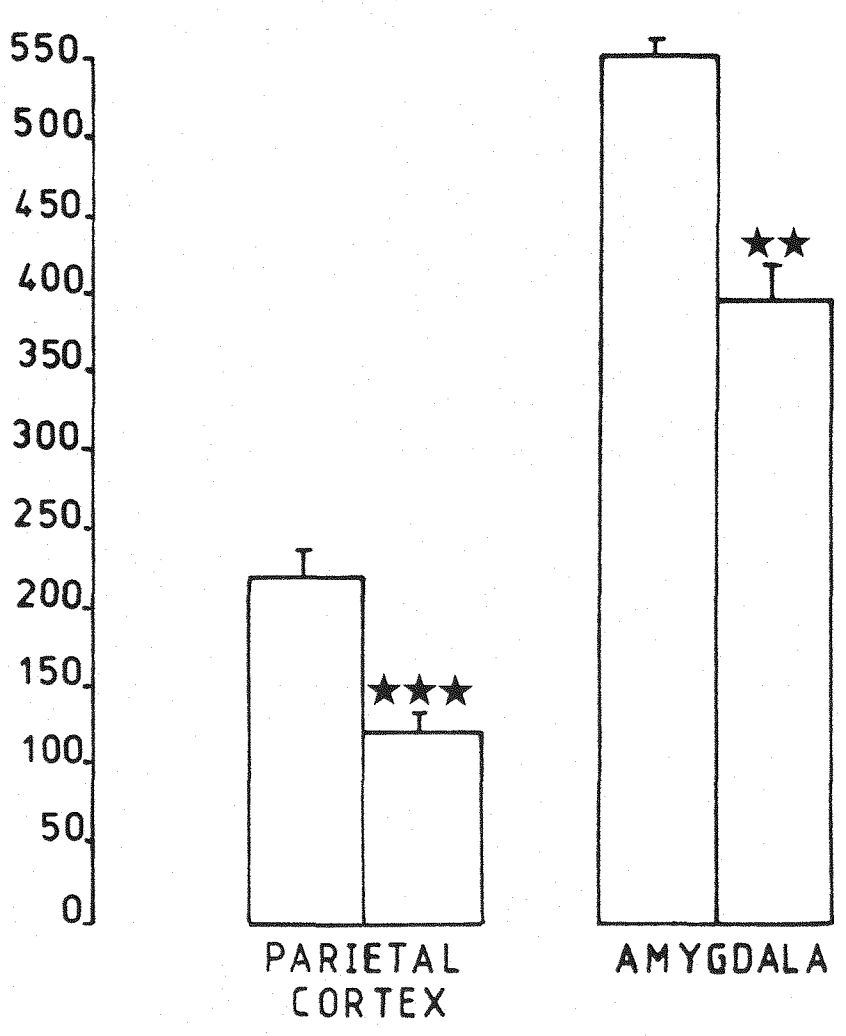

CAT (nmols.mg tissue.hr.)
11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
★★
★★
PARIETAL CORTEX
TEMPORAL HIPPOCAMPUS
SEPTAL HIPPOCAMPUS
AMYGDALA

Figure 27. Choline acetyltransferase and acetylcholinesterase activity in the amygdala and parietal cortex following unilateral ibotenic acid (20 μg) lesions of the nucleus basalis. Results are means ± S.E. of triplicate determinations within 5 animals. Analysis by Student's t-test, *** $p < 0.001$, ** $p < 0.01$.


CHOLINE ACETYLTRANSFERASE
( nmols.mg tissue . hr. )
11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
★★★
★★
PARIETAL CORTEX
AMYGDALA

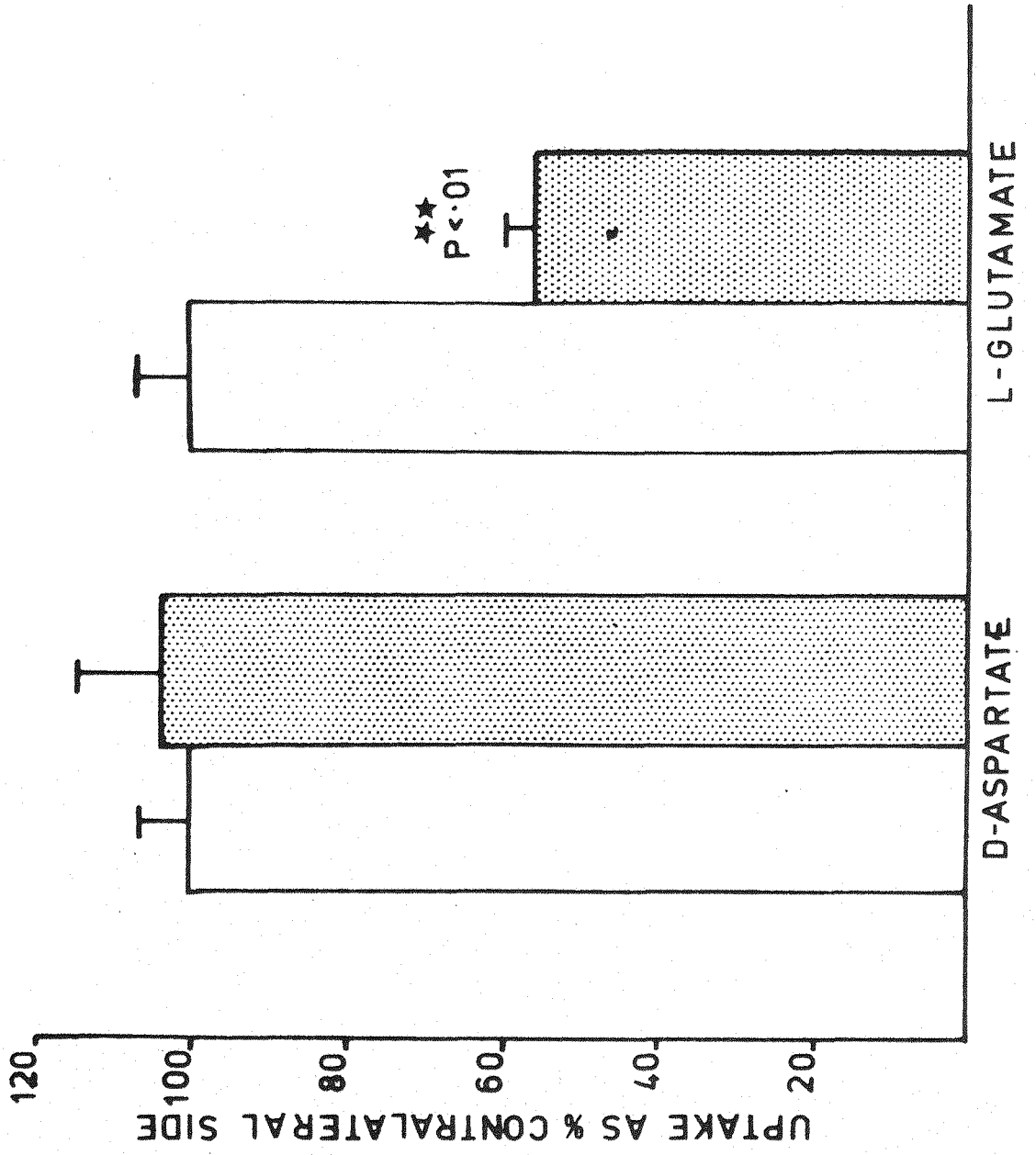

ACETYLCHOLINESTERASE
( nmols.mg tissue . hr. )
550
500
450
400
350
300
250
200
150
100
50
0
★★★
★★
PARIETAL CORTEX
AMYGDALA

| GABAergic Marker | PARIETAL CORTEX | |
|---|---|---|
| | CONTRALATERAL | IPSILATERAL |
| L-Glutamate Decarboxylase nmols. mg tissue. hr | 22.83 ± 1.07 | 24.85 ± 1.06 |
| γ-Aminobutyric acid levels nmols. mg tissue | 5.00 ± 0.38 | 5.35 ± 0.38 |
| γ-Aminobutyric acid uptake pmols. mg tissue. min | 14.99 ± 1.11 | 12.07 ± 0.85 |

Table 7. L-glutamate decarboxylase activity, high affinity GABA uptake and GABA levels within the parietal cortex following ibotenic acid lesions of the nucleus basalis. Results for GAD activity and GABA levels are means ± S.E. of triplicate determinations within 6 animals. [$^3$H] GABA uptake results are means ± S.E. of quadruplicate determinations in 5 animals. Uptake of 1 μM [$^3$H] GABA was determined in the presence of 50 μM β-alanine. Uptake in sham operated animals is 12.58 ± 0.89, contralateral and 12.89 ± 0.62 ipsilateral (n = 5). Uptake at 4°C represented 9.5% of values at 37°C with filter blanks (GF/C) representing 3% of control values.

Figure 28. High affinity uptake of [$^3$H] D-aspartate and [$^3$H] L-glutamate into a synaptosomal preparation of parietal cortex, from animals lesioned within the nucleus basalis with 20 μg ibotenic acid.

Results are means ± S.E. fo quadruplicate determinations within 6 animals. Analysis is by Student's t-test.


UPTAKE AS % CONTRALATERAL SIDE
120
100
80
60
40
20
D-ASPARTATE
L-GLUTAMATE
P<·01

| EXCITATORY AMINO ACID UPTAKE | | | |
|---|---|---|---|
| | CONTROL | LESION | |
| [$^3$H] L-GLUTAMATE (pmols/mg/min) | 26.53 ± 2.89 | 21.17 ± 1.92 | NS |
| [$^3$H] D-ASPARTATE (pmoles/mg/min) | 35.56 ± 1.76 | 30.37 ± 2.76 | NS |
| D-ASPARTATE/ L-GLUTAMATE RATIO | 1.38 ± 0.01 | 1.44 ± 0.07 | NS |

Table 8. L-glutamate and D-aspartate uptake into a washed $P_2$ synaptosomal preparation from parietal cortex, following unilateral ibotenic acid lesions of the ventral globus pallidus (nucleus basalis). Results are means ± S.E. from 5 rats determined in quadruplicate. D-aspartate:L-glutamate ratio is similar to that determined in septum, mamillary body and in hippocampus (CAI oriens plus radiatum) by Storm-Mathisen and Woxen Opsahl (1978).

group. This would, however, then raise the possibility of an ascending glutamatergic cortical innervation.

It is interesting to note that differences between the uptake of glutamate and aspartate after lesions have been reported previously (Biziere and Coyle, 1979).

Based on the findings for GABA-containing neurones (uptake and GAD levels) and for the later experiments on glutamatergic neurones (L-glutamate and D-aspartate uptake) it is apparent that ibotenic acid lesions of the globus pallidus have no direct effect on intrinsic cortical cells.

b) Ascending Cortical Innervation.

The parietal cortex receives a noradrenergic innervation from fibres originating within the cells of the locus coeruleus (Coyle, 1982). These fibres, contained in the median forebrain bundle, pass within close proximity to the ventral globus pallidus (the site of injection of ibotenic acid) (Konig and Klippel, 1963). Neurotoxic amino acid induced lesions are believed to destroy neuronal perikarya whilst leaving axons of passage undamaged (Peterson and Moore, 1980; Coyle et al., 1978) although this has been disputed (Mason and Fibiger, 1979; Meibach et al., 1978; Friedle et al., 1978). We therefore investigated the selectivity of ibotenic acid lesions of the ventral globus pallidus by measuring the uptake and levels of noradrenaline within the parietal cortex. No significant differences between the control and lesioned sides were found for either parameter (Table 9).

These findings are supported by the results of catecholamine histofluorescence within the median forebrain bundle in the vicinity of the globus pallidus lesion. No differences in either the intensity or extent of fluorescence was found on either the lesioned or control sides (Figs. 29, 30, 31).

c) Cholinergic Innervation.

In an attempt to extend our understanding of the cholinergic innervation of the parietal cortex we investigated the effects of 1) ibotenic acid lesions of the parietal cortex and 2) unilateral fronto-parietal cortex ablation.

| NORADRENALINE LEVELS | | |
|---|---|---|
| IPSILATERAL | CONTRALATERAL | |
| 555.64 ± 124.66 | 577.97 ± 144.40 | NS |

| [$^3$H] NORADRENALINE UPTAKE (fmol./mg. tissue/min.) | | |
|---|---|---|
| IPSILATERAL | CONTRALATERAL | |
| 17.20 ± 0.28 | 17.21 ± 0.38 | NS |

Table 9. Noradrenaline levels and noradrenaline uptake within the parietal cortex after lesions of the nucleus basalis. Results are means ± S.E. from 6 animals, triplicate determinations for levels, and 4 animals quadruplicate determinations for uptake. Inclusion of 10 μM imipramine reduced uptake to 15% of control values. Levels of noradrenaline uptake within the control cortex (68.84 ± 1.52 fmol/mg tissue/4 minutes) are in accord with published data for this area (60 ± 6.0, Johnson et al., 1981).

Figures 29 - 31:

Histofluorescence of catecholamine containing fibres within the median forebrain bundle, 8 - 10 days following a unilateral ibotenic acid lesion of the ventral globus pallidus. Figure 29 demonstrates location of sections presented in Figures 30 and 31. Lesion is on the right hand side in all sections. Scale bar represents 1 mm.

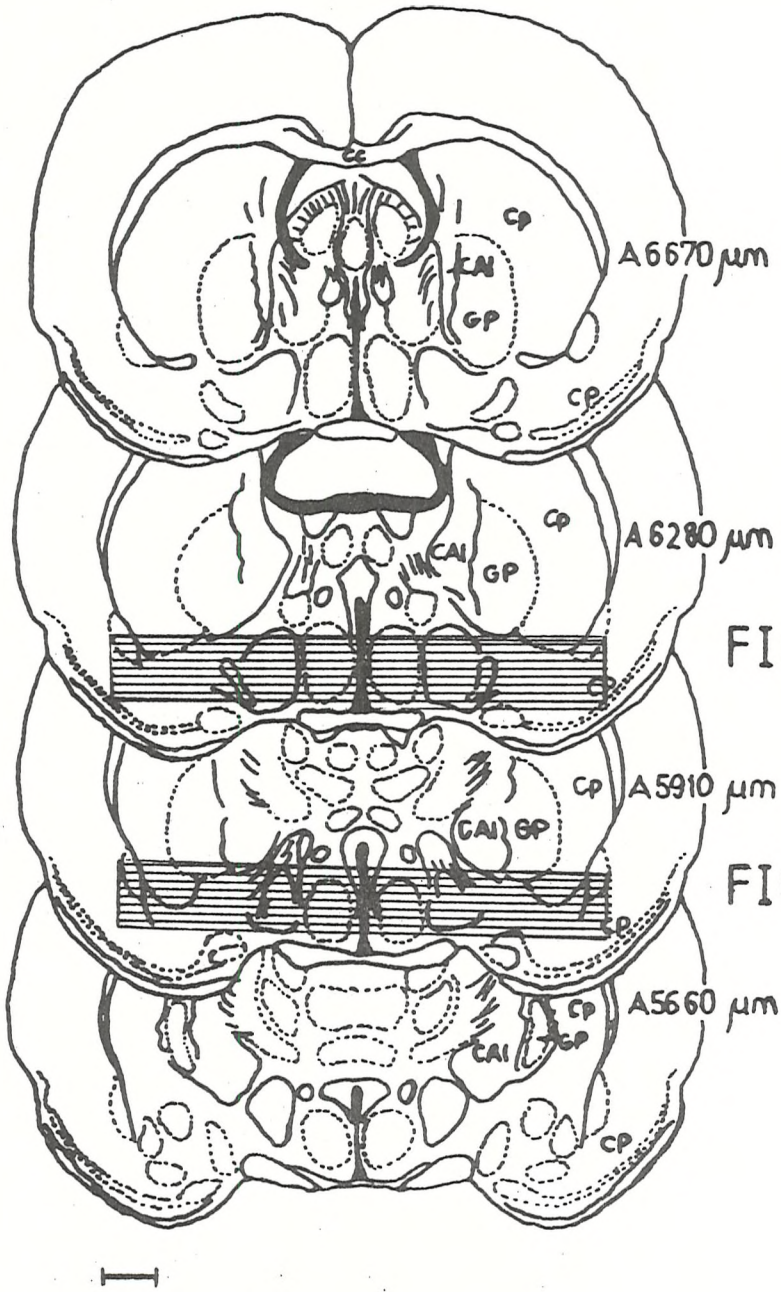
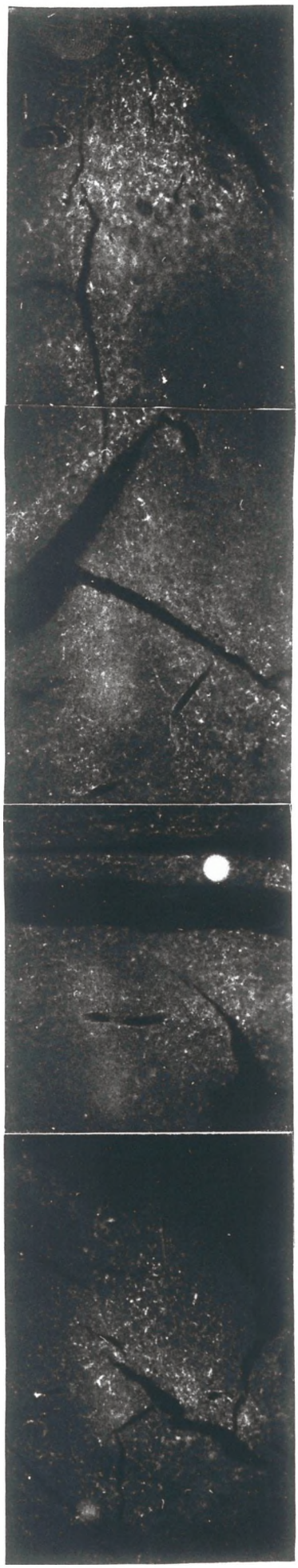

A6670 µm
A6280 µm
A5910 µm
A5660 µm
Cp
CAI
GP
IN
FIGURE 30
FIGURE 31

Figure 29.

200 µM

Figure 30.



200 µM

Figure 31.

Injection of 20 μg of ibotenic acid into the parietal cortex resulted in a significant decrease in GAD activity measured four days later (Table 10). The decrease was significant if GAD activity was expressed as either 'per mg of tissue' or as 'per mg of protein'. A small reduction in CAT activity was found if the results were expressed as 'per mg tissue'; this decrease was not apparent when results were expressed as 'per mg protein'. These findings therefore suggest that any intrinsic cortical cholinergic cells are a very small percentage of the total cortical cholinergic innervation, if indeed they exist at all.

Destruction of cortical cholinergic nerve endings was achieved by aspiration of the fronto-parietal cortex (see Fig. 32). Analysis of GABAergic and cholinergic markers in the striatum and ventral globus pallidus 28 days later reveal possible retrograde degeneration of ascending pathways. Measurement of GAD in both the striatum and ventral globus pallidus (see Fig. 33 for dissection) showed no significant differences between GAD activity on the control and lesioned side within the two structures (Table 11).

CAT activity, however, is decreased both in the striatum and the globus pallidus on the lesioned side (Table 11). The decrease in the striatum is not significant if the results are expressed 'per mg protein'' (156.44 $\pm$ 3.15, control; 141.56 $\pm$ 6.65, lesion), whilst those in the ventral globus pallidus remain significant (60.92 $\pm$ 1.89, control; 38.64 $\pm$ 0.86, lesion). The results for GAD activity remain unchanged for striatum (210.4 $\pm$ 7.34, control; 209.4 $\pm$ 4.16, lesion) and for the ventral globus pallidus (210.0 $\pm$ 12.29, control; 208 $\pm$ 10.57, lesion) when expressed 'per mg protein'.

These experiments therefore corroborate the results from experiments using amino acid lesions of cholinergic perikarya within the ventral globus pallidus in that a direct pallido-cortical cholinergic pathway has been demonstrated neurochemically by means of both retrograde and anterograde degeneration studies.

These findings are further supported by the acetylcholinesterase histochemistry in lesioned animals (Figs. 34 - 40) where a group of magnocellular acetylcholinesterase-positive cells can be seen within the ventro-medial globus pallidus (arrowed) on the control, but not

| | LESION | CONTROL |
|---|---|---|
| CAT (nmols/mg tissue/hour) | 3.92 ± 0.15 | 4.64 ± 0.20 * |
| GAD (nmols/mg tissue/hour) | 8.50 ± 0.21 | 22.96 ± 0.65 *** |
| PROTEIN (μg/mg tissue) | 125.9 | 142.8 |

| | LESION | CONTROL |
|---|---|---|
| CAT (nmols/mg protein/hour) | 31.14 ± 1.19 | 32.49 ± 1.40 NS |
| GAD (nmols/mg protein/hour) | 67.51 ± 1.67 | 160.78 ± 4.55 *** |

Table 10. Choline acetyltransferase and L-glutamate decarboxylase activities in parietal cortex one week after unilateral ibotenic acid lesions of the parietal cortex. The lesion was produced by the injection of 20 μg ibotenic acid in 1 μl sodium phosphate buffered sterile saline (pH 7.4). Results are means ± S.E. of triplicate determinations in six animals, and are expressed either per mg tissue or per mg protein. Analysis is by Student's unpaired t-test, *** $p < 0.001$, * $p < 0.5$.

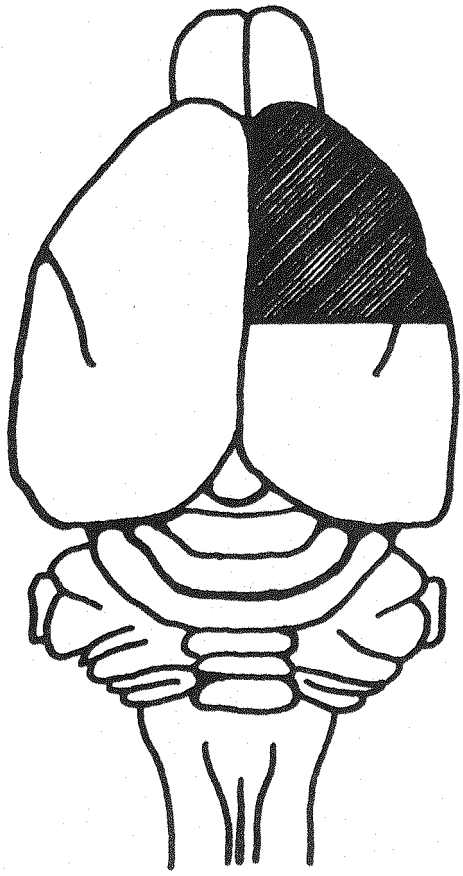


Figure 32. Rats (200 - 250 g Wistar) were anaesthetised with pentobarbitone (60 mg/kg i.p.) and the cortical surface exposed by the use of a hand-held drill. Frontal and part of the parietal cortex tissue from the righthand side of the brain were removed lateral to the sagital, and anterior to the coronal sutures. Cortical grey matter was removed by suction under vacuum to expose the underlying white matter. The wound was packed with 'Sterispon' before closure and the animal left for 28 days before sacrifice. All rats were injected with 'Ampliclox (50 mg i.m.) to prevent infection.

Figure 33. Dissection of ventral globus pallidus/nucleus basalis. Tissue samples (31.83 ± 1.8 mgs, lesioned; 33.24 ± 3.1 mgs, control) were rapidly dissected on an ice cold glass plate with the aid of a Zeiss binocular dissecting microscope. Anterior-posterior co-ordinates refer to the Atlas of Konig and Klippel (1963).

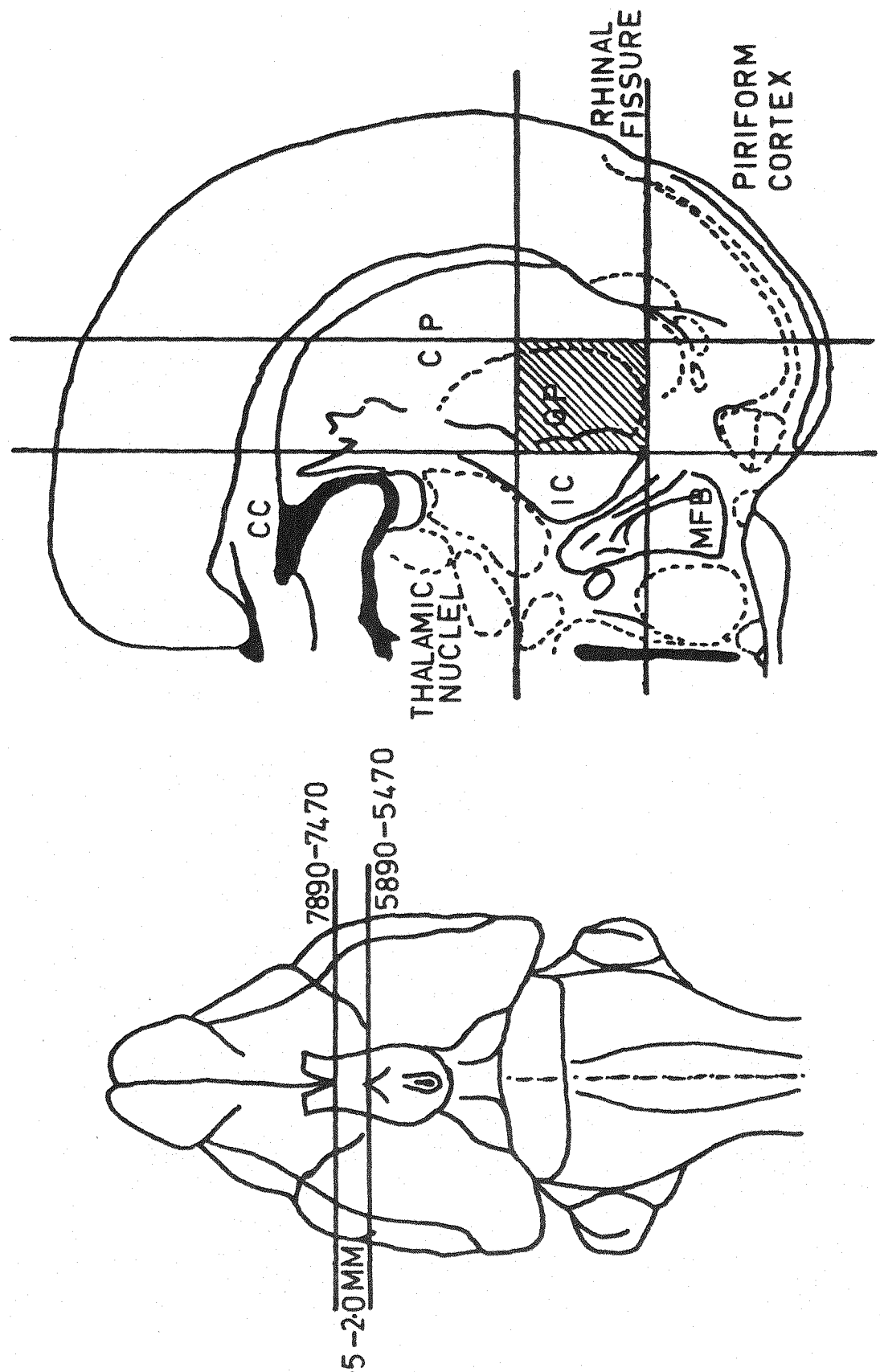

7890-7470
5890-5470
1·5-2·0MM
CC
CP
GP
IC
MFB
THALAMIC NUCLEI
RHINAL FISSURE
PIRIFORM CORTEX

Table 11. Choline acetyltransferase and L-glutamate decarboxylase activities within the ventral globus pallidus and corpus striatum 28 days after unilateral fronto-parietal cortex ablation. Results are means ± S.E. of triplicate determinations from 6 animals. Analysis is by Student's t-test, *** $p < 0.001$, ** $p < 0.01$.

| CORPUS STRIATUM | | VENTRAL GLOBUS PALLIDUS | |
|---|---|---|---|
| L-GLUTAMATE DECARBOXYLASE (nmols/mg tissue/hour) | | | |
| IPSILATERAL | CONTRALATERAL | IPSILATERAL | CONTRALATERAL |
| $27.70 \pm 0.37_{NS}$ | $30.03 \pm 1.04$ | $29.41 \pm 1.72_{NS}$ | $29.81 \pm 1.51$ |
| CHOLINE ACETYLTRANSFERASE (nmols/mg tissue/hour) | | | |
| IPSILATERAL | CONTRALATERAL | IPSILATERAL | CONTRALATERAL |
| $18.72 \pm 0.73_{**}$ | $22.34 \pm 0.45$ | $5.41 \pm 0.12_{***}$ | $8.70 \pm 0.27$ |

the lesioned, side (Figs. 34 - 36). Acetylcholinesterase positive fibres and terminals are also absent from the parietal cortex (Figs. 37 - 39) but not from either the piriform or limbic cortex on the lesioned side (Fig. 37).

A quantitative evaluation of the loss of acetylcholinesterase staining fibres from the various cortical laminae of Area 2 of the parietal cortex (area postcentralis caudalis, Krieg, 1946) was determined by scanning micro-densitometry of 40 μm acetylcholinesterase stained sections, from an ibotenic acid lesioned rat. The results are presented in Figure 40, and show a significant loss of acetylcholinesterase positive staining from laminae II - VI.

III. 4. Metabolic Changes Resulting from Ibotenic Acid Lesions of the Ventral Globus Pallidus

[$^3$H]-2-deoxyglucose uptake studies in four animals revealed decreases in uptake within the medial cortex, amygdala and striatum, and to a lesser extent in the frontal cortex. These areas correspond to those already investigated neurochemically and to where a decrease in innervation has been demonstrated. No decrease in [$^3$H]-2-deoxyglucose uptake into the hippocampus was found. Dissection of the corpus striatum in these studies includes by definition a substantial proportion of the globus pallidus. Decreased [$^3$H]-2-deoxyglucose uptake in this area may therefore reflect neuronal degeneration in the direct area of injection (see Fig. 41). Similar studies performed in animals lesioned with kainic acid (1 μg) or NMDLA (60 μg), showed no differences either quantitatively or qualitatively in the uptake of 2-deoxyglucose (results not shown) within the areas studied.

III. 5. Afferent Connections of the Ventral Globus Pallidus

The ventral globus pallidus, or ventral pallidum of Heimer and Wilson (1975; Heimer et al., 1982) is known to receive an afferent projection from the nucleus accumbens (Williams et al., 1977; Nauta et al., 1978; Troiano and Siegel, 1978). It has been suggested that this projection uses GABA as its transmitter (Walaas and Fonnum,

Figures 34 - 39:

Acetylcholinesterase histochemistry after *in vivo* pretreatment with an irreversible cholinesterase inhibitor, di-isopropylfluorophosphate (DFP).

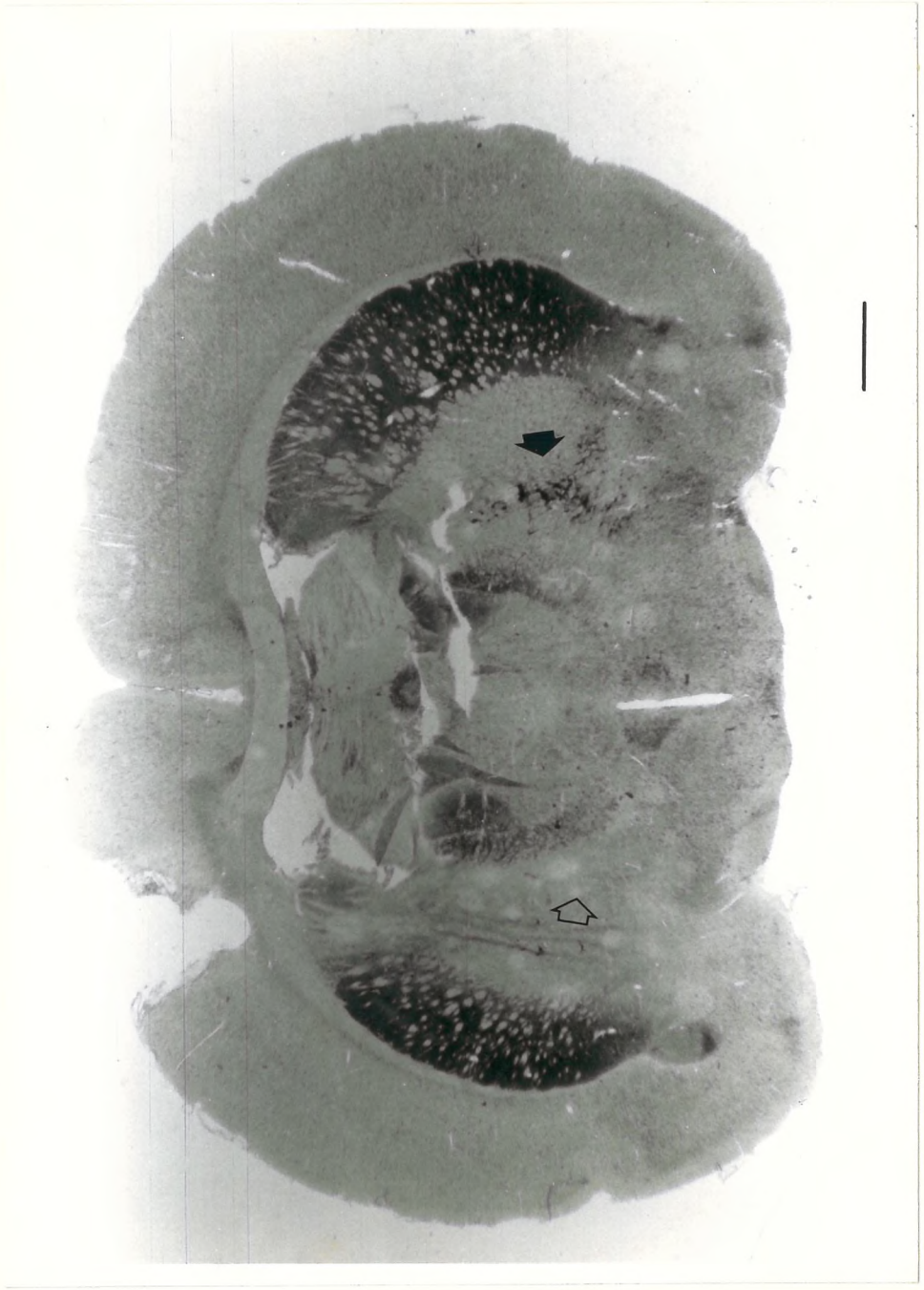


Figure 34. Acetylcholinesterase histochemistry of a rat lesioned in the left globus pallidus with 20 μg ibotenic acid 8 - 10 days previously. The 40 μm section was processed for acetylcholinesterase 6 hours after DFP treatment. Magnocellular cholinesterase-positive perikarya are seen within, and on the boundary of, the ventral globus pallidus and correspond to the nucleus basalis magnocellularis of Lehman et al. (1980). Scale bar represents 1 mm.

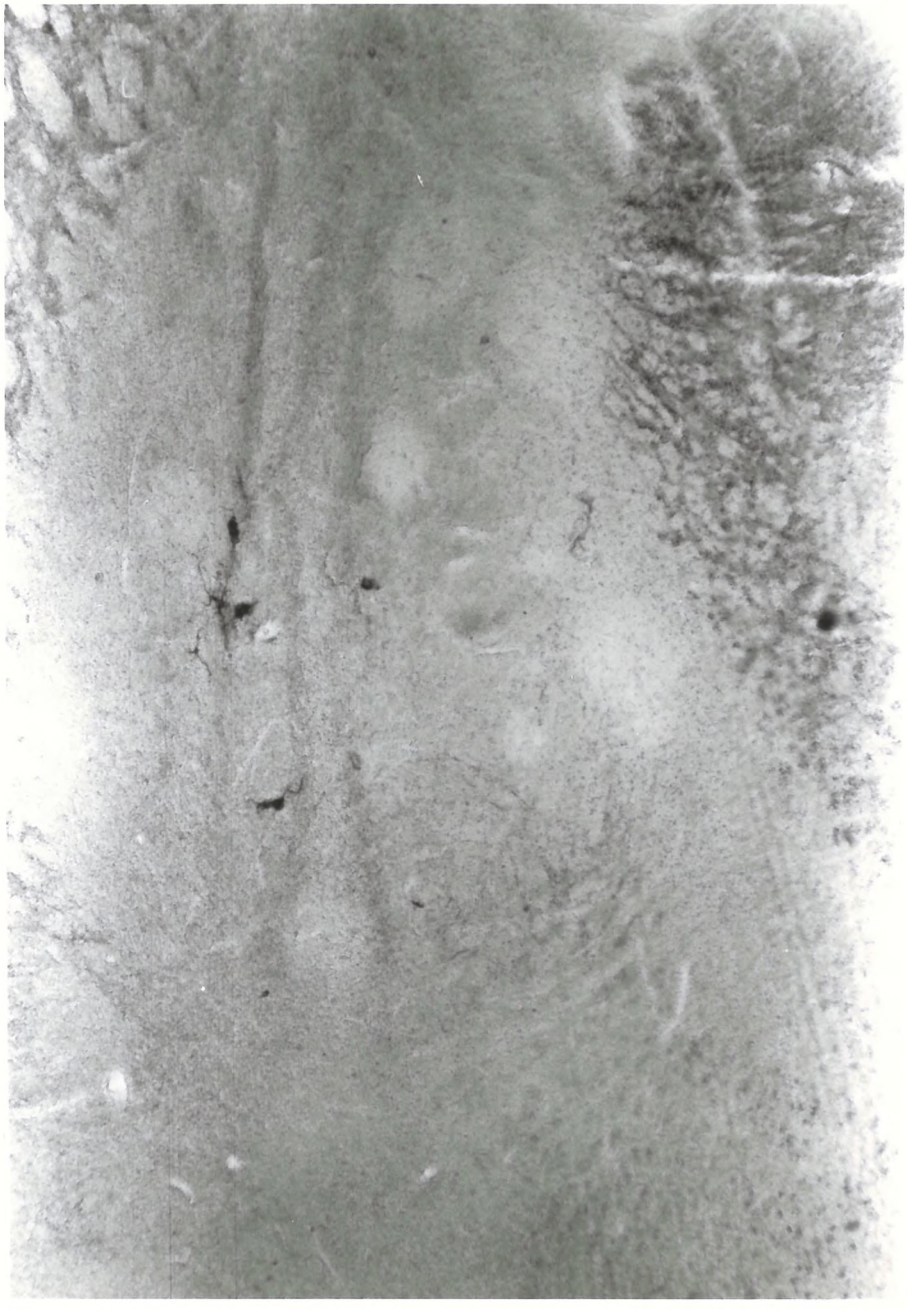


Figure 35. High power magnification of ventral globus pallidus from lesioned side of previous section (Fig. 34). Note almost total absence of any acetylcholinesterase-positive perikarya within the globus pallidus. Scale bar represents 200 μM.

Figure 36. High power magnification of ventral globus pallidus from control side of previous section (Fig. 34). Large (35 - 40 μm), intensely acetylcholinesterase-positive perikarya can be seen on the boundary between the ventral globus pallidus and the internal capsule. Scale bar represents 200 μM.

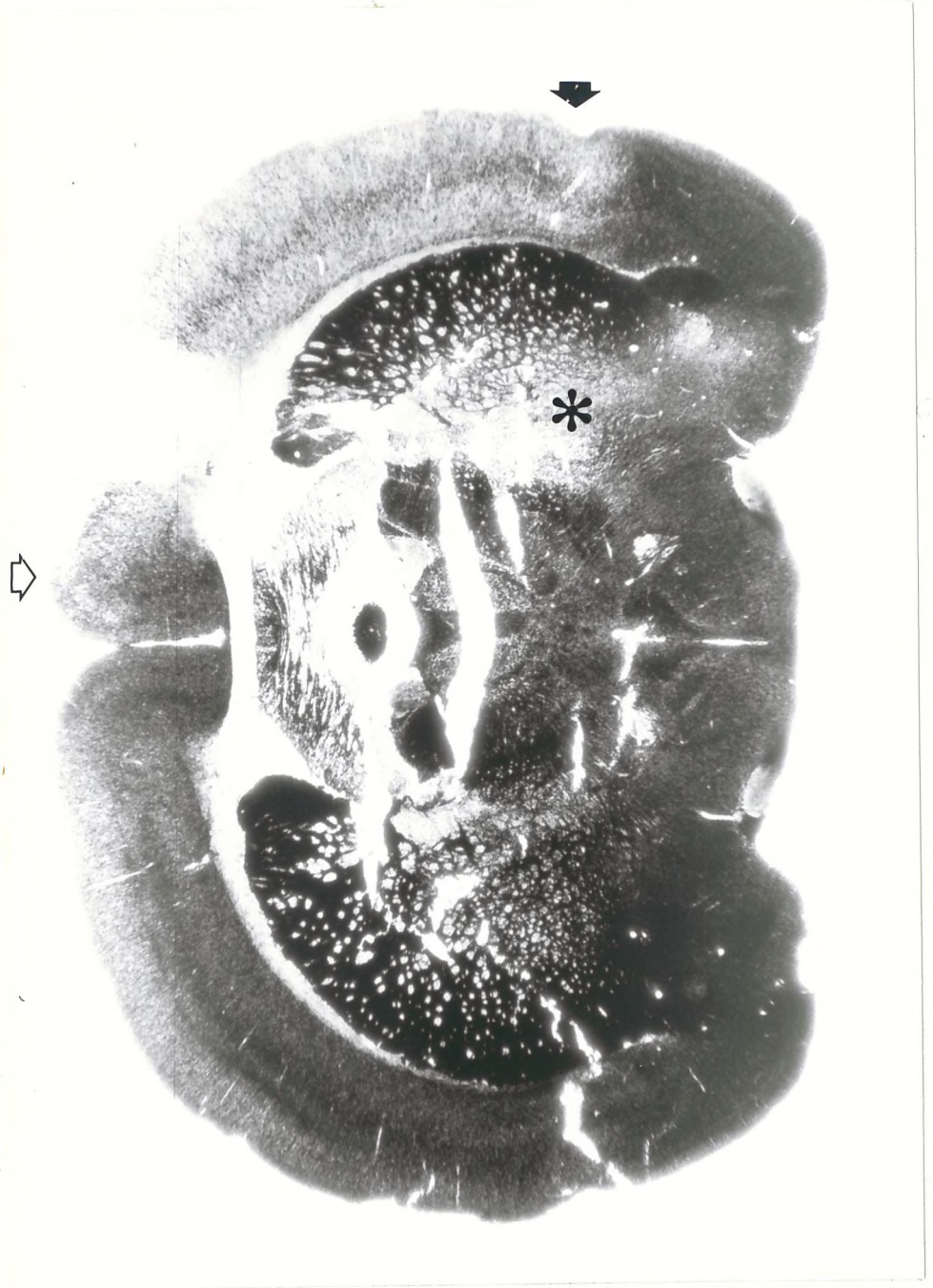


Figure 37. Acetylcholinesterase histochemistry in a rat lesioned in the right globus pallidus 8 - 10 days previously with 20 μg ibotenic acid. This 40 μm section was processed for acetylcholinesterase 24 hours after injection of DFP. Both cholinesterase-positive perikarya, fibres and terminals stain intensely. Lesioned side shows extensive loss of acetylcholinesterase-positive neurones from within the globus pallidus (Asterisk) and from within the parietal cortex (Between arrows). No loss of acetylcholinesterase fibres is apparent from either the limbic (Below white arrow) or piriform cortex (Below dark arrow, which indicates position of the rhinal fissure). Scale bar represents 1 mm.

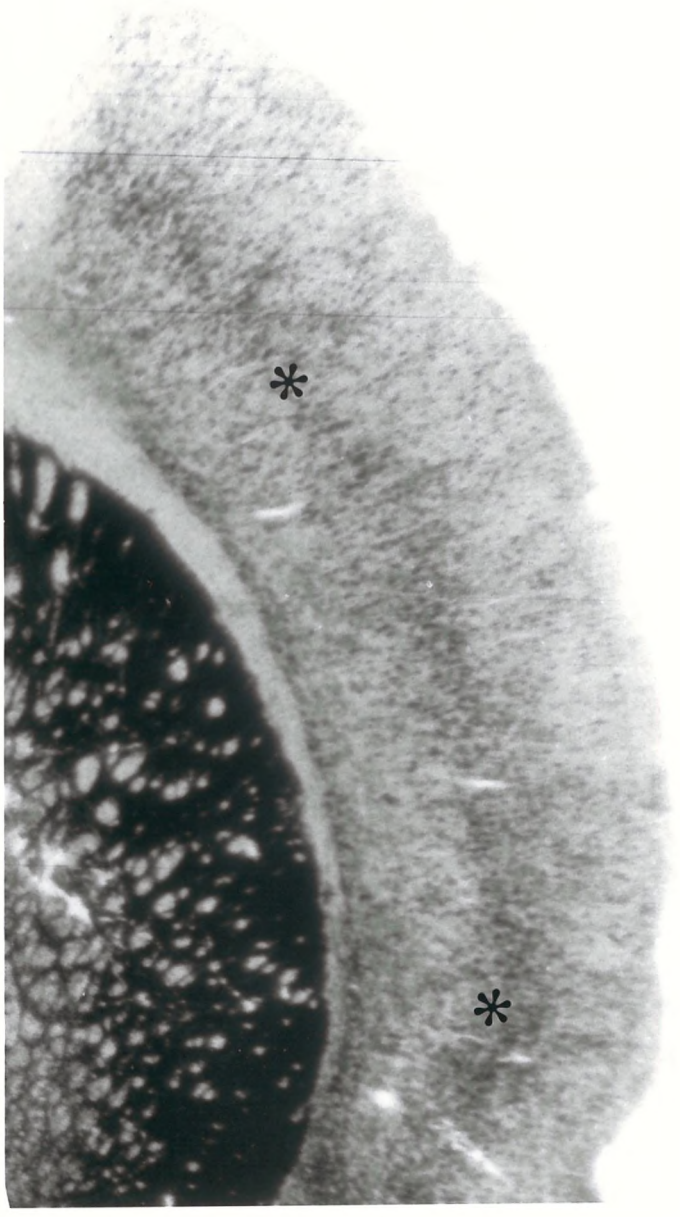


Figure 38. High power magnification of parietal cortex of rat brain from the side ipsilateral to an ibotenic acid lesion of the nucleus basalis. Almost complete loss of acetylcholinesterase-positive fibres and terminals is noted on the lesioned side, apart from a dark staining band within cortical layer V (asterisk). No loss of staining is seen within the dorso-lateral striatum. Scale bar represents 200 μM.

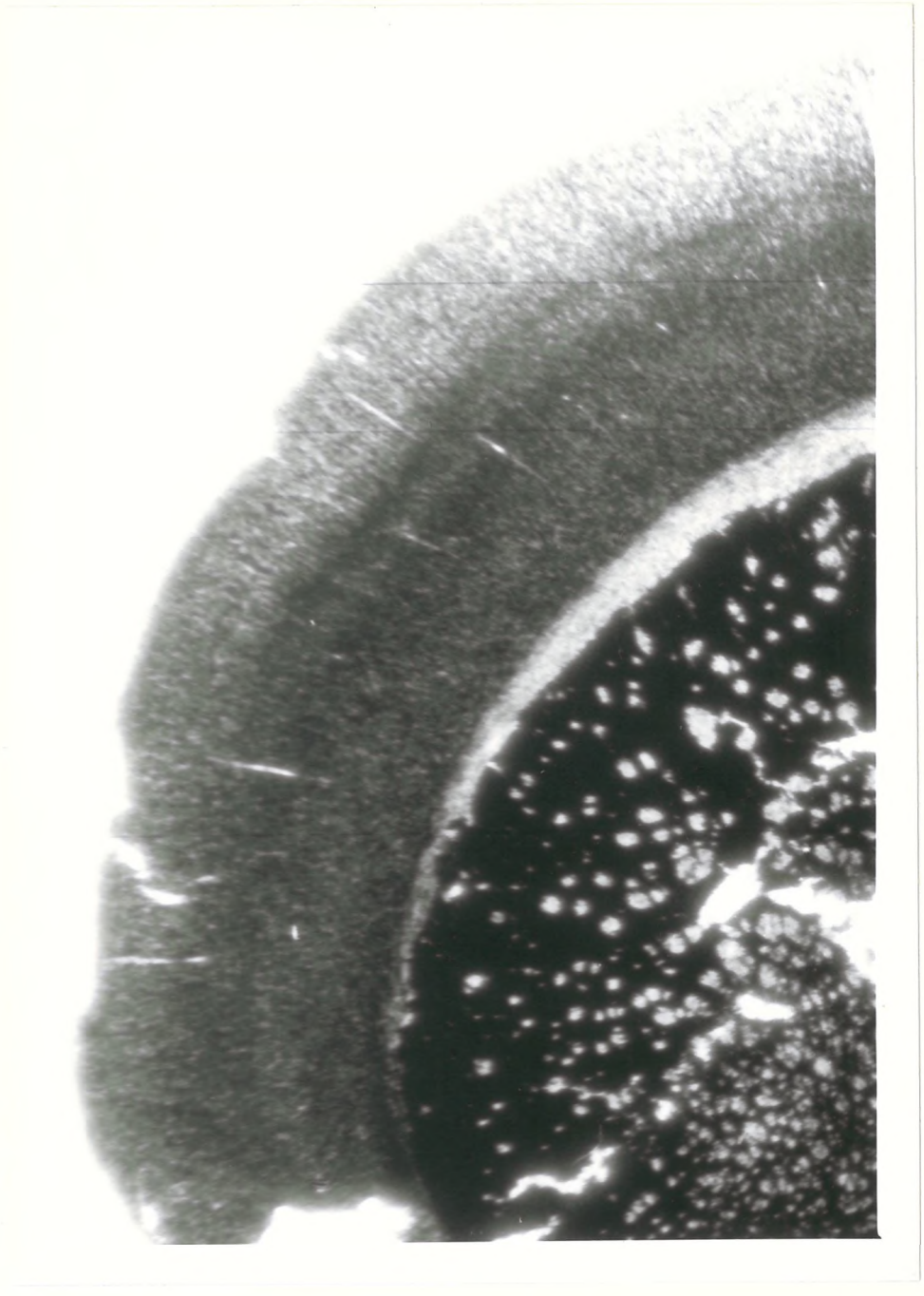
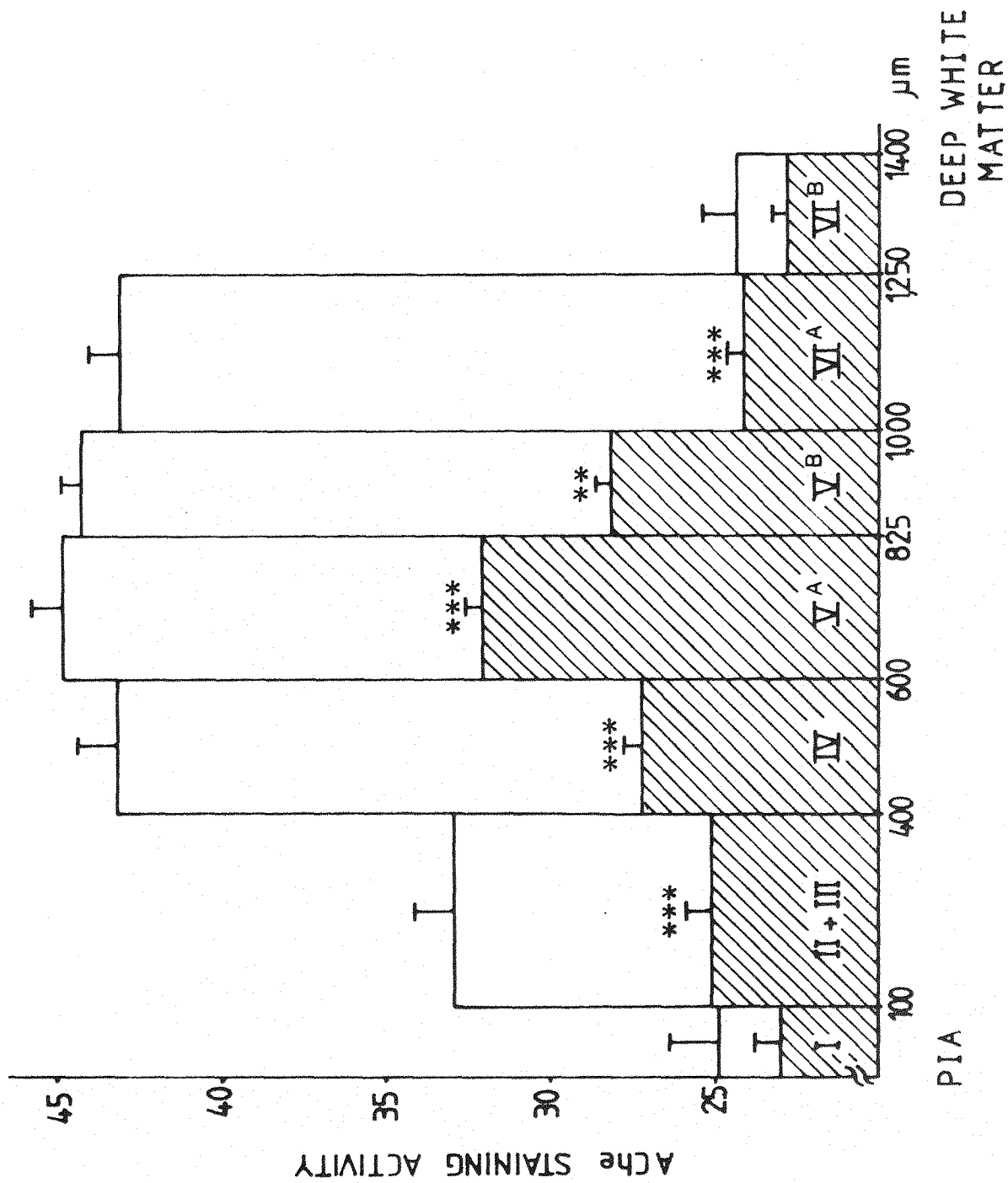


Figure 39. High power magnification of parietal cortex from control side. Scale bar represents 200 μM.

Figure 40. Laminar distribution of acetylcholinesterase staining within the control (open bars) and lesioned (hatched bars) parietal cortex. Results are obtained by scanning microdensitrometry. The number of observations within each lamina are I (10), II and III (35), IV (30), VA (20), VB (15), VIA (25), and VIB (20). An 100 point random staining density analysis within all layers gives $46.3 \pm 0.2$ for the non-lesioned side and $27.6 \pm 0.2$ for the lesioned side ($p < 0.001$). All results are analysed by signed rank test where *** $p < 0.001$, ** $p < 0.002$.


AChe STAINING ACTIVITY
45
40
35
30
25
PIA
I
II + III
IV
V^A
V^B
VI^A
VI^B
DEEP WHITE MATTER
100
400
600
825
1000
1250
1400
µm
***
**

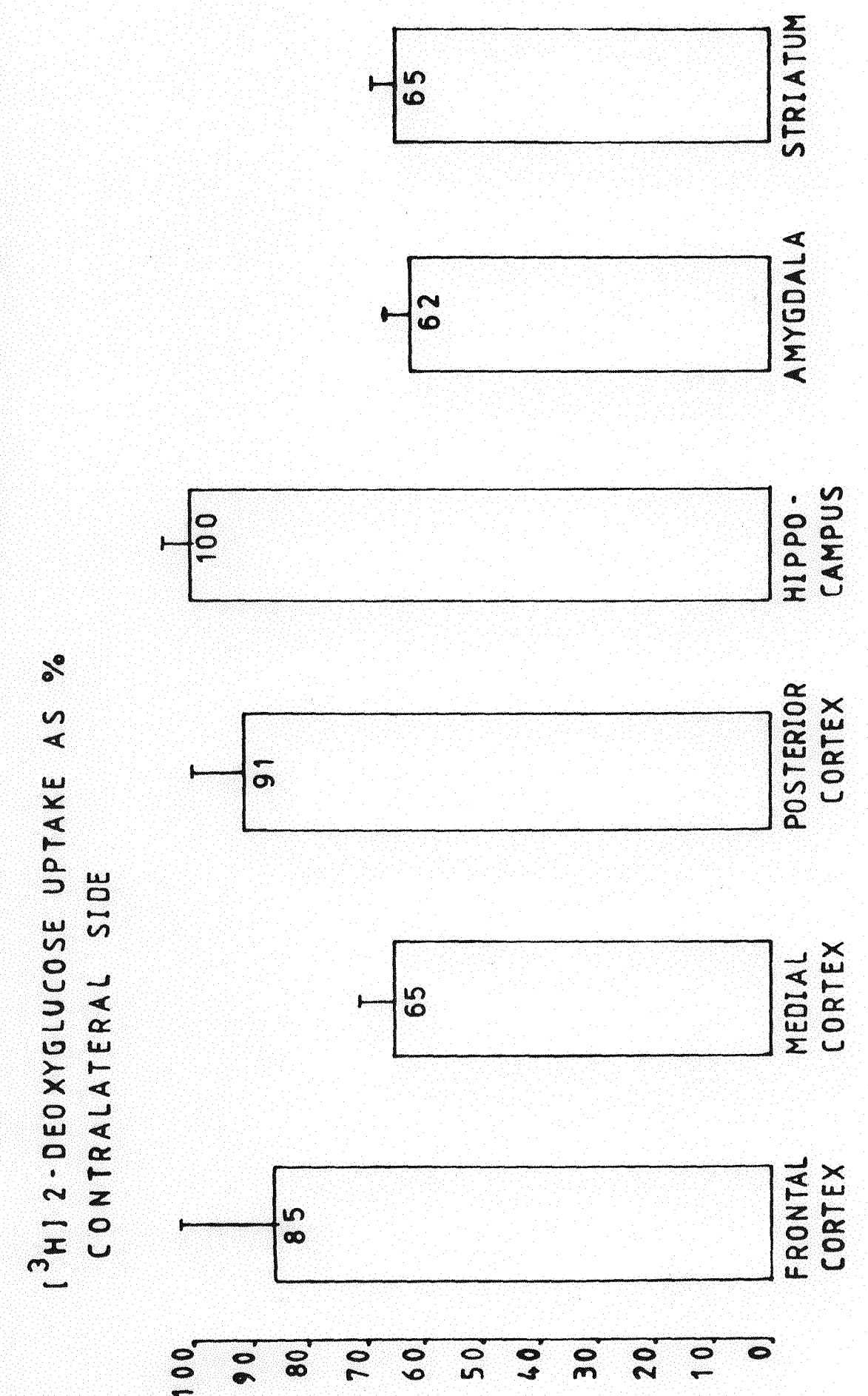

[3H] 2-DEOXYGLUCOSE UPTAKE AS %
CONTRALATERAL SIDE
100
90
80
70
60
50
40
30
20
10
0
85
65
91
100
62
65
FRONTAL CORTEX
MEDIAL CORTEX
POSTERIOR CORTEX
HIPPO-CAMPUS
AMYGDALA
STRIATUM

Figure 41. [$^{3}$H] 2-deoxyglucose uptake within several areas of the CNS of a rat lesioned within the nucleus basalis with 20 μg ibotenic acid. 10 μCi [$^{3}$H] 2-deoxyglucose was injected intravenously into a conscious rat, brain areas being removed and microdissected 30 minutes later. Results are from 4 animals and are expressed as a percentage of the control side.

1979a), although the same group also propose GABA as a transmitter contained exclusively within neuronal systems intrinsic to the nucleus accumbens (Walaas and Fonnum, 1979b). This suggestion of a GABA containing pathway between the nucleus accumbens and the ventral pallidum, and the possibility of a cholinergic pathway from the ventral pallidum to the nucleus accumbens was investigated.

Bilateral lesions were induced within the globus pallidus using the method of electrolytic coagulation. CAT and GAD were assayed within the nucleus accumbens 28 days later. Unilateral lesions of the nucleus accumbens were performed using 2 μg kainic acid injected in a volume of 1 μl (co-ordinates AP 9.2, ML 2.4 at an angle of 12° from the vertical, DV -0.5, Konig and Klippel, 1963; Mustapha, 1982) and CAT and GAD activity assayed within the ventral globus pallidus (see Tables 12 and 13) eight days later. Electrolytic lesions of the globus pallidus produces a 20% decrease in GAD activity within the nucleus accumbens. No change in CAT activity within this nucleus was observed. Kainic acid lesions of the nucleus accumbens produce a 45% decrease in GAD activity in the globus pallidus, again no change in CAT activity is seen. The existence of a GABAergic projection from the nucleus accumbens to the ventral pallidum was thus confirmed, but no evidence for any cholinergic link between these two structures within the basal ganglia was provided by these studies.

## III. 6. The Actions of Neurotoxic Amino Acids on Cholinergic Neurones

The neurotoxic action of kainic acid has been studied in considerable detail in the corpus striatum. Decortication, which causes degeneration of the cortico-striatal glutamatergic pathway (Divac et al., 1977; Kim et al., 1977; Rowlands and Roberts, 1980), protects against the neurotoxic action of kainic acid injected into the adult striatum (Biziere and Coyle, 1978, 1979; McGeer et al., 1978). This lesion (decortication) does not confer immediate protection, but becomes effective over the successive 24 - 48 hours (Biziere and Coyle, 1979). Lesions of areas of the cerebral cortex that do not project to the head of the striatum, as well as lesions of the substantia

| Region | choline acetyltransferase nmoles/mg tissue/hour | | L-glutamate decarboxylase nmoles/mg tissue/hour | |
|---|---|---|---|---|
| | Control | Lesioned | Control | Lesioned |
| Nucleus accumbens | 16.00 ± 0.77 | 16.07 ± 0.27 | 43.76 ± 1.12 | 35.38 ± 1.40 ** |

Table 12. Enzyme activities in the nucleus accumbens 1 week after bilateral electrolytic lesion of the globus pallidus (Mustafa, 1982). Results are means ± S.E. of triplicate determinations with 4 lesioned and 4 control animals. Analysis by Student's unpaired t-test, ** $p < 0.01$.

| Region | choline acetyltransferase nmoles/mg tissue/hour | | L-glutamate decarboxylase nmoles/mg tissue/hour | |
|---|---|---|---|---|
| | Left (Control) | Right (Lesion) | Left (Control) | Right (Lesion) |
| Globus pallidus | 8.26 ± 0.28 | 7.32 ± 0.86 N.S. | 31.70 ± 2.89 | 17.28 ± 1.04 ** |

Table 13. Enzyme activities in the globus pallidus on the control and lesioned sides of the brain 1 week after unilateral kainic acid lesion of the nucleus accumbens (2 μg kainic acid in 1 μl PBSS, co-ordinates AP 9.2, ML 2.4, DV 6.5 at an angle of 12°, from Mustafa, 1982). Results are means ± S.E. of triplicate determinations from 4 animals. Analysis by Student's unpaired t-test, ** $p < 0.01$.

nigra or thalamus, do not confer protection against kainate neurotoxicity (Coyle, 1983). In order to extend these observations to the two other amino acids used to produce lesions of the ventral globus pallidus, namely NMDA and ibotenic acid, their neurotoxic actions in the decorticate and intact adult striatum were investigated.

Animals received either a fronto-parietal cortex ablation (Biziere and Coyle, 1979), or a sham operation, 4 days prior to intrastriatal injection of the amino acids. This procedure was found to produce no substantial change in CAT and GAD activity (intrinsic markers within the striatum) (Table 14) but did however produce a 46% decrease in high affinity amino acid uptake (L-Glu and D-Asp) within the decorticate striatum (see Table 14). The results compare favourably with those of Biziere and Coyle (1979) who found a 48% reduction in the uptake of L-glutamate following decortication (control side 11.20 $\pm$ 1.0 pmols. mg tissue. minute). No indication of any differential effects between the uptake of D-Aspartate or L-Glutamate were observed however (uptake ratio of D-Aspartate:L-Glutamate, 1.46 $\pm$ 0.09 and 1.21 $\pm$ 0.13 for control and lesioned sides respectively, uptake ratios not significantly different by Student's t-test). A difference between the uptake of L-Glutamate and L-Aspartate within the striatum following decortication has been reported (Biziere and Coyle, 1979).

Following intrastriatal injection of 1 µg kainic acid, 20 µg ibotenic acid and 30 µg N-methyl-D-aspartic acid (and not 60 µg NMDLA as had been used in previous experiments) CAT and GAD activity were assayed in the ipsilateral and contralateral striatum (8 - 10 days after injection). Whilst decortication clearly protects against kainic acid induced neurotoxicity (Table 15) towards both cholinergic and GABAergic neurones, in agreement with previous reports (Biziere and Coyle, 1978, 1979; McGeer et al., 1978), no such protective effect towards either ibotenic acid or NMDA toxicity was found (Table 15). To extend this study of the relative sensitivities of cholinergic neurones to these three amino acids, we examined the effects of injections of kainic acid, ibotenic acid and NMDA (1 µg, 20 µg and 30 µg respectively) into the medial septal complex (vertical limb

| Neurochemical Marker | CONTROL STRIATUM | DECORTICATE STRIATUM |
|---|---|---|
| Choline acetyltransferase (nmoles/mg protein/hr) | 156.40 ± 3.15 | 141.50 ± 6.65 NS |
| L-glutamate decarboxylase (nmoles/mg protein/hr) | 210.40 ± 7.34 | 209.40 ± 4.16 NS |
| L-glutamate uptake (pmol/mg tissue/min) | 13.46 ± 0.79 | 7.93 ± 0.49 *** |
| D-aspartate uptake (pmol/mg tissue/min) | 19.39 ± 0.31 | 9.42 ± 0.72 *** |
| D ASP:L GLU RATIO | 1.46 ± 0.09 | 1.21 ± 0.13 NS |

Table 14. Neurochemical markers for cholinergic and GABAergic interneurones and glutamatergic terminals within the control (left) and lesioned (right) corpus striatum, 28 days after removal of the right fronto parietal cortex by aspiration. Results are means ± S.E. of triplicate determinations in 6 animals for enzyme assays and quadruplicate determinations in 5 animals for amino-acid uptake. Cortical ablation is found to produce a 41% decrease in L-glutamate uptake (51% decrease in D-aspartate uptake) whilst having no significant effect on intrinsic neuronal markers (CAT and GAD). Analysis is by Student's unpaired t-test, *** $p < 0.001$.

Choline acetyltransferase (nmoles/mg tissue/hour)

| Neurotoxin | Control Striatum | | Decorticate striatum | |
|---|---|---|---|---|
| | Lesion | Control | Lesion | Control |
| KA | 4.87 ± 0.66 | 19.24 ± 0.71*** | 15.51 ± 1.19 | 19.18 ± 1.01 NS |
| IBO | 7.99 ± 0.65 | 20.03 ± 0.76*** | 8.45 ± 0.42 | 19.02 ± 0.99*** |
| NMDA | 4.92 ± 1.03 | 21.39 ± 1.34*** | 6.67 ± 0.78 | 19.23 ± 0.78*** |

L-glutamate decarboxylase (nmoles/mg tissue/hour)

| Neurotoxin | Control striatum | | Decorticate striatum | |
|---|---|---|---|---|
| | Lesion | Control | Lesion | Control |
| KA | 10.22 ± 0.39 | 28.44 ± 0.98*** | 26.16 ± 2.10 | 31.57 ± 1.42 NS |
| IBO | 12.79 ± 1.02 | 30.79 ± 0.82*** | 13.06 ± 0.75 | 30.23 ± 0.82*** |
| NMDA | 9.46 ± 0.89 | 29.98 ± 1.01*** | 11.42 ± 0.98 | 31.13 ± 1.27*** |

Table 15. The effect of fronto-parietal cortex ablation on the neurotoxicity of kainic acid (KA), ibotenic acid (IBO) and N-methyl-D-aspartic acid (NMDA) in the corpus striatum. Choline acetyltransferase and L-glutamate decarboxylase activities were assayed within the striatum 8-10 days after stereotaxic injection of the neurotoxic amino acids. Results are means ± S.E. of triplicate determinations in six animals for each group. Analysis is by Student's t-test, *** $p < 0.001$.

of the nucleus of the diagonal band of Broca and the medial septal nucleus). The medial septal complex is the major source of the cholinergic innervation of the hippocampus (Storm-Mathissen, 1978; Fonnum and Walaas, 1978). 8 - 10 days after a unilateral lesion of the medial septal complex (AP 9.0, ML 0.0, DV - 2.3, Konig and Klippel, 1963), CAT and GAD activity were assayed within the septal and temporal halves of the hippocampus. The results are presented in Table 16 and show that in contrast to the two areas previously studied (corpus striatum and ventral pallidum), kainic acid is relatively far less potent as a toxin than either NMDA or ibotenic acid. GABA-ergic interneurones within the hippocampus are unaffected by any lesion of the medial septum (Table 16). This finding confirms the specificity of the lesion, since the GABA containing cells of the hippocampal formation are amongst the most sensitive to 'distant' lesions produced by neurotoxic amino acids (Malthe-Sørenssen et al., 1980). The results for this series of experiments on the regional differences in amino acid toxicity are summarised in Figs. 42, 43 and 44. This clearly shows that the cholinergic perikarya of the medial septal complex and the decorticate striatum exhibit a similar profile of sensitivity to these three neurotoxic amino acids. Whilst the cholinergic cells within the ventral globus pallidus exhibit a sensitivity far more similar to that of the intact striatum.

## III. 7. Post Lesion Adaptation in Cholinergic Neurones

In addition to the previously determined presynaptic cholinergic markers (choline acetyltransferase and acetylcholinesterase) the high affinity uptake of choline (HACU) was also determined. That the uptake of choline into cholinergic neurones is related to the neuronal activity within these cells is demonstrated by the reduction (67.6%) in HACU 30 minutes after administration of pentobarbital (60 mg/Kg i.p.) (Table 17). A reduction in uptake of a similar magnitude has been shown by Simon et al. (1976) to occur in synaptosomes prepared from the cerebral cortex but not from the striatum. These findings are in agreement with those of Trabucchi et al. (Trabucchi et al., 1975) who found that pentobarbital does not alter acetylcholine

Choline acetyltransferase (nmoles/mg tissue/hour)

| Neurotoxin | Septal Hippocampus | Temporal Hippocampus |
|---|---|---|
| CONTROL | 5.09 ± 0.49 | 6.66 ± 0.44 |
| KA | 4.87 ± 0.45 NS | 5.93 ± 0.39 NS |
| IBO | 3.21 ± 0.27 ** | 4.02 ± 0.32 *** |
| NMDA | 3.25 ± 0.21 ** | 4.11 ± 0.40 *** |

L-glutamate decarboxylase (nmoles/mg tissue/hour)

| Neurotoxin | Septal Hippocampus | Temporal Hippocampus |
|---|---|---|
| CONTROL | 28.00 ± 2.11 | 35.63 ± 3.58 |
| KA | 29.17 ± 2.02 NS | 33.66 ± 3.27 NS |
| IBO | 27.35 ± 1.96 NS | 31.97 ± 4.01 NS |
| NMDA | 28.16 ± 2.31 NS | 33.35 ± 3.42 NS |

Table 16. The neurotoxicity of kainic acid (1 μg), ibotenic acid (20 μg) and N-methyl D-aspartic acid (30 μg) to medial septal cholinergic neurones. Choline acetyltransferase and L-glutamate decarboxylase activities were assayed within the septal and temporal halves of the hippocampus 8 - 10 days after stereotaxic injection of the neurotoxic amino acids. Results are means of triplicate determinations in four animals for each group. Analysis is by Student's t-test, *** $p < 0.001$, ** $p < 0.02$.

Figure 43. Choline acetyltransferase and L-glutamate decarboxylase within the septal and temporal halves of the hippocampus following lesions of the medial septal complex. Results are expressed as a percentage of the contralateral side and are those presented in Table 16. Analysis is by Student's unpaired t-test on the original data.

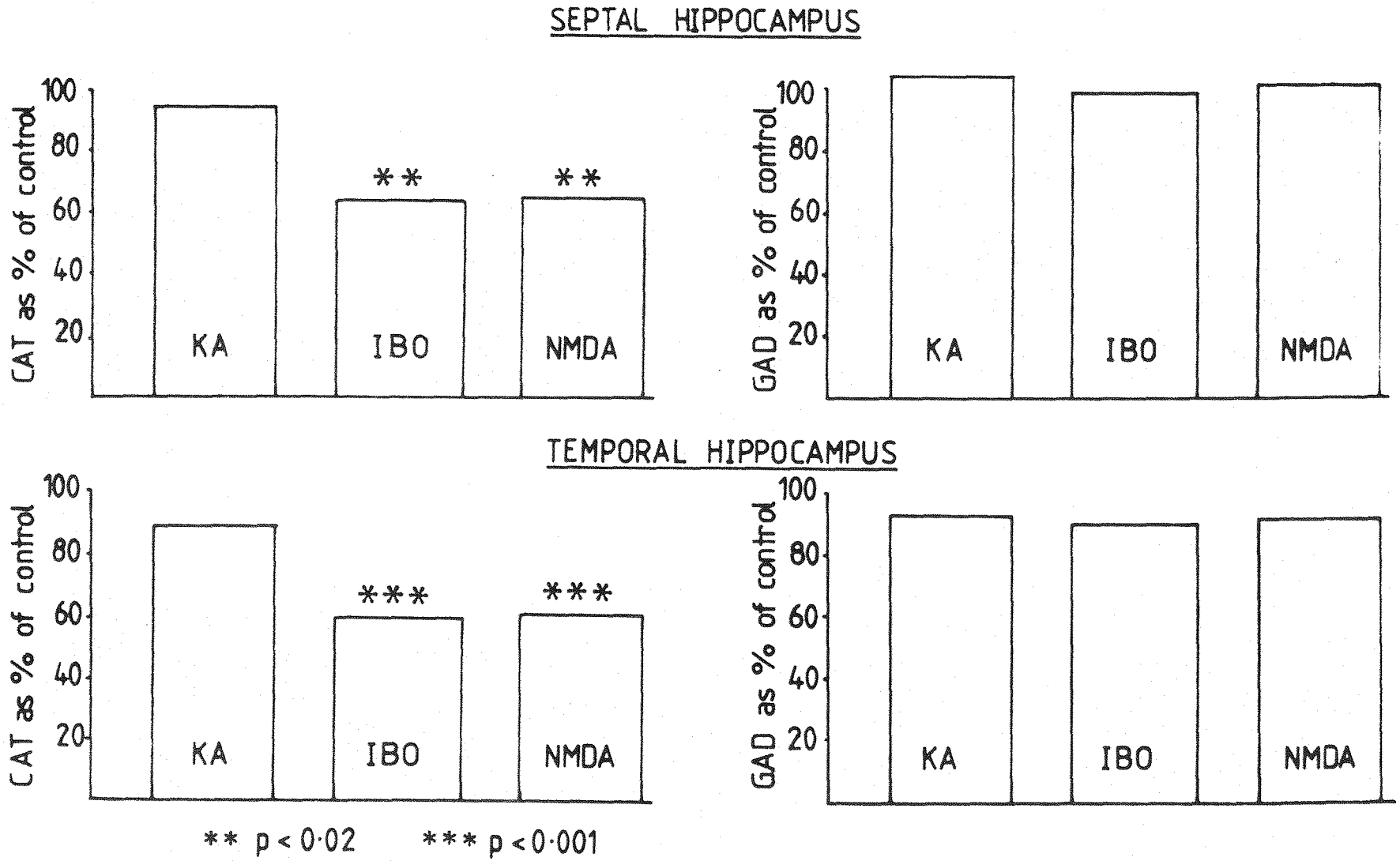

SEPTAL HIPPOCAMPUS
CAT as % of control
100
80
60
40
20
KA
IBO
NMDA
**
**
GAD as % of control
100
80
60
40
20
KA
IBO
NMDA
TEMPORAL HIPPOCAMPUS
CAT as % of control
100
80
60
40
20
KA
IBO
NMDA
***
***
GAD as % of control
100
80
60
40
20
KA
IBO
NMDA
** p < 0·02
*** p < 0·001

Figure 42. Choline acetyltransferase and L-glutamate decarboxylase within the parietal cortex after neurotoxic amino acid induced lesions of the nucleus basalis. Results are expressed as a percentage of the contralateral side and are those presented in Figures 17 and 18. Analysis is by Student's unpaired t-test on the original data.

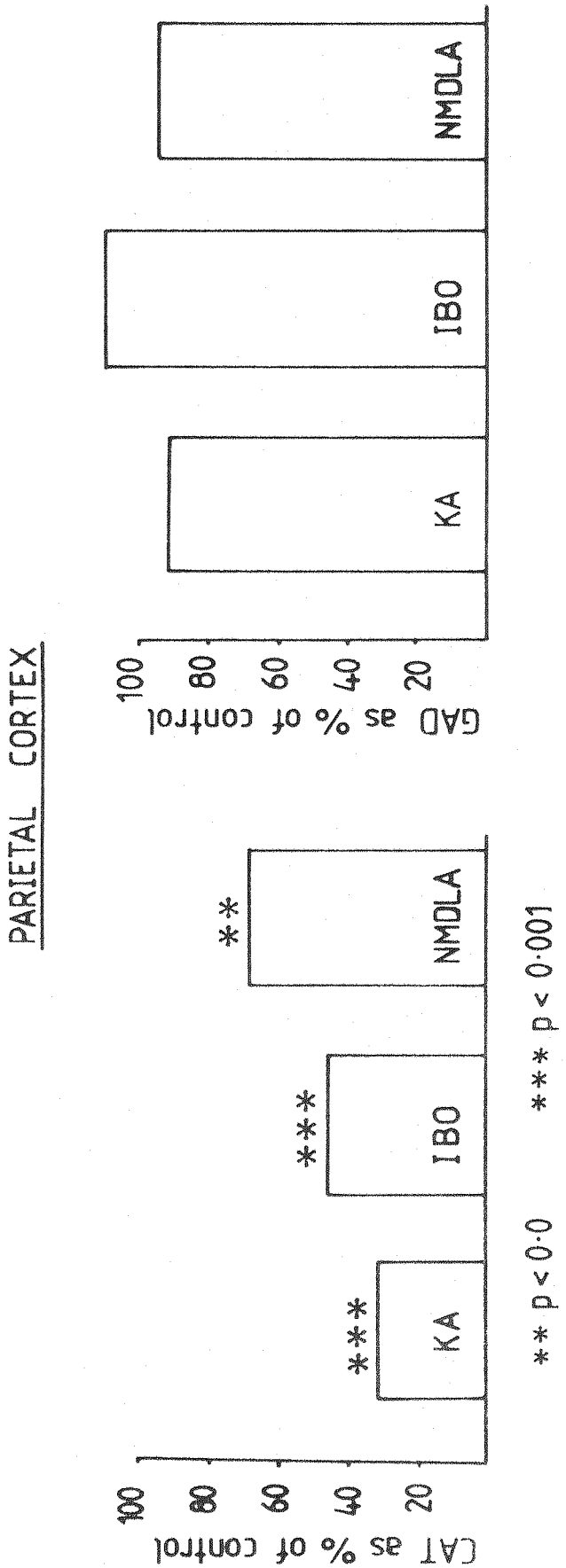
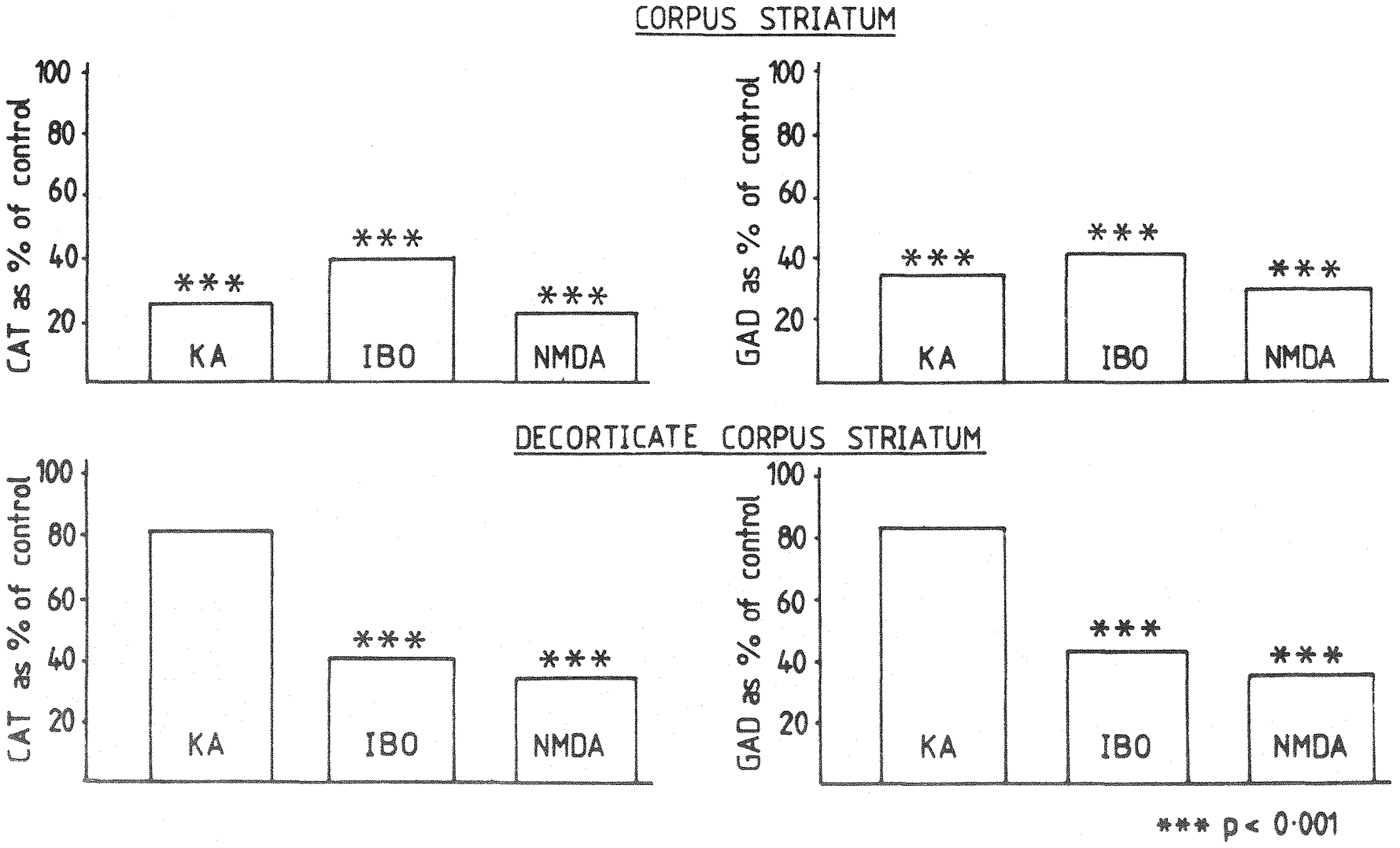

PARIETAL CORTEX
GAD as % of control
100
80
60
40
20
KA
IBO
NMDLA
CAT as % of control
100
80
60
40
20
****
****
**
KA
IBO
NMDLA
* p < 0·0
*** p < 0·001

Figure 44. Choline acetyltransferase and L-glutamate decarboxylase within the control and decorticate neostriatum, following neurotoxic amino acid induced lesions of this structure. Results are expressed as a percentage of the contralateral side and are those presented in Table 15. Analysis is by Student's unpaired t-test on the original data.


CORPUS STRIATUM
CAT as % of control
GAD as % of control
100
80
60
40
20
***
KA
IBO
NMDA
DECORTICATE CORPUS STRIATUM
*** p < 0·001

turnover in rat striatum but decreases turnover in rat cerebral cortex. Addition of pentobarbital *in vitro* to the incubation does not decrease HACU (Simon et al., 1976).

Results for HACU, CAT and AChE activity assayed in the parietal cortex at various time intervals after lesion of the ventral globus pallidus are presented in Tables 18 - 20 and summarised in Figure 45. A significant decrease in both CAT activity and HACU occurs after 1 day, whilst AChE activity is not significantly reduced until 10 days post-lesion. At 10 weeks post-lesion both CAT and AChE remain significantly reduced; HACU, however, is no longer significantly reduced on the lesioned side. The results for the high affinity uptake of choline have been expressed as 'f. mol[$^3$H] choline taken up per p. mol [$^3$H]acetylcholine synthesised' and suggest a significant increase in the amount of choline taken up per remaining cholinergic neurone (Cooper and Schmidt, 1980); see Table 21.

## III. 8. The Actions of Isoxsuprine on Metabolism and Acetylcholine Synthesis in the Cerebral Cortex

### a) Oxygen uptake in cerebral cortex slices *in vitro*.

Investigations carried out within the Department of Physiology at Southampton University during 1979 (Horn and Williams, unpublished observations), suggested that chronic pretreatment of rats with isoxsuprine HCl causes a significant increase in oxygen uptake in hand-cut cerebral cortex slices. This effect reaches its maxima at 8 - 10 days and constitutes an approximate 30% increase in either basal or succinate stimulated oxygen uptake (Williams, unpublished observations). The inclusion of 1, 10 or 100 μm isoxsuprine in the *in vitro* incubation was found to have no effect on the rate of oxygen uptake in slices from untreated animals. 1 mM isoxsuprine, however, produced a 60% inhibition of uptake (Results not shown). The original experiments of Williams and Horn were repeated investigating the effects of pretreatment for 8 - 10 days with either 0.02 mg B.I.D. or 0.2 mg B.I.D. isoxsuprine, injected intraperitoneally. The results (see ~~Fig~~ Tab. 22) show a maximal 35% increase in succinate stimulated oxygen uptake with 0.2 mg isoxsuprine. The lower dose (0.02mg

| HIGH AFFINITY CHOLINE UPTAKE (fmol/mg/min) | |
|---|---|
| CONTROLS | |
| 81.00 ± 5.6 | 80.25 ± 2.80 |
| Na PENTOBARBITONE PRETREATED (60 mg/kg I.P. for 30 mins) | |
| 27.17 ± 1.30 | 25.14 ± 1.67 *** |

Table 17. The effects of in vivo sodium pentobarbitone (60 mg/kg, 30 mins) on the high affinity uptake of choline (0.06 μm) into synaptosomes prepared from the rat brain parietal cortex. Results are mean ± S.E. of quadruplicate determinations in 6 animals. Analysis is by Student's t-test, *** $p < 0.001$.

| CHOLINE ACETYLTRANSFERASE (NMOLS/MG/HOUR) | | | |
|---|---|---|---|
| | CONTROL | LESION | |
| CONTROLS | 5.36 ± 0.35 | 5.47 ± 0.23 | NS |
| 1 DAY | 5.37 ± 0.40 | 2.78 ± 0.23 | ** |
| 10 DAYS | 5.14 ± 0.22 | 2.26 ± 0.24 | *** |
| 10 WEEKS | 6.21 ± 0.57 | 1.78 ± 0.32 | *** |

Table 18. Time course of changes in choline acetyltransferase within the parietal cortex after ibotenic acid lesion of the nucleus basalis. Results are means ± S.E. of triplicate determinations from 6 animals.
*** $p < 0.001$, ** $p < 0.01$; analysis by Student's unpaired t-test.

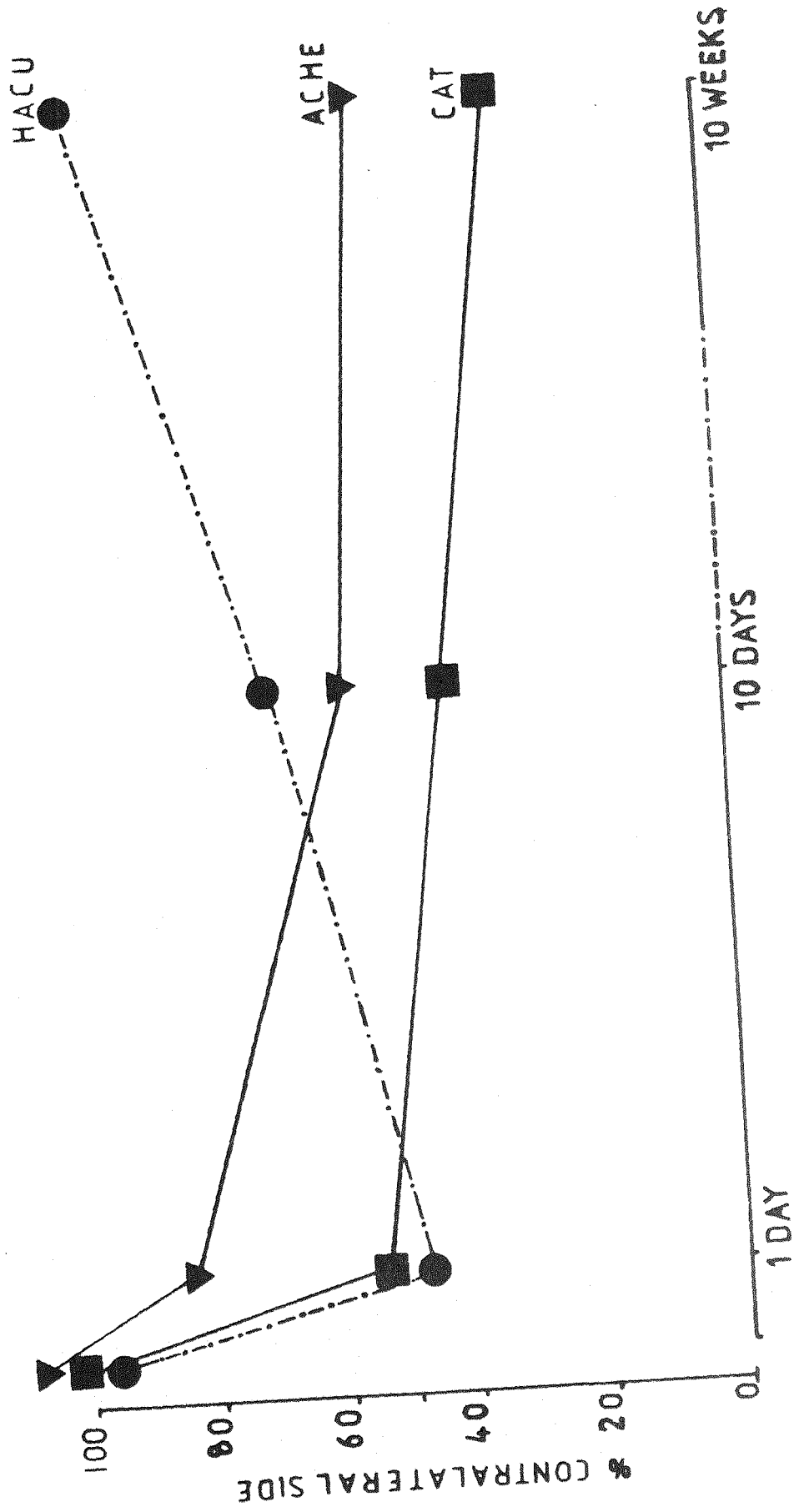
| ACETYLCHOLINESTERASE (NMOLS/MG/HOUR) | | | |
|---|---|---|---|
| | CONTROL | LESION | |
| CONTROLS | 269.98 ± 22.03 | 276.58 ± 18.96 | |
| 1 DAY | 283.43 ± 24.67 | 236.68 ± 12.77 | |
| 10 DAYS | 248.79 ± 24.63 | 141.34 ± 23.80 | ** |
| 10 WEEKS | 260.00 ± 15.98 | 137.44 ± 23.30 | ** |

Table 19. Time course of changes in acetylcholinesterase within the parietal cortex after ibotenic acid lesions of the nucleus basalis. Results are means ± S.E. of triplicate determinations from 6 animals. Analysis by Student's unpaired t-test, ** $p < 0.01$.

| HIGH AFFINITY CHOLINE UPTAKE (FMOLS/MG/MIN) | | | |
|---|---|---|---|
| | CONTROL | LESION | |
| CONTROLS | 80.25 ± 2.80 | 81.00 ± 5.68 | NS |
| 1 DAY | 78.89 ± 5.23 | 39.13 ± 1.87 | *** |
| 10 DAYS | 80.72 ± 4.22 | 57.28 ± 1.55 | *** |
| 10 WEEKS | 96.86 ± 2.84 | 95.10 ± 8.86 | NS |

Table 20. Time course of changes in high affinity choline uptake within the parietal cortex after ibotenic acid lesions of the nucleus basalis. Results are means ± S.E. of quadruplicate determinations from 6 animals. Analysis is by Student's unpaired t-test, *** $p < 0.001$.

Figure 45. Choline acetyltransferase activity, acetylcholinesterase activity and high affinity choline uptake within the parietal cortex at various time intervals after lesion of the nucleus basalis. This figure summarises the results presented in Tables 18, 19 and 20.


HACU
ACHE
CAT
10 WEEKS
10 DAYS
1 DAY
% CONTRALATERAL SIDE
100
80
60
40
20
0

HIGH AFFINITY CHOLINE UPTAKE

(fmol choline taken up/pmol Acetylcholine synthesised)

| | CONTROL | LESION | |
|---|---|---|---|
| CONTROLS | 0.90 ± 0.09 | 0.89 ± 0.10 | |
| 1 DAY | 0.88 ± 0.12 | 0.85 ± 0.16 | |
| 10 DAYS | 0.98 ± 0.08 | 1.52 ± 0.18 | ** |
| 10 WEEKS | 0.94 ± 0.12 | 3.20 ± 0.48 | *** |

Table 21. High affinity choline uptake expressed 'per cholinergic neurone' (Cooper and Schmidt, 1980). Statistical analysis of the results for HACU and CAT have been performed more correctly using the methods of Colquhoun (D. Colquhoun, Lectures in Biostatistics, publ. Oxford University Press, 1971) analysing the variance of the ratio of two variables in terms of the coefficients of variation. The uptake per cholinergic neurone remains significant at both 10 days and 10 weeks post-lesion when analysed by Student's unpaired t-test. Original data is that presented in Tables 18 and 20.

| OXYGEN UPTAKE ($\mu$l $O_2$ uptake/mg. dry weight. hr at 25°C) | | | |
|---|---|---|---|
| Isoxsuprine pretreatment regime (I.P., B.I.D.) | sham injected | isoxsuprine pretreated | % change |
| 0.02 mg | 3.55 ± 0.08 (20) | 4.00 ± 0.12 (30) ** | 13% |
| 0.20 mg | 3.45 ± 0.10 (30) | 4.65 ± 0.25 (30) *** | 35% |

Table 22. In vitro oxygen uptake by cerebral cortex slices following in vivo isoxsuprine pretreatment. Results are means ± S.E., no. of experiments are given in parentheses and were performed in triplicate. Analysis by Student's unpaired t-test, *** $p < 0.001$, ** $p < 0.01$.

isoxsuprine) produces a 13% increase in uptake. These results confirm the preliminary findings of Williams and Horn, showing a 30 - 35% increase in oxygen uptake into cerebral slices under optimal conditions (0.2 mg B.I.D. for 8 - 10 days). Williams has previously shown that increasing the dose from 0.2 to 2.0 mg B.I.D. produces no additional increase in uptake (unpublished observations).

The effect of this pretreatment regime (0.2 mg B.I.D., 8 - 10 days) on the rate of oxygen uptake into hand cut rat brain cerebellar cortex slices was investigated. Isoxsuprine pretreatment caused a 20% increase in uptake (control 5.10 $\pm$ 0.4 $\mu$l $O_2$/mg dry weight/hour; pretreated 6.12 $\pm$ 0.2 $\mu$l $O_2$/mg dry weight/hour, not significantly different by Student's unpaired t-test) although this was not significantly different to sham injected control values. In an attempt to extend our understanding of this effect, to include several regions within the rat central nervous system, we investigated the <u>in vivo</u> uptake of [$^3$H] -2-deoxyglucose.

b) [$^3$H] -2-deoxyglucose uptake into the CNS <u>in vivo</u>.

The [$^{14}$C] deoxyglucose technique developed by Sokoloff et al. (Sokoloff, 1977; Sokoloff et al., 1974, 1977) has become widely accepted among neuroscientists. In several recent studies, however, [$^{14}$C] deoxyglucose has been substituted by [$^3$H] deoxyglucose, with brain regions being dissected and counted in a liquid scintillation counter (Delanoy and Dunn, 1978; Glick et al., 1979; Reinstein et al., 1979; Meibach et al., 1980). Further modifications employing different routes of administration of the label have also been reported (Delanoy and Dunn, 1978; Reinstein et al., 1979; Meibach et al., 1980). It has been suggested that the combination of peripheral administration of [$^3$H] -2-deoxyglucose with the microdissection technique provides a readily quantifiable method for the sensitive determination of subtle changes in metabolic rate (Delanoy and Dunn, 1978). The effect of isoxsuprine pretreatment on the uptake of [$^3$H] deoxyglucose was therefore investigated. Following either subcutaneous or intraperitoneal injection of 10 $\mu$Ci [$^3$H] deoxyglucose, uptake was determined in cerebral cortex (fronto parietal), cerebellar cortex, hippo-

campus, corpus striatum, superior colliculus, inferior colliculus, thalamic nuclei, pons and hypothalamus. Whilst these two routes of administration gave a similar level and distribution of radioactivity throughout the brain regions studied, the variation between individual animals was sufficient to obscure any possible changes between treatment groups (sham injected and isoxsuprine pretreated). In an attempt to minimise these intra-group variations we investigated the intravenous route of administration of [$^3$H] deoxyglucose, in unanaesthetised animals with minimal restraint, via an indwelling right internal jugular vein cannula. All animals were cannulated 2 days prior to the injection of [$^3$H] deoxyglucose. Using this technique the plasma levels of [$^3$H] were detected (see Fig. 16, page 43 ). Plasma radioactivity was found to reach a peak in 1 - 2.5 minutes, whilst at 30 minutes after injection plasma levels were stable at 10% of peak levels. A 30 minute survival period following [$H^3$] deoxyglucose injection was therefore used for all further studies. Neither chronic (8 days pretreatment, see Table 23) nor acute (30 minute pretreatment; results not shown) pretreatment with isoxsuprine (0.2 mg B.I.D.) produced any significant increase in [$^3$H] deoxyglucose uptake in any brain area studied. Indeed all areas showed a small but insignificant decrease in [$^3$H] deoxyglucose uptake. One possible method of minimising the variations in uptake between animals is to express the results in relation to a common reference. Results are therefore expressed as Relative specific activity (Delanoy and Dunn, 1978) or relative to the plasma levels of [$^3$H] deoxyglucose (see Table 23). These data transformations, however, make no significant difference to the results. The sensitivity of this modified, intravenous technique was assessed by determining the effects of amphetamine (5 mg/Kg i.v.) and sodium pentobarbitone (60 mg/kg i.p.) on regional [$^3$H] deoxyglucose uptake. Administration of these two drugs, which result in gross behavioural changes, namely hyperexcitation and sedation respectively, produced significant changes in [$^3$H] deoxyglucose uptake within several areas of the rat brain (see Table 24). The smooth muscle relaxant, papaverine which causes a marked vasodilation,

Table 23. *In vivo* [$^{3}$H] 2-deoxyglucose uptake in various regions of the rat brain following isoxsuprine pre-treatment. Results are means ± S.E. from 6 animals which received an intra-venous injection of 10 μCi [$^{3}$H] 2-deoxyglucose. Isoxsuprine pretreatment regime was 0.2 mg isoxsuprine injected intra-peritoneally, twice daily.

| Micro dissected Brain Region | TISSUE UPTAKE | | RELATIVE SPECIFIC ACTIVITY | | TISSUE TO PLASMA RATIO | |
|---|---|---|---|---|---|---|
| | Isoxsuprine Pre-treated | Sham injected controls | Isoxsuprine Pre-treated | Sham injected controls | Isoxsuprine Pre-treated | Sham injected controls |
| Cerebral cortex | 108.1 ± 12.0 | 122.7 ± 17.7 | 1.03 ± 0.05 | 1.01 ± 0.04 | 3.84 ± 0.43 | 3.73 ± 0.34 |
| Cerebellar cortex | 100.9 ± 10.4 | 109.2 ± 15.6 | 0.93 ± 0.05 | 0.90 ± 0.02 | 3.59 ± 0.37 | 3.32 ± 0.47 |
| Hippocampus | 76.8 ± 7.3 | 100.3 ± 16.4 | 0.74 ± 0.03 | 0.81 ± 0.04 | 2.73 ± 0.26 | 3.05 ± 0.50 |
| Striatum | 111.8 ± 11.4 | 150.7 ± 24.2 | 1.07 ± 0.06 | 1.11 ± 0.05 | 3.98 ± 0.41 | 4.58 ± 0.74 |
| Superior colliculi | 117.4 ± 7.3 | 135.6 ± 19.7 | 1.16 ± 0.07 | 1.11 ± 0.07 | 4.18 ± 0.26 | 4.12 ± 0.60 |
| Inferior colliculi | 136.6 ± 15.1 | 175.2 ± 13.6 | 1.30 ± 0.08 | 1.50 ± 0.08 | 4.86 ± 0.54 | 5.32 ± 0.41 |
| Thalamic nuclei | 109.9 ± 9.7 | 150.6 ± 19.7 | 1.07 ± 0.05 | 1.26 ± 0.07 | 3.91 ± 0.35 | 4.58 ± 0.60 |
| PONS | 88.3 ± 13.3 | 101.9 ± 14.7 | 0.84 ± 0.07 | 0.84 ± 0.05 | 3.14 ± 0.47 | 3.10 ± 0.45 |
| Hypothalamus | 90.6 ± 7.5 | 105.3 ± 12.8 | 0.89 ± 0.05 | 0.88 ± 0.02 | 3.22 ± 0.27 | 3.20 ± 0.39 |

Table 24. [$^{3}$H] 2-deoxyglucose uptake into various areas of the rat CNS following (i) Amphetamine pretreatment, 5 mg/kg intravenously and (ii) Sodium pentobarbitone pretreatment, 60 mg/kg intraperitoneally. Results are means $\pm$ S.E. for 6 animals in each group. Analysis is by Student's unpaired t-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

| Micro dissected Brain region | [$^3$H] 2-deoxyglucose uptake DPM mg tissue/DPM mg plasma | | |
|---|---|---|---|
| | CONTROLS | AMPHETAMINE | PENTOBARBITONE |
| Cerebral cortex | 4.31 ± 0.64 | 8.76 ± 1.38* | 2.32 ± 0.29* |
| Cerebellar cortex | 4.15 ± 0.78 | 7.84 ± 0.64** | 2.35 ± 0.28$^{NS}$ |
| Hippocampus | 4.00 ± 0.62 | 6.15 ± 0.90$^{NS}$ | 1.87 ± 0.45$^{NS}$ |
| Striatum | 4.91 ± 0.39 | 8.89 ± 1.13** | 2.21 ± 0.47*** |
| Superior colliculus | 5.75 ± 0.82 | 9.48 ± 1.33* | 2.94 ± 0.62$^{NS}$ |
| Inferior colliculus | 6.20 ± 0.54 | 9.07 ± 1.30$^{NS}$ | 3.28 ± 1.04$^{NS}$ |
| Thalamic nuclei | 6.10 ± 0.61 | 10.53 ± 1.20** | 2.75 ± 0.50** |
| PONS | 3.50 ± 0.60 | 7.08 ± 1.14* | 2.34 ± 0.49$^{NS}$ |
| Hypothalamus | 4.27 ± 0.42 | 5.62 ± 0.50$^{NS}$ | 1.76 ± 0.08*** |

produced decreases in [$H^3$] deoxyglucose uptake in all areas studied, these were however statistically insignificant.

c) CAT and HACU in the cerebral cortex, the effects of choline and isoxsuprine.

Impairing the oxidation of pyruvate to acetyl coenzyme A leads to a proportional impairment of the synthesis of acetylcholine even though less than 1% of the acetyl coenzyme A formed is incorporated into acetylcholine (Blass et al., 1980). This relationship holds both in vivo and in vitro. In vitro it has been demonstrated using non-competitive or competitive inhibitors of the pyruvate dehydrogenase complex (PDHC), inhibitors of the tricarboxylic acid cycle; inhibitors of electron transport, and by reducing the concentrations of glucose or oxygen in the media. In vivo it has been shown in hypoglycemia, anaemic hypoxia, histotoxic hypoxia and hypoxic hypoxia, and in thiamine deficiency. Hypoxia too mild to alter significantly the levels of cyclic AMP or AMP, or lactate - all sensitive metabolic indicators of hypoxia - did reduce the synthesis of acetylcholine. Hypoxic hypoxia induced with 12.5% $O_2$ in nitrogen reduced acetylcholine synthesis in hippocampus to less than 10% of normal, (Blass et al., 1979). The actions of isoxsuprine on choline acetyltransferase activity were therefore studied, using the same dosing regime employed in the oxygen uptake studies. Pretreatment of rats for 8 - 10 days with isoxsuprine (10 mg/Kg i.p. B.I.D. for 8 - 10 days) produced no significant increase in CAT activity in either the hippocampus, striatum or cortex (see Table 25). This pretreatment schedule was also shown not to alter the total body weight of the animals (control 251.67 $\pm$ 8.38 g; isoxsuprine pretreated 249.17 $\pm$ 5.58 g). A more detailed analysis of the effects of isoxsuprine (10 mg/Kg i.p. B.I.D. for 10 days), choline chloride (60 mg/Kg i.p. for 10 days) and these two treatments in combination on choline acetyltransferase activity in fronto-parietal cortex was carried out (see Table 26). None of the pretreatment protocols either singularly or in combination was found to produce any change in CAT activity.

There is considerable experimental evidence which indicates

CHOLINE ACETYLTRANSFERASE ACTIVITY
(nmoles/mg tissue/hour)

| | Hippocampus | Striatum | Cortex |
|---|---|---|---|
| pretreated | 5.85 ± 0.19 | 19.85 ± 1.08 | 5.97 ± 0.39 |
| sham injected controls | 5.96 ± 0.23 | 20.41 ± 1.29 | 5.66 ± 0.13 |

Table 25. Choline acetyltransferase activity in hippocampus, striatum and cortex after *in vivo* isoxsuprine pretreatment. Animals recieved 10 mg/kg isoxsuprine intraperitoneally for 8 - 10 days. Results are means ± S.E. of sextuplicate determinations for groups of 6 animals.

Fronto-Parietal Cortex Choline acetyltransferase activity
(nmoles/mg. tissue/hour)

| Controls | Isoxsuprine Pretreated | Choline Pretreated | Isoxsuprine + Choline Pretreatment |
|---|---|---|---|
| 5.26 ± 0.34 | 5.70 ± 0.34 | 5.01 ± 0.31 | 5.56 ± 0.31 |

Table 26. Choline acetyltransferase activity in parietal cortex after in vivo pretreatment with isoxsuprine and choline chloride, both singularly and in combination. Isoxsuprine preptreatment, 10 mg/kg Intraperitoneally for 10 days; choline pretreatment, 60 mg/kg Intraperitoneally for 10 days. Results are means ± S.E. fo triplicate determinations within 6 animals in each group.

that the regulation of the rate of synthesis of acetylcholine in response to altered demand is governed, at least in part, by the sodium dependent high-affinity uptake system for choline (HACU), and that this system is confined exclusively to cholinergic neurones (for reviews see, Kuhar and Murrin, 1978; Jope, 1979). Furthermore, in vivo changes in the rate of HACU are evident in in vitro synaptosomal fractions, thus, measurement of the rate of in vitro HACU can be used to investigate changes in in vivo cholinergic activity (Kuhar and Murrin, 1978). In making these measurements, however, uptake values have classically been expressed on the basis of tissue protein. Whilst such experiments yield results which appear to reflect cholinergic function, the amount of protein in the crude synaptosomal fraction, used for choline uptake, is not reliably related to the number of cholinergic synaptosomes within a given preparation, and reflects primarily noncholinergic synaptosomes, glial elements, and other subcellular organelles. A more rational system would be to calculate choline uptake values on the basis of a parameter that is related to the number of cholinergic synaptosomes present in an area, rather than on an independent variable, such as total tissue protein. Such a synaptosomal marker for cholinergic neurones is choline acetyltransferase since CAT activity in the CNS is confined exclusively to cholinergic neurones (for a review, see Rossier, 1977). The measurement of HACU on the basis of CAT activity has thus been suggested as a sensitive index of cholinergic function (Cooper and Schmidt, 1980).

The effects of isoxsuprine pretreatment on CAT activity and HACU were determined (see Table 27). Since isoxsuprine pretreatment has previously been shown not to alter CAT activity (see Tables 25 and 26) any change in the HACU:CAT ratio would reflect changes within the rate limiting uptake system.

Again however no significant effects of in vivo isoxsuprine pretreatment on either choline acetyltransferase activity, or HACU, or the ratio between these two variables were observed (Table 27).

| | Isoxsuprine | CONTROLS |
|---|---|---|
| High Affinity Choline Uptake fmols/mg tissue/minute | 78.14 ± 2.39 | 80.89 ± 3.70 |
| Choline acetyltransferase activity pmols/mg tissue/minute | 89.83 ± 5.17 | 90.50 ± 4.50 |
| HACU:CAT Ratio fmol choline taken up per pmol ACh synthesised | 0.87 ± 0.075 | 0.89 ± 0.079 |

Table 27. Choline acetyltransferase activity and high affinity choline uptake following in vivo pretreatment with isoxsuprine (0.2 mg. intraperitoneally, B.I.D., for 8 - 10 days). Results are means ± S.E. of quadruplicate determinations in groups of 6 animals.

# CHAPTER IV

# DISCUSSION

## IV. 1. Neurochemical studies after lesions of the nucleus basalis

On the basis that acetylcholinesterase (AChE)- rich axons projecting to the neocortex appeared to originate from the pallidum, Shute and Lewis (1967) originally proposed the existence of a cholinergic pallido-neocortical projection. This suggestion was subject to question, however, since it was well known at that time that AChE content alone was not a sufficient criterion to characterise a projection as cholinergic (Koelle, 1955). Furthermore, there was no anatomically recognised projection from the globus pallidus to the cortex. Indeed, several studies had shown that chronic isolation of the cortex produced no decrease in cortical choline acetyltransferase (McGeer et al., 1977; Ulmar, Ljungdahl and Hökfelt, 1975). Studies that had shown some decrease in cortical choline acetyltransferase (65% - 80% depletion) following similar operations (Hebb et al., 1963; Green et al., 1970a, b), have been explained on the basis of retrograde degeneration of cortical cholinergic perikarya (McGeer et al., 1977), or a "secondary effect of denervation" (Ulmar, Ljungdahl and Hökfelt, 1975). The globus pallidus was also known to have extremely low levels of AChE and choline acetyltransferase (McGeer et al., 1973). Yet in 1976, Kelly and Moore found that pallidal lesions did indeed result in substantial decreases of choline acetyltransferase in large areas of neocortex.

In reporting retrograde transport of HRP injected into the cortex, Divac (1975) speculated that what Shute and Lewis (1967) had identified as neurones in the globus pallidus were actually the rat's homologue of the primate nucleus basalis of the substantia innominata, which also projects to the neocortex (Kievet and Kuypers, 1975; Mesulam and van Hoesen, 1976; Jones et al., 1976; Parent and Butcher, 1976). Mesulam and van Hoesen (1976) demonstrated that in the primate, cortically injected HRP was transported to AChE-rich neurones of the nucleus basalis of the substantia innominata, and joined Divac (1975) in speculating that these neurones were the source of a cholinergic projection to the neocortex. The preliminary findings presented in this thesis, which utilise electrolytic lesions of the globus pallidus, confirm the original findings of Kelly and Moore (1976) (see Table 3), whilst the results obtained using neurotoxic amino acids substantiate the anatom-

ical localisation of the cholinergic perikarya whose axons innervate the neocortex (Lehmann et al., 1979, 1980). Indeed, the specific localisation of the cell group shown to innervate the parietal cortex (Area postcentralis caudalis, Krieg, 1946) by means of retrograde fluorescent traces and acetylcholinesterase histochemistry (Bigl et al., 1982), exactly matches the cell group localised in Figs. 34 and 36, and lesioned in these studies. A recent report, however, (Jerusalinsky et al., 1983) finds that lesions placed some distance below the ventral pallidum produce a significant decrease (53%) in cortical acetylcholinesterase. The discrepancy between these obviously different locations of the nucleus basalis cannot be reconciled with the findings presented in this study. The picture of a specific neurochemical deficit within the cerebral cortex following amino acid lesions of the ventral globus pallidus is in good agreement with the results of Johnston et al. (1979a, 1981). Whilst the neuroanatomical specificity of these lesions is in agreement with Hartgraves (1982), it is in disagreement with those of Burton et al. (1980). These authors suggest that the rat has no projection from the substantia innominata to the dorsal cortex, and that this finding is consistent with the reported absence of magnocellular cell groups in the substantia innominata of the rat (Burton and Fitzgerald, 1980). This report is clearly not in accord with the present findings, which demonstrate,

(i) a magnocellular cell group within the pallidal/innominata region (acetylcholinesterase histochemistry, Figs. 34 to 36),

(ii) the removal of which produces a selective decrease in presynaptic cholinergic markers within the ipsilateral parietal cortex (Tables 18, 19 and 20) and

(iii) perhaps most importantly (Doty, 1983), cholinergic neurones within the pallidal/innominata area undergo severe retrograde degeneration following neocortical lesions (Table 11, and see Lehmann et al., 1980).

The possible existence of cholinergic perikarya within the neocortex is in no way answered by the results obtained in this study (see Table 10). The existence of these neurones is suggested both by

nucleus basalis lesions (Johnston et al., 1979a, 1981) and by the use of methylazoxymethanol treatment of foetal rats (Johnston et al., 1979b, 1980). Their presence however cannot be confirmed either by direct injection of kainic acid into the neocortex (Lehmann and Fibiger, 1979) or by acetylcholinesterase histochemistry (Fibiger, 1982).

The difficulties encountered in interpreting these conflicting results are reviewed in detail by Fibiger (1982) and Lehmann and Fibiger (1979). They may in some part be resolved by the findings of Eckenstein and Thoenen (1983), who demonstrate the presence of cholinergic neurones within the neocortex using immunohistochemical methods, with a sensitivity greater than previously published reports (Sofroniew et al., 1982). The results presented in this study are in accord with these histological findings (Eckenstein and Thoenen, 1983) in that they suggest that intrinsic cortical cholinergic cells represent a very small percentage of the cortical cholinergic innervation.

When the distribution of cholinergic terminals within the cortex is studied by means of acetylcholinesterase histochemistry (see Figs. 37, 38, 39 and 40) a laminar pattern of staining is obtained with the densest staining in layers IV - VI. The cortical area studied in detail is parietal Area 2 (Krieg, 1946) - the area postcentralis caudalis - which 'dominates the parietal cortex and may be taken as the prototype of the parietal pattern' (Krieg, 1946). The cortical laminae are determined by comparison with the studies of Krieg (1946), Kristt (1979) and Morrison et al. (1983). The distribution pattern obtained for acetylcholinesterase is similar to that obtained by Beesley and Emson (1975), who also studied the distribution of choline acetyltransferase, and obtained a fairly even distribution throughout all cortical layers. The distribution of acetylcholinesterase in both control and deafferentated (cholinergic) cortex is very different from that obtained by Johnston, Young and Coyle (1981), who found an even distribution of the enzyme throughout all cortical layers, in both the control and denervated cortex. It is interesting to note that the cortical layers which show the greatest reduction in acetylcholinesterase staining, layers IV and VI contain the highest density of muscarinic cholinergic recep-

tors (Wamsley et al., 1980; Kuhar and Yamamura, 1976), whilst cortical layer V contains the lowest muscarinic receptor density of any cortical layer (Wamsley et al., 1980) but is high in nicotinic receptor binding sites (Emson and Lindvall, 1979) and appears to retain a band of high cholinesterase staining following lesions of the nucleus basalis (Figs. 39 and 40).

A similar dichotomy between intrinsic versus extrinsic cholinergic innervation exists for the amygdaloid complex. McCaughran et al. (1980) suggest on the basis of kainic acid lesions, that the amygdala contains of population of cholinergic interneurones. The original studies of Emson et al. (1979), followed by the elegant histochemical studies of Woolf and Butcher (1982) and Nagai et al. (1982), reviewed by Fibiger (1982), however, comprehensively describe the cholinergic innervation of the amygdala from neurones within the nucleus basalis/substantia innominata complex. The decreases in CAT and AChE (Fig. 27) within the amygdala following ibotenic acid lesions of the ventral globus pallidus are entirely consistent with these findings. The current understanding of the cholinergic innervation of the neocortex is best summarised in Figure 46, adapted from the review of Wenk et al. (1981) and exhibits a marked difference to that suggested by Karczmar in 1969 (Fig. 47).

Having discussed some of the initial experiments performed in this study, which utilise neurotoxic amino acid induced lesions, some comment on the nature of kainic acid and ibotenic acid neurotoxicity is required.

Since the introduction of kainic acid, a conformationally restricted analogue of glutamate, as a tool in neurobiology (McGeer et al., 1978; McGeer and McGeer, 1982), considerable discussion has surrounded some characteristics of the drug which are intimately involved in its specific lesioning qualities. Whilst some investigations (Meibach et al., 1978; Butcher and Rogers, 1978; Mason and Fibiger, 1979) have questioned the ability of kainic acid to specifically lesion cell bodies whilst sparing axons 'en passage', the evidence in support of a selective action is considerable (Peterson and Moore, 1980; Coyle et al., 1978; reviewed by McGeer, 1982). Some factors that

Figure 46. Schematic drawing of the cholinergic projections from the basal nucleus of Meynert to cortical regions in rats. Neurones of the NBM are indicated by black dots. Defined nuclei within this area are (1) nucleus tractus diagonalis of Broca, (2) nucleus basalis/ substantia innominata and (3) nucleus preopticus magnocellularis. Sm = medial septum, Am = amygdala and Hi = hippocampus, Diagram adapted from Wenk et al. (1981).

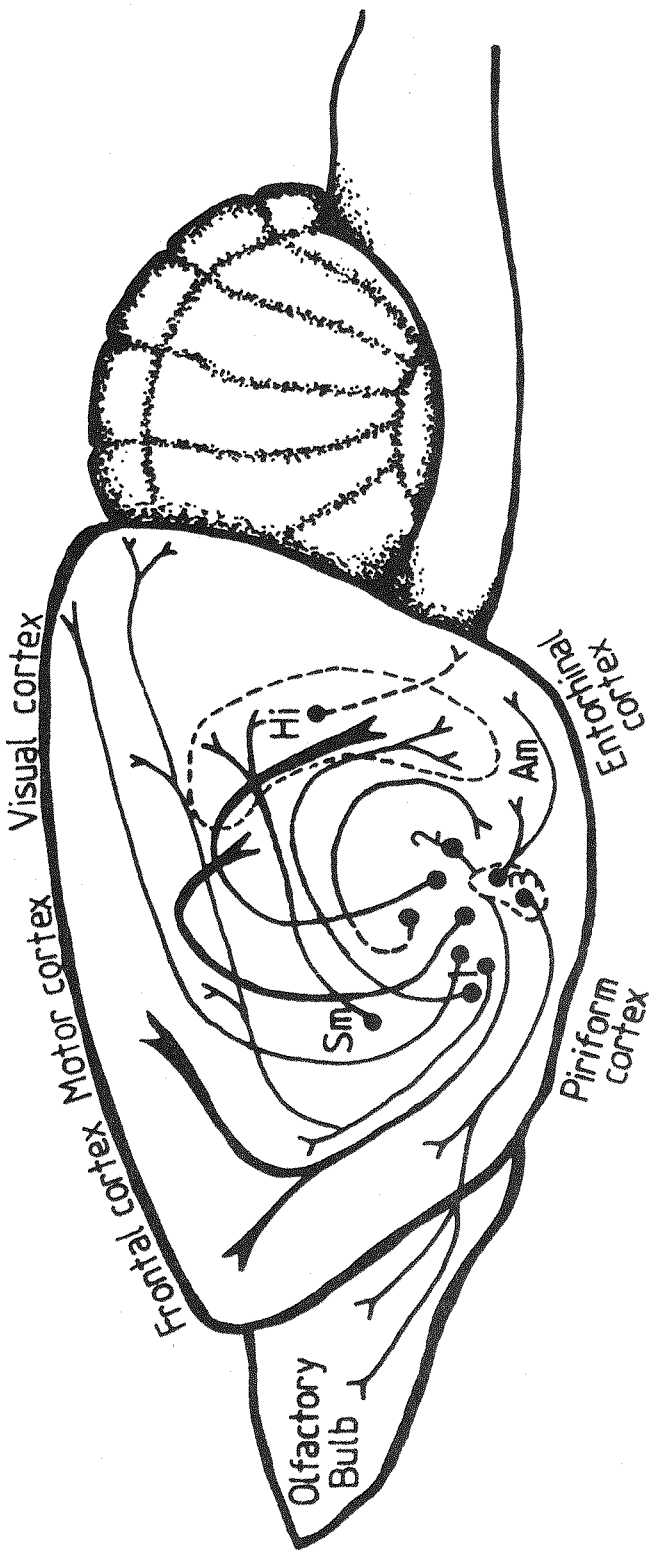
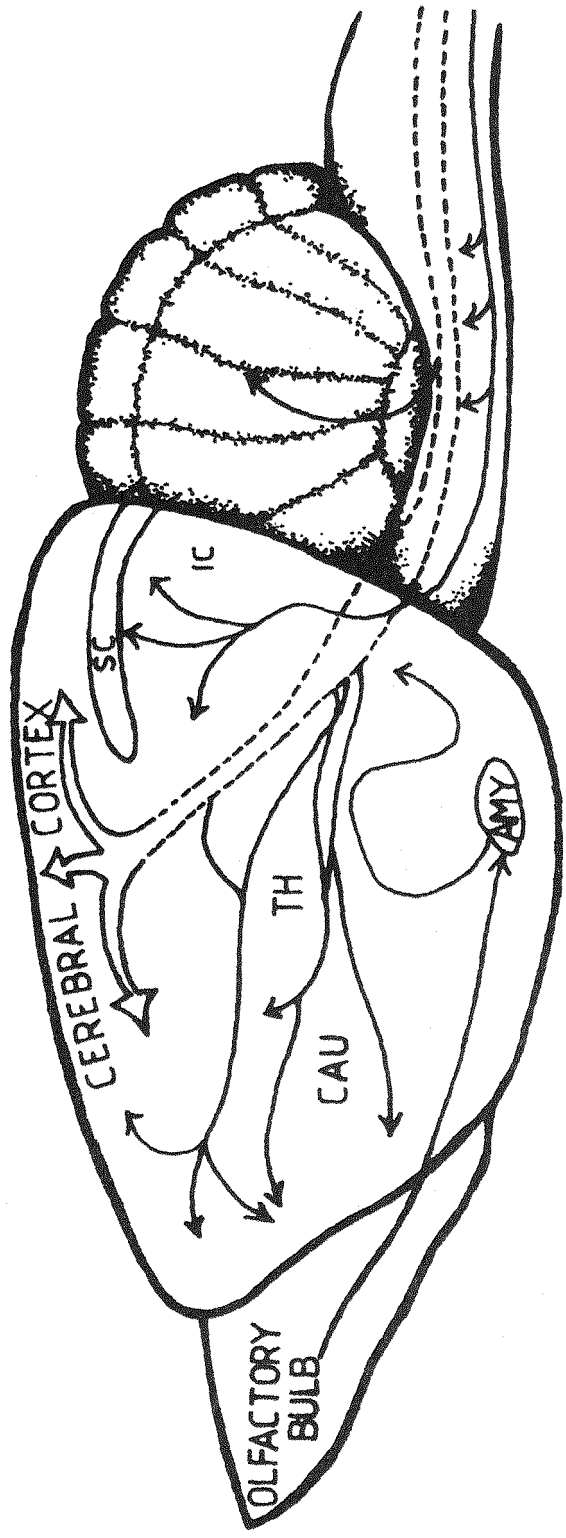

Frontal cortex
Motor cortex
Visual cortex
Hi
Am
Sm
Entorhinal cortex
Piriform cortex
Olfactory Bulb

Figure 47. Schematic drawing of the cholinergic projections to cortical regions. The main localisation of ascending cholinergic pathways was suggested by the distribution of acetylcholinesterase. CAU = caudate, AMY = amygdala, TH = thalamic nuclei, SC = superior colliculus, IC = inferior colliculus. Diagram adapted from Karczmar (1969).


CEREBRAL CORTEX
SC
IC
TH
CAU
AMY
OLFACTORY BULB

have been previously shown to affect the neurotoxicity of kainic acid are:- the dose, volume, infusion rate, and stability of the solution (McGeer and McGeer, 1978), the batch of kainic acid (Gottesfeld and Jacobowitz, 1979; Moore, 1982), the rat strain (Sanberg et al., 1979), and age (Gaddy et al., 1979) and the nature and amount of anaesthetic (Zaczek et al., 1978). These factors have been reviewed by Sanberg et al. (1981) who suggest that "those investigators that have questioned the specificity of kainic acid . . . . . have used from 4 to 15 times the dose (2.5 μg - 9.0 μg) of often unbuffered or un-neutralised kainic acid in at least twice the volume (1 μl) and with faster injection rates than those utilised in the present study".

In association with these methodological aspects relating to the specificity of kainic acid, two intrinsic properties of the drug severely hinder its use as a neurotoxin in neurochemical or neuroanatomical studies. These are firstly, the occurrence of neuronal loss distant from the injection site after intracerebral application (Wuerthele et al., 1978; Zaczek et al., 1980) and secondly, the powerful epileptogenic action of the drug (Ben-Ari et al., 1979; Pisa et al., 1980). It was recently reported that intracerebral injections of ibotenic acid, a naturally occurring excitatory amino acid (Eugester, 1968), can reproduce the specific axon-sparing local neuronal degeneration known from previous work with kainic acid (Schwarcz et al., 1979). It appeared, however, that ibotenic acid lesions were more circumscribed than those produced by kainic acid and that this compound was also far less convulsive than kainate (Aldinio et al., 1981). Experiments conducted _in vivo_ and _in vitro_ indicated that ibotenic acid's obvious practical advantages as a lesioning tool may be related to pronounced differences in the mechanism of action of ibotenic acid and kainic acid (Foster and Roberts, 1978; Köhler et al., 1979a, b; McLennan and Lodge, 1979; Nadler et al., 1978, 1981; Schwarcz and Fuxe, 1979; Schwarcz and Köhler 1980; Köhler and Schwarcz, 1983). The present study therefore concentrated on using ibotenic acid as the 'axon sparing neurotoxin', in preference to the more commonly used kainic acid. Several observations on the nature of the ibotenic acid induced degeneration of the ventral globus pallidus can be made,

(i) Cresyl violet/luxol fast blue histology reveals the circumscribed area of degeneration extending spherically from the tip of the injection cannula, a finding in concordance with that of Kohler and Schwarcz (1983). This is in contrast to the larger area of degeneration produced by kainic acid (see Figs. 21 and 22).

(ii) Ibotenic acid produces characteristic axon-sparing lesions. Neither catecholamine induced histofluorescence within the medial forebrain bundle (MFB) (Figs. 29, 30 and 31), nor noradrenaline levels and noradrenaline uptake (Table 9) within the ipsilateral neocortex are significantly reduced when compared with control, suggesting no damage to the fine unmyelinated fibres of the MFB passing through, or in close proximity to, the lesion site. This clearly differentiates this study from those of Kelly and Moore (1976), Wenk et al. (1980), Pedata et al. (1982) and Jerusalinsky et al. (1983), where electrolytic lesions of the nucleus basalis included severe damage to the ascending catecholamine fibres innervating the neocortex.

(iii) No evidence of neuronal loss distant to the site of injection was observed. GABAergic markers within the neocortex were unchanged (Figs. 18, 19, 20 and Table 7) as were cholinergic markers within the hippocampus (Fig. 25) following ibotenic acid injection into the ventral globus pallidus. Injection of ibotenic acid (also kainic acid and N-methyl-D-aspartic acid) into the medial septum, produced no changes in GAD activity within the hippocampus (see Table 16). This is taken to indicate the absence of distant lesions with all three amino acids, since the GABA containing cells of the hippocampal formation are amongst the most sensitive to "distant lesions produced by neurotoxic amino acids" (Malthe-Sørenssen et al., 1980).

The present study therefore supports the suggestion that ibotenic acid has several properties which render it a more selective lesioning agent than kainic acid (Kohler and Schwarcz, 1983). The conclusions are supported by the findings of Guldin and Markowitsch (1981), who report that intrastriatal injections of up to 100 µg ibotenic acid, produce no evidence of distant lesions, epileptiform activity or damage to 'axons of passage' through the lesion site.

The third neurotoxic acid used in this study N-methyl-DL-aspartic acid (and N-methyl-D-aspartic acid in later studies) has rarely been used as a neurotoxin previously (Olney, 1981; Zaczek et al., 1981), and was found to be considerably less potent than either kainic acid or ibotenic acid (Fig. 17), and more similar to ibotenic acid than kainic acid in its mode of action (Figs. 42 - 44). Whilst Zaczek et al. (1981) have reported that N-methyl-D-aspartic acid is a convulsant with weak neurotoxic properties, Olney (1981) has suggested that N-methyl-DL-aspartic acid will induce typical neurotoxin type lesions whenever it is introduced, but does not induce distant lesions nor does it produce the temporal lobe status epilepticus associated with kainic acid injection; findings more in accord with observations made in this study, than with those of Zaczek et al. (1981).

The use of several neurotoxic amino acids with differing properties and possibly, differing mechanisms of action (Köhler and Schwarcz, 1983) to produce lesions, may further our understanding of the synaptic connections within a given area. Malthe-Søressen et al. (1980) have suggested that the cholinergic cells within the medial septum seem to be resistant to kainic acid and that by inference this indicates that they are probably not innervated by glutamatergic fibres. This is consistent with the low activity for high affinity glutamate uptake within this region (Fonnum and Walaas, 1978; but see Fonnum et al., 1979). Cholinergic cell bodies within the diagonal band were lesioned by kainic acid suggesting a glutamatergic input onto these cells (Malthe-Sørenssen et al., 1980). The resistance of the cholinergic cells of the medial septum to the neurotoxic actions of kainic acid have been confirmed (Köhler and Schwarcz, 1983; Brunello and Cheney, 1981). Conversely, the cholinergic cells of the nucleus basalis/substantia innominata are particularly sensitive to kainic acid (McGeer et al., 1983) and it has been suggested that they receive a "massive glutamate input, perhaps as a feedback loop from cortical areas to which they project" (McGeer et al., 1983). Studies by Campochiaro and Coyle (1978) and Lehmann and Fibiger (1979) on the develomental profile of kainic acid neurotoxicity in the striatum have further supported the role for endogenous glutamate in kainic acid toxicity. Campochiaro and Coyle (1978)

reported that kainic acid injected into the striatum of 10-21 day old rats preferentially depletes choline acetyltransferase, compared to the GABAergic marker glutamic acid decarboxylase. This preferential depletion of choline acetyltransferase at early postnatal times has been explained by the earlier formation of contact between the cortico-striatal glutamatergic pathway with the cholinergic neurones, than that with other neurones within the striatum (Lehamnn and Fibiger, 1979).

The comparison of the neurotoxicity of the three previously studied amino acids (IBO, KA, NMDA) was therefore extended to include the cholinergic cells of the medial septum and the striatum (in intact and decorticate form). The neurotoxicity of these three amino acids within the medial septum is remarkably similar to that within the decorticate striatum (Figs. 43 and 44), whilst that within the nucleus basalis more closely resembles that seen within the intact striatum (Figs. 42 and 44). The independence of ibotenic acid and N-methyl-D-aspartic acid neurotoxicity within the striatum from an intact glutamatergic innervation has not previously been reported. The effects of removal of the glutamatergic innervation on the neurotoxicity of ibotenic acid in the hippocampus have, however, been investigated (Schwarcz et al., 1979; Köhler et al., 1979a, b), whilst the dependence of kainic acid neurotoxicity on this parameter is well established (Biziere and Coyle, 1979).

Injection of 1 μg kainic acid within the nucleus basalis was found to produce a 68.5% reduction in choline acetyltransferase within the cortex. The injection of 20 μg ibotenic acid produced a 55.7% reduction within the same area (Fig. 42). Thus injection of 24 times the dose (molar ratio) produced only 83% of the reduction seen with kainic acid. These results support the suggestion that the cholinergic cells of the magnocellular nuclei of the basal forebrain exhibit a differential sensitivity to kainic acid. The rostrally located cells of the medial septum are resistant to kainic acid whilst those of the nucleus basalis are exquisitely sensitive to this amino acid's neurotoxic effects. The suggestion that this sensitivity reflects differences in the glutamatergic innervation of these nuclei is also supported.

The other afferent connection of the nucleus basalis investigated in this study was the nucleus accumbens - ventral pallidal pathway. The existence of this pathway has previously been suggested by Williams et al. (1977) and Nauta et al. (1978) although these two studies differ from several others (Swanson and Cowan, 1975; Conrad and Pfaff, 1976; Powel and Leman, 1976) in the extent to which the nucleus accumbens projects to the ventral pallidum (Heimer and Wilson, 1975). The two studies using autoradiographic techniques suggest that the 'ventral pallidum' is the brain region within which accumbens efferents terminate in greatest density.

The transmitter within this pathway may be GABA (Walaas and Fonnum, 1979) but is believed not to be acetylcholine (Hartgraves et al., 1982). Immunohistochemical studies have indicated a heavy density of GAD-containing axosomatic and axodendritic synapses with neurones of the substantia innominata (Perez de la Mora et al., 1981). These GABAergic nerve endings do not appear to be from local interneurones (Perez de la Mora, 1981) but originate from cell bodies in the nucleus accumbens (Walaas and Fonnum, 1979). The results presented in this study confirm the presence of a nucleus accumbens - substantia innominata/nucleus basalis GABAergic pathway, using both retrograde and orthograde degeneration studies. The absence of any cholinergic link between these two nuclei is in accord with the observations of Hartgraves (1983). The recent findings of Wood and Richard (1982), studying turnover rates of acetylcholine in the cortex, support both the neurochemical and immunohistochemical evidence showing an inhibitory function for the accumbens - innominata pathway in the regulation of the substantia innominata - cortical cholinergic projection.

The changes in presynaptic cholinergic markers (HACU, CAT, AChE) seen after lesions of the nucleus basalis (Tables 18, 19 and 20) have recently been reported (Pedata et al. 1982; de Belleroche, 1983). The changes noted by Pedata (1982) follow electrolytic lesions of the magnocellular forebrain nuclei, which must involve damage to the fibre systems of the internal capsule and the median forebrain bundle. The results obtained for HACU and CAT are, however, extremely similar to those reported in this study (Tables 18 and 20). de Belle-

roche (1983) obtains similar findings for acetylcholinesterase within the neocortex following kainic acid lesions of the nucleus basalis (Table 19). The results of Jerusalinsky et al. (1983) are however somewhat at variance with those reported in this thesis. The location of their lesion site (as discussed previously) may help to explain these discrepancies. No differences in the extent of the decrease in CAT within the ipsilateral cortex was found between the rats killed 1 day, 10 days or 10 weeks after nucleus basalis lesion, an indication that no reparative process takes place in the cortical cholinergic fibres destroyed by the lesion. Therefore the recovery in ipsilateral HACU activity 10 days and 10 weeks after the lesion would conceivably be due to an increase in the metabolic and firing activity of the residual cholinergic neurones. 10 weeks after the lesions there is a moderate increase in CAT activity (16%, not significant by Student's t-test, Table 18) and in HACU (21%, $p < 0.01$, by Student's t-test, Table 20) within the contralateral cortex. This increase is similar to that found in the contralateral hippocampus after a septal lesion (Wieraszko et al., 1977) or in the contralateral cerebral cortex following lesions of the ascending noradrenergic innervation (Reis and Ross, 1973). Although a sprouting of ipsilateral cholinergic axons cannot be excluded (Lynch et al., 1972); the present results and those of Hartgraves (1982) suggest that there is no contralateral projection of cholinergic neurones within the nucleus basalis (see Table 5). The remaining possibility for both the changes seen in the ipsilateral and contralateral cortex, after lesions of the nucleus basalis, might be attributable to plastic changes within intrinsic cortical cholinergic neurones.

Functional recovery after denervating lesions in the central nervous system is particularly prominent if part of the lesioned projection is spared (Gage et al., 1983). The ascending cholinergic innervation of the neocortex must be a system in which functional recovery will be prominent following lesions. This is due to the extended neuroanatomical distribution of its cells of origin. Maximal decreases in cortical CAT activity after lesions of the nucleus basalis are 70 - 80% (Johnston et al., 1979b, 1981). A more detailed study of the adaptive changes occurring within the cortex, after lesions of

the ascending cholinergic innervation, might determine the extent to which collateral sprouting, hyperactivity of remaining axons and development of receptor supersensitivity contribute to functional recovery after subtotal lesions.

## IV.2. Pharmacological Investigations

Isoxsuprine is a compound with a structural resemblance not only to sympathomimetic amines, but also to compounds chemically related to the smooth muscle relaxant papaverine (Blicke, 1944) (For structure and pharmacological properties see Appendix I). Indeed it has been shown to exhibit direct β-adrenoceptor stimulant effects (Lish et al., 1960; Farrar and Verity, 1976) as well as a papaverine like action on smooth muscle (Lish et al., 1960). Isoxsuprine has also been shown to have α-adrenoceptor blocking actions (Lish et al., 1960; N. Horn, unpublished observations).

The β-adrenergic receptor stimulation produced by isoxsuprine has been shown to increase intracellular levels of cyclic adenosine monophosphate (c.AMP) both in vitro and in vivo (Amer et al., 1974). Both the stimulation of adenylate cyclase and the in vivo stimulation of c.AMP levels produced by isoxsuprine are blocked by a β-antagonist (Amer et al., 1974). Most of the β-adrenergic receptor agonists available are phenolic amine derivatives, which do not effectively reach the brain. However clenbuterol (4-amino-3,5-dichloro-2 (tertbutyl-amino-methyl)benzyl alcohol) is a very lipophilic and potent β-receptor agonist (Engelhardt, 1976; O'Donnell, 1976), which readily penetrates the blood brain barrier (Ross, 1980). Isoxsuprine is a potent β-adrenoceptor agonist with a high lipid solubility, thirteen times higher than that of clenbuterol (n-octanol: Buffer partition coefficients:- isoxsuprine, 56- clenbuterol, 4.3; salbutamol, 0.053; terbutaline, 0.025, measured at 37°C, pH 7.4). Den Hertog (1976) has shown that isoxsuprine has significant local anaesthetic action, and that this action is more pronounced than that of lidocaine. Indications of its central nervous system activity are its ability to reverse reserpine induced hypothermia (where it is as potent as clenbuterol, S. Davies, unpublished observations) and its ability to inhibit spontan-

eous locomotor activity (S. Davies, unpublished observations). Quach et al. (1978) have shown that noradrenaline stimulates glycogenolysis in mouse brain cortical slices, and that this response is mediated via β rather than α-adrenoceptors. These results confirm previous investigations linking cerebral β-receptor activation with increasing cyclic nucleotide levels, and increasing rates of glycogenolysis (Nahorski et al., 1975; Nahorski and Rogers, 1975; Edwards et al., 1974). The ability of noradrenaline to stimulate cerebral glycogenolysis is found throughout the rodent cerebral cortex (Morrison and Magistretti, 1983). The observed increases in cerebral oxygen consumption following chronic isoxsuprine pretreatment (see Table 22) are therefore consistent with isoxsuprine's action as a potent centrally acting β-adrenoceptor agonist. The failure of in vitro isoxsuprine to stimulate oxygen uptake may be due to the duration of the pre-incubation period which is known to be critical (Quach et al., 1978) in allowing the post-mortem rise in c.AMP levels to disappear (Edwards et al., 1974).

Several recent publications have advocated the use of either intraperitoneal (i.p.) (Meibach et al., 1980) or subcutaneous (s.c.) (Delanoy and Dunn, 1978; Reinstein et al., 1979) administration of [$^3$H]2-deoxyglucose as an improvement on the original intravenous [$^{14}$C] method of Sokoloff (Savaki, 1980). The use of both these techniques in this study, however, produced considerable variation in [$^3$H]2-deoxyglucose uptake between individual animals. The quantitative assessment of changes due to drug treatment (which produced a 30% increase in oxygen consumption) is therefore likely to be extremely difficult by this method. Reported changes in [$^3$H]-2-deoxyglucose uptake using these methods, following severe surgical lesions in cortical and hippocampal areas produced only marginal changes (Reinstein et al., 1979). The major practical difficulty inherent in these methods is likely to be variability in activity of the injected bolus of radioactivity (Meibach et al., 1980). The use of chronic indwelling venous cannulae, in conscious animals with minimal physical restraint, was therefore employed. The advantages of this method are:-

i) the bolus of isotope is injected directly into the circulation, differences in absorption rates from peripheral injection sites are therefore removed, and

ii) the measurement of plasma [$^3$H] levels provides a means of standardising data within groups of animals.

Despite these modifications, [$^3$H] -2-deoxyglucose uptake values between animals showed significant variation. Although many studies employing the 2-deoxyglucose technique attempt quantification of their data on the basis of deistrometry alone, or from liquid scintillation counting of dissected regions, it would appear from the operational equation that these methods cannot be employed for comparing 2-deoxyglucose incorporation among different animals. This is due to that fact that plasma glucose levels play a significant role in determining the $K_1$, $K_2$ and $K_3$ rate constants (Savaki et al., 1980). Therefore since plasma glucose levels probably vary among animals (especially under experimental conditions) it would be incorrect to assume that the differences seen either in the autoradiograms or in the counts are relative to actual differences in 2-deoxyglucose incorporation. Whilst several investigators have not found any significant variation in any structure among the groups of animals studied, suggesting that possible variations in plasma glucose levels do not measurably alter 2-deoxyglucose incorporation (Glick et al., 1979; Meibach et al., 1979, 1980) the present study did observe variation between animals in both groups. Meibach et al. (1979, 1980) did, however, observe differences in actual counts, although these were always found to be attributable to variability of activity in the injected bolus of isotope (Meibach et al., 1980).

The intravenous method of [$^3$H] -2-deoxyglucose administration produces results consistent with published results following either amphetamine pretreatment (Wechsler et al., 1979) or pentobarbital pretreatment (Sokoloff et al., 1977) (see Table 24). The results for the isoxsuprine pretreated animals show very little difference from sham injected controls (see Fig. 23). Any differences that are apparent are small reductions in the pretreated group. These cannot be explained in relation to the increases seen in the <u>in vitro</u> uptake studies,

and are more consistent with the suggestion that isoxsuprine is a more powerful peripheral than cerebral vasodilator and may decrease cerebral blood flow (Fazekas and Alman, 1964).

A number of studies indicate that graded impairments of cerebral carbohydrate metabolism impair the synthesis of a number of neurotransmitters, including particularly acetylcholine (Blass and Gibson, 1979a, b). Thus acetylcholine synthesis has been demonstrated to be tightly linked to carbohydrate utilisation both in vitro and in vivo (Blass et al., 1979). Despite this evidence for a direct relationship between decreased carbohydrate metabolism and decreased acetylcholine synthesis, no study, as yet, has demonstrated an increased rate of carbohydrate metabolism leading to an increased synthesis of acetylcholine (Blass et al., 1979). When evaluating reports of biochemical studies on acetylcholine synthesis in the CNS, it is worth noting that the majority of studies concentrate on either whole brain or striatal, slices or homogenates. Wecker and Dettbarn (1979) recently demonstrated that there may be differences between acetylcholine synthesis in the striatum, and in the cerebral cortex and hippocampus. A similar difference exists for the effects of pentobarbitone on high affinity choline uptake (Simon et al., 1976) and acetylcholine turnover (Trabucchi et al., 1975) within these areas. Gibson and Peterson (1983) studied acetylcholine synthesis within the medial septum and the hippocampus (the cell bodies and terminals of the septo-hippocampal cholinergic pathway, respectively). They present several interesting differences between these two areas:-

1. Anoxia (0% $O_2$) inhibits acetylcholine synthesis (-77%) and its $Ca^{2+}$ dependent release (-87%) from hippocampal slices, but has no effect on synthesis or release in septal slices.
2. Citrate incorporation into acetylcholine was higher in septum then in hippocampus, suggesting that citrate metabolism differs regionally.
3. (-) hydroxycitrate reduced acetylcholine synthesis in septum more than in hippocampus.
4. $^{14}CO_2$ production from glucose or citrate was similar in control and experimental conditions in the two regions.

5. U-$^{14}$C glucose incorporation into acetylcholine was 1.4 times higher in hippocampus than in septum, although $^{14}CO_2$ production was similar. Release of [$^{14}$C] acetylcholine was 8.3 times greater from hippocampus than from septum.

The conclusions drawn from these studies, coupled with the experimental results obtained with oxygen uptake after isoxsuprine pretreatment, prompted the studies reported in this thesis. The effects of isoxsuprine on CAT activity, and high affinity choline uptake, and the ratio between these two variables were studied. Isoxsuprine pretreatment was found to be without effect on any of these parameters, either alone or in combination with choline (choline and isoxsuprine) (Tables 25, 26 and 27). The combination of choline with a pharmacological agent capable of increasing acetyl CoA availability has been suggested as a novel rationale for the treatment of cholinergic dysfunction (Bartus et al., 1982). Equivocal results have been obtained with choline precursor treatment alone (Jenden, 1979) prompting the suggestion that choline might function directly as a cholinergic agonist (Jenden, 1979; Krnjevic et al., 1979; Ladinsky et al., 1979). The lack of effect of isoxsuprine on CAT activity is not unexpected, since CAT is not the rate limiting step in acetylcholine synthesis (Jope, 1979). The lack of effect of this drug on HACU or HACU:CAT ratio (a more sensitive index of cholinergic function, Cooper and Schmidt, 1980) is, however, more surprising (Table 27).

Sodium-dependent high affinity choline uptake has been reported to be decreased approximately 20% under basal conditions in the hippocampus of aged rats (Sherman et al., 1981). No age related differences were observed in either choline or acetylcholine levels, or in choline acetyltransferase (Sherman et al., 1981; Bartus et al., 1982). The possibility that isoxsuprine would stimulate acetylcholine synthesis in a partially denervated system (following nucleus basalis lesions) remains an interesting area for future research.

## IV. 3. The relevance of the foregoing animal studies to Alzheimer's dementia

One of the most consistent neurochemical findings in the aged human brain is that the activity of choline acetyltransferase is markedly reduced in the brains of Alzheimer's disease patients, when compared with age-matched controls (Davies and Maloney, 1976; Perry et al., 1977; Davies, 1978; Yates et al., 1979; Bowen and Davison, 1980; Rossor et al., 1980; Sims et al., 1980; Davies and Feisullin, 1981; Perry et al., 1981; Rossor et al., 1981). Because CAT is far from saturated under normal circumstances (Haubrich and Chippendale, 1977), the functional relevance of these decreases in Alzheimer's disease has been questioned. Acetylcholine synthesis in biopsy samples from Alzheimer's patients, however, has been reported to be less than that in samples from age matched controls (Sims et al., 1981; Bowen et al., 1982; Sims et al., 1983). Indeed the decreases in choline acetyltransferase seen in the Alzheimer brain are not ubiquitous but are restricted to the cerebral cortex, hippocampus and amygdala (Rossor et al., 1980; Perry et al., 1980). Several groups have found no significant decreases in CAT within the caudate nucleus (Bowen et al., 1976; Perry et al., 1977; Davies et al., 1978; Rossor et al., 1980) or globus pallidus (Rossor et al., 1982) in Alzheimer's autopsy brain. Initially the possibility was entertained that neocortical cholinergic innervation might be intrinsic; that it could originate within the cortex itself. This possibility was especially attractive, with respect to Alzheimer's disease, a condition in which neocortical neurones are consistently involved. However this hypothesis about the origin of cortical cholinergic innervation had to be modified substantially in view of two observations. Firstly, damage to subcortical neuronal populations produced a greater loss of choline acetyltransferase than lesions which destroyed cortical neurones (Johnston et al., 1979, 1980; Lehmann et al., 1980; Wenk et al., 1980; and results presented in this thesis) for studies in rodents, and in primates by either anterograde degeneration (Struble et al., 1982) or retrograde degeneration studies (Pearson et al., 1983). Secondly, immunohistochemical observations indicated that cortical neurones do not

appear to contain appreciable amounts of CAT, so that they could not be expected to give rise to cholinergic exons (Kimura et al., 1981; Mesulam et al., 1983). These observations are consistent with the now widely accepted conclusion that the cholinergic innervation of the amygdala, hippocampus and cerebral cortex originate predominantly outside these structures, from cellular groups within the basal forebrain (Mesulam et al., 1983). Since quantitative cell counts in the neocortex of patients with Alzheimer's disease have not demonstrated major reductions in numbers of neurones (Terry and Davies, 1980), the loss of presynaptic cholinergic markers may result from losses in these basal forebrain neurones. A severe loss of neurones from the nucleus basalis of Meynert has been reported in Alzheimer autopsy brain (Whitehouse et al., 1981, 1982; Wilcock et al., 1983; Tagliavini and Pilleri, 1983; Price et al., 1982), although these cell losses have been questioned (Perry et al., 1982; Candy et al., 1983). Nagai et al. (1983) have recently reported a loss of immunohistochemically identified cholinergic neurones from the nucleus basalis of Meynert, in Alzheimer's autopsy brain. There was no loss of choline acetyltransferase containing cells from the putamen. The use of a specific antibody to choline acetyltransferase for identification of cholinergic neurones within the nucleus basalis, overcomes several of the problems inherent in morphometric analysis of cell numbers. Both acetylcholinesterase and choline acetyltransferase have been shown to be decreased within the nucleus basalis of Meynert in Alzheimer autopsy brain (Rossor et al., 1982; Candy et al., 1983) although these results have similarly been challenged (Henke and Lang, 1983). The losses of choline acetyltransferase from the neocortex have been correlated with the number of cortical plaques (Perry et al., 1978), the number of neurofibrillary tangles (Wilcock et al., 1982) and the extent of the dementia as assessed by mental test score (Perry et al., 1978; Wilcock et al., 1982, 1983). The clear correlation between neuritic plaques and clinical dementia, and the inverse correlation between plaque density and cholinergic deficit, prompted studies into the relationships between neuritic plaques and the cholinergic system (Perry et al., 1978; Struble et al., 1982; Price et al., 1982). Price

et al. (1982) have demonstrated the presence of acetylcholinesterase rich neurites within neuritic plaques in aged rhesus monkey brain. Whilst this is no proof for the inclusion of dystrophic cholinergic terminals in Alzheimer's plaques, it does indicate that cholinergic terminals do participate in the formation of neuritic plaques within ageing brain. Accumulating evidence therefore suggests a significant role for acetylcholine containing neurones in Alzheimer's disease. More recent evidence strongly suggests a specific involvement of ascending cholinergic projections from the basal forebrain to the neocortex, hippocampus and amygdala; areas exhibiting the highest extent of pathological abnormalities (Price et al., 1982). A considerable number of studies have concentrated on investigating other neurotransmitter candidates in Alzheimer's dementia. Somatostatin immunoreactivity and markers for ascending noradrenergic innervation are reduced, but not to the extent of CAT and AChE activities (Davies et al., 1980; Davies and Terry, 1981; Rossor et al., 1980; Cross et al., 1981; Perry et al., 1981). It has been suggested that both the decreases in noradrenergic (dopamine-β-hydroxylase) and somatostatin immunoreactive markers in Alzheimer's dementia are secondary to the cholinergic abnormality (Perry et al., 1981; Perry and Perry, 1982). In contrast, muscarinic cholinergic receptor binding sites, concentrations of GABA, the activity of the GABA synthesising enzyme, glutamic acid decarboxylase (GAD) and levels of the neuropeptides, cholecystokinin (CCK), vasoactive intestinal peptide (VIP), methionine enkephalin, thyroid releasing hormone (TRH), substance P, angiotensin converting enzyme, and neurotensin are not significantly decreased in Alzheimer's disease (Bowen et al., 1976; Perry et al., 1977; White et al., 1977; Davies and Verth, 1978; Reisine et al., 1978; Perry et al., 1978; Davies, 1979; Rossor et al., 1980; Perry et al., 1981; Rossor et al., 1982; Perry and Perry, 1982; Arregui et al., 1982). In summary, these neurochemical studies suggest that while there are modest changes in somatostatin and noradrenergic systems, the most consistent and severe neurochemical deficiency in Alzheimer's disease is a reduction in cholinergic markers within the cortex.

In view of this apparently straightforward neurochemical pathology forming the primary deficit in Alzheimer's dementia, an animal model involving degeneration of ascending cholinergic pathways appeared feasible. A model based on the use of neurotoxic amino acids to lesion the nucleus basalis has recently been proposed (Coyle, 1982; Price et al., 1982; de Belleroche, 1983). The results presented in this study for the neurochemical studies following amino acid lesions of the ventral globus pallidus (nucleus basalis) suggest that this animal model displays a similar specificity for the cholinergic system as that currently believed to occur in Alzheimer's dementia. The anatomical selectivity for ascending cholinergic projections is again compatible with current post-mortem studies on Alzheimer brain. Comparison of Alzheimer's dementia with the proposed animal model highlights several areas of interest.

Rats lesioned within the nucleus basalis either unilaterally or bilaterally fail to eat or drink for up to three days after lesioning. If these animals are not tube fed for this period they rapidly lo se weight and eventually die. The failure of these animals to eat normally has been related to deficits in oro-ingestive behaviour caused by globus pallidus lesions (Labuszewski et al., 1981). Recent investigations, however, have suggested that cholinergic neurones of the nucleus basalis project to the lateral hypothalamus (Troiano and Siegel, 1978; Haring and Davis, 1983). An intriguing observation that implies the interaction of the nucleus basalis with the lateral hypothalamus is that dysphagia is observed in Alzheimer's disease (a characteristic neuropathological change in Alzheimer's disease is loss of cells from the cerebellar vermis, which is invariably found in a variety of malnutrition states). Although this observation may be merely coincidental the correlation of the loss of cholinergic nucleus basalis neurones with the apparent dysfunction of the lateral hypothalamus, recently reported as having a potential influence on food intake behaviours (Rolls et al., 1976; Rolls, 1983), provides at least anecdotal evidence linking the functions of the nucleus basalis with those of the lateral hypothalamus. This hypothesis of a nucleus basalis-lateral hypothalamus interaction in food intake behaviour is directly supported by

the observation that substantia innominata and lateral hypothalamus neurones fire in association with the sight of food (Rolls et al., 1977; De Long, 1971). A possible behavioural analogy between Alzheimer's dementia and the proposed 'animal model' is therefore apparent. This behavioural characteristic of lesions of the nucleus basalis may be easier to study than measures of memory, memory retrieval or cognitive impairment.

In an analysis of choline acetyltransferase activity in the hippocampus of Alzheimer post-mortem brain, Rossor et al. (1980) have described a differential regional loss of enzyme activity. They describe a significant loss of CAT from the posterior hippocampus (46% of control values, $p < 0.01$) with an insignificant loss from the anterior hippocampus (87% of control values). The absence of change in anterior hippocampal CAT with reduced activity in posterior hippocampus may relate to previous reports that the posterior hippocampus shows more profound histological changes in SDAT than the anterior hippocampus (Ball, 1977, 1978). Analysis of neuroanatomical studies in the rat suggest that there is a crude topographical projection of neurones within the medial septal complex (Lynch et al., 1978; Meibach and Siegal, 1977). The cholinergic cells of the medial septum proper project predominantly to the anterio (dorsal) hippocampus. The cells of the more ventero-laterally located nucleus of the diagonal band project to the posterior (ventral) hippocampus (Meibach and Siegal, 1977). Differences between the properties of the cholinergic cells of the medial septum and the diagonal band have previously been commented upon (see page 152).

The preferential degeneration of diagonal band cholinergic cells in Alzheimer's dementia suggests that is is the 'kainic acid sensitive' cells of this area which degenerate. It is these cells which are believed to receive a glutamatergic innervation (Malthe-Sørenssen et al., 1980) as are the cells of the nucleus basalis (McGeer et al., 1983). These observations may have relevance to the role of endogenous excitotoxins in Alzheimer's dementia (Coyle, 1982; Schwarcz and Kohler, 1983).

The adaptive mechanisms seen within presynaptic cholinergic function following lesions of the nucleus basalis are highly reminiscent of those seen both within idiopathic Parkinson's disease and its respective experimental animal model. These changes have been reviewed extensively by Schultz (1982). It is generally accepted that a depletion of striatal dopamine is a pathological substrate of idiopathic Parkinson's disease. Furthermore, an experimental depletion of dopamine in the striatum of mammals is widely regarded as a valid model of Parkinsonism. The immediate neurochemical effect of a lesion of the nigrostriatal dopamine system consists of an increase in dopamine synthesis. This is probably due to decreased end-product inhibition of the synthetic enzyme tyrosine hydroxylase, after the arrest of impulse flow. Increased amounts of dopamine enter the extraneuronal space while the membranes disintegrate during the death of neurones. Two or three days after the lesion the dopamine terminals, and dopamine itself, disappear from the striatum and the degeneration has reached its final state. Striatal dopamine content never recovers afterwards.

Shortly after the lesion, probably during the actual degeneration, compensatory reactions begin to occur. Even with minor striatal dopamine depletions nigro-striatal dopamine neurones increase the metabolic activity in their terminals, as evidenced by increased synthesis,metabolism and turnover (Schultz, 1982). Increased dopamine turnover within the Parkinsonsian striatum is marked by a significant increase in the homovanillic acid to dopamine (HVA:DA) ratio (Hornykiewicz, 1983). Although a similar process appears to occur in the cerebral cortex following lesions of the nucleus basalis in the rat, no evidence exists for its occurrence in the Alzheimer brain. In a recent study, however, Sims et al. (1983) have shown a smaller decrease in high affinity choline uptake (43%) than in choline acetyltransferase activity (57%). Since choline acetyltransferase is not the rate limiting step in acetylcholine synthesis (Haubrich and Chippendale, 1977), whilst high affinity choline uptake might well be (Jope, 1979), this may indicate some increase in choline uptake within the remaining neurones. Further studies are obviously required both in the animal

model and the Alzheimer brain before any confident correlation between Alzheimer and Parkinsonian adaptation can be made.

As previously discussed (Section IV.2) a clear correlation between decreased carbohydrate utilisation and acetylcholine synthesis clearly exists both in vitro and in vivo (Blass et al., 1980) in animal studies. In the Alzheimer brain, however, a decrease in acetylcholine synthesis has been demonstrated without a decrease in glucose utilisation (Sims et al., 1980). Decreased cortical metabolic activity may well be the consequence rather than the cause of a decreased cholinergic innervation (see Fig. 41, where nucleus basalis lesions produce a decrease in [$^3$H]-2-deoxyglucose uptake in parietal cortex). Walker et al. (1983) have recently commented on the abundant cytoplasm of nucleus basalis cells 'teeming' with organelles. This agrees well with enzyme histochemical studies that have shown that the nucleus basalis is characterised by a high metabolic rate (Iijima et al., 1968). The marked proliferation of lipofuscin seen in nucleus basalis neurones may be related to their high level of oxidative metabolism (Friede, 1962). Briggs (1980) has studied the actions of several drugs reputed to have effects on glucose metabolism on the flux of glucose into acetylcholine, using the methods of Sims et al. (1980). The drug meclofenoxate was found to increase both acetylcholine synthesis and $CO_2$ production. Unfortunately the actions of the drug in vitro at only one concentration (500 μmol) were studied. Attempts to increase acetylcholine synthesis using choline precursor therapy have proved singularly unsuccessful (reviewed by McGeer, 1981; Bartus et al., 1982). This is in marked contrast to the effectiveness of Dopa precursor therapy in Parkinson's disease (Hefti and Melamed, 1980).

There are at least two theoretical problems with attempts to promote cholinergic activity with agents such as choline or lecithin:-

1. There is very little evidence that supplements of choline or its analogues can raise acetylcholine levels in the brain of animals on a normal diet (Wecker and Schmidt, 1980). Dopa supplements on the other hand, will raise brain dopamine levels many-fold (Wada et al., 1966).

2. DOPA can be converted to dopamine by an enzyme which is not specific to dopaminergic neurones and is generally present in excess; the conversion of choline to acetylcholine, on the other hand, is dependent on an enzyme specific to cholinergic neurones and present in relatively small amounts.

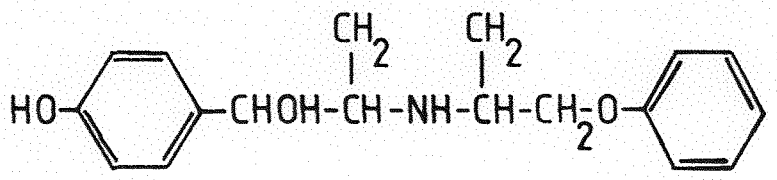
Some have argued that supplementation with choline may be effective only in certain areas and particularly when cholinergic systems are defective (Wurtman, 1979), but the rationale seems weak and clinical trials of choline and its analogues have not so far proven very effective in Alzheimer's disease (Christie et al., 1979; Etienne et al., 1979).

The finding of a large loss of cholinergic terminals in Alzheimer's brain with the remaining terminals showing an essentially normal response to $K^+$ depolarisation suggests that propogation of nerve impulses via these terminals will be little assisted by increased production of transmitter (Sims et al., 1983). Thus, major improvement would probably be achieved only if excess acetylcholine could interact with receptor sites normally associated with terminals which had become nonfunctional. The likelihood of this would depend on a variety of factors including the distribution of receptor sites, the activity of acetyl cholinesterase, the amounts of excess transmitter produced, and the rate of diffusion of this transmitter. Moreover, even if impulse generation were achieved at postsynaptic sites lacking appropriate inputs, this would occur at the expense of some specificity, which is likely to be important in the complex higher mental functions affected in dementia.

The development of the proposed animal model of Alzheimer's dementia is an attempt to provide a means of investigating some of these possibilities.

APPENDIX I

## ISOXSUPRINE



1-(p-hydroxyphenyl)-2-(1'-methyl-2'-phenoxyethylamino) propranol-1-hydrochloride.

I. β-Adrenoceptor Agonist Activity

Lish, P.M., Dungan, K.W. and Peters, E.L., (1960)

Farrar, D.G. and Verity, A., (1976)

*Ariens, E.J., Waelen, M.J.G.A., Sonneville, P.F. and Simonis, A.M., (1963).

$PD_2$ value on isolated tracheal muscle of the calf; 6.2 ± 0.3

II. α-Adrenoceptor Antagonist Activity.

Lish et al., (1960).

*Ariens et al., (1963).

$PA_2$ value on isolated vas deferens of the rat; 5.9 ± 0.4.

III. "Papaverine like" direct smooth muscle relaxant activity

Lish et al., (1960).

*Ariens et al., (1963).

$PD_2$ value on isolated vas deferens of the rat; $3.8 \pm 0.2$.

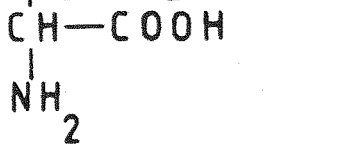
IV. Local Anaesthetic Activity.

Den Hertog (1976).

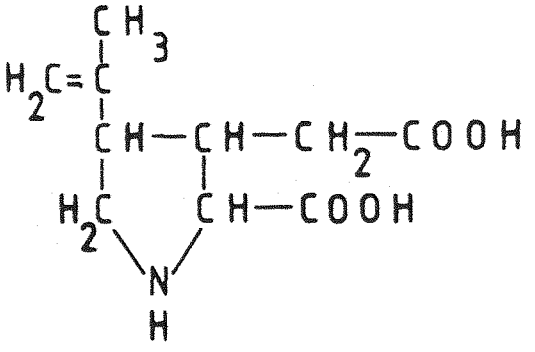
*Ariens, E.J., Waelen, M.J.G.A., Sonnerville, P.F. and Simonis, A.M., (1963).
The pharmacology of catecholamines and their derivatives.
Part I: Relationship between structure and activity especially as far as the vascular system is concerned.
Arznien Forsch., 13, 541 - 546.

## APPENDIX II

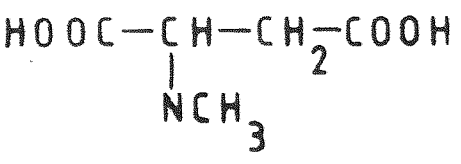
The structures of the neurotoxic amino acids kainate, ibotenate and N-methyl-DL-aspartate with the related putative endogenous neurotransmitters L-glutamate and L-aspartate.



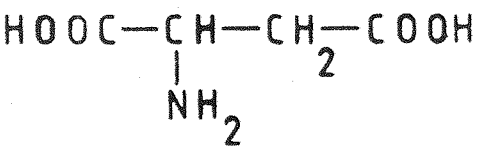
GLUTAMIC ACID



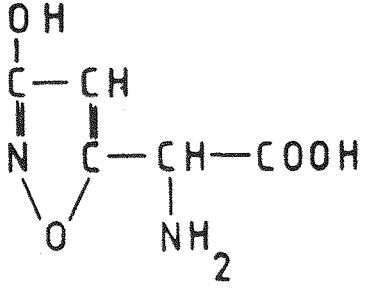
KAINIC ACID



N-METHYL ASPARTIC ACID



ASPARTIC ACID



IBOTENIC ACID

# REFERENCES

Aldinio, C., French, E.D. and Schwarcz, R., (1981).
Seizures and neuronal degeneration: relationships investigated by intra-hippocampal kainic and ibotenic acid.
Soc. Neuroscience Abstracts, 7, 589.

Amer, M., Dungan, K.W. and McKinney, G.R., (1974).
The effects of isoxsuprine hydrochloride on the cyclic nucleotide system in the rat uterus.
J. Pharm. Exp. Ther. 190(2), 243-248.

Ball, M.J. (1977).
Histopathology of cellular changes in Alzheimer's disease.
In: Senile dementia: a biomedical approach.
Eds: Nandy, K., Elsevier, North Holland, 89-105.

Ball, M.J., (1977).
Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia.
Acta. Neuropahtol. (Berl.) 37, 111-118.

Ball, M.J., (1978).
Topographic distribution of neurofibrillary tangles and granulovacuolar degeneration in hippocampal cortex of ageing and demented patients. A quantiative study.
Acta. Neuropathol. (Berl.) 42, 73-80.

Barbeau, A., (1973).
Biochemistry of Huntington's chorea.
Adv. Neurol. 1, 473.

Barbeau, A., (1973).
The Biochemistry of Huntington's Chorea.
Psychiatr. Forum. 3, 8-12.

Bartus, R.T., Dean, R.L., Beer, B. and Lippa, A.S., (1982).
The cholinergic hypothesis of geriatric memory dysfunction.
Science. 217, 408-417.

Beesley, P.W. and Emson, P.C., (1975).
Distribution of transmitter-related enzymes in the rat sensorimotor cortex.
Biochem. Soc. Trans. 3, 936-939.

Ben-Ari, Y., Lagowska, J., Tremblay, E. and Le Gal Le Salle, G. (1979).

A new model of focal status epilepticus: intra-amygdaloid application of kainic acid elicits repetitive secondarily generalised convulsive seizures.

Brain Res. 163, 176 - 180.

Bigl. V., Woolf, N.J. and Butcher, L.L. (1982).

Cholinergic projections from the basal forebrain to frontal parietal, temporal, occipital, and cingulate cortices: A combined fluorescent tracer and acetylcholinesterase analysis.

Brain Research Bulletin. 8, 727 - 749.

Biziere, K. and Coyle, J.T. (1978).

Influence of corticostriatal afferents on striatal kainic acid neurotoxicity.

Neuroscience Letters, 8, 303 - 310.

Biziere, K. and Coyle, J.T., (1979).

Effects of cortical ablation on the neurotoxicity and receptor binding of kainic acid in striatum.

J. Neuroscience Research. 4, 383 - 398.

Björklund, A., Falck, B. and Lindvall, O., (1975).

Microspectrofluorometric analysis of cellular monoamines after formaldehyde or glyoxylic acid condensation.

In: Methods in Brain Research, (ed. Bradley, P.B.), pp. 249 - 294. New York, London, Wiley.

Blass, J.P. and Gibson, G.E., (1979).

Consequences of mild, graded hypoxia.

Adv. Neurol. 26, 229 - 253.

Blass, J.P. and Gibson, G.E., 1979).

Carbohydrates and acetylcholine synthesis: implications for cognitive disorders.

In: Brain acetylcholine and neuropsychiatric disease.

Eds. Davis, K.L. and Berger, P.A.

Plennum Press, New York, pp. 215 - 236.

Blass, J.P., Gibson, G.E., Shimada, M., Kihara, T., Watanabe, M., and Kurinioto, K., (1980).

Brain carbohydrate metabolism and dementias.

In: Biochemistry of dementia. Ed. Roberts, P.J.

J. Wiley

Blessed, G., Tomlinson, B.E. and Roth, M. (1968).

The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects.

Brit. J. Psychiat., 114, 797 - 811.

Blicke, F.F., (1944).

Synthetic drugs - antispasmodics.

Ann. Review Biochem., 13, 549 - 574.

Blocq, P. and Marinesco, G. (1893).

Sur un cas de tremblement parkinsonien hemipleqique. Symptomatique d'une tremeur du pedoncule cerebral.

C.R. Soc. Biol. (Paris)., 5, 105 - 111.

Bowen, D.M., (1980).

Biochemical evidence for nerve cell changes in senile dementia.

In: Ageing of the Brain and Dementia. (Aging Volume 13).

Ed. Amaducci, L. et al., Raven Press.

Bowen, D.M., Benton, J.S., Spillane, J.A., Smith, C.C.T. and Allen, S.J. (1982).

Choline acetyltransferase activity and histopathology of frontal neocortex from biopsies of demented patients.

J. Neurological Sciences, 57, 191 - 202.

Bowen, D.M. and Davison, A.N., (1980).

Biochemical changes in the cholinergic system of the ageing brain and in senile dementia.

Psychol. Med. 10(2), 315 - 319.

Bowen, D.M., Sims, N.R. and Davison, A.N., (1982).

Neurochemistry of Alzheimer's Disease: An Update.

Exp. Brain Research, Suppl. 5, 127 - 132.

Bowen, D.M., Smith, C.B., White, P. and Davison, A.N., (1976).

Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies.

Brain. 99, 459 - 496.

Bowen, D.M., Smith, C.B., White, P., Goodhardt, M.J., Spillane, J.A., Flack, R.H.A. and Davison, A.N., (1977).

Chemical pathology of the organic dementias. Part I, Vailidity of biochemical measurements in human post-mortem brains.

Brain, 100, 397-426.

Bradford, H.F., (1973).

Cerebral cortex slices and synaptosomes: in vitro approaches to brain metabolism.

In: Methods in Neurochemistry, 3, Ed: Fried, R., pp. 155-202.

Branconnier, P. and Cole, J.O., (1976).

Senile dementia and drug therapy.

Boston State Hospital Report, Boston, Mass.

Briggs, R.S., (1980).

Effects of drugs on acetylcholine synthesis.

Clinical Science, 59, 19P.

Brunello, N. and Cheney, D.L., (1981).

The septo-hippocampal cholinergic pathway. Role in antagonism of pentobarbital anaesthesia and regulation by various afferents.

J. Exp. Pharm. and Ther. 219(2), 489-495.

Burton, M. and Fitzgerald, R.E., (1980).

Failure to find projections from substantia innominata to dorsal neocortex in the rat.

Neuroscience letters, Suppl. 5, S189.

Butcher, L.L. and Rogers, R.C., (1978).

Histochemical effects of kainic acid on neostriatal dopamine and acetylcholinesterase.

European J. Pharmacol., 50, 287-289.

Butcher, L.L., Talbot, K. and Bilezikjian, L., (1975).

Acetylcholinesterase neurones in dopamine containing regions of the brain.

J. Neural. Trans., 37, 127-153.

Campochiaro, P. and Coyle, J.T., (1978).

Ontogenetic development of kainate neurotoxicity: correlates with glutamatergic innervation.

Proceedings of the National Academy of Sciences, 75, 2025-2029.

Candy, J.M., Perry, R.H., Perry, E.K., Irving, D., Blessed, G., Fairbairn, A.F. and Tomlinson, B.E., (1983).

Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases.

J. Neurological Sciences, 54:59, 277-289.

Charington, C.B. and Taberner, P.V., (1981).

Effects of antioxidants on glutamate decarboxylase activity.

Biochemical Pharmacology, 30(13), 1855-1856.

Christie, J.E., Balckburn, I.M., Glen, A.I.M., Zeisel, S., Shering, A. and Yates, C.M., (1979).

Effects of choline and lecithin on CSF choline levels and on cognitive function in patients with presenile dementia of the Alzheimer type.

In: Nutrition and the Brain, Volume 5,

Ed. Barbeau, A., Growdon, J.H. and Wurtman, R.J.

pp. 377-388, Raven Press, Now York.

Conrad, L.C.A. and Pfaff, D.W., (1976).

Autoradiographic tracing of nucleus accumbens efferents in the rat.

Brain Research, 113, 589-596.

Cooper, D.O. and Schmidt, D.E., (1980).

The use of choline acetyltransferase as a cholinergic marker in the determination of high affinity choline uptake.

J. Neurochem., 34,(6), 1553-1556.

Coyle, J.T., (1981).

In Excitotoxin models of Huntington's disease: Report of a Mary Jennifer Selznick workshop.

Ed. Ferkany, J.W. pp. 7-12.

Coyle, J.T., (1982).

Development of neurotransmitters in the neocortex.

Neurosciences Research Program Bulletin, 20, (4), 479-491.

Coyle, J.T., (1983).

Neurotoxic action of kainic acid.

J. Neurochem., 41 (1), 1-11.

Coyle, J.T., Molliver, M.E. and Kuhar, M.J., (1978).

In situ injection of kainic acid: a new method of selectively

lesioning neuronal cell bodies while sparing axons of passage.
J. Comp. Neurol., 180, (2), 301 - 325.

Coyle, J.T. and Schwarcz, R., (1976).
Lesion of striatal neurones with kainic acid provides a model of Huntington's chorea!
Nature, 263, 244 - 246.

Cross, A.J., Crow, T.J., Perry, E.K., Perry, R.H., Blessed, G. and Tomlinson, B.E., (1981).
Reduced dopamine-β-hydroxylase activity in Alzheimer's disease.
Brit. Med. J., 282, 93 - 94.

Davies, P., (1978).
Studies on the neurochemistry of central cholinergic systems is Alzheimer's disease.
In: Alzheimer's disease: senile dementia and related disorders.
Eds: Katzman, R., Terry, R.D. and Blick, K.L.
Raven Press, New York, pp. 453 - 459.

Davies, P., (1979).
Neurotransmitter-related enzymes in senile dementia of the Alzheimer type.
Brain Res., 171, 319 - 327.

Davies, P. and Feisullin, S., (1981).
Post-mortem stability of alpha-bungarotoxin binding sites in mouse and human brain.
Brain Research., 216, 449 - 454.

Davies, P. and Feisullin, S., (1982).
A search for discrete cholinergic nuclei in the human ventral forebrain.
J. Neurochem., 39(6), 1743 - 1747.

Davies, P., Katzman, R. and Terry, R.D., (1980).
Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia.
Nature, 288, 279 - 280.

Davies, P. and Maloney, A.J.F., (1976).
Selective loss of central cholinergic neurones in Alzheimer's disease.
Lancet, II, 1403.

Davies, P. and Terry, R.D., (1981).

Cortical somatostatin-like immunoreactivity in cases of Alzheimer's disease and senile dementia of the Alzheimer type.

Neurobiol. Ageing. 2 (1),

Davies, P. and Verth, A.H., (1977).

Regional distribution of muscarinic acetylcholine receptors in normal and Alzheimer's-type demented brains.

Brain Reseach, 138, 385 - 392.

Davies, S. W. and Horn, N.M., (1982).

Selective amino acid lesions can differentiate cholinergic pathways from rat basal forebrain.

J. Physiol. (Lond.), 332, 109 - 110P.

De Long, M.R., (1971).

Activity of pallidal neurones during movement.

J. Neurophysiol. 34, 414 - 427.

Divac, I., (1975).

Magnocellular nuclei of the basal forebrain project to neocortex, brain stem and olfactory bulb. Review of some functional correlates.

Brain Research, 93, 385 - 398.

Divac, I., Fonnum, F. and Storm-Mathisen, J. (1977).

High affinity uptake of glutamate in terminals of cortical striatal axons.

Nature, 266, 377 - 378.

de Belleroche, J., (1983).

Receptor and behavioural changes following lesion of the substantia innominata of the rat; a model for Alzheimer's disease.

In: Research progress in dementia. (in press).

Delanoy, R.L. and Dunn, A.J., (1978).

Mouse brain deoxyglucose uptake after footshock, ACTH analogues, $\alpha$-MSH, corticosterone or lysine vasopressin.

Pharmacology, Biochemistry and Behaviour, 9, 21 - 26.

Den Hertog, A., (1976).

The effect of isoxsuprine on non-myelinated nerve fibres.

Eur. J. Pharmacol., 39, 283 - 286.

De Silva, L., (1977).

Biochemical mechanisms and management of choreiform movement disorders.

Drugs, 14 (4), 300 - 310.

Doty, R. W., (1983).

Nongeniculate afferents to striate cortex in Macaques.

J. Comp. Neurol., 218, 159 - 173.

Earley, C. J. and Leonard, B. E., (1978).

Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine, and several metabolites from brain tissue using disposable Bio-Rad columns packed with Sephadex G-10.

J. Pharmacological Methods, 1, 67 - 79.

Early, S. L., Michaelis, E. K. and Mertes, M. P., (1981).

Pharmacological specificity of synaptosomal and synaptic membrane γ-aminobutyric acid (GABA) transport processes.

Biochem. Pharmacol., 30 (10), 1105 - 1113.

Eckenstein, F. and Thoenen, H., (1983).

Cholinergic neurones in the rat cerebral cortex demonstrated by immunohistochemical localisation of choline acetyltransferase.

Neuroscience Letters, 36, 211 - 215.

Edwards, S. C., Nahorski, S. R. and Rogers, K. J., (1974).

In vivo changes of cerebral cyclic adenosine 3'5'-monophosphate induced by biogenic amines: Association with phosphorylase activation.

J. Neurochem., 22, 563 - 572.

El Badawi, A. and Schenk, E. A., (1967).

Histochemical methods for separate, consecutive and simultaneous demonstration of acetyl cholinesterase and norepinephrine in cryostat sections.

J. Histochem. Cytochem., 15, 580 - 588μ

Emson, P. C. and Lindvall, O. (1979).

Distribution of putative neurotransmitters in the neocortex.

Neuroscience, 4, 1 - 30.

Emson, P.C., Paxinos, G., Le Gal La Salle, G., Ben-Ari, Y. and Silver, A., (1979).

Choline acetyltransferase and acetylcholinesterase containing projections from the basal forebrain to the amygdaloid complex of the rat.

Brain Research, 165, 271 - 282.

Engelhardt, G., (1976).

Pharmacologisches Wirkungsprofil von NAB 365 (clenbuterol) einem neven Broncholyticum mit einer selectiven wirkung auf die adrenergen $\beta_2$-receptoren.

Arzenmittelforsch, 26, 1404 - 1420.

Etienne, P., Gauthier, S., Dastoor, D., Collier, B. and Ratner, J., (1979).

Alzheimer's disease: clinical effects of lecithin treatment.

In: Nutrition and the Brain, Vol. 5, pp. 389 - 396.

Eds: Barbeau, A., Growdon, J.H. and Wurtman, R.J.

Raven Press, New York.

Eugster, C.H., (1968).

Wirkstoffe aus dem Fliegenpolz.

Naturwissen-Schaften, 55, 305 - 313.

Falck, B., (1962).

Observations on the possibilities of the cellular localisation of monoamines by a fluorescence method.

Acta Physiol. Scand., 56, Suppl. 197.

Falck, B., Hillarp, N.A., Thieme, G. and Torp, A., (1962).

Fluorescence of catecholamines and related compounds condensed with formaldehyde.

J. Histochem. Cytochem., 10, 348 - 354.

Farrar, D.G. and Verity, A., (1976).

Analysis of the effects of isoxsuprine on guinea-pig atria and trachea.

Brit. J. Pharmacol., 48, 432P.

Fazekas, J.F. and Alman, R.W., (1964).

Comparative effects of isoxsuprine and carbon dioxide on cerebral haemodynamics.

Am. J. Med. Sci., 248, 16 - 19.

Fibiger, H.C., (1982).

The organisation and some projections of cholinergic neurones of the mammalian forebrain.

Brain Research Reviews. 4, 327 - 388.

Fibiger, H.C. and Lehmann, J., (1981).

Anatomical organisation of some cholinergic systems in the mammalian forebrain.

In: Advances in behavioural Biology, 25, Cholinergic Mechanisms, 663 - 672.

Fonnum, F., (1975a).

A rapid radiochemical method for the determination of choline acetyltransferase.

J. Neurochem., 24, 407 - 409.

Fonnum, F., (1975b).

Radiochemical assay for choline acetyltransferase and acetylcholinesterase.

In: Research Methods in Neurochemistry, Volume 3.

Eds: Marks and Rodnight, pp. 253 - 274.

Raven Press, New York.

Fonnum, F., Karlsen, R.L., Malthe-Sørenssen, D., Skerde, K.K. and Walaas, I., (1979).

Localisation of neurotransmitters, particularly glutamate, in hippocampus, septum, nucleus accumbens and superior colliculus.

Progress In Brain Research, 51, 167 - 191.

Fonnum, F. and Walaas, I., (1978).

The effect of intrahippocampal kainic acid injections and surgical lesions on neurotransmitters in hippocampus and septum.

J. Neurochem., 31, 1173 - 1181.

Foster, A.C. and Roberts, P.J., (1978).

High affinity[L-$^{3}$H]glutamate binding to post-synaptic receptor sites or rat cerebellar synaptic membranes.

J. Neurochem., 31, 1467 - 1477.

Foster, G.A., (1983).

Post synaptic effects of excitatory amino acids in the rat cerebellum and cyclic GMP.

Ph.D. Thesis, Southampton University.

Friede, R.L., (1962).

The relation of the formation of lipofuscin to the distribution of oxidative enzymes in the human brain.

Acta. Neuropathol., 2, 113 - 125.

Friedle, N.M., Kelly, P.H. and Moore, M.E., (1978).

Regional brain atrophy and reductions in glutamate release and uptake after intrastriatal kainic acid.

Brit. J. Pharmacol., 63, 151 - 159.

Gaddy, J.R., Britt, M.D., Neill, D.B. and Haigler, H.J., (1979).

Susceptibility of rat neostriatum to damage by kainic acid: age dependence.

Brain Research. 176, 192 - 196.

Gage, F.H., Bjorklund, A. and Stenevi, U., (1983).

Local regulation of compensatory noradrenergic hyperactivity in the partially denervated hippocampus.

Nature, 303, 819 - 921.

Gibson, G.E. and Peterson, C., (1983).

Acetylcholine and oxidative metabolism in septum and hippocampus in vitro.

J. Biol. Chem. 258, (2), 1142 - 1145.

Glick, S.D., Meibach, R.C., Cox, R.D. and Maayani, S., (1979).

Multiple and interrelated function asymmetries in rat brain.

Life Science, 25, 395 - 400.

Gottesfeld, Z. and Jacobowitz, D.M., (1979).

Kainic acid-induced neurotoxicity in the striatum, a histofluorescent study.

Brain Research, 169, 513 -

Green, J.R., Halpern, L.M. and Van Niel, S., (1970).

Alterations in the activity of selected enzymes in chronic isolated cerebral cortex of the rat.

Brain, 93, 57 - 64.

Guldin, W.O. and Markowitsch, H.J., (1981).

No detectable remote lesions following massive intra-striatal injections of ibotenic acid.

Brain Research, 225, 446-451.

Guyenet, P., Lefresne, P., Rossier, J., Beaujovan, J.C. and Glowinski, J., (1973).

Inhibition by hemicholinium-3 of [$^{14}$C] acetylcholine synthesis and [$^{3}$H] choline high affinity uptake in rat striatal synaptosomes.

J. Molec. Pharmacol., 9, 630-639.

Haring, J.H. and Davis, J.N., (1983).

Acetylcholinesterase neurones in the lateral hypothalamus project to the spinal cord.

Brain Research, 268, 275-283.

Hartgraves, S.L., Mensah, P.L. and Kelly, P.H., (1979).

Effects of subcortical lesions on neocortical cholinergic markers.

Neuroscience Abstracts, 5, 590.

Hartgraves, S.L., Mensah, P.L. and Kelly, P.H. (1982).

Regional decrease of cortical choline acetyltransferase after lesions of the septal area and in the area of nucleus basalis magnocellularis.

Neuroscience, 7 (10), 2369-2376.

Hattori, T. and McGeer, E.G., (1977).

Fine structural changes in the rat striatum after local injections of kainic acid.

Brain Research, 129, 174-186.

Haubrich, D.R. and Chippendale, T.J., (1977).

Regulation of acetylcholine synthesis in nervous tissue.

Life Science, 20, 1465-1478.

Hebb, C.O., Krnjevic, K. and Silver, A., (1963).

Effect of undercutting on the acetylcholinesterase and choline acetyltransferase activity in the cats cerebral cortex.

Nature (Lond.), 198, 692.

Hejti, F. and Melamed, E., (1980).

L-Dopa's mechanism of action in Parkinson's disease.

Trends in Neurosciences, 3, 229-232.

Heimer, L., Switzer, R.D. and Van Hoesen, G.W., (1982).
Ventral striatum and ventral pallidum: components of the motor systems.
Trends in Neurosciences. 5 (3), 83 - 88.

Heimer, L. and Wilson, R.D., (1975).
In: Golgi Centennial Symposium
Eds: Santini, M., pp. 177 - 193. Raven Press, New York.

Henke, H. and Lang, W., (1983).
Cholinergic enzymes in neocortex, hippocampus and basal forebrain of non-neurological and senile dementia of Alzheimer type patients.
Brain Research, 267, 281 - 291.

Hersh, L.B., (1980).
Studies on the kinetic mechanism and salt activation of bovine brain choline acetyltransferase.
J. Neurochem., 34 (5), 1077 - 1081.

Hornykiewicz, O., (1966).
Dopamine (3-hydroxytyramine) and brain function.
Pharmacol. Rev., 18, 925 - 964.

Hornykiewicz, O., (1973).
'Parkinson's disease: from brain homogenates to treatment'.
Federation Proceedings. 32, 183 - 190.

Hornykiewicz, O., (1983).
Ageing and Brain dopamine.
In: Research Progress in dementia. (in press).

Iijima, K., Shantha, T.R. and Bourne, G.H., (1968).
Histochemical studies on the nucleus basalis of Meynert of the squirrel monkey (Saimiri Saureus).
Acta Histochem., 30, 96 - 108.

Iversen, L.L. and Kelly, J.S., (1973).
Uptake and metabolism of γ-aminobutyric acid by neurones and glial cells.
Biochem. Pharmacol., 24, 933 - 938.

Jenden, D.J., (1979).

The neurochemical basis of acetylcholine precursor loading as a therapeutic strategy.

In: Brain acetycholine and neuropsychiatric disease.

Eds: Davis, K.L. and Berger, P.A.

Plenum Press, New York, 483-513.

Jerusalinsky, D., Medina, J.H. and De Robertis, E., (1983).

Lesion of forebrain nuclei reveals possible presynaptic cholinergic muscarinic receptors in rat cerebral cortex.

Neuropharmacology, 22 (7), 835-838.

Johnson, J.L., (1978).

The excitant amino acids glutamic and aspartic acid as transmitter candidates in the vertebrate central nervous system.

Prog. Neurobiol. 10, 155-202.

Johnston, M.V., Carman, A.B. and Coyle, J.T., (1981).

Effects of fetal treatment with methylazoxymethanol acetate at various gestational dates on the neurochemistry of the adult neocortex of the rat.

J. Neurochem., 36, 124-128.

Johnston, M.V. and Coyle, J.T., (1979).

Laminar distribution of cholinergic innervation in rat neocortex. Lesions of extrinsic and intrinsic components.

Neuroscience Abstracts, 5, 116.

Johnston, M.V. and Coyle, J.T., (1979).

Histological and neurochemical effects of fetal treatment with methylazoxymethanol on rat neocortex in adulthood.

Brain Research, 170, 135-155.

Johnston, M.V. and Coyle, J.T., (1980).

Ontogeny of neurochemical markers for noradrenergic, GABA-ergic and cholinergic neurones in neocortex lesioned with methylazoxymethanol acetate.

J. Neurochem., 34, 1429-1441.

Johnston, M.V., McKinney, M. and Coyle, J.T., (1979).
Evidence for a cholinergic projection to neocortex from neurones in basal forebrain.
Proc. Natl. Adac. Sci., 7 (10), 5392 - 5396.

Johnston, M.V., McKinney, M. and Coyle, J.T., (1981).
Neocortical cholinergic innervation: a description of extrinsic and intrinsic components in the rat.
Exp. Brain Research., 43, 159 - 172.

Johnston, M.V., Young, A.C. and Coyle, J.T., (1981).
Laminar distribution of cholinergic markers in neocortex: effects of lesions.
J. Neuroscience Research, 6, 597 - 607.

Jones, E.G., Burton, H., Saper, C.B. and Swanson, L.W., (1976).
Midbrain diencephalic and cortical relationships of the basal nucleus of Meynert and associated structures in primates.
J. Comp. Neurol., 167, 385 - 420.

Jonnson, G., (1980).
Chemical neurotoxins as denervation tools in Neurobiology.
Annual. Rev. Neurosci., 3, 169 - 187.

Jope, R.S., (1979).
High affinity choline transport and acetyl CoA production in brain and their roles in the regulation of acetylcholine synthesis.
Brain Research Reviews. 1, 313 - 344.

Kanazawa, I., Iversen, L.L. and Kelly, J.S., (1976).
Glutamate decarboxylase activity in the rat posterior pituitary, pineal gland, dorsal root ganglion and superior cervical ganglion.
J. Neurochem., 27, 1267 - 1269.

Karczmar, A.G., (1969).
Is the central cholinergic nervous system over exploited?
Fed. Proc., 28, 147 - 157.

Karnovsky, M.J. and Roots, L., (1964).
A direct colouring thiocholine method for cholinesterases.
J. Histochem. Cytochem., 12, 219 - 221.

Kelly, P.H. and Moore, K.E., (1978).
Decrease of neocortical choline acetyltransferase after lesion of the globus pallidus in the rat.
Exp. Neurol., 61, 479-484.

Kievit, J. and Kuypers, H.G.J.M., (1975).
Basal forebrain and hypothalamic connections to frontal and parietal cortex in the rhesus monkey.
Science, 187, 660-662.

Kim, J.S., Hassler, R., Haug, P. and Paik, K.S., (1977).
The effect of frontal cortex ablation on striatal glutamic acid level in the rat.
Brain Research, 132, 370-374.

Kimura, H., McGeer, P.L., Peng, J.H. and McGeer, E.G., (1981).
The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat.
J. Comp. Neurol., 200, 151-201.

Klawans, H.L. and Weiner, W.J., (1976).
The pharmacology of choreatic movement disorders.
Prog. Neurobiol., 6, 49-81.

Koelle, G.B., (1955).
Histochemical identification of acetylcholinesterase in cholinergic adrenergic and sensory neurones.
J. Pharmacol. Exp. Ther., 114, 167-184.

Kohler, C., Schwarcz, R. and Fuxe, K., (1979).
Hippocampal lesions indicate differences between the excitotoxic properties of acidic amino-acids.
Brain Research, 175, 366-371.

Kohler, C., Schwarcz, R. and Fuxe, K., (1979).
Intrahippocampal injections of ibotenic acid provide histological evidence for a neurotoxic mechanism different from kainic acid.
Neuroscience Letters, 15, 223-228.

Kohler, C. and Schwarcz, R., (1983).
Comparison of ibotenate and kainate neurotoxicity in rat brain: a histological study.
Neuroscience, 8 (4), 819-835.

Konig, J.F.R. and Klippel, R.A., (1967).

The Rat Brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem.

Robert E. Krieger Publishing Co. Inc. New York.

Krieg, W.J.S., (1946).

Connections of the cerebral cortex. I. The Albino Rat.

A. Topography of cortical areas.

J. Comp. Neurol., 84, 221-227.

Krieg, W.J.S., (1946).

Connections of the cerebral cortex. I. The Albino Rat.

B. Structure of the cortical areas.

J. Comp. Neurol., 84 (3), 277-323.

Kristt, D.A., (1979).

Development of neocortical circuitry: Histochemical localisation of acetylcholinesterase in relation to the cell layers of rat somatosensory cortex.

J. Comp. Neurol., 186, 1-16.

Krnjevic, K. and Reinhardt, W., (1979).

Choline excites cortical neurones.

Science, 206, 1321-1323.

Kuhar, M.J. and Murrin, L.C., (1978).

Sodium-dependent high affinity choline uptake.

J. Neurochem., 30, 15-21.

Kuhar, M.J. and Simon, J.R., (1974).

Acetylcholine uptake: lack of association with cholinergic neurones.

J. Neurochem., 22, 1135-1137.

Kuhar, M.J. and Yamamura, H.I., (1976).

Localisation of cholinergic muscarinic receptors in rat brain by light microscopic radioautography.

Brain Research, 110, 229-243.

Labuszewski, T., Lockwood, R., McManus, F.E., Edelstein, L.R. and Lidsky, T.I., (1981).

Role of postural deficits in oro-ingestive problems caused by globus pallidus lesions.

Experimental Neurology, 74, 93-110.

Ladinsky, H., Consolo, S. and Pugnetti, P., (1979).
A possible central muscarinic receptor agonist role for choline in increasing rat striatal acetylcholine content.
In: Choline and Lecithin in brain disorders.
Eds: Barbeau, A., Growdon, J.H. and Wurtman, R.J.
Raven Press, New York, pp. 227-232.

Lehman, J. and Fibiger, H.C., (1979).
Acetylcholinesterase and the cholinergic neurone.
Life Sciences, 25, 1939-1947.

Lehman, J., Nagy, J.I., Atmadja, S. and Fibiger, H.C., (1980).
The nucleus basalis magnecellularis: The origin of a cholinergic projection to the neocortex of the rat.
Neuroscience, 5, 1161-1174.

Lindvall, O., Björklund, A., Falck, B. and Loren, I., (1981).
Recent developments in aldehyde-induced monoamine fluorescence: the aluminium-formaldehyde (ALFA) method applied to immature and adult central nervous tissue.
Histochemical J., 13, 583-598.

Lish, P.M., Dungan, K.W. and Peters, E.L., (1960).
A survey of the effects of isoxsuprine on nonvascular smooth muscle.
J. Pharmac. Exp. Ther., 129, 191-199.

Loren, I., Björklund, A., Falck, B.O. and Lindvall, O., (1976).
An improved histofluorescence procedure for freeze-dried paraffin-embedded tissue based on combined formaldehyde glyoxilic acid perfusion with high magnesium content and acid pH.
Histochemistry, 49, 177-192.

Loren, I., Björklund, A., Falck, B. and Lindvall, O., (1980).
The aluminum-formaldehyde (ALFA) histofluorescence method for improved visualisation of catecholamines and indoleamines.
I. A detailed account of the methodology for central nervous tissue using paraffin, cryostat or vibratome sections.
J. Neuroscience Methods, 2, 277-300.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951).

Protein measurement with Folin phenol reagent.

J. Biol. Chem., 193, 263-275.

Lynch, G., Matthews, D.A., Mosko, S., Parks, T. and Cotman, C., (1972).

Induced acetylcholinesterase-rich layer in rat dentate gyrus following entorhinal lesions.

Brain Research, 42, 311-318.

Lynch, G.S., Rose, G. and Gall, C.M., (1978).

Anatomical and functional aspects of the septo-hippocampal projections. In: Functions of the septo-hippocampal system.

CIBA Foundation Symposium, 58, 5-25.

McCaughran, J.A., Genovese, F.L. and Schecter, N., (1980).

The effect of kainic acid on cholinergic enzymes and receptors in the amygdala complex of the rat.

Brain Research, 199, 127-133.

McGeer, E.G., (1981).

Neurotransmitter systems in ageing and senile dementia.

Prog. NeuroPsychoPharmacol., 5, 435-445.

McGeer, E.G. and McGeer, P.L., (1976).

Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids.

Nature, 263, 517-519.

McGeer, E.G. and McGeer, P.L., (1978).

Some factors influencing the neurotoxicity of intrastriatal injections of kainic acid.

Neurochem. Res., 3, 501-517.

McGeer, E.G., McGeer, P.L. and Singh, K., (1978).

Kainate-induced degeneration of neostriatal neurones: Dependency on cortico-striatal tract.

Brain Res., 139, 381-383.

McGeer, P.L. and McGeer, E.G., (1982).

Kainic acid: the neurotoxic breakthrough.

CRC Critical reviews in toxicity, 1-26.

McGeer, P.L., McGeer, E.G. and Fibiger, H.C., (1973).
Choline acetylase and glutamic acid decarboxylase in Huntington's chorea, a preliminary study.
Neurology, 23, 912 - 917.

McGeer, P.L., McGeer, E.G. and Nagai, T., (1983).
GABAergic and cholinergic indices in various regions of rat brain after intracerebral injections of folic acid.
Brain Research., 260, 107 - 116.

McGeer, P.L., McGeer, E.G., Scherer, V. and Singh, K., (1977).
A glutamatergic corticostriatal path?
Brain Research, 128, 369 - 373.

McLennan, H., and Lodge, D., (1979).
The antagonism of amino acid-induced excitation of spinal neurones in the cat.
Brain Research., 169, 83 - 90.

Malthe-Sørensson, D.,Odden, E. and Walaas, I., (1980).
Selective destruction by kainic acid or neurones innervated by putative glutamatergic afferents in septum and nucleus of the diagonal band.
Brain Research, 182, 461 - 465.

Marsden, C.D., (1980).
Animal models of extrapyramidal disease.
In: Animal models of Neurological Disease, pp. 269 - 278.
Eds: Clifford, F. and Behan, P.O., Pitman Medical.

Mason, S.T., (1981).
'Animal models of Huntington's chorea'.
Trends in Neurosciences, 4, 10 - 11.

Mason, S.T. and Fibiger, H.C., (1979).
On the specificity of kainic acid.
Science, 204, 1339 - 1341.

Meibach, R.C., Brown, L. and Brooks, F.H., (1978).
Histofluorescence of kainic acid induced striatal lesions.
Brain Research., 148, 219 - 223.

Meibach, R.C., Glick, S.D., Cox, R. and Maayani, S., (1979).
Localisation of phencyclidine-induced changes in brain energy

metabolism.

Nature (Lond.), 282, 625-626.

Meibach, R.C., Glick, S.D., Ross, D.A., Cox, R.D. and Maayani, S., (1980).

Intraperitoneal administration and other modifications of the 2-Deoxy-D-Glucose technique.

Brain Research., 195, 167-176.

Meibach, R.C. and Siegel, A., (1977).

Efferent connections of the septal area in the rat: An analysis utilising retrograde and anterograde transport methods.

Brain Research, 119, 1-20.

Mesulam, M.M., (1982).

In: Tracing Neural Connections with Horseradish peroxidase.

IBRO Handbook Series: Methods in the Neurosciences, Volume 2

J. Wiley and Sons.

Mesulam, M.M., Mufson, E.J., Levey, A.I. and Wainer, B.H., (1983).

Alzheimer's disease and the cholinergic innervation of neocortex by the nucleus basalis of Meynent (Ch 4).

In: Biological aspects of Alzheimer's disease, Ed: Katzman, R.

Cold Spring Harbour Laboratories, Cold Spring Harbour.

Mesulam, M.M., Mufson, E.J., Levey, A.I. and Wainer, B.H. (1983).

Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey.

J. Comp. Neurol., 214, 170-197.

Mesulam, M.M. and Van Hoesen, C.W., (1976).

Acetylcholinesterase-rich projections from the basal-forebrain of the rhesus monkey to neocortex.

Brain Research, 109, 152-157.

Moore, R.Y., (1982).

In: Neuroanatomical tract tracing methods.

Eds: Heimer, L. and Robards, M.J., Plennum Press.

Morrison, J.H., Benoit, R., Magistretti, P.J. and Bloom, F.E., (1983).

Immunohistochemical distribution of pro-somatostatin-related peptides in cerebral cortex.

Brain Research, 262, 344 - 351.

Morrison, J.H. and Magistretti, P.J., (1983).

Monoamines and peptides in cerebral cortex - contrasting principles of cortical organisation.

Trends in Neurosciences, 6 (4), 146 - 151.

Mustafa, A.A., (1982).

Behavioural studies on dopamine receptors in the central nervous system of the rat.

Ph.D. Thesis, University of Southampton.

Nadler, J.V., Evenson, D.A. and Cuthbertson, G.J., (1981).

Comparative toxicity of kainic acid and other acidic amino acids towards rat hippocampal neurones.

Neuroscience, 6, 2505 - 2517.

Nadler, J.V., Perry, B.W. and Cotman, C.W., (1978).

Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells.

Nature (Lond.), 271, 676 - 677.

Nagai, T., Kimura, H., Maeda, T., McGeer, P.L. and McGeer, E.G., (1982).

Cholinergic projections from the basal forebrain of rat to the amygdala.

J. Neuroscience, 2 (4), 513 - 520.

Nagai, T., McGeer, P.L., Peng, J.H., McGeer, E.G. and Dolman, C.E., (1983).

Choline acetyltransferase immunohistochemistry in brains of Alzheimer's disease patients and controls.

Neuroscience letters, 26, 195 - 199.

Nahorski, S.R. and Rogers, K.J., (1975).

The role of catecholamines in the action of amphetamines and L-Dopa on cerebral energy metabolism.

Neuropharmacology, 14, 283 - 290.

Nahorski, S.R., Rogers, K.J. and Edwards, C., (1975).
Cerebral glycogenolysis and stimulation of β-adrenoceptors and histamine $H_2$ receptors.
Brain Research, 92, 529-533.

Nauta, W.J., Smith, G.P., Faull, R.L. and Domesick, V.B., (1978).
Efferent connections and nigral afferents of the nucleus accumbens septi in the rat.
Neuroscience, 3, 385-401.

O'Donnell, S.R., (1976).
Selectivity of clenbuterol (NAB 365) in guinea-pig isolated tissues containing β-adrenoceptors.
Arch. Int. Pharmacocyn, 224, 190-198,

Olney, J.W., (1981).
Kainic acid and other excitotoxins: a comparative analysis.
In: Glutamate as a transmitter, Eds: Di Chiara and Gessa.
Raven Press, New York, pp. 375-382.

Parent, A. and Butcher, L.L., (1976).
Organisation and morphologies of acetylcholinesterase-containing neurones in thalamus and hypothalamus of the rat.
J. Comp. Neurol., 170, 205-226.

Pearson, R.C.A., Gatter, K.C. and Powell, T.P.S., (1983).
Retrograde cell degeneration in the basal nucleus in monkey and man.
Brain Research, 261, 321-326.

Pedata, F., Lo Conte, G., Sorbi, S., Marconcini-Pepeu, I. and Pepeu, G., (1982).
Changes in high affinity choline uptake in rat cortex following lesions of the magnocellular forebrain nuclei.
Brain Research, 223, 359-367.

Perez de la Mora, M., Possani, L.D., Tapia, R., Teran, L., Palacios, R., Fuxe, K., Hökfelt, T. and Ljungdahl, A., (1981).
Demonstration of central γ-aminobutyrate-containing nerve terminals by means of antibodies against glutamate decarboxylase.
Neuroscience, 6, 875-895.

Perry, E.K., (1980).

The cholinergic system in old age and Alzherimer's disease.

Age and Ageing, 9 (1), 1 - 8.

Perry, E.K. and Perry, R.H., (1980).

The cholinergic system in Alzheimer's disease.

In: Biochemistry of dementia, pp. 135 - 183.

Ed: Roberts, P.J., J. Wiley, Chichester.

Perry, E.K. and Perry, R.H., (1982).

Neurotransmitter and Neuropeptide systems in Alzheimer-type dementia .

Exp. Brain Res., Suppl. 5, The Ageing Brain, 141 - 145.

Perry, E.K., Perry, R.H., Blessed, G. and Tomlinson, B.E. (1977).

Necroscopy evidence of central cholinergic deficits in senile dementia.

Lancet, I, 189.

Perry, E.K., Tomlinson, B.E., Blessed, G., Bergmann, K., Gibson, P.H. and Perry, R.H., (1978).

Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia.

Br. Med. J., 2, 1427 - 1429.

Perry, E.K., Tomlinson, B.E., Blessed, G., Perry, R.H., Cross, A.J. and Crow, T.J., (1981).

Neuropathological and biochemical observations on the noradrenergic system in Alzheimer's disease.

J. Neurological Sciences, 51, 279 - 287.

Perry, R.H., Blessed, G., Perry, E.K. and Tomlinson, B., (1980).

Histochemical observations on cholinesterase activities in the brains of elderly normal and demented (Alzheimer-type) patients.

Age Ageing, 9, 9 - 16.

Perry, R.H., Candy, J.M., Perry, E.K., Irving, D., Blessed, G., Fairbairn, A.F. and Tomlinson, B.E., (1982).

Extensive loss of choline acetyltransferase activity is not reflected by neuronal loss in the nucleus of Meynert in

Alzheimer's disease.

Neuroscience letters, 33, 311 - 315.

Pert, C.B. and Snyder, S.H., (1974).

High affinity transport of choline into the myenteric plexus of guinea pig intestine.

J. Pharmac. Exp. Ther., 191, 102 - 108.

Peterson, G.M. and Moore, R.Y., (1980).

Selective effects of kainic acid on diencephalic neurones.

Brain Research, 202, 165 - 182.

Pisa, M., (1982).

In: 'Excitotoxin models of Huntington's Disease': Report of Jennifer Selznick Workshop.

Ed. Ferrany, J.W. pp. 11 - 12.

Pisa, M., (1982).

'Kainate model of Huntington's Disease: chorea or no chorea?

Trends in Neurosciences, 5, 36.

Pisa, M., Sanberg, P.R., Corcoran, M.E. and Fibiger, H.C., (1980).

Spontaneously recurrent seizures after intra cerebral injections of kainic acid in rat: a possible model of temporal lobe epilepsy.

Brain Research, 200, 481 - 487.

Price, D.L., Whitehouse, P.J., Struble, R.G., Clark, A.W., Coyle, J.T., De Long, M.R. and Hedreen, J.C., (1982).

Basal forebrain cholinergic systems in Alzheimer's disease and related dementias.

Neuroscience Commentaries, 1 (2), 84 - 92.

Quach, T.T., Rose, C. and Schwarcz, J.C., (1978).

[$^3$H] Glycogen hydrolysis in brain slices: responses to neurotransmitters and modulation of noradrenaline receptors.

J. Neurochem., 30, 1335 - 1341.

Reinstein, D.K., Isaacson, R.L. and Dunn, A.J., (1979).

Regional changes in 2-deoxyglucose uptake after neocortical and hippocampal destruction.

Brain Research, 175, 392 - 397.

Reis, D.J. and Ross, R.A., (1973).

Dynamic changes in brain dopamine-β-hydroxylase activity during anterograde and retrograde reactions to injury of central noradrenergic axons.

Brain Research, 57, 307 - 326.

Reisine, T.D., Yamamura, H.I., Bird, E.D., Spokes, E. and Enna, S.J., (1978).

Pre- and postsynaptic neurochemical alterations in Alzheimer's disease.

Brain Research, 159, 477 - 481.

Rolls, E.T., (1983).

The initiation of movements in neural coding of motor performance.

Experimental Brain Research Suppl. 7, 97 - 114.

Rolls, E.T., Burton, M.J. and Mora, F., (1976).

Hypothalmic neuronal responses associated with the sight of food.

Brain Research, 111, 53 - 66.

Rolls, E.T., Roper-Hall, A. and Sanghera, M.K., (1977).

Activity of neurones in the substantia innominata and lateral hypothalamus during the initiation of feeding in the monkey.

J. Physiol. (Lond.), 272, 24P.

Rolls, E.L., Sanghera, M.K. and Roper-Hall, A., (1979).

The latency of activation of neurones in the lateral hypothalamus and substantia innominata during feeding in the monkey.

Brain Research, 164, 121 - 135.

Ross, S.B., (1980).

Antagonism of reserpine-induced hypothermia in mice by some β-adrenoceptor agonists.

Acta. Pharmacol. et Toxicol., 47, 347 - 350.

Rossier, J., (1977).

Choline acetyltransferase, a review with special reference to its cellular and subcellular localisation.

Int. Rev. Neurobiol., 20, 283 - 337.

Rossier, J., Spantidakis, Y. and Benda, P., (1977).

The effect of $Cl^-$ on choline acetyltransferase kinetic parameters and a proposed role for $Cl^-$ in the regulation of acetylcholine synthesis.

J. Neurochem., 29, 1007 - 1012.

Rossor, M.N., Emson, P.C., Mountjoy, C.Q., Roth, M. and Iversen, L.L., (1980).

Reduced amounts of immunoreactive somatostatin in the temporal cortex in senile dementia of Alzheimer type.

Neuroscience letters, 20, 373 - 377.

Rossor, M.N., Emson, P.C., Mountjoy, C.Q., Roth, M. and Iversen, L.L., (1982).

Neurotransmitters of the cerebral cortex in senile dementia of Alzheimer cortex.

Exp. Brain Res. Suppl. 5, 153 - 157.

Rossor, M.N., Fahrenkrug, J., Emson, P.C., Mountjoy, C.Q., Iversen, L.L. and Roth, M., (1980).

Reduced cortical choline acetyltransferase activity in senile dementia of Alzheimer type is not accompanied by changes in vasoactive intestinal polypeptide.

Brain Research, 201, 249 - 253.

Rossor, M.N., Garrett, N.J., Johnson, A.L., Mountjoy, C.Q., Roth, M. and Iversen, L.L., (1982).

A post-mortem study of the cholinergic and GABA systems in senile dementia.

Brain, 105, 313 - 330.

Rossor, M.N., Rehfeld, J.F., Emson, P.C., Mountjoy, C.Q., Roth, M. and Iversen, L.L., (1981).

Normal cortical concentration of cholecystokinin-like immunoreactivity with reduced choline acetyltransferase activity in senile dementia of Alzheimer type.

Life Sciences, 29, 405 - 410.

Rossor, M.N., Svendsen, C., Hunt, S.P., Mountjoy, C.Q., Roth, M. and Iversen, L.L., (1982).

The substantia innominata in Alzheimer's disease: an histochemical and biochemical study of cholinergic marker enzymes.

Neuroscience letters, 28, 217-222.

Rowlands, G.J. and Roberts, P.J., (1980).

Specific calcium-dependent release of endogenous glutamate from rat striatum is reduced by destruction of the cortico-striatal tract.

Exp. Brain Res., 39, 239-240.

Sachs, C. and Jonsonn, G., (1975).

Mechanisms of action of 6-hydroxy-dopamine .

Biochem. Pharmacol., 24, 1-8.

Sanberg, P.R., Pisa, M. and Fibiger, H.C., (1981).

Kainic acid injections in the striatum alter the cataleptic and locomotor effects of drugs influencing dopaminergic and cholinergic systems.

Eur. J. Pharmacology, 74, 347-357.

Sanberg, P.R., Pisa, M. and McGeer, E.G., (1979).

Strain differences and kainic acid neurotoxicity.

Brain Res., 166, 431-435.

Savaki, H.E., Davidsen, L., Smith, C. and Sokoloff, L., (1980).

Measurement of free glucose turnover in brain.

J. Neurochem., 35 (2), 495-502.

Schmued, L.C., Swanson, L.W. and Sawchenko, P.E., (1982).

Some fluorescent counterstains for neuroanatomical studies.

J. Histochem. Cytochem., 30 (2), 123-128.

Schon, F. and Kelly, J.S., (1975),

Selective uptake of [$^3$H] β-alanine by glia: association with the glial uptake system of GABA.

Brain Research., 86, 243-257.

Schultz, W., (1982).

Depletion of dopamine in the striatum as an experimental model of parkinsonism: direct effects and adaptive mechanisms.

Progress in Neurobiology, 18, 121-166.

Schwarcz, R., (1983).

Neurotoxic properties of ibotenate and endogenous acidic amino acids.

In: Excitotoxins. Proceeding of the Werner-Gren Symposium, Eds: Fuxe, K., Schwarcz, R. and Roberts, P.J.

Pergammon Press, Oxford.

Schwarcz, R. and Fuxe, K., (1979).

[$^3$H] kainic acid binding: relevance for evaluating the neurotoxicity of kainic acid.

Life Sciences, 24, 1471 - 1480.

Schwarcz, R. and Köhler, C., (1980).

Evidence against an exclusive role of glutamate in kainic acid neurotoxicity.

Neuroscience Letters, 19, 243 - 249.

Schwarcz, R., and Köhler, C., (1983).

Differential vulnerability of central neurones of the rat to Quinolinic acid.

Neuroscience Letters, 38, 85 - 90.

Schwarcz, R., Scholtz, D. and Coyle, J.T., (1978).

Structure-activity relations for the neurotoxicity of kainic acid derivatives and glutamate analogues.

Neuropharmacology, 17, 145 - 151.

Sherman, K., Kuste, J.E., Dean, R.L., Bartus, R.T. and Friedman, E., (1981).

Presynaptic cholinergic mechanisms in brain of aged rats with memory impairments.

Neurobiol. Ageing, 2 (2), 99 - 104.

Shute, C.C.D. and Lewis, P.R., (1967).

The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections.

Brain, 90, 497 - 522.

Simon, J.R., Atweh, S. and Kuhar, M.J., (1976).

Sodium dependent high affinity choline uptake: a regulatory step in the synthesis of acetylcholine.

J. Neurochem., 26, 909 - 922.

Sims, N.R., Bowen, D.M., Allen, S.J., Smith, C.C.T., Neary, D., Thomas, D.J. and Davison, A.N., (1983).

Presynaptic cholinergic dysfunction in patients with dementia.

J. Neurochem., 40 (2), 503 - 509.

Sims, N.R., Bowen, D.M., Smith, C.C.T., Flack, R.H.A., Davison, A.N., Snowden, J.S. and Neary, D., (1980).

Glucose metabolism and acetylcholine syntheis in relation to neuronal activity in Alzheimer's disease.

Lancet, I, 333 - 335.

Sims, N.R., Bowen, D.M., and Davison, A.N., (1981).

[$^{14}$C] acetylcholine synthesis and [$^{14}$C] carbon dioxide production from U - $^{14}$C glucose by tissue prisms from human neocortex.

Biochem. J., 196, 867 - 876.

Sofroniew, M.V., Eckenstein, F., Thoenen, H. and Cuello, A.C., (1982).

Topography of choline acetyltransferase-containing neurones in the forebrain of the rat.

Neuroscience letters, 33, 7 - 12.

Sokoloff, L., (1977).

Relation between physiological function and energy metabolism in the central nervous system.

J. Neurochem., 29, 13 - 26.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O. and Shinohara, M., (1977).

The [$^{14}$C] deoxyglucose method for the measurement of local cerebral glucose utilisation: theory, procedure and normal values in the conscious and anaesthetised albino rat.

J. Neurochem., 28, 897 - 916.

Sokoloff, L., Reivich, M., Patlak, C.S., Pettigrew, K.D., Des Rosiers, M. and Kennedy, C., (1974).

The [$^{14}$C] deoxyglucose technique for the quantiative determination of local cerebral glucose consumption.

Proc. 5th Meet. Am. Soc. Neurochem., p. 86.

Spokes, E.G.S., (1981).

The neurochemistry of Huntington's chorea .

Trends in Neurosciences, 4, 115 - 118.

Storm-Mathisen, J., (1978).

Localisation of putative transmitters in the hippocampal formation (with a note on the connections to septum and hypothalamus).

In: Functions of the Septo-Hippocampal system.

Elsevier, North-Holland, pp. 49 - 87.

Storm-Mathisen, J. and Woxen-Opsahl, M., (1978).

Aspartate and/or glutamate may be transmitters in hippocampal efferents to septum and hypothalamus.

Neuroscience Letters. 9, 65 - 70.

Struble, R.G., Cork, L.C., Whitehouse, P.J. and Price, D.L., (1982).

Cholinergic innervation in neuritic plaques.

Science, 216, 413 - 415.

Struble, R.G., McKinney, M., Sanberg, P.R., Kitt, C.A., De Long, M.R., Coyle, J.T. and Price, D.L., (1982).

Ibotenic acid lesions of the primate nucleus basalis of Meynert result in decreased cortical choline acetyltransferase.

Neuroscience Abstracts, 8, 211.

Swanson, L.W. and Cowan, W.M., (1975).

A note on the connections and development of the nucleus accumbens.

Brain Research, 105, 389 - 403.

Tagliavini, F. and Pilleri, G., (1983).

Neuronal counts in Basal Nucleus of Meynent in Alzheimer's Disease and in simple senile dementia.

Lancet, I, 469 - 470.

Terry, R.D. and Davies, P., (1980).

Dementia of the Alzheimer type.

Ann. Rev. Neuroscience, 3, 77 - 95.

Tomlinson, B.E., Blessed, G. and Roth, M., (1970).

'Observations on the brains of demented old people'.

J. Neurol. Sci., 11, 205 - 242.

Trabucchi, M., Cheney, D.L., Racagni, G. and Costa, E., (1975).
Pentobarbital and in vivo turnover rate of acetylcholine in mouse brain and in regions of rat brain.
Pharmacol. Res. Commun., 7, 81 - 94.

Troiano, R. and Siegel, A., (1978).
Efferent connections of the basal forebrain in the cat: the nucleus accumbens.
Experimental Neurology, 61, 185 - 197.

Troiano, R. and Siegel, A., (1978).
Efferent connections of the basal forebrain in the cat: the substantia innominata.
Exp. Neurol., 61, 198 - 213.

Uchida, T. and O'Brien, R.D., (1964).
The effects of hydrazines on rat brain 5-hydroxytryptamine, norepinephrine and GABA.
Biochem. Pharmacol., 13, 725 - 730.

Ulmar, G., Ljungdahl, Å. and Hökfelt, T., (1975).
Enzyme changes after undercutting of cerebral cortex in the rat.
Exp. Neurology, 46, 199 - 208.

Ungerstedt, U., (1968).
6-Hydroxydopamine induced degeneration of central monoamine neurones.
Eur. J. Pharmac., 5, 107 - 110.

Ungerstedt, U., (1971).
'Stereotaxic mapping of the monoamine pathway in rat brain'.
Acta. Physiol. Scand. Suppl. 367, 1 - 48.

Ungerstedt, U., Ljungberg, T., Hoffer, B. and Siggins, G., (1975).
Dopaminergic supersensitivity in the striatum.
In: Adv. Neurol., Vol. 9, pp. 57 - 65.
Eds: Calne, D.B., Chase, T.N. and Barbeau, A.,
Raven Press, New York.

Uretsky, N.J. and Iversen, L.L., (1970).
Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain .
J. Neurochem., 17, 269 - 278.

Wada, J.A. and McGeer, E.G., (1966).

Central aromatic amines and behaviour.

III. Correlative analysis of conditioned approach behaviour and brain levels of serotonin and catecholamines in monkeys.

Arch. Neurol., 14, 129 - 142.

Walaas, I. and Fonnum, F., (1979).

The distribution and origin of glutamate decarboxylase and choline acetytransferase in ventral pallidum and other basal forebrain regions.

Brain Research, 177, 325 - 336.

Walaas, I. and Fonnum, F., (1979).

The effects of surgical and chemical lesions on neurotransmitter candidates in nucleus accumbens.

Neuroscience, 4, 209 - 216.

Walker, L.C., Tigges, M. and Tigges, J., (1983).

Ultrastructure of neurones in the nucleus basalis of Meynert in squirrel monkey.

J. Comp. Neurol., 217, 158 - 166.

Wamsley, J.K., Zarbin, M.A., Birdsall, J.M. and Kuhar, M.J., (1980).

Muscarinic cholinergic receptors: autoradiographic localisation of high and low affinity agonist binding sites.

Brain Research, 200, 1 - 12.

Wechster, L.R., Savak, H.E. and Sokoloff, L., (1979).

Effects of D- and L-amphetamine on local cerebral glucose utilisation in the conscious rat.

J. Neurochem., 32, 15 - 22.

Wecker, L. and Dettbarn, W.D., (1979).

Relationship between choline availability and acetylcholine synthesis in discrete regions of rat brain.

J. Neurochem., 32, 961 - 967.

Welch, A.S. and Welch, B.L., (1969).

Solvent extraction method for simultaneous determination of norepinephrine, dopamine, serotonin and 5-hydroxyindolacetic acid in a single mouse brain.

Anal. Biochem., 30, 161 - 179

Wenk, H., Bigl. V. and Meyer, U., (1980).

Cholinergic projections from magnocellular nuclei of the basal forebrain to cortical areas in rats.

Brain Research Reviews, 2, 295 - 316.

Wenk, H., Bigl, V., Meyer, U. and Biesold, D., (1981).

Cholinergic pathways to the cerebral cortex in rats.

In: Advances in Behavioural Biology, 25, Cholinergic mechanisms. 673 - 683.

Eds: Pepeu, G. and Ladinsky, H., Plennum Press.

Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T. and De Long, M.R., (1981).

Alzheimer disease: evidence for selective loss of cholinergic neurones in the nucleus basalis.

Ann. Neurol., 10, 122 - 126.

Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T. and De Long, M.R., (1982).

Alzheimer's disease and senile dementia: loss of neurones in the basal forebrain.

Science, 215, 1237 - 1239.

White, P., Goodhardt, M.J., Keet, J.P., Hiley, C.R., Carrasco, L.H., Williams, I.E.I. and Bowen, D.M., (1977).

Neocortical cholinergic neurones in elderly people.

Lancet, i, 668 - 671.

* Wilcock, G.K., Esiri, M.M., Bowen, D.M. and Smith, C.C.T., (1982).

Alzheimer's disease, correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities.

J. Neurological Sciences, 57, 407 - 417.

Wilcock, G.K. and Esiri, M.M., (1983).

Age and Alzheimer's disease.

Lancet, II, 346.

Wilcock, G.K., Esiri, M.M., Bowen, D.M. and Smith, C.C.T., (1983).

The nucleus basalis in Alzheimer's disease: cell counts and

cortical biochemistry.

Neuropathology and Applied Neurobiology, 9, 175 - 179.

Williams, D.J., Crossman, A.R. and Slater, P., (1977).

The efferent projections of the nucleus accumbens in the rat.

Brain Research, 130, 217 - 227.

Wood, P.L. and Richard, J., (1982).

GABAergic regulation of the substantia innominata - cortical cholinergic pathway.

Neuropharmacology, 21, 969 - 972.

Woolf, N.J. and Butcher, L.L., (1982).

Cholinergic projections to the basolateral amygdala: a combined Evans blue and acetylcholinesterase analysis.

Brain Research Bulletin, 8, 751 - 763.

Wuerthele, S.M., Lovell, K.M., Jones, M.Z. and Moore, K.E., (1978).

A histological study of kainic acid-induced lesions in the rat brain.

Brain Res., 149, 489 - 497.

Wurtman, R.J., (1979).

Precursor control of transmitter synthesis.

In: Nutrition and the Brain, Volume 5, pp. 1 - 12.

Eds: Barbeau, A., Growdon, J.H. and Wurtman, R.J.

Raven Press, New York.

Yamamura, H.I. and Snyder, S.H., (1973).

High affinity transport of choline into synaptosomes of rat brain.

J. Neurochem., 21, 1335 - 1374.

Yates, C.M., Allison, Y., Simpson, J., Maloney, A.F.J. and Gordon, A., (1979).

Dopamine in Alzheimer's disease and senile dementia.

Lancet, ii, 851.

Yesavage, J.A., (1979).

Vasodilators in senile dementias. A review of the literature.

Arch. Gen. Psychiatry, 36, 220 - 223.

Zaczek, R., Collins, J. and Coyle, J.T., (1981).
N-Methyl-D-aspartic acid: a convulsant with weak neurotoxic properties.
Neuroscience Letters, 24, 181 - 186.

Zaczek, R., Nelson, M.F., and Coyle, J.T., (1978).
Effects of anaesthetics and anticonvulsants on the action of kainic acid in the rat hippocampus.
Eur. J. Pharmacology, 52, 323 - 327.

Zaczek, R., Simonton, R. and Coyle, J.T., (1980).
Local and distant neuronal degeneration following intrastriatal injection of kainic acid.
J. Neuropath. Exp. Neurol., 39, 245 - 264.

*Wieraszko, A., Oderfeld-Nowak, B. and Narkiewicz, O. (1977).
Ipsi and contralateral changes in acetylcholinesterase and choline acetyltransferase activities in the hippocampus following unilateral septal lesions in the rat.
Neuroscience, 2, 649 - 654.